Preparative Enantioselective Chromatography

Edited by Geoffrey B. Cox

Blackwell Publishing

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Preface

GEOFFREY B. COX

Life is chiral. The peptides, proteins and carbohydrates from which we are all made are generally available in only one configuration, made up of enantiopure building blocks. It is not so surprising that the enantiomers of even small molecules have different properties (physiological or pharmacological) when placed in the chiral environment of living organisms. This fact, underlined by the thalidomide tragedy played out in Europe some years ago, is at the basis of the current regulatory environment where the enantiomers of any drug candidate have to be tested individually for toxicity, activity and side effects and where, for the many cases in which the properties differ, only an enantiopure drug is deemed to be acceptable.

Enantioselective chromatography is generally accepted to be the most rapid route to the preparation of small quantities of pure enantiomers. In recent years, it has begun to be realised that chromatographic processing does not have to be the excessively expensive and wasteful process that many chemists and engineers have been educated to believe; given careful optimisation, chromatographic processes can be cost-competitive with the other routes to enantiopure materials.

Against this background of the expansion of use of preparative enantioselective chromatographic techniques throughout the industry, the need is clear for a book that brings together experiences from people who have been involved in the development, improvement and implementation of chromatographic purification at all scales from laboratory to production and using all techniques, HPLC, SFC and multicolumn operation. The book is not written by academics for teaching, although one can argue the benefits of having fresh graduates who understand something of the preparative separations they will be asked to perform. It has been written by those with a job to do for those who will work with them today and who tomorrow will have to follow on after them. These scientists will need the depth of understanding and practical experience that is contained within these pages. From a wider viewpoint, and especially at the larger scale of operation, there are many who are looking for answers about preparative and production-scale chromatography. In the scope of the various chapters, from dealing with the smallest to the largest scale, these people, too, can find the information they seek.

Many of the authors who have contributed to this book have been in and around the pharmaceutical industry at the cutting edge of the development of enantioselective preparative chromatography. All are recognised by their peers as being experts in their field. The aim of the book is to bring this expertise to answer many of the questions that are often raised:

- What is going on in the preparative column?
- What chiral stationary phase should one use?

- How can one develop preparative separation methods; are they different from analytical methods?
- How does one carry out preparative chromatography; what equipment should be used?
- What is involved in recycling, in SFC and multicolumn techniques such as SMB?
- What about production-scale chromatography; is it possible, economic and should it be done in-house or outsourced?

The book contains many case studies to illustrate the principles, although as these studies are from work carried out in the pharmaceutical industry the true identities of the compounds concerned are often shrouded in secrecy. This does not matter greatly, since the purpose is to illustrate what to do and how to do it rather than discuss the intimate details of molecular interactions (which in any case only too often descends into optimistic arm-waving when attempting to find explanations of why an enantioselective separation occurs – or does not occur). Collectively, we have tried to address the needs of most of the people involved in enantioselective chromatography, from the analytical or discovery chemist who needs to isolate a few milligrams of a new compound, through those who need to isolate the few grams or few tens of grams for further study, to the development chemists and engineers who have the job of isolating materials for clinical trials and eventually taking the decision if the chromatographic route is viable for manufacturing.

My thanks go to the contributors to the book who have somehow fitted this extra task into their very busy lives and who have accepted the prolonged gestation period as I have fitted my tasks as editor into mine. My thanks also go to Blackwell's editor, Paul Sayer, without whose patience and encouragement this book would still be something necessary but unavailable.

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1 Chiral chromatography in support of pharmaceutical process research

CHRISTOPHER J. WELCH

1.1 Introduction

Preparative chiral chromatography has recently become a preferred method for rapidly accessing enantiopure compounds in the pharmaceutical industry [1–8]. While preparative chromatographic enantioseparation has been practiced for a number of years by specialized researchers, the current widespread interest in the approach can be attributed in part to advances in equipment and stationary phases, but more importantly, to an increasingly widespread realization of the cost-effectiveness of this technique. In many instances, developing and executing a chromatographic enantioseparation is faster and less labour-intensive than more traditional approaches for accessing enantiopurity. Consequently, preparative chiral chromatography is increasingly used in place of, or in conjunction with, the more traditional methods of organic synthesis. We herein present a general introduction that focuses on some of the current areas of interest in the field of preparative chromatographic enantioseparation, which we hope will be useful to newcomers and experienced practitioners alike.

1.2 A brief introduction to chirality

If one imagines the set of all possible organic molecules with molecular weight less than 1000, it can readily be appreciated that most of these structures are *chiral*, i.e. they cannot be superimposed on their mirror images. Only those comparatively simple and symmetrical structures that show a plane, centre, or alternating axis of symmetry are *achiral*. The two mirror image forms of a chiral molecule are termed *enantiomers*, and a mixture composed of equal proportions of the two enantiomers is termed a *racemic mixture* or *racemate*. A mixture containing an excess of one enantiomer is said to be *enantioenriched*, while a mixture containing exclusively one enantiomer is said to be *enantiopure*. *Enantioenrichment* is typically reported in terms of *% e.e.* or *enantiomeric excess*. This is a somewhat antiquated term indicating the excess of pure enantiomer relative to the racemate, and given by the formula e.e. = (major − minor)/(major + minor), where major and minor denote the relative amounts of the more and less prevalent enantiomers. Enantioenrichment is also sometimes expressed in terms of *e.r.* or *enantiomeric ratio*, which is simply the ratio of the major to the minor enantiomer. Clearly, enantiopurity is an ideal that is only approximated in the real world, although the term 'enantiopure' is routinely used somewhat loosely to denote substances that are >98% e.e. A process whereby a racemic mixture is separated into its two component enantiomers is termed a *resolution*, and a process where enantioenriched mixture is converted to a racemate is termed a *racemization*. The interested reader is directed to Eliel's *Stereochemistry of Organic Compounds* [9] for a more comprehensive description of key stereochemical terminology.

1.3 Why chirality is important

Most of the molecules of importance to living systems are chiral, e.g. amino acids, sugars, proteins and nucleic acids. An interesting feature of these chiral biomolecules is that in nature they usually exist in only one of the two possible enantiomeric forms. When a chemist synthesizes a chiral molecule in an achiral environment using achiral starting materials, an equal mixture of the two possible enantiomers (i.e. a racemic mixture) is produced. In order to make just one enantiomer, some enantioenriched starting material, reagent, catalyst, or template must be present in the reaction medium. Oftentimes, only a single enantiomer of a chiral molecule is desired, as is the case when the target molecule is a chiral drug that will be used in living systems. Drug molecules can be likened to tiny keys that fit into locks in the body and elicit a particular biological response. Since the 'locks' in living organisms are chiral, and exist in only one of the two possible enantiomeric forms, only one enantiomer of the 'key' molecule should be used (the mirror image of our car key will not start our car). In general, the use of both enantiomers in a racemic formulation of a chiral drug may be wasteful, and sometimes even introduces extraneous material that may lead to undesired side effects or adverse reactions.

The importance of chirality has been appreciated and addressed by the pharmaceutical industry for decades. As technologies for measuring and making enantiopure materials have improved, the production of enantiopure pharmaceuticals has become commonplace, with many of the top selling drugs in the world now being sold in enantiopure form. Consequently, the subject of chirality and the pharmaceutical industry is a topic of considerable recent interest and importance [10–12].

1.4 Accessing enantiopurity: a brief overview of approaches

Chiral chromatography is but one of a number of methods for providing enantiopure compounds. While more detailed descriptions of each of these approaches can be found in individual textbooks, or in a generalized text on the subject of stereochemistry, some discussion of the *pros* and *cons* of these various approaches is appropriate for placing chromatographic enantioseparation in the appropriate context.

1.4.1 *Enantiopure starting materials: the chiral pool*

The most straightforward approach to accessing enantiopure materials is to use starting materials that are available from the 'chiral pool' of naturally occurring enantiopure materials (e.g. amino acids, sugars, terpenes). This approach is preferable when the starting materials are inexpensive and readily available, and when the synthetic sequence from the chiral pool material to the target compound is direct and straightforward. Synthesis from chiral pool starting materials is a tried and true approach that has been used for many years. The approach sometimes suffers from the disadvantage of requiring numerous synthetic steps to achieve the goal of proper insertion of stereochemistry into the target molecule, and so it is not practical for all syntheses. The art of transforming the chirality of natural materials into the required stereochemistry of biologically relevant target molecules has a long and rich history, which is evidenced in much of the body of classical natural product

synthesis. The subject is specifically treated in Hanessian's classic, *The Chiron Approach to Natural Product Synthesis* [13].

It is worth noting here that the chiral pool of naturally occurring enantiopure starting materials is continually being augmented by newly available commodity chemicals that are produced via new chirotechnology approaches. Consequently, much of modern enantioselective synthesis can be viewed as drawing from an 'expanded chiral pool' [14]. When viewed in this light, the importance of keeping up to date with newly emerging chirotechnology approaches and their associated enantiopure products can readily be appreciated.

1.4.2 *Removable enantioenriched auxiliaries*

An alternative approach to synthesis from chiral pool starting materials, also with a decades-long history, involves the use of removable enantioenriched auxiliaries to influence the stereochemistry at a newly formed stereogenic centre. After removal of the auxiliary, an enantioenriched product is obtained. Classic examples of this approach can be found in the work of chemists such as Evans [15,16], Meyers [17,18] and Seebach [19]. A wide variety of chiral auxiliaries have been used, but they most often are derived from enantiopure chiral pool starting materials such as amino acids. Advantages of the chiral auxiliary approach include predictability and dependability. Major disadvantages include the need for a full equivalent of the chiral auxiliary and the need to attach the auxiliary to the substrate and subsequently remove it after reaction. This requires a minimum of two additional steps, and sometimes considerably more. Consequently, this technique can be rather ungainly, especially at industrial scale, and has been criticized as an approach with poor *atom economy* [20,21] since a full equivalent of auxiliary is used and converted to waste for each stereocentre that is set. This problem can sometimes be addressed through recovery and recycling of the auxiliary. Nevertheless, the auxiliary approach is less frequently used today than in the past.

1.4.3 *Enantioselective catalysis*

Most of the disadvantages of the auxiliary approach are overcome with enantioselective catalysis [22] – a substoichiometric amount of an enantioenriched material is used to control product stereochemistry, and, in contrast to the auxiliary approach, the chemical steps needed for appending and then later removing the stereochemically determining component are eliminated. With enantioselective catalysis, the stereochemical outcome of the new bond formation is determined in the catalytic reaction that leads to the newly formed stereocentre. In addition, a single catalyst molecule can effect multiple transformations, millions in the best cases [23]. In such cases with high catalyst turnover, even relatively expensive catalysts can be used for economical production of enantiopure products. Although enzymes remain the prototypical enantioselective catalysts, and are extensively used in enantioselective synthesis, considerable progress in the development of *synthetic* enantioselective catalysis has occurred in recent years [24]. A wide variety of such catalysts are now available for many different kinds of synthetic transformations. In many cases these catalysts show a degree of substrate generality that renders them quite useful and predictable tools for enantioselective synthesis.

1.4.4 *Resolution technologies: introduction*

Resolution techniques in which the two enantiomers comprising a racemic mixture are physically separated are an important and extensively utilized family of approaches for accessing enantiopure materials on a large scale. The major resolution approaches, classical resolution via diastereomeric salt formation, resolution of conglomerate crystals, kinetic resolution and chromatographic resolution, will be described in more detail below. Initial considerations suggest that all resolution approaches suffer from a common fundamental drawback of being inherently wasteful, as at most only half of the material is recovered (the half corresponding to the desired enantiomer, the other half being 'waste'). However, recycling of the undesired enantiomer is sometimes possible, enabling higher yield and reduction of waste. Coupling of such resolution and racemization approaches can lead to truly impressive processes for generating enantiopurity [25], and such approaches have long been a mainstay of successful industrial-scale synthesis of enantiopure materials. The most widely utilized resolution technologies are summarized below.

1.4.4.1 *Resolution technologies: classical resolution* Classical resolution of enantiomers via diastereoselective salt formation is an important technique with a long history [26–28]. In this approach, a single enantiomer of a resolving agent is mixed with the two enantiomers of a racemate, leading to two diastereomeric salts with differing solubility properties, which are subsequently separated by crystallization. As resolving agents, naturally occurring acids or bases from the chiral pool have traditionally been used (e.g. tartaric acid, quinine), although in recent years, reagents derived from the 'expanded chiral pool' have also been used. Classical resolution remains one of the preferred methods for industrial-scale resolution. In a related technique, a chiral derivatization reagent can be used to prepare covalent diastereomeric derivatives, which are then separated by crystallization, chromatography or other means, and which afford the purified enantiomer upon deprotection.

1.4.4.2 *Resolution technologies: conglomerate resolution* Racemic materials can crystallize in several ways, as illustrated in Fig. 1.1. Most racemates crystallize as racemic compounds in which the two component enantiomers (R and S in the figure) are regularly arrayed within the crystal lattice in equal numbers. Thus, analysis of any single crystal always reveals a 1:1 ratio of the two component enantiomers. A few percent of all racemates

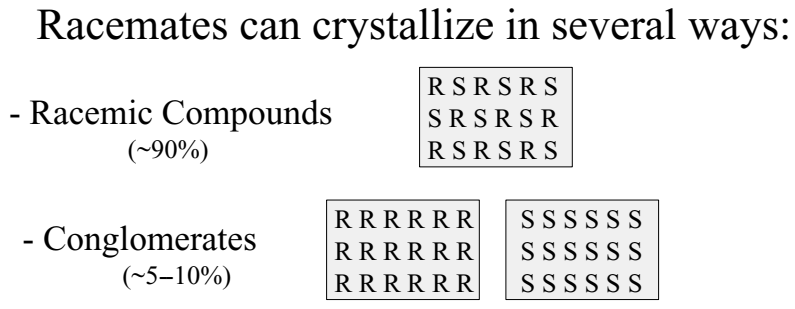

Figure 1.1 Racemates can crystallize as either racemic compounds, where both enantiomers are present in the crystal lattice, or more rarely as conglomerates, where two enantiomorphous crystal types are formed.

crystallize as conglomerates, in which two enantiomorphous crystals are present, each containing only one of the component enantiomers [26]. Consequently, analysis of any single crystal of a conglomerate shows a very high level of enantioenrichment.

The technique of *triage*, the manual sorting of conglomerate crystals, is the method by which Pasteur initially separated the enantiomers of the conglomerate, sodium ammonium tartrate [25]. Manual sorting of conglomerate crystals is, of course, an unsuitable technique for generating enantiopure materials on large scale (although some interesting physical sorting devices have been proposed [29,30]). Large-scale resolution processes are sometimes based on preferential crystallization approaches, where a supersaturated solution of the racemate is seeded with a single enantiomorphous form of a conglomerate crystal, with the ensuing kinetically controlled crystallization affording the desired crystals of a single enantiomorphous form. It is important to understand that this is a *kinetically* controlled process, and that if the contents of the crystallization vessel were allowed to come to equilibrium, a 1:1 mixture of the two enantiomorphous crystal forms would result. Seeding with a single crystal form of the conglomerate and harvesting the product crystals before the undesired enantiomorphous crystal begins to form can afford material with very high enantiopurity.

As with any resolution technology, recycling the undesired enantiomer is an important consideration for process economy. The industrial process developed more than 30 years ago for preparation of the drug, Aldomet^{TM}, is based on a conglomerate resolution that takes place in a continuous crystallization reactor with periodic crystal harvesting and a recycling of the undesired enantiomer via racemization [31]. While conglomerate resolutions can be attractive for commercial processes, most compounds are unsuitable for resolution with this technique.

1.4.4.3 *Resolution technologies: kinetic resolution* Kinetic resolution is another resolution technology that is frequently used to access enantiopure materials at industrial scale [32]. In this approach, the enantiomers of a racemic mixture undergo reaction with a chiral reagent or catalyst at differing rates, leading to a build-up of one of the enantiomers of either product or starting material over time. In kinetic resolution, frequently carried out with enzyme catalysts, the enantiopurity of the product varies with time, and the reaction must be harvested at the appropriate point to afford maximum yield and enantioselectivity of the desired enantiomer. Consequently, method development is often not as straightforward as with other resolution technologies, making kinetic resolution generally less well suited for small-scale, short-term needs. However, kinetic resolution can be very useful for industrial-scale manufacturing, provided that the economics are favourable. The all-important parameter for kinetic resolutions is the stereoselectivity factor (s), which is the ratio of the rate constants for reaction of the two enantiomers. While in principle, enantiopure material can be generated whenever $s > 1$, in practice high enantiopurity can only be achieved at the expense of greatly reduced yield when s is small. On the other hand, high s values allow access to enantiopure material with very little loss in yield. As with all resolution approaches, recovery and recycling of the undesired enantiomer is important. However, unlike other resolution approaches, the two enantiomers produced in a kinetic resolution are chemically differentiated. For example in the kinetic resolution of a chiral ester, one enantiomer remains unchanged while the other is hydrolysed to the corresponding alcohol. In cases where stereoselectivity is very high, it may be possible to obtain both of these materials with high enantiopurity, and it is sometimes possible to convert *both* of

these materials to a common downstream product (e.g. using one sequence that affords inversion of the alcohol, and another sequence that affords retention with the ester). In the best examples of this approach, the two reactions can be executed simultaneously in the same pot without the need for any physical separation [33].

1.4.4.4 *Resolution technologies: chromatographic resolution* The technique of chromatographic enantioseparation depends upon the differential adsorption of enantiomers by an enantioenriched adsorbent material called the chiral stationary phase (CSP) (Fig. 1.2), which is packed into a column through which an eluent flows [34]. Selective adsorption of one enantiomer results in increased retention of that enantiomer on the column. The degree of adsorption of each enantiomer by the column is described by the retention factor k', which equals $[(t - t_0)/t_0)]$, where t is the retention time of the enantiomer and t_0 is the retention time of an unretained analyte or void marker [35]. The retention factor k' is proportional to the equilibrium adsorption constant, and is in effect a ratio of the time that the analyte spends adsorbed to the CSP relative to the time spent in the eluent.

The ratio of retention factors for the two enantiomers is given by α, the separation factor, also called the chromatographic selectivity. A complete lack of separation of the two enantiomers corresponds to a selectivity of 1.0. Separation factors of 1.05 or even a little less can be measured using highly efficient analytical columns; however, preparatively useful HPLC separations typically have α of at least 1.2, and hopefully 1.5 or better. Very large separation factors in excess of 100 are possible, although rare. In general, bigger separation factors are advantageous for preparative resolution of enantiomers, although other factors come into play, as will be discussed in a following section. Since the separation factor is the ratio of two numbers that are each proportional to adsorption equilibrium constants, α can be directly related to $\Delta\Delta G$, the difference in free energy of adsorption for the two enantiomers, by the equation $\Delta\Delta G = -RT \ln \alpha$, where R is the universal gas constant and T is absolute temperature in Kelvin. Variable temperature chromatographic studies are sometimes used to further extract the enthalpic ($\Delta\Delta H$) and entropic ($\Delta\Delta S$) contributions to this free energy term, which are sometimes of value in studies of the mechanism of enantioselective adsorption [36].

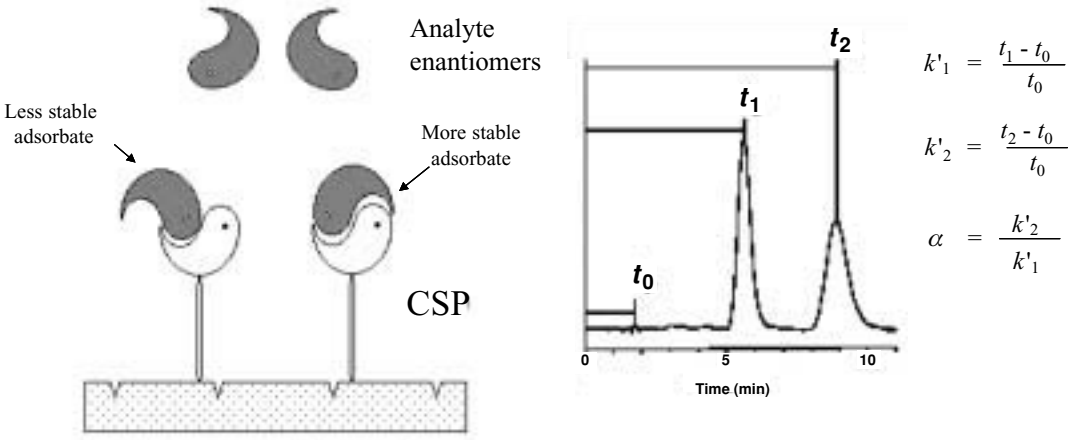

Figure 1.2 Chromatographic resolution of enantiomers depends upon differential adsorption of enantiomers by an enantioenriched adsorbent material called the chiral stationary phase (CSP).

The chromatographic parameter N, or *efficiency*, is given in terms of *theoretical plates*, and is a measure of the ability of the column to elute a nice, sharp, chromatographic peak. Most preparative chromatography takes place with columns that are considerably less efficient than those used for analytical HPLC [37]. Nevertheless, chromatographic efficiency is quite important for preparative separations. It should also be noted that in contrast to analytical HPLC, symmetrical peaks are no longer the goal. Small columns are routinely used for small-scale chromatographic enantioseparation [38,39], and because of the ready scalability of chromatography, such studies can be used to accurately model separations at larger scale.

1.4.5 *Chromatographic productivity is the key metric for preparative chromatography*

In addition to the familiar parameters describing any chromatographic separation, preparative chromatography is most concerned with the additional parameter, productivity, which measures how much purified material can be prepared with a given quantity of stationary phase per unit time. Owing to the predictable scalability of chromatographic processes, a measurement of productivity developed using a small column can accurately predict the requirements for separation on a much larger scale. Productivity is typically expressed with units of kg/kg/day (kilograms of purified enantiomer per kilogram of stationary phase per day), with a poor separation having a productivity of about 0.1 kg/kg/day or lower, and a good separation having a productivity in the range of 1 kg/kg/day. A truly remarkable separation might have a productivity greater than 10 kg/kg/day. Chromatographic productivity can easily be estimated by carrying out loading studies, where injection of progressively larger amounts of material onto an analytical HPLC column packed with the adsorbent of interest is performed. In the example illustrated below, the production rate (the rate at which enantiopure material can be produced) for various sizes of HPLC column can easily be calculated for a separation with a given productivity (Fig. 1.3). Estimates of chromatographic

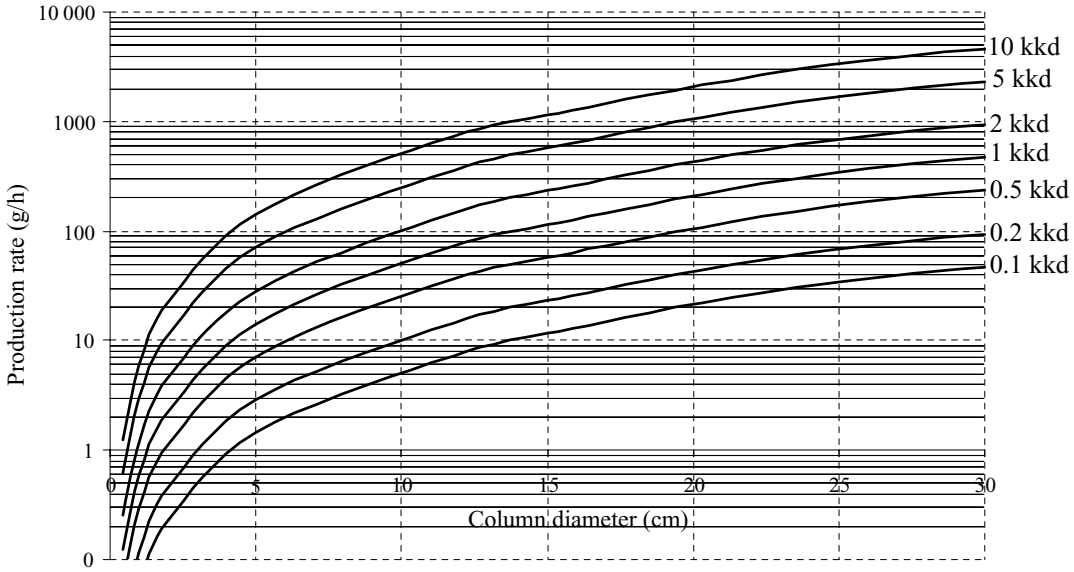

Figure 1.3 Production rate depends upon column size and chromatographic productivity. kkd = kg/kg/day.

productivity obtained from modelling with small columns allow the user to estimate the equipment, labour, and materials that will be required for carrying out a separation at any given scale.

An additional parameter to keep in mind when developing a chromatographic resolution is specific solvent consumption, which measures the amount of solvent required to purify a given amount of material. A low productivity resolution might require the use of as much as 10 000 L of solvent to obtain a single kilogram of purified material, whereas an outstanding resolution might require only a few hundred litres. Not surprisingly, solvent is generally recycled in larger scale resolutions, and solvent distillation and recycling is an important parameter contributing to the overall economics of a preparative chromatographic resolution.

1.4.6 *Stationary phases for preparative chiral chromatography*

Although many CSPs are available for use in analytical columns, only a few materials are available or widely used for preparative enantioseparation. By far, the most commonly used preparative CSPs at this time are the modified cellulose and amylose CSPs invented by Yoshio Okamoto and commercialized by Daicel Chemical Industries, Ltd. [40]. One of these materials, Chiralpak AD, based on the 3,5-dimethylphenylcarbamate derivative of amylose, is by far the most versatile preparative CSP on the market.

Several other types of CSP are routinely used for preparative chiral chromatography, and there are new commercial CSPs introduced each year. In addition, there are ongoing advances in the technology of CSP design and development [41–48]. While most commercial CSPs are used because they demonstrate some general ability to separate enantiomers, the chemist considering an industrial-scale resolution may be more interested in the ability of a CSP to separate the enantiomers of one particular compound, and there has been considerable recent interest in the preparation and evaluation of tailor-made CSPs for carrying out given separation tasks [49–51].

1.4.7 *Advantages of preparative chiral chromatography over other approaches for accessing enantiopure materials*

Preparative chromatography offers a number of advantages over competing techniques for rapidly accessing enantiopure materials, these advantages stemming from the speed and the relatively small amount of labour required to develop a chromatographic resolution method. Each of the various approaches for accessing enantiopure material (chiral pool, catalysis, auxiliary, classical resolution, preferential crystallization) employs a search protocol which first requires that the researcher develop a chiral assay, and then subsequently use this assay to screen for the appropriate combination of catalyst, conditions, reagent or resolving agent to achieve the desired result. Following the identification of a lead, the process is gradually optimized, and then investigated on pilot scale before going to large-scale production.

Chiral assays are typically performed using chiral chromatography, and method development often relies on automated or semi-automated analysis of a number of different commercial chiral chromatography columns and solvent combinations for their ability to separate the target enantiomers [52,53]. Such method development screening tools often turn up column and mobile phase combinations with a reasonably large separation factor

that can readily be translated, with just a few hours of labour, into a preparative chromatographic resolution. In such instances, the chromatographic option for accessing a few grams or even a few kilograms of material can already be in place before the development work for the competing approaches has even begun. When only small amounts of material are required, this method development advantage often makes chromatographic resolution the fastest and the most cost-effective option.

Clearly, there are more important considerations than speed and labour economy in the development of a process that will be implemented on an industrial scale, where overall process economy dictates which approach will be used. In such instances, an approach that requires much greater development labour may be selected if it can provide resolved material at a saving of just a few cents per kilogram.

1.4.8 *Simulated moving bed enantioseparation*

Most enantioseparation demands for pharmaceutical development can be met with traditional elution chromatography approaches utilizing 'touching band' separations (i.e. separations in which the two enantiomers are still baseline or nearly baseline resolved). Moving from the touching band situation to the sample overload situation results in a complex peak, where peaks become merged to a degree. Chromatographic productivity using such an approach can be superior to the touching band method, provided one has investigated the appropriate place for fraction cutting within the complex chromatographic peak. In addition, the solvent requirements for such separations can be reduced, although a considerable disadvantage is the need to collect and reprocess a middle fraction consisting of a mixture of the two peaks. The technique of simulated moving bed (SMB) chromatography, by using multiple columns, can allow for continuous chromatography with continuous reprocessing of the mixed fraction. SMB chromatography has recently gained attention as a useful tool for industrial-scale chromatographic separations [3–5,54], and can often lead to gains in productivity and reduced solvent consumption, which can improve the economics of a separation process at the appropriate scale [55]. A single column innovation has been developed [56] that utilizes recycling of the mixed fraction with augmentation with fresh feed solution so that a 'steady-state recycling' situation can be attained.

It should be noted that the increase in productivity and the solvent savings of SMB chromatography relative to touching band elution chromatography is most dramatic for those separations having poor chromatographic selectivity. The 'SMB advantage' is much reduced for the most enantioselective, highly productive separations. As a general rule, highly productive chromatography requires either highly selective separation media or high-performance separation equipment, but not both.

1.5 Green enantioseparation

The subject of 'Green Chemistry' has recently received considerable attention [57], although many of the central tenets of waste reduction, process economy, and elimination of risks and hazards have been embraced by industrial chemists for many decades. First impressions might incline one to the conclusion that chromatography, with its intensive use of solvent, is decidedly 'un-green'. However, industrial-scale separations, in order to

be economically viable, almost always utilize solvent recycling; so in order to be fair, one should focus only on waste solvent, and not recycled solvent [58]. Smaller scale preparative chromatography utilized in developmental research may eliminate the need for carrying out development using more traditional approaches, thereby saving considerable labour, and sometimes even resulting in a net decrease in waste generation.

The use of supercritical fluid chromatography (SFC) for preparative enantioseparation has enjoyed considerable recent attention [59–61], and is the method of first choice in our own laboratories. In this technique, supercritical or subcritical carbon dioxide replaces petrochemical derived hydrocarbons, resulting in reduction in solvent utilization by as much as 90% or more. Preparative SFC is not a net generator of carbon dioxide, a known greenhouse gas. Instead, it utilizes carbon dioxide which is condensed from the atmosphere, and then later returned to the atmosphere. Furthermore, with preparative SFC the product is recovered in a more concentrated form relative to HPLC, greatly reducing the amount of solvent that must be evaporated, and resulting in considerable savings in labour. In addition, because of the low viscosity of the supercritical fluid eluent, separations may be conducted at flow rates that would be impossible with liquid solvents, an advantage that can contribute to the oftentimes higher productivity of preparative SFC enantioseparations relative to HPLC methods. Cumulatively, these advantages make preparative SFC enantioseparation an attractive and potentially 'greener' addition to conventional HPLC approaches, and a technique with a promising future.

1.6 What is the appropriate role of preparative chromatography in organic synthesis?

The incorporation of preparative HPLC into organic synthesis is by no means a new phenomenon. R.B. Woodward, perhaps the most famous synthetic chemist of the twentieth century, was an early advocate of preparative HPLC, using some of the very first commercial HPLC equipment to separate complex mixtures of closely related isomeric intermediates required for his famous vitamin B_{12} synthesis. Woodward's experience prompted him to state in 1973 that

> *The power of these high pressure liquid chromatographic methods hardly can be imagined by the chemist who has not had experience with them; they represent relatively simple instrumentation and I am certain that they will be indispensable in the laboratory of every organic chemist in the near future.* [62]

Interestingly, it is only within the last few years that Woodward's prediction of the widespread adoption of preparative HPLC as an enabling technique for organic synthesis has begun to be borne out. The biochemical and natural product fields have embraced preparative HPLC for a number of years, and the HPLC purification of peptides, recombinant proteins, natural products and oligonucleotides has become commonplace [63]. The delayed realization of the Woodward prediction of widespread use of preparative HPLC within the field of organic synthesis may be in part due to Woodward being significantly ahead of his time, but can also be traced, in part, to a historical reluctance of synthetic chemists to utilize preparative chromatography. Many causes underlie this reluctance, ranging from

unavailability of equipment and expertise to a matter of aesthetic appeal, with the utilization of chromatography being viewed as 'inelegant' – a 'crutch' that helps an incomplete synthesis to cross the finish line, and something to be avoided when possible.

To be fair, most synthetic chemists have embraced the use of preparative chromatography in the form of flash chromatography, especially since the 1978 publication of Still's influential paper on the topic [64]. Nevertheless, despite its routine use in medicinal chemistry and early development, the use of flash chromatography in a final process came to be viewed (rightly so in many cases) as a hallmark of an inferior process. Thus, a generalized rule of thumb of 'first, eliminate all chromatography' came to be held by those engaged in developing large-scale processes from medicinal chemistry syntheses, and the 'eliminate chromatography at all costs' outlook is still occasionally encountered. Clearly, such shorthand notions are of limited use, and the question of whether or not to use preparative chromatography within a synthesis is purely an economic one, where the costs of different competing processes are calculated based on assumed production scales over time.

In recent years there has been a growing appreciation of the value that preparative chromatography can bring to organic synthesis, and rather than a crutch, the technique can be more of a 'fulcrum', empowering and enabling a synthesis. While industrial-scale processes which will be repeated on multi-ton scale over many years are still generally chromatography-free, there have been a growing number of examples in recent years where large-scale chromatography has proven to be the preferred approach on a production-cost basis.

Furthermore, the vast majority of the syntheses developed in academia and industry are performed on a relatively small scale, and destined to be repeated infrequently, if ever. This includes early preclinical drug candidates, the large majority of which are unsuccessful. In such cases, there is little practical advantage to developing a chromatography-free process, particularly when one considers the developmental labour required to do so. Understandably, the use of the enabling technology of preparative chiral chromatography is becoming increasingly accepted in pharmaceutical development. Given the fact that only a small fraction of all lead compounds in early pharmaceutical development survive the increased scrutiny of later stage preclinical investigation, a high value is placed on methods that can provide the compound needed for preclinical investigation quickly and with minimum labour and materials costs. A downside to this chromatographic shortcut is that should the candidate survive preclinical evaluation, a route with potential for larger scale manufacturing will be needed, and quickly. Thus, a balance between synthetic quality and speed often characterizes modern pharmaceutical process research, where needs to provide material quickly are weighed against needs to develop a process that is suitable for industrial scale.

1.7 Fording the river at the easiest point: some observations on the appropriate placement of a chromatographic resolution within a chiral synthesis

While the paradigm for developing and carrying out an enantioselective synthesis is now quite well established, strategies for the efficient use of preparative chiral chromatography within a synthesis are still evolving. As with the planning of any resolution, a certain

degree of flexibility should be maintained regarding placement within the overall synthetic scheme. Even more so than with enantioselective synthesis or non-chromatographic resolution approaches, seemingly minor changes in compound structure, for instance a change in protecting group, can dramatically influence chromatographic resolution and productivity. Consequently, a development strategy that examines a variety of intermediates and structural variants is more likely to afford a convenient and highly productive enantioseparation than a strategy that demands the resolution of one particular compound.

Developing a synthetic process can be likened to navigating in an unknown territory, with the objective of proceeding from starting materials to product with a minimum of effort. If we imagine a river dividing this territory, with racemates and achiral compounds on one bank, and enantioenrichment on the other, then a resolution can be likened to a river crossing. And, just as a navigator would never attempt a difficult river crossing without first scouting upstream and down, the sensible chemist closely examines the available options for chromatographic enantioseparation within a synthetic scheme, ever watchful for a place where the river can be forded with a single step. Clearly, the person deciding where the river should be crossed should have a firm grasp of the subject of preparative chromatography, otherwise forced crossings at rapids or canyons can inevitably lead to disaster and wasted effort.

1.8 Origins of preparative chiral chromatography

The year 2003 marked the centennial of Tswett's invention of chromatography, a technique that plays an essential supporting role in most areas of chemistry and biochemistry [65,66]. It is interesting to note that chromatography began life as a preparative method, and only later evolved into what would become the most widely used method for chemical purity analysis. Given the current pivotal role of chromatography throughout the chemical sciences [67], it is somewhat surprising to find that it was initially quite slow to 'catch on'. There was very little utilization of the technique from the time of Tswett's original work with separation of plant pigments until about 1940, when 'rediscovery' of the technique led to rapid developments in modern liquid and gas chromatographic techniques and theory [52]. Pumped flow liquid chromatography systems with UV detection were becoming known by the early 1960s, and preparative liquid chromatography using automated fraction collection and solvent recycling was known by the mid-1970s [68]. Since that time, preparative chromatography has grown in importance for conducting laboratory and industrial-scale purifications of organic compounds and biomolecules.

Like chromatography itself, chiral chromatography also has a long and rich history. It is interesting to note that much of the initial research into chiral chromatography was motivated by the desire to resolve enantiomers *preparatively*, with the adoption of chiral chromatography as the preferred method for measuring enantiopurity coming only later. Willstätter initially proposed the idea that the two enantiomers of a racemic dye might be differentially adsorbed by a biopolymer such as wool or silk in 1904 [69], and there followed several subsequent reports dealing with this phenomenon [70,71]. It was not until 1938 that the first chromatographic separations of enantiomers were reported by two different groups: one reporting a partial separation of the enantiomers of a camphor derivative using a lactose stationary phase [72], and the other reporting a partial separation of the enantiomers of an organometallic chromium complex using a stationary phase consisting of optically

active quartz powder [73]. Subsequently, Prelog and Wieland described the preparative separation of the enantiomer of Tröger's base using starch as a stationary phase [74], and Senoh and co-workers reported the chromatographic separation of amino acid enantiomers using paper chromatography [75]. Pauling's idea that polymerization in the presence of a 'template' molecule could lead to a stationary phase possessing some selectivity for the template molecule was demonstrated for enantiomers in 1952 [76], and the first 'brush type' bonded phase consisting of a chiral selector immobilized on a silica support was described by Klemn and Reed in 1960 [77]. The first separation of amino acid enantiomers using ligand-exchange chromatography was reported by Davankov and Rogozhin in 1971 [78], the same year that the first attempt at the development of a CSP designed specifically for the enantioseparation of a particular target molecule (3,4-dihydroxyphenylalanine) was reported [79]. In 1974, Cram and co-workers described the preparation of a completely synthetic CSP that showed high enantioselectivity for amino acid enantiomers [80]. As an outgrowth of studies on NMR chiral solvating agents [81], Pirkle and co-workers reported the first in a long line of CSPs in 1979 [82]. The first commercial CSP was introduced in 1980, followed rapidly by widespread general acceptance and utilization of chiral chromatography, and by the introduction of a number of commercial CSPs. Interestingly, the explosion of general research interest in chirotechnology [83] (the science of making and measuring enantiopure materials), which began in the early 1980s and continues unabated to this day, can in some measure be attributed to the availability of tools for the rapid and reliable quantitation of enantiopurity. Where it once took days or weeks to obtain oftentimes questionable results, the ready availability of commercial CSPs meant that accurate and reliable enantiopurity measurement could be obtained in a matter of minutes.

1.9 Practical tips for preparative chromatographic enantioseparation

(1) Screen broadly: A broad selection of high-quality stationary phases will increase the chances of developing a straightforward resolution for any compound.
(2) Invest in good chromatographic equipment: Quality equipment will pay for itself in labour savings over time.
(3) Invest in automation: Automated sample injection and fraction collection are a must – even in academic laboratories.
(4) Monitor and maximize productivity: The productivity of any separation method is easily measured, and developing a sense of how to maximize productivity is the key to successful preparative chromatography.
(5) Preparative chromatography begins where the pretty peaks end: beginners often inject too little sample onto the column. Do not be afraid to push injection size to the limit.
(6) Maximize the use of the column: Ideally, one should endeavour to use most of the column most of the time. For enantiomer separations the strategy of overlapping injections is frequently used so that *something* (either peak A or peak B) is always eluting from the column.
(7) Maximize flow rate: Increased productivity will result when columns are operated towards the upper end of their specified safe flow rate or pressure range. Less

viscous solvents allow greater flow, and are thus preferred as eluents. Operating large columns at low flow rates is a common beginner's mistake, which is unproductive and wasteful.

(8) Analyte solubility: Solubility of the analyte in the chromatographic eluent is an important factor in preparative separation. Poor solubility can render even a highly enantioselective separation unproductive. Make sure to consider solubility when selecting a chromatographic method, and when selecting the appropriate intermediate for chromatographic resolution.

(9) Compound stability: Compounds in solution are more prone to undesired reaction and decomposition than are compounds in the solid state. If chromatographic fractions are to remain in solution for prolonged periods of time, make sure that the chemical and stereochemical stability studies have been performed to ensure that there will be no surprises with formation of new impurities or with racemization or epimerization. Esterification, transesterification and ketal formation reactions are frequent problems when using alcohol modifiers, especially when strong acids such as trifluoroacetic acid are present.

(10) Beware crystallization of feed: By necessity, chromatographic feed solutions are highly concentrated, usually near the saturation point. As such solutions sit for prolonged periods of time, it is not uncommon to observe formation of crystals, even in cases where the compound has never before been crystallized. Formation of crystals in the feed mixture not only disrupts chromatography and potentially damages injector pumps or other hardware, but once crystallization has taken place it may be virtually impossible to return to the original operating conditions. It is therefore often prudent to prepare feed solution only as needed.

(11) Beware solvent impurities: Since large quantities of solvent are evaporated in product recovery, impurities in the solvent can become concentrated in the recovered sample and may be difficult to remove. Evaporation and analysis of bulk solvent allows one to anticipate this problem.

(12) Look for upgrade: Many compounds can be 'upgraded' by crystallization, meaning that a sample of lower enantiopurity can be crystallized to afford material with the desired enantiopurity. When such an upgrade can be accomplished simply and with minimal loss of material, it can greatly increase the overall productivity of a resolution. Chromatographic productivity for producing 95% ee material can be several-fold the productivity for producing 99% ee material.

(13) Beware of temperature effects: Chromatography can be very dependent upon temperature, and sometimes temperature can dramatically influence preparative separations. If operating in an environment that could experience temperature swings, it may be a good idea to investigate temperature effects. Temperature effects can sometimes be used to advantage in preparative chromatography [84].

(14) Tell vendors what you like, what you don't like, and what you need: Materials and equipment for preparative chromatography are still at an early stage of evolution. You can help to move the process along by interacting with the vendors and suppliers.

(15) When all else fails, consider derivatization: although seldom required today, the old trick of derivatization often renders compounds more easily separated [85,86].

(16) If you cannot find the right CSP, consider making your own.

1.10 Conclusion

Preparative chiral chromatography has become a preferred method for rapidly accessing enantiopure compounds in the pharmaceutical industry, largely owing to the speed with which a chromatographic method can be developed and executed as well as the comparatively small labour requirements of the chromatographic approach. As Woodward predicted, the use of preparative chromatography within the field of organic synthesis can be expected to increase as the technique becomes more familiar to synthetic chemists. As this occurs, it can be expected that there will be an increasing prevalence of syntheses that are designed from the outset with a view toward utilization of the best advantages that preparative enantioseparation has to offer.

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Preparative Enantioselective Chromatography
Edited by Geoffrey B. Cox

2 Introduction to preparative chromatography

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2.1 Introduction

The practice of preparative chromatography in the isolation of enantiomeric species is in some ways a little different from most preparative chromatographic processes. The products of the separation are chemically identical and are usually present in the sample in equal concentrations (although this may not be always the case, since preparative separations can equally be applied to the end product of enantioselective syntheses when the final enantiomeric purity is insufficiently high). Usually there are few impurities and the aim of the separation often is not to remove these – they disappear in later synthetic steps, crystallisation or even subsequent achiral preparative chromatographic steps. Notwithstanding these differences, the basis of preparative enantioselective separations is firmly rooted in the basic theory of mass-overloaded chromatography and the treatment here can be applied to both chiral and achiral separations. Where there are significant differences between chiral and achiral chromatography, these are noted.

For many years, preparative chromatography was a black art. Even as recently as 1987 [1], there was, in general, insufficient understanding of the basic processes involved in the technique. Peak shapes remained unpredictable and mysterious and the isolation of products in unexpected parts of the chromatogram was a continual problem. However, in 1986 a seminal paper that began to address these issues appeared from the laboratories of Prof. John Knox in Edinburgh [2]. Although some of the issues had been addressed previously, this was the first paper that introduced simple concepts that described what was occurring in mass-overloaded separations. At the same time as this work, other groups, notably those of Snyder [3] and of Guiochon [4], were working along similar lines, the latter two using computer simulations rather than the simple mathematics resulting from the simplifications employed by Knox. By 1994, when the definitive work by Georges Guiochon was published [5], preparative chromatographic theory had reached a mature state. With the understanding gained from these workers, preparative chromatography is now largely predictable.

2.2 Adsorption isotherms

The study of preparative chromatography is the study of adsorption isotherms, whether the practitioners are aware of this or not. The adsorption isotherm relates the concentration of a product in the stationary phase of a chromatographic system to that in the mobile phase; graphically it is represented as a plot of the concentration in the stationary phase as a function of the mobile phase concentration. The isotherm is important because as the sample load on the column is increased, so is its mobile phase concentration increased. This, in turn, increases the concentration of the products in the stationary phase. The quantity of the stationary phase in the column is limited and there comes a point when the sample load is high enough to overload it. At this point, what happens is dictated by the shape and

the curvature of the adsorption isotherm. If we know – or if we can find out – something about the isotherm, then we are well on the way to understanding what is happening in the preparative separation. When sample concentrations are very low, for example in analytical chromatography, the isotherm is a simple, linear one. When concentrations are high, it is a complex function of the interactions between the solute molecules and the stationary phase adsorption sites (of which there may be several different types), the solute with the mobile phase and of adsorbed solute molecules with each other. In practice, the simplest case can often be applied, at least semi-quantitatively, and is frequently sufficiently close to reality for separations to be understood and planned.

2.2.1 *The simple case – the Langmuir isotherm*

The distribution between two phases is described by a simple equilibrium relation

$$A_{(\text{phase 1})} = A_{(\text{phase 2})} \tag{2.1}$$

which is defined by a distribution coefficient,

$$K = [A_{(\text{phase 2})}]/[A_{(\text{phase 1})}] \tag{2.2}$$

In this case, there is a constant, linear relation between the two concentrations and the concentration in one phase is always given by a simple multiple of the concentration in the other.

In the case of a chromatographic system, however, we have generally a liquid phase (the mobile phase) and a solid, adsorbing phase (the stationary phase). The stationary phase is usually characterised by a finite number of adsorption sites – in the case of an ion exchanger, for example, these sites would be the ionic groups situated on the surface. Other phases are less well defined, but all have a finite surface area on which the solutes can adsorb or have a finite number of defined sites where differences in the enantioselective adsorption of the solutes is found (in the case of the chiral polymers often used for enantioselective separations this is represented by the total quantity of chiral polymer coated or bonded on the surface of the support). This means that the simple equilibrium equation must be changed to reflect these adsorption sites:

$$A_{(\text{mp})} + \text{solvent-free sites} = A_{(\text{sp})} - (\text{total sites} - \text{free sites}) + \text{solvent} \tag{2.3}$$

The distribution between the phases is more complex here, but with a few simple approximations (which one should do well to remember, as they are perhaps important in understanding why this equation does not always fit the experimental data) it is easy to arrive at a relation (the Langmuir isotherm) between the stationary phase and mobile phase concentrations. One has to assume that the solvent and the solute molecules are of similar size, each taking the same surface area on the chiral stationary phase (CSP) and that the concentrations are low enough that the ratio of mole fractions can be approximated by the ratio of concentrations. The Langmuir isotherm takes the form

$$C_\text{s} = aC_\text{m}/(1 + bC_\text{m}) \tag{2.4}$$

where a is the distribution coefficient at infinite dilution (sometimes called the Henry constant) and the ratio a/b describes the total number of adsorption sites available for the solute – in fact here it is given in terms of the stationary phase concentration of the solute

when the surface of the stationary phase is saturated with solute. This ratio is usually called the *saturation capacity* of the phase. It is useful to remember that saturation capacity may also be calculated as the column saturation capacity, which is a weight (the weight of a monolayer of solute on the packing material in the column under consideration) rather than a concentration. Discussion of the measurement of the saturation capacity (and other isotherm parameters) is beyond the scope of this chapter and the reader is referred to the appendix for a discussion of the relevant equations and methods. Only qualitative and semi-quantitative aspects of this are important for an understanding of the processes occurring in the mass-overloaded column. Other forms of this isotherm (e.g. the 'modified' Langmuir isotherm) exist; these are also discussed in the appendix.

The relation expressed in Equation (2.4) has some chromatographic consequences. The concentration in the stationary phase is no longer a linear function of that in the mobile phase. We know, however, that the retention time of a chromatographic peak is obtained through the capacity factor (latterly called retention factor), k', which is given as the ratio of the quantity of solute in the stationary phase to that in the mobile phase. If on increasing the sample load the ratio of the concentrations between the two phases changes, then clearly the retention of the peak will also change. A plot of the concentration in the stationary phase versus that in the mobile phase illustrates this point. This is shown for the isotherm derived above in Fig. 2.1. The slope of the curve at any one point of the curve gives the ratio of the two concentrations, from which the capacity factor may be derived. It is obvious that the capacity factor changes with solute concentration, from a high initial value (quasi-linear at very low concentrations) to zero at very high concentrations when the stationary phase is saturated. To determine what happens to the peak shape, it is necessary to imagine a chromatographic experiment

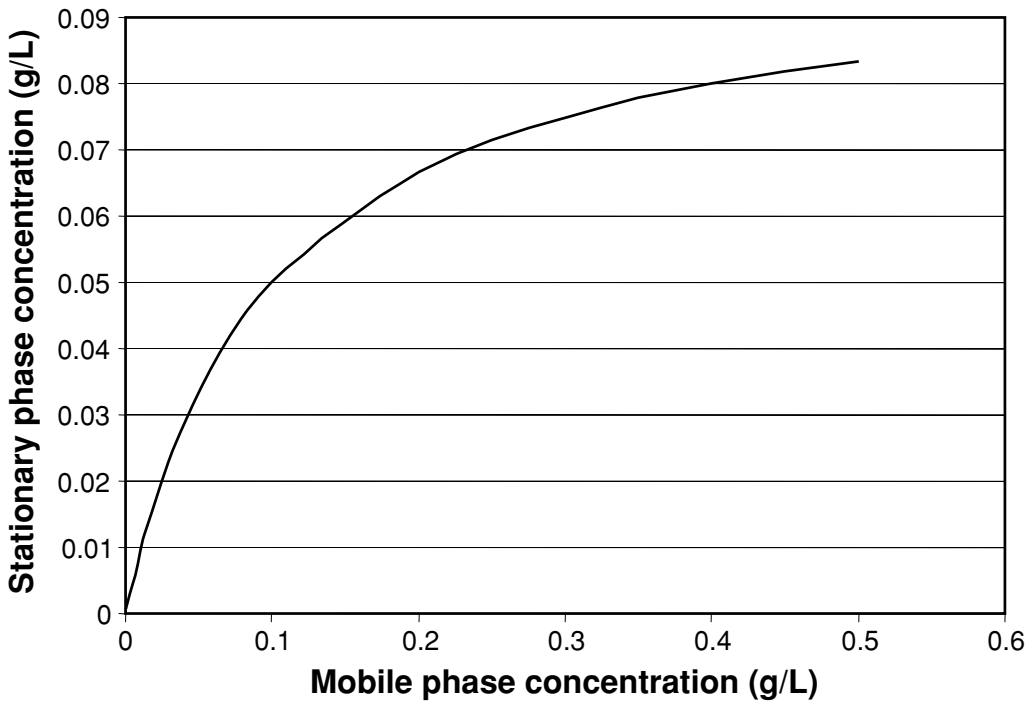


Figure 2.1 Plot of stationary phase vs mobile phase concentrations: Langmuir isotherm.

where a certain volume of a high concentration sample is injected. If there were no band spreading, the peak would move as a square wave of concentration through the column, its speed determined by the band concentration; each injection at a different concentration would result in a rectangular peak eluting at different retention time. In practice, there is band spreading. Solute molecules at the edges of the band diffuse out of the main peak, leading to the formation of more dilute zones at the leading and trailing edges of the band. From the above discussion, it is clear that these dilute zones will move more slowly than the main (undiluted) part of the peak owing to the higher retention factor. At the trailing edge, these molecules are simply left behind by the high concentration zone. As the band develops, the molecules diffusing out of the trailing edge move more slowly, those that move the slowest are those at the lowest concentration. These ultimately elute with the same retention time as a peak from an analytical injection. There is, of course, a continuum of concentrations between this lowest one and the peak maximum and there is a consequent continuous increase in concentration from the slowest part of the zone to the peak maximum. This is self-regulating, since if more molecules move more slowly, the concentration in the slower parts of the peak increase, thus reducing the retention and bringing these molecules back to their original position in the peak envelope. This implies that the peak tail for all mass-overloaded injections of a solute will follow the same curve; superposition of several injections of different loads will give a nested family of curves. The reader should see Chapter 4 for examples of these sets of chromatograms generated in loading studies. The situation on the front of the zone is similar. Those solute molecules that diffuse forward out of the front of the band also find themselves in a low concentration zone, which therefore moves more slowly than the high concentration parts of the band. In this case, they are rapidly overtaken by the high concentrations immediately behind them in the column. This means that the front of the band maintains a high concentration, since molecules cannot escape from the zone front. This is said to be a self-sharpening or 'shock' zone in the chromatographic band. From the foregoing, it is possible to determine the band shape. The front of the band will be steep while the tail will run down from the maximum concentration to a point corresponding to the analytical retention time. Such a band (derived from a computer simulation – see the appendix – using the Langmuir isotherm) is shown in Fig. 2.2.

Langmuir isotherms can be normalised to one another by dividing both sides of Equation (2.4) by the saturation capacity. The left-hand side then becomes the ratio of the solute concentration in the stationary phase to the saturation capacity, a ratio defined as the loading factor (L_f) [6]. Equation (2.4) then becomes

$$L_f = \frac{bC_m}{1 + bC_m} \tag{2.4a}$$

2.2.2 *Other isotherms*

The Langmuir isotherm is the simplest relation that occurs in chromatography. It assumes – amongst other things – that the surface of the adsorbent is homogeneous. This may not be true. It has been shown, for example [7], that protein-based CSPs have two different types of adsorption site, one which is achiral and retains both enantiomers equally, and another, chiral, site that gives the differential retention necessary for the enantioseparation. Each site has a characteristic Henry's constant and each gives rise to its own saturation capacity.

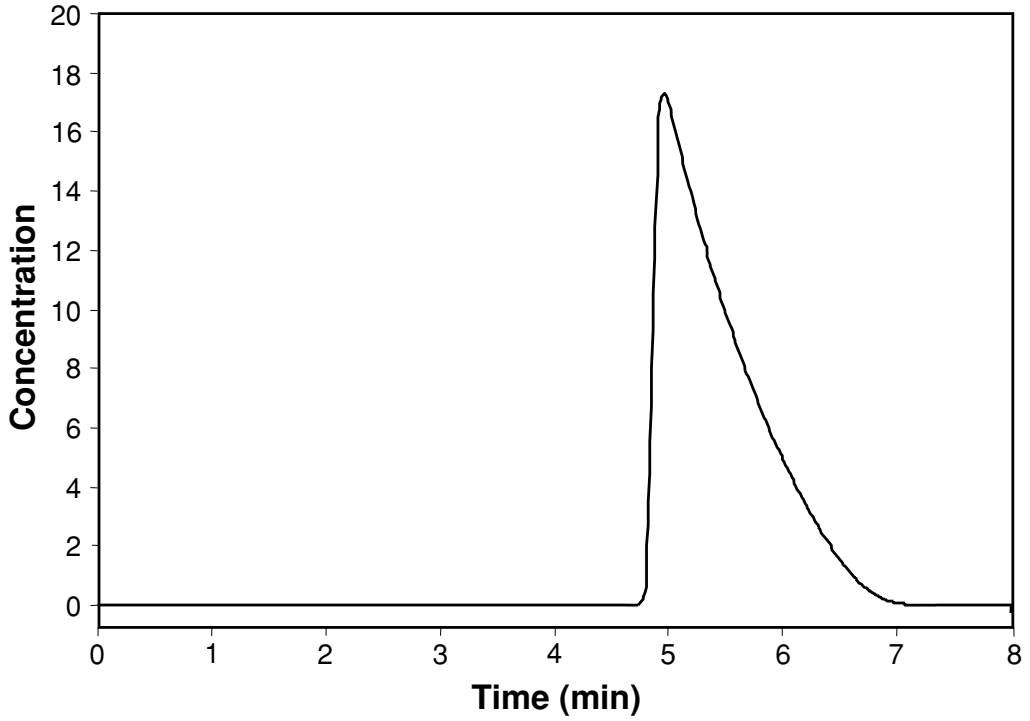


Figure 2.2 Band shape for a mass overloaded peak: Langmuir isotherm.

Thus, the adsorption on each site can be given by an individual Langmuir isotherm. If the two sites do not interact in any way, then these two isotherms can be added to describe the total system. This gives the bi-Langmuir isotherm,

$$C_s = C_m a_1/(1 + b_1 C_m) + C_m a_2/(1 + b_2 C_m) \tag{2.5}$$

The analytical retention may be obtained from the sum of the two Henry's constants. The usual situation is that one of the sites is present in small concentration and is the more retentive. As the sample load increases, this site overloads more rapidly than the other, leading to a much more rapid reduction in retention and thus a more rapid degradation of peak shape relative to a simple Langmuir isotherm. Normally there is little that can be done to improve the situation (other than finding a different CSP) although where the strong adsorption is achiral it can be masked by use of a suitable additive. This is often done when the solutes are basic and interact strongly with the acidic sites on the silica base particle of the CSP. An amine additive is often used to improve the peak shape by preferentially filling these strong adsorption sites, thus preventing their interaction with the solute.

As the surface becomes more complex, the isotherms themselves may also become complex and many isotherm equations have been proposed. Some, such as the Freundlich isotherm, are intellectually difficult as they predict an infinite retention of everything at low enough concentration. Others have been developed to fit special cases. It is merely surprising that the Langmuir isotherm fits so much of the data to a good enough extent to be useful.

The CSP surface is not the only factor that influences the isotherms. Where the solutes are not very soluble in the mobile phase or where they can self-associate strongly, the stationary

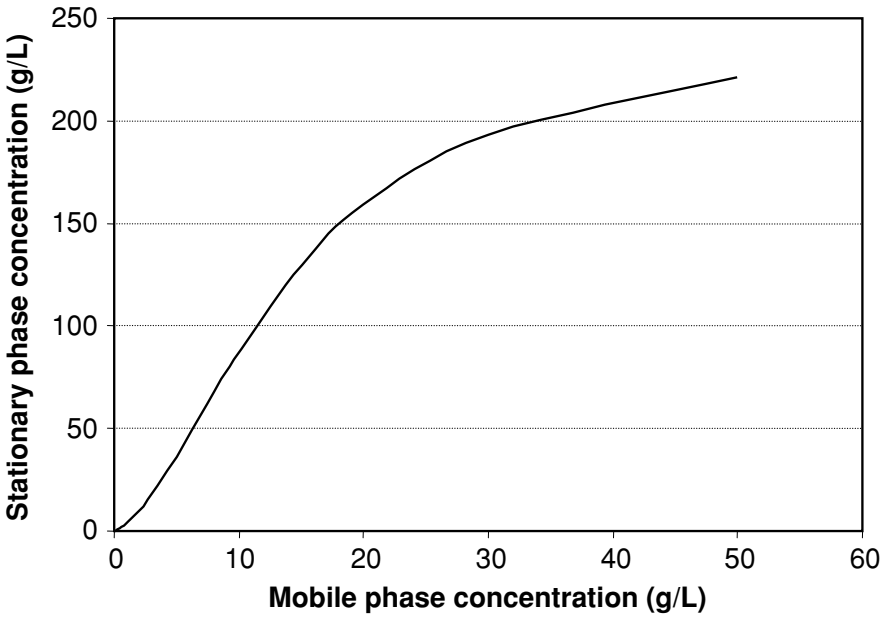
phase concentration can build more rapidly than predicted by the Langmuir isotherm, to the extent that the retention time of the overloaded peaks first increases with increasing load rather than decreasing. This is usually attributed to stacking of the solute molecules on the surface, with each addition of a molecule leading to a stronger adsorption of the next. Eventually, the surface starts to fill with the adsorbed solute and the decrease in number of adsorption sites becomes controlling and the retention decreases with further increases in solute concentration. These isotherms are called 'S-shaped' and are usually quadratic in nature. The typical behaviour of solutes with such isotherms was described by Guiochon [8]. A typical representation of such an isotherm is shown in Equation (2.6):

$$C_s = (aC_m + cC_m{}^2)/(1 + bC_m + dC_m{}^2) \tag{2.6}$$

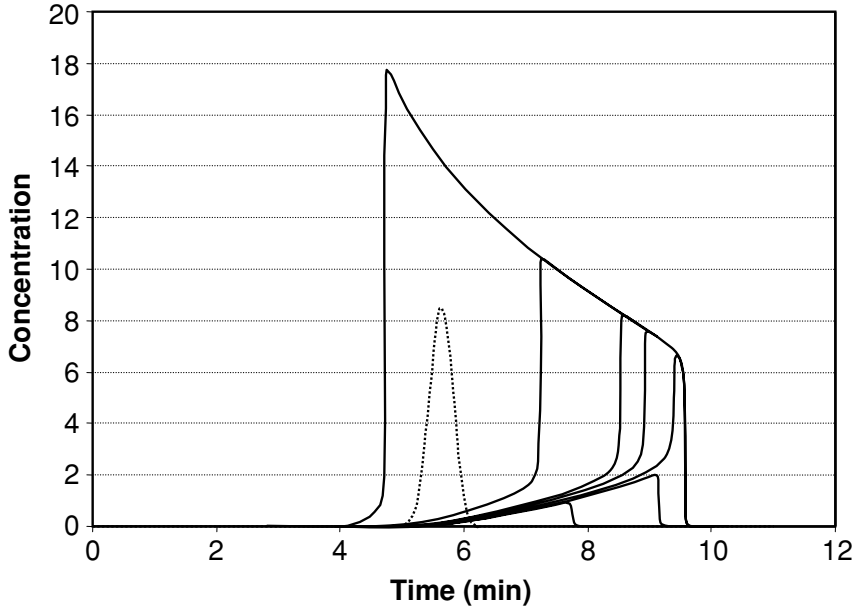
An isotherm according to this equation is shown in Fig. 2.3a, while the chromatograms of a solute conforming to this isotherm are shown in Fig. 2.3b for several loads. The form of the peaks for such compounds is distinctive, although an extreme case is shown here for illustrative purposes. The advantage for preparative chromatography occurs in those rare cases where the second component has such an isotherm, since instead of the peaks both moving to shorter retention time and beginning to merge, in this case the second peak moves away from the first and the separation improves with increasing load. This has been observed in SFC separations (R.W. Stringham, personal communication, 2003), but is rarely seen in HPLC separations of enantiomers.

2.2.3 *Competitive isotherms*

The foregoing discussion is related to single component separations. It is, of course, rare for preparative chromatography to be carried out using a single compound, although it is sometimes a good approximation for separations where the main component is well separated from minor impurities. What makes the situation complex is the addition of one or more other components to the mixture to be separated. If the two (or more) solutes being separated adsorb on different types of site on the surface of the packing material, then they are separated exactly as if the other were not there. This has been observed by Katti [9], but is extremely rare. Usually the solutes interact with the same adsorption sites and this, not surprisingly, complicates the equilibrium. If two solutes are adsorbed on the same sites, then there will be mass action effects; the solute at higher concentration will tend to displace that at lower concentration from the site. Imagine, for the moment, the zone of overlap between two solutes, each having a Langmuir isotherm (Fig. 2.4). The trailing edge of the first peak is at low concentration relative to that of the front of the second peak. This implies that all other things being equal, the high concentration front of the second peak will tend to displace the first solute from the adsorption sites by simple mass action. Thus, the tail of the first peak will elute more quickly than would be expected from the behaviour of the component injected alone. The presence of the low concentration of the first peak will have some lesser effect on the high concentration zone of the second and this will thus elute a little earlier than it would were the first component not present. These effects will have the end result of distorting the individual peak shapes, pushing the first peak a little earlier and reducing the overlap zone while bringing the overlapped portion of the second peak to shorter retention time. Figure 2.5 shows the result of these interactions on the hypothetical separation shown in Fig. 2.4.



(a)



(b)

Figure 2.3 (a) Band shape for the isotherm of Equation (2.6) with $a = 2.61, b = 0.261, c = 1.31, d = 0.0052$. (b) Computer-simulated chromatograms arising from the isotherm of Figure 2.3a. Dotted line = analytical injection, other curves are from sample loads from 0.001 to 0.05 g (25 × 0.46 cm column, 1500 plates.)

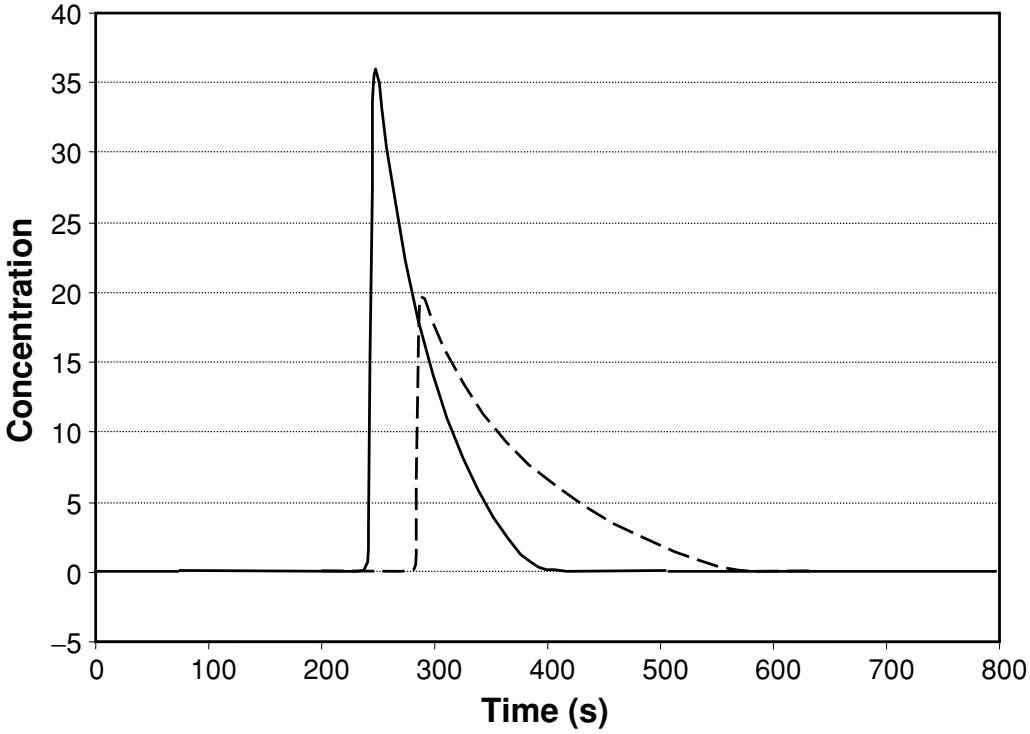


Figure 2.4 Zone of overlap between Langmuir isotherm peaks with zero inter-component interactions.

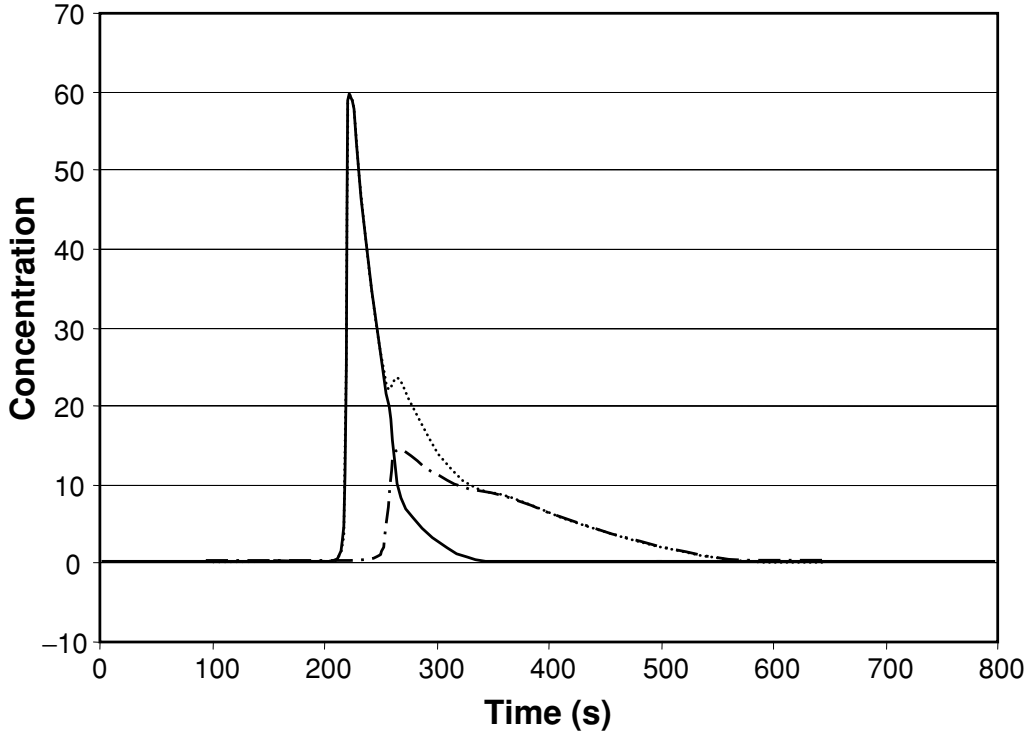


Figure 2.5 Effects of displacement on the hypothetical chromatogram of Fig. 2.4.

These effects are far more profound when the concentrations are very different. Figure 2.6 shows the consequences of two situations where the solute concentrations are in a 20:1 ratio. When the first eluting peak is at low concentration, it is almost entirely displaced from under the second and elutes mainly as a very narrow band on the main peak front (Fig. 2.6a). When it is the second peak which is at low concentration, it is also displaced from the adsorption sites by the high concentration of the first peak. In this case, as the second component is now moving more quickly than it would otherwise, it moves into the tail of the first component. This results in a far worse separation than expected and explains much of the frustration of early preparative chromatographers as well as that of analytical chemists who wish to analyse trace components that elute immediately after the major component in the sample. In both cases the minor peak is drawn into the major, sometimes disappearing almost completely (Fig. 2.6b).

All this can, of course, be described mathematically and is exemplified by the competitive isotherms. The competitive Langmuir isotherm is typical and illustrates that the stationary phase concentration of the component of interest is influenced by the mobile phase concentration of all other species present in the sample:

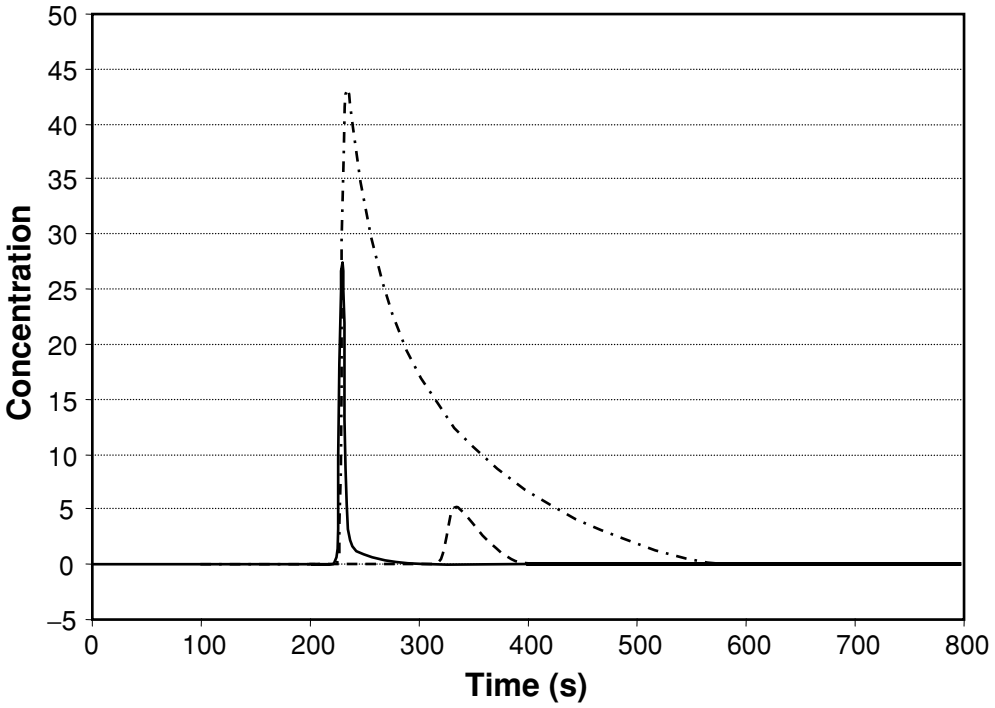
$$C_{s,i} = a_i C_{m,i}/(1 + \Sigma b_j C_{m,j}) \tag{2.7}$$

Another problem is that it is possible to obtain simple equations for competitive isotherms only under special circumstances. Competitive isotherm equations can be derived for the Langmuir and bi-Langmuir isotherms. For other isotherms this is not possible, and other methods (beyond the scope of this chapter, but see the section on the Ideal Adsorbed Solution Theory in the appendix) have to be used.

It is a fundamental law in most sciences that nothing is actually as simple as is described by the simple theories. One of the assumptions in deriving the Langmuir isotherm is that all species have the same dimensions and take up the same space on the surface of the stationary phase. This is the same as saying that the saturation capacities of all species must be equal. The competitive Langmuir isotherm assumes this and strictly should be applied only to cases where the saturation capacities are equal.

It was shown experimentally some time ago by Cox and Snyder [10] that displacement effects very much larger than those predicted from the competitive Langmuir equation were observed for certain separations. For example, as shown in Fig. 2.7, the separation of benzyl alcohol and phenol was shown to have a profound displacement such that the two components could be recovered at very high purity and recovery; only a very small overlap zone was seen. Calculation of the saturation capacities of the solutes revealed a great difference, one being approximately 50% of the other. In the inverse case, with *p*-cresol taking the place of phenol, where the component of lower saturation capacity elutes second, the separation of the components is lost very quickly with the second eluting component merging with the first. Even more spectacular displacements can be found for peptides and proteins, where very sharp cut-offs between components are seen [11,12].

These larger displacements can be understood on considering the energetics of the system. If we suppose that one component has a saturation capacity twice the other, then it will use 50% of the surface, and two times the number of these molecules can adsorb on the same surface area as for the other. If we assume similar adsorption energies for the two species, then consideration of a small part of the surface reveals the picture shown in Fig. 2.8. The two solutes are competing for a site on the surface of the adsorbent. Adsorption of one



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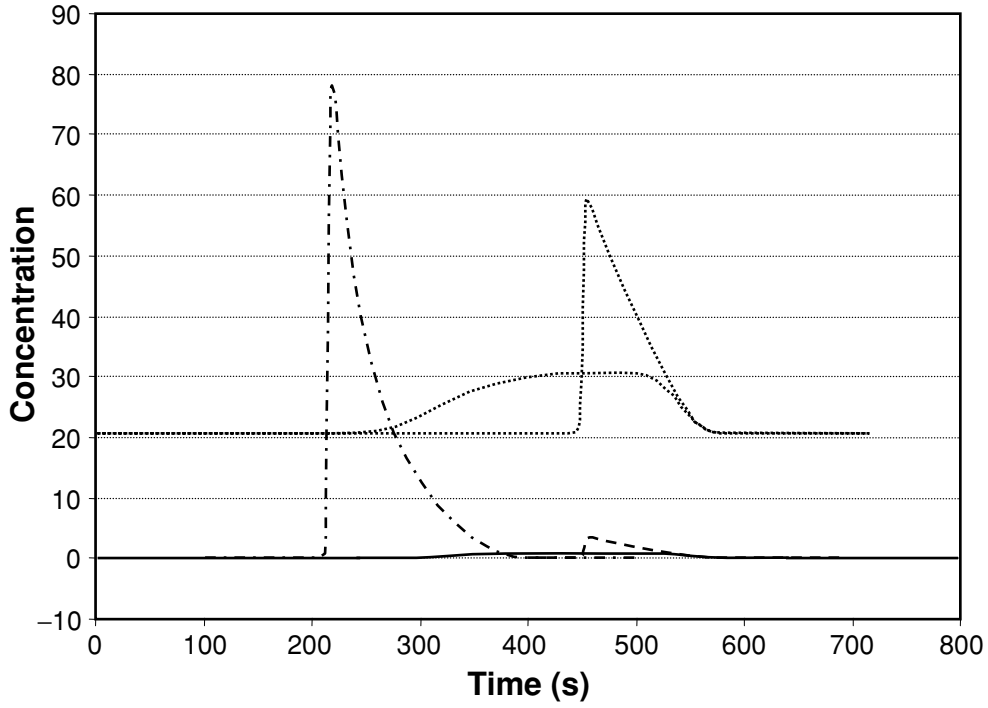


(b)

Figure 2.6 (a) Displacement effects for 1:20 ratio peaks: Langmuir isotherm. (b) Displacement effects for 20:1 ratio peaks: Langmuir isotherm. Dashed lines: minor component injected alone. Dotted lines: expansion of minor component peaks.

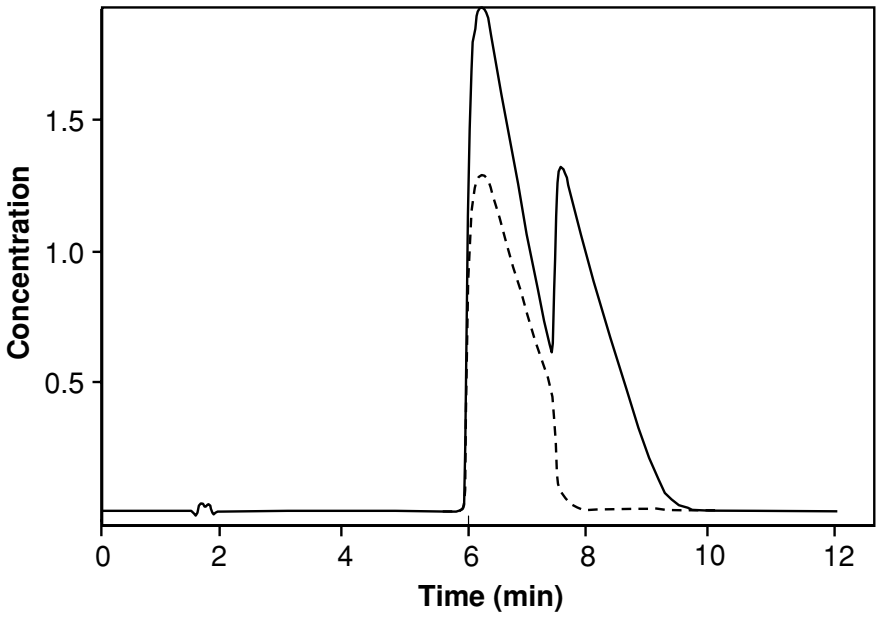


Figure 2.7 Displacements effects for phenol and benzyl alcohol. Reproduced from Ref. [10].

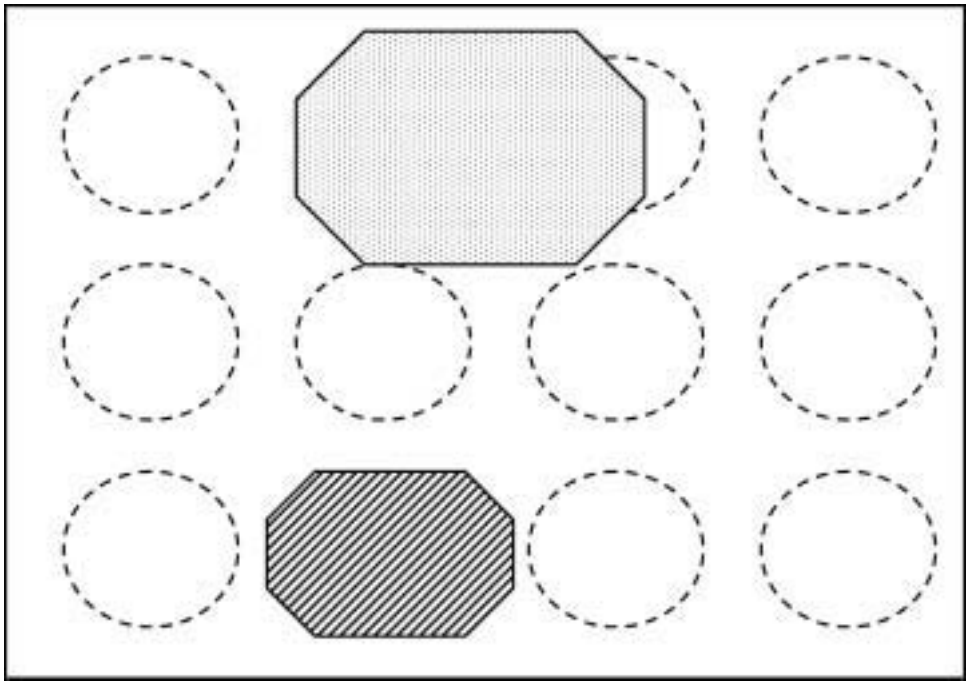


Figure 2.8 Surface showing competitive adsorption.

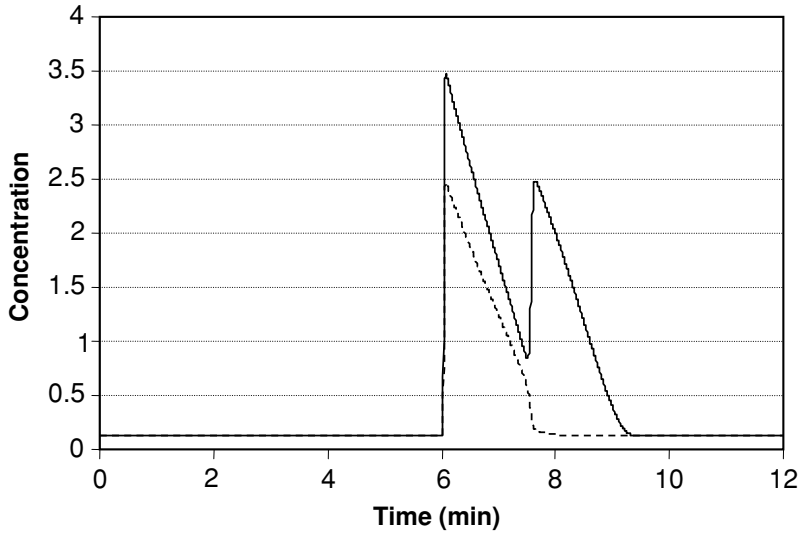


Figure 2.9 Computer simulation of separation in Fig. 2.7 using IAS theory.

solute will give the same (more or less) adsorption energy and the relative concentrations will be determined by the relative concentrations of the solute. But when one molecule with the larger footprint (smaller saturation capacity, solute 'A') is adsorbed, it takes up (but does not use) two adsorption sites on the surface. At high concentrations of adsorbed solute, it will have to displace not one but two of the competing solute molecules, requiring twice the energy of desorption. Equally, the molecule with smaller saturation capacity (solute 'B') displaces one of the larger molecules and at the same time frees a second site for adsorption of another molecule. Because of the extra energy involved, it is much less likely that B will displace A, and more likely that A will displace B.

This can again be demonstrated by the results of computer simulation. Figure 2.9 shows the simulation of the separation of Fig. 2.7. The sharp displacement seen experimentally is seen, as expected. In these computer simulations, the simple competitive Langmuir isotherms cannot be used because of the different saturation capacities. In this case the Ideal Adsorbed Solution Theory (see the appendix) was used to generate the competitive data from the individual isotherms.

There are consequences for preparative chromatography in general and chiral chromatography in particular. In enantioselective separations, the more retained enantiomer has more specific interactions with the CSP than that less retained. It is not surprising that because of these differing interactions the second eluting component generally uses a higher surface area for adsorption than the less retained enantiomer. Thus, the second eluting enantiomer often has a lower saturation capacity than the first and the inevitable consequence is that the separation degrades quickly with increasing load. This is one reason for the lower loadings often seen in enantioselective preparative chromatography than in achiral separations.

Where the 'normal' state of affairs in enantioseparations exists (lower saturation capacity for the second component), there are limits to the extent that sample loading is possible. The solute concentrations in the sample are equal so mass action displacements are weak

Table 2.1 Effect of increasing load on recovery at 98% purity

	Recovery (%)	
L_f	Peak 1	Peak 2
0.017	100.0	100.0
0.025	100.0	100.0
0.033	98.6	99.9
0.042	94.5	83.0
0.046	91.7	75.9
0.050	88.7	69.9

and small degrees of overlap rapidly degrade the separation to the point where the desired result (often the isolation of a reasonable recovery yield of both enantiomers at high enantiomeric excess) is compromised. Table 2.1 shows the results of increasing load on the recovery of the two enantiomers at a desired purity of 98% ee. For these reasons, preparative enantioselective separations at small scale are usually carried out at relatively low load with so-called 'touching band' separations. Under certain circumstances, where only one of the enantiomers is needed, the separation of large quantities is required and the unwanted enantiomer can be racemised and recycled, much larger overloads are possible. The reader is referred to Chapter 5 for some examples of such separations.

2.3 Kinetics

The isotherms are a thermodynamic property of the chromatographic system (that is to say they are related to the energies of adsorption, solvation, etc.) and as such are of paramount importance in the design of the separations. Band spreading in the chromatographic system is of lesser importance under conditions of mass overload, but it is necessary to address this issue in order in part to dispel some miscomprehensions and in part to demonstrate under which circumstances it is important to have high column efficiencies and where one can trade performance for speed.

It is not the intention here to discuss band-broadening mechanisms, the derivation of the plate height or the van Deemter (or Knox) equation. The reader is referred to a standard chromatographic text (e.g. [13]) for details.

When confronted with the broad, mass-overloaded bands found in preparative chromatography, the usual initial reaction is that column efficiency is lost on mass overload and is not therefore very important. What should be realised, however, is that the band spreading due to mass overload is a thermodynamic function while that due to the various column parameters (particle size, how well the column is packed, the state of the inlet and outlet frits and the extra-column volume) is kinetic in nature. The two band-spreading mechanisms are therefore independent of each other and it is normally true to say that a poorly performing column will give poor preparative separations.

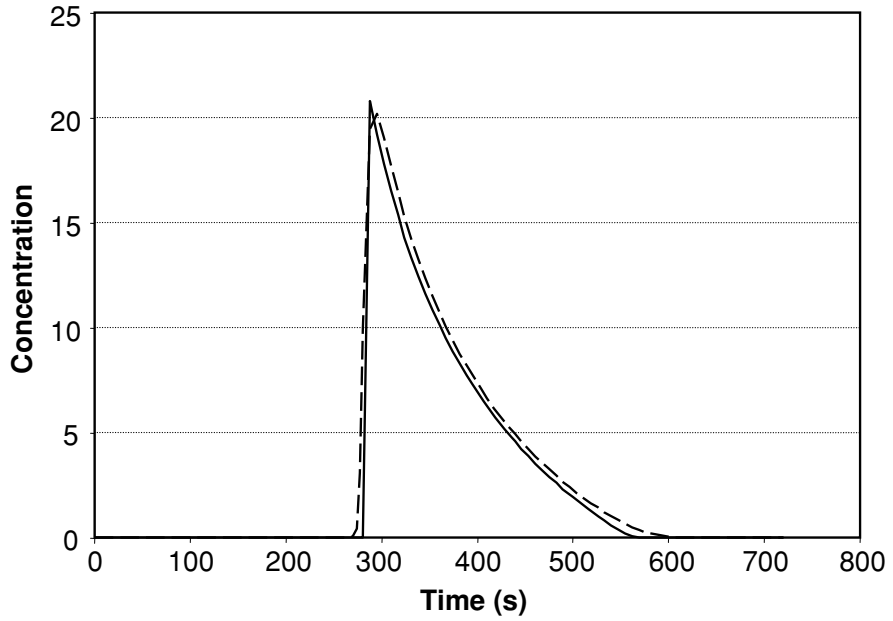
The effect of efficiency can readily be seen in considering the peak shapes obtained with columns of different efficiencies. Because we wish to change only one parameter, it is

Table 2.2 Recovery at 98% ee as a function of efficiency

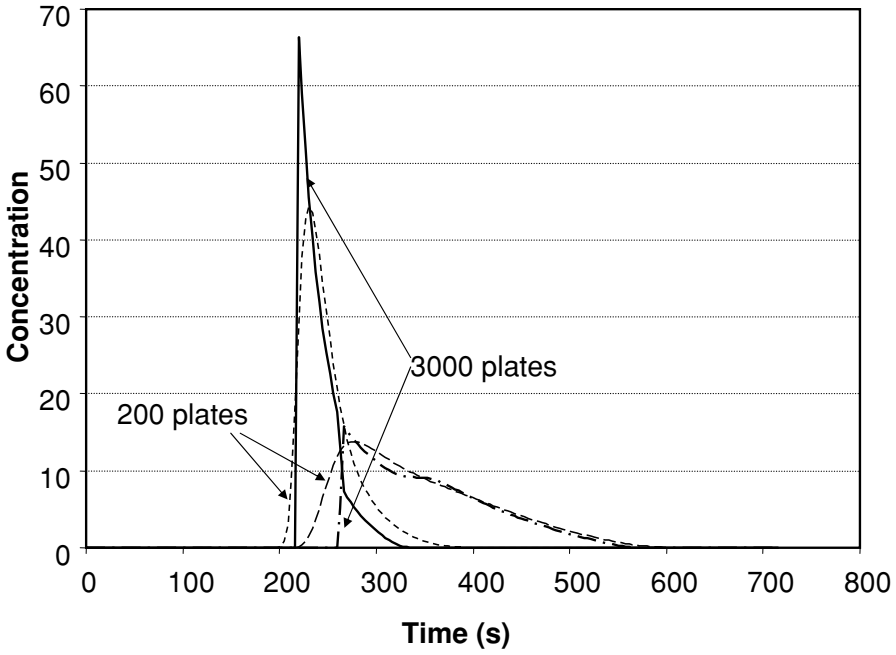
	Recovery (%)	
Plates	Peak 1	Peak 2
200	38.1	43.9
500	75.8	61.9
1000	85.6	66.6
2000	89.1	70.7
3000	89.7	72.2
5000	90.2	73.5

convenient to use computer simulations to carry this out. Although similar experiments can be run in practice, it is difficult to find columns of different efficiencies that are identical in all other properties. Figure 2.10a shows the effect of efficiency on the shape of a single overloaded peak. This is not especially profound and the peak shape is similar over a wide range of plate counts. It is only when we consider multi-component separations that the effects of efficiency are seen. Figure 2.10b shows some separations of two-component mixtures on the same 'columns' as used in Fig. 2.10a. Table 2.2 shows the recovery of the components at the desired purity specification (98% ee = 99% optical purity). Here the benefits of the higher plate number are clearly seen.

When the bands are not significantly overlapped, however, the situation is a little different, as shown in Fig. 2.11. For these touching band separations, the column efficiency required is much less important since only the overlap at the lower concentration zones is affected by the plate count. Clearly, at least some minimum number of plates is required; in the case of the data of Fig. 2.11 more than 500 plates are needed. Once the column has an efficiency above this value, there is little if no benefit of higher efficiency. In such cases, we can take advantage of using high efficiency columns to operate the separation faster. The Knox equation relates the theoretical plate height to the flow velocity and it is apparent that the faster the column is operated, the lower its efficiency. If we have too many plates for the separation, therefore, we can calculate the flow rate that will give just enough efficiency for the separation and (providing that the operating pressure is reasonable) we can then run at this high flow rate, thus improving the throughput of the separation. For the separation depicted in Fig. 2.11, an example for such a column with 2000 plates would be a column 50 × 5 cm, packed with 20 μm particles and operated at 120 mL/min. This could be operated at a flow rate 5.7 times greater before the efficiency drops to the 500 plates necessary for a good purity and recovery, increasing the production rate by a similar figure. Use of this finding can be illustrated by the separation of guaiphenesin enantiomers using a CHIRALCEL® OD® column. The separation conditions are shown in Table 2.3 and the effect of flow rate on the separation is shown in the chromatograms in Fig. 2.12. As can be seen, the increase in flow rate from 50 to 400 mL/min (this higher flow rate reached the maximum 200 psi allowed for the column hardware) caused no degradation of the separation, as could be predicted from the considerations above. The 'S–shaped' character of the isotherm can be noted from the sharp drop in the tail of the second component at the lower flow rates. This feature is degraded at higher flow rates by the lower column efficiency.



(a)



(b)

Figure 2.10 (a) Effect of efficiency on a single peak. (b) Effect of efficiency on multi-component separations.

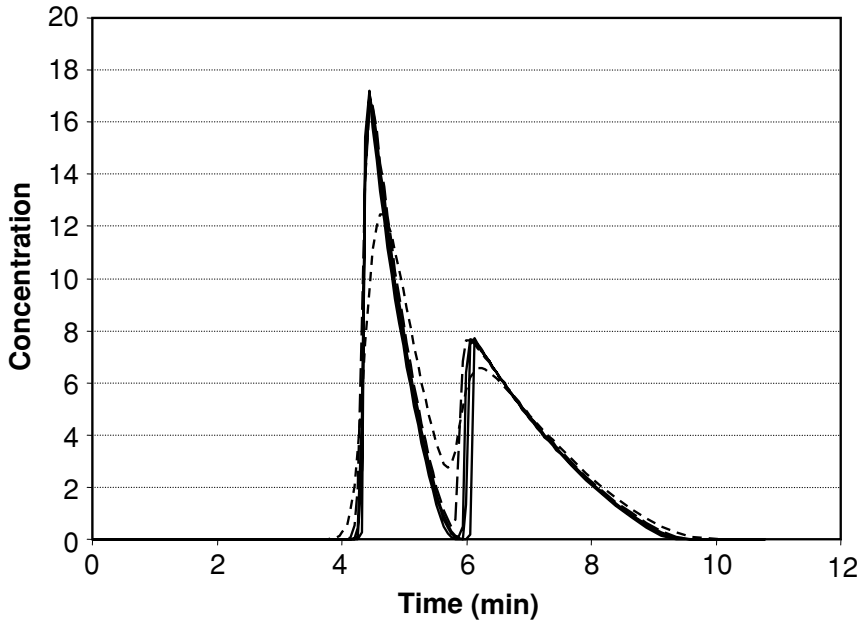


Figure 2.11 Touching band separations. Short dashed line: 200 plates; long dashed line: 500 plates; other curves: 750–2000 plates.

2.4 Metrics for preparative operations

Many ways have been proposed to quantify the output of a preparative separation process. These range from use of simple parameters to the derivation of complex equations. Some of the more useful are outlined below.

2.4.1 *Throughput*

This is the use of feed, usually in terms of mass per unit time (g/h, kg/day). Because it concentrates on the feed stock, this is not so useful in letting us know how much product can be obtained, because it is rare that one obtains 100% purity with 100% recovery. Throughput is, of course, useful when one asks the question of how much feed needs to be prepared for a given period of separation.

Table 2.3 Preparative separation of the enantiomers of guaiphenesin (operating conditions)

Column size	50 × 5 cm
Packing material	CHIRALPAK OD, 20 μm
Mobile phase	20% ethanol in hexane
Flow rate	50, 100, 200 & 400 mL/min
Sample	Guaiphenesin, 20 g/L
Injection volume	550 mL
Load	11 g

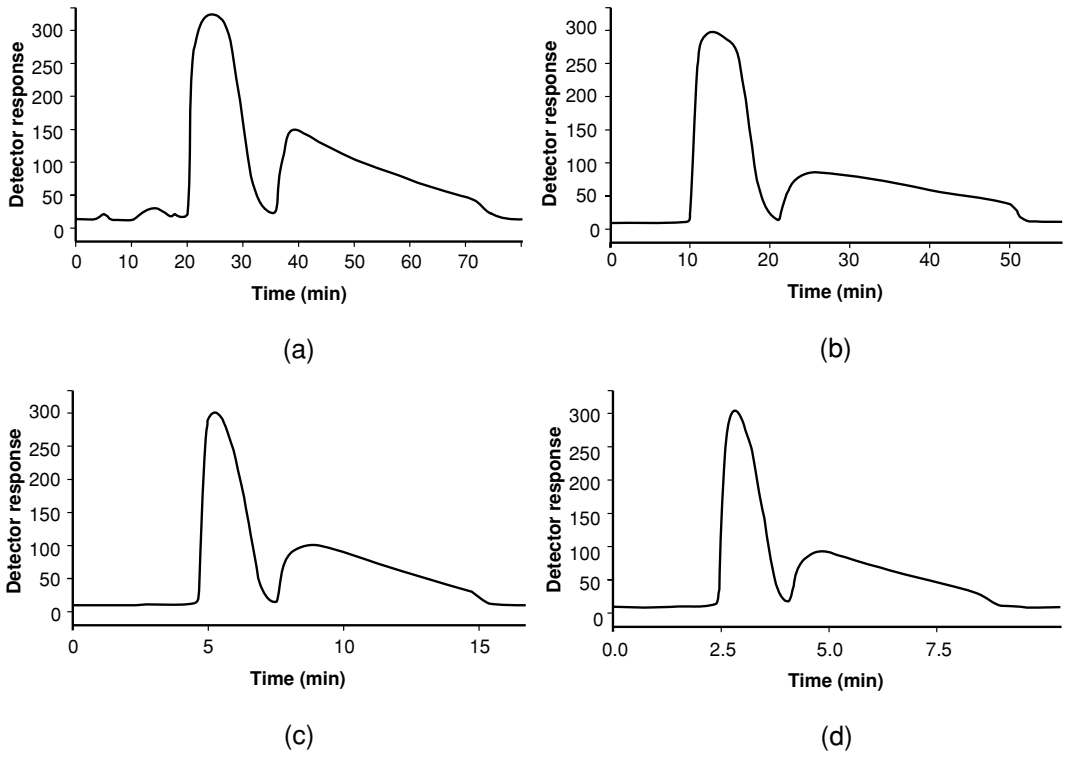


Figure 2.12 Separation of guaiphenesin enantiomers on CHIRALPAK OD, 50 × 5 cm, flow rates: 50, 100, 200 and 400 mL/min. Injected quantity: 11 g.

2.4.2 *Production rate*

This is the rate of production of the desired product, again usually in terms of mass per unit time. This takes into account the recovery yield losses and also the purity of the final product.

Both the above parameters are dependent upon the details of the preparative separation. Each is clearly dependent upon the column diameter and the flow rate. As such, they are useful when comparing data for specific equipment but are less useful as a generic metric for the operation. Other parameters have been suggested that are more general.

2.4.3 *Productivity*

This relates the quantity of product that can be purified on a unit quantity of packing material in unit time, often quoted as kg/kg/day. Again, this does not take into account the flow rate for the system. In addition, for this parameter to be useful, the separation must be optimised. One can find many articles in the literature where productivities of unoptimised separations are compared and conclusions (often erroneous) are drawn. This parameter also emphasises the separation in terms of the stationary phase and its cost, which is usually not the most important expense in the operation of the preparative process.

2.4.4 *Specific productivity*

If the productivity (kg/kg/day) is divided by the solvent consumption (L/day) then one can derive the specific productivity (kg/kg/L). This takes into account the constant costs of a

separation in terms of the use of the packing material and solvent per kilogram of product. These are constant whatever the scale of the operation and can be used to find a 'limiting cost' where the effects of scale are supposed to be so large that one can neglect the cost of equipment, labour and installation. Since the solvent cost is an important part of preparative liquid chromatography (though less so in simulated moving bed chromatography or in supercritical fluid chromatography – see Chapters 7 and 8, respectively) this is a useful parameter. Sometimes the productivity and the solvent use (L/kg) are quoted together instead of specific productivity.

2.4.5 *Cost*

As one moves to larger scale production, the cost per kilogram of final product becomes all-important. It can easily be argued that the cost of preparation of small quantities of product for preclinical testing and for Phase I and II trials is totally unimportant relative to the cost of the total operation (the cost of bringing a new drug to market is estimated to be around $500–$600 million) and to the importance of making product to meet the tight timelines. Once one reaches a manufacturing operation, however, the most important question is: How much does the product cost to make?

The cost of production is made up of many contributions – of the CSP, the solvent (both recovery and replacement of losses), the equipment, site, labour, utilities, raw materials, maintenance, etc. These are difficult to quantify in part because no two companies use the same values for many of their internal costs. Nevertheless, it is possible to make some assumptions that allow the comparison of different processes.

2.5 The influence of chromatographic parameters on preparative chromatography

Based on the general principles laid out in the earlier sections of this chapter, it is now possible to assess the influence of the various parameters upon the performance of preparative chromatography systems. These influences are usually measured by one or more of the metrics above.

2.5.1 *Effect of particle size on preparative performance*

Given that one needs a certain efficiency to achieve a given separation, the question then arises as to what particle size should be used. In theory this does not matter so very much since it is possible to achieve a desired efficiency for any particle size, supposing that one is free to choose the column dimensions and flow rate. In practice, some solutions may be sufficiently absurd to be avoided at all costs – a large particle may need to be in a column many metres in length for high plate counts, while a 3 μm particle may require a column only one or two centimetres in length, neither of which is very practical. If one assumes that the preparative LC unit will be operated at the maximum allowed pressure (a fair assumption for a production unit, less so for a lab system) then the consequences of particle size can be readily calculated, making some reasonable assumptions for the various parameters required for the plate height calculations.

Table 2.4 Production and productivity as a function of particle size

d_p (μm)	Length (cm)	Flow rate (mL/min)	Relative production rate	Diameter for constant production rate (cm)	Quantity of CSP (g)	Relative productivity
5	5.54	510.5	0.77	5.69	84.6	237.7
7	10.26	540.1	0.82	5.54	148.2	135.8
10	19.9	567.3	0.86	5.40	273.6	73.5
15	42.9	593.5	0.90	5.28	563.8	35.7
20	74.3	609.0	0.92	5.21	951.6	21.1
25	114.1	619.6	0.94	5.17	1436.4	14.0
30	162.3	627.2	0.95	5.14	2018.4	10.0
40	283.7	638.0	0.96	5.09	3468.5	5.8
50	438.3	645.1	0.97	5.07	5299.7	3.8
75	970	655.9	0.99	5.02	11535.6	1.7
100	1708	662.1	1.00	5.00	20121.9	1.0

Note: Knox equation parameters used in the calculations: $A = 1.3$, $B = 2.0$; $C = 0.1$.

This can be illustrated by supposing, for example, that one needs 1000 theoretical plates to achieve a specific separation (a given recovery and product purity at a given specific load) and one has a maximum operating pressure of 40 bar. If we assume for the moment that the performance of columns packed with particles of diameter between 5 and 100 μm can be described by the Knox equation, then we can calculate the length and flow rate for such columns as a function of the particle size. The results in Table 2.4 assume a column diameter of 5 cm and methanol as solvent.

In this example, the production rate is related to the flow rate through the column; as all columns have identical efficiency, the separation is achieved within a given number of column volumes of solvent – the faster the solvent flow, the greater the number of injections that can be made per unit time.

The consequence is that for a given operating pressure and column diameter, a larger particle will always give a higher production rate than a small one, although it should be noted that the column length and the quantity of stationary phase are much greater with the larger particles. This implies a higher equipment cost and higher packing cost. It should also be borne in mind that the larger particles operating at very high reduced velocities will no longer be at complete equilibrium with the mobile phase as assumed for the simple relations used for the above calculations. The finite intraparticle diffusion rates and the differential rates of adsorption/desorption will increase the peak skew and reduce their effectiveness under such conditions.

If we correct the column diameter to achieve constant production rate from all columns then it is clear that because the smaller particles require short columns, the productivity for these materials will be much higher than that for the larger particle packing materials: by a factor of 21 in changing from 100 to 20 μm CSP and by a factor of 10 in using 5 μm in place of 20 μm material.

For achiral separations where the cost of the packing is a strong function of the particle size, there is an optimum particle size that minimises the total cost of the production. This is usually found to be between 10 and 15 μm. In the case of enantioselective separations, however, the cost of the chiral selector and its introduction to the CSP is often much greater

than the cost of the silica base particle. In such cases, the cost of the CSP is not a strong function of the particle size and simple economics dictates using the smallest particle size possible consistent with maintaining reasonable pressure drops and column dimensions.

In laboratory-scale operation, the choices are often different, resulting from the constraints of existing equipment and available column size. Often the requirement is to make the maximum quantity in the minimum time. This is reasonable, considering that laboratory overheads and operator costs are usually the controlling factors in the separation cost. Frequently the time spent in finding the optimum column and particle size exceeds that needed for performing the separation on the existing column and equipment, which means that almost by definition the laboratory-scale separations are not carried out under optimum conditions. This is not at all important in the general case, but where the separation is to be scaled to larger quantities, one can end up in the difficult situation of needing to meet ever-increasing demands with a non-optimum method and insufficient time to devote to its improvement.

2.5.2 *Effects of pressure*

It is clear from the foregoing that the production rate achievable for a column is dependent upon the flow rate passing through it. This, in turn, is a function of the pressure available to drive the solvent, the required efficiency and the column length necessary to achieve that efficiency. It is interesting to consider the effects of increasing the operating pressure in a system. Table 2.5 shows the effects of pressure on the production rate and productivity for a separation requiring 2000 plates using 15 μm particles.

Standardising on 40 bar (more or less the current practice in large-scale chromatography – at least in SMB systems), we can see that increasing the operating pressure to 300 bar increases the productivity by only 20%. Decreasing the operating pressure to 2 bar decreases the productivity by a factor of 1.75. There is a cost in moving to high pressures; the column hardware, valves and tube fittings have to be significantly stronger and the pumps have to be larger. These increases may not be economically viable and it is difficult to imagine that operating pressures in HPLC will significantly increase much beyond the present levels. There is

Table 2.5 Effect of pressure on productivity and production rate

Pressure (bar)	Length (cm)	Flow (mL/min)	Relative production rate	Relative productivity	Diameter for constant production rate (cm)	Relative CSP quantity
2	18.64	68.25	0.17	0.57	12.17	1.75
5	26.39	120.55	0.30	0.71	9.15	1.40
10	34.87	182.4	0.45	0.82	7.44	1.23
20	46.6	272.9	0.68	0.91	6.08	1.10
30	55.5	343.7	0.85	0.97	5.42	1.04
40	63	404.1	1.00	1.00	5.00	1.00
75	83.3	572.7	1.42	1.07	4.20	0.93
100	94.9	670.5	1.66	1.10	3.88	0.91
150	114.2	835.7	2.07	1.14	3.48	0.88
200	130.4	975.9	2.41	1.17	3.22	0.86
300	157.4	1212	3.00	1.20	2.89	0.83

a significant reduction in hardware (investment) costs if the pressure is reduced. Operating at 2 bar in this instance, while increasing the CSP requirement by a factor of 1.75, will allow the use of low-pressure columns, valves and pumps that may compensate for the increased CSP cost. Equally, operation at such low pressures will also reduce the stress of operation on the CSP and thus will probably increase its lifetime, again reducing the operating costs. Clearly, any decisions on choices of operating pressures are closely tied in with the economics of operation and the capital expense of the chromatographic units, and a careful analysis has to be made in assessing the actual operating parameters adopted for the separation.

2.5.3 *Effects of column efficiency*

In practice, there is usually not a wide range of particle sizes available for optimisation of the separation, nor is there an infinite variety of column hardware from which to choose. At the semi-preparative level, there are perhaps three particle sizes and two different column lengths commercially available from which one may choose. The question of which is appropriate depends – as always – on the objective of the separation. If the objective is to maximise the production rate, which is often the case since the pressures to produce the first few grams for testing are high, then one needs to have a good idea of the influence of the column efficiency on the production rate of a separation in order to make the appropriate choice. There are a number of ways to do this, however, since there are several parameters at play in the definition of column efficiency. At the small scale, the operating pressure is not very important and semi-preparative columns are usually run at a certain flow rate, irrespective of operating pressure, so long as it does not exceed the maximum allowed for the column hardware. As the scale increases, however, the pressure becomes a limiting factor, since the maximum allowable pressure is a function of the mechanical strength of the packing material and of the pressure capability of the column hardware.

If the separation is constrained to operate at a certain maximum pressure, then the maximum production rate attainable is always at this maximum pressure, providing the column and packing can be optimised. Such a case is shown in Fig. 2.13, where a maximum pressure of 20 bar is assumed and the column dimension is fixed at 25 $\times$ 2 cm. The particle size is varied to reach the desired efficiency. Three separations with different selectivities are modelled. There is, under these conditions, an optimum number of theoretical plates that allows one to perform the separation at a maximum production rate. As the efficiency is increased (by reducing the particle size) so the flow rate through the column decreases. At the same time, the load increases since the bands become narrower and the amount of overlap due to the band spreading is reduced, thus allowing an increase in sample load. The production rate in this case is calculated for the second enantiomer. At low selectivity (solid line) the efficiency required for the separation is high. The maximum in the curve is broad and the same production rate can be achieved with efficiency anywhere from 2500 to 4000 plates. As selectivity increases, the optimum plate count decreases, and the production rate increases. For a selectivity of 2, the optimum plate number is small, around 250–300.

If the operating pressure is not a constraint, then the picture is a little different. If one supposes that a column 2 cm in diameter will be operated at a constant flow rate (say approximately 18–20 mL/min, equivalent to 1 mL/min in a 4.6 mm diameter analytical column on which the separation is first developed) then the efficiency will depend on the particle size and so increase with decreasing particle diameter, but the overall separation

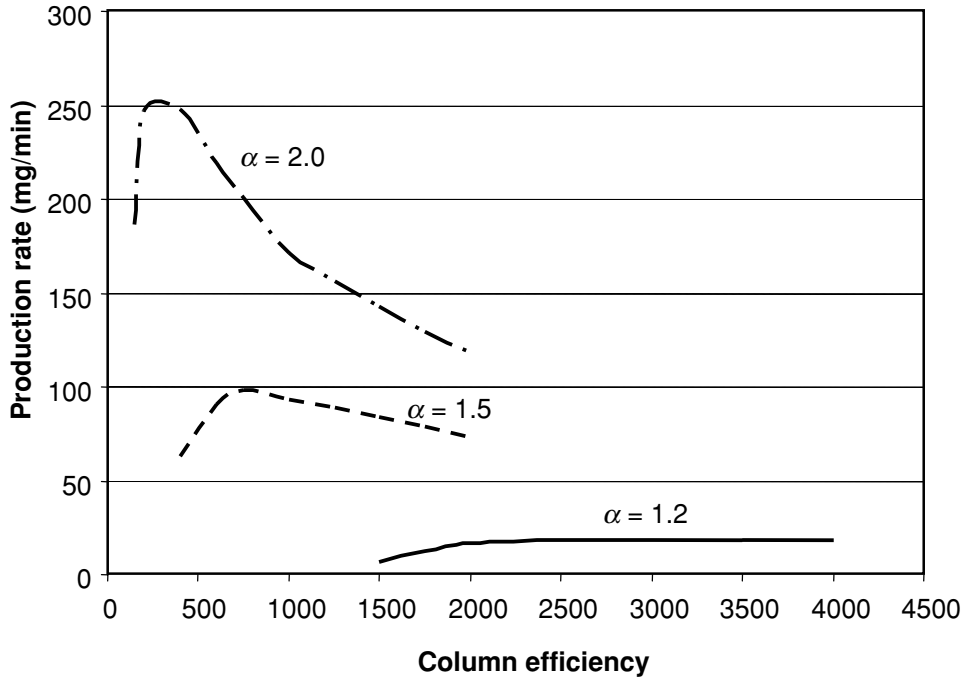


Figure 2.13 Effect of column efficiency on production rate at constant pressure.

time will remain constant if one ignores for the instant the effects of peak broadening. In this case, it can be expected that the column with higher plate count will always give the higher production rate, up to the point that the equipment can no longer produce the flow at the pressure required to maintain the desired flow rate. Figure 2.14 shows this situation for comparison with that where pressure can be limiting. In this case, the higher the performance of the column, the more load can be applied and the greater is the production rate. Beyond a certain point, there is marginal improvement in the production rate for any given selectivity. This is around 1500 plates for the larger selectivity separations and over 4000 for the low-selectivity separations. Thus, in a laboratory carrying out semi-preparative separations, if only one column can be purchased (semi-preparative chiral columns can be expensive) it is better to purchase one with high efficiency that will be capable of performing all separations. Where the scale is larger and there is scope for optimisation, there is clearly an important place for the lower efficiency, larger particle size columns since these can be operated at higher flow rates without incurring the penalty of a high operating pressure.

2.5.4 *Effect of column length*

At first sight, the length of the preparative column does not seem to be too important. This is because the column saturation capacity is a function of the quantity of CSP in the column, which in the case of a fixed diameter, is proportional to the length. If the length is doubled, so is the column saturation capacity and therefore so too is the total quantity of sample that can be injected, supposing that there are sufficient theoretical plates to allow the separation to proceed. With the doubling of the length, the retention time (at constant flow rate) is

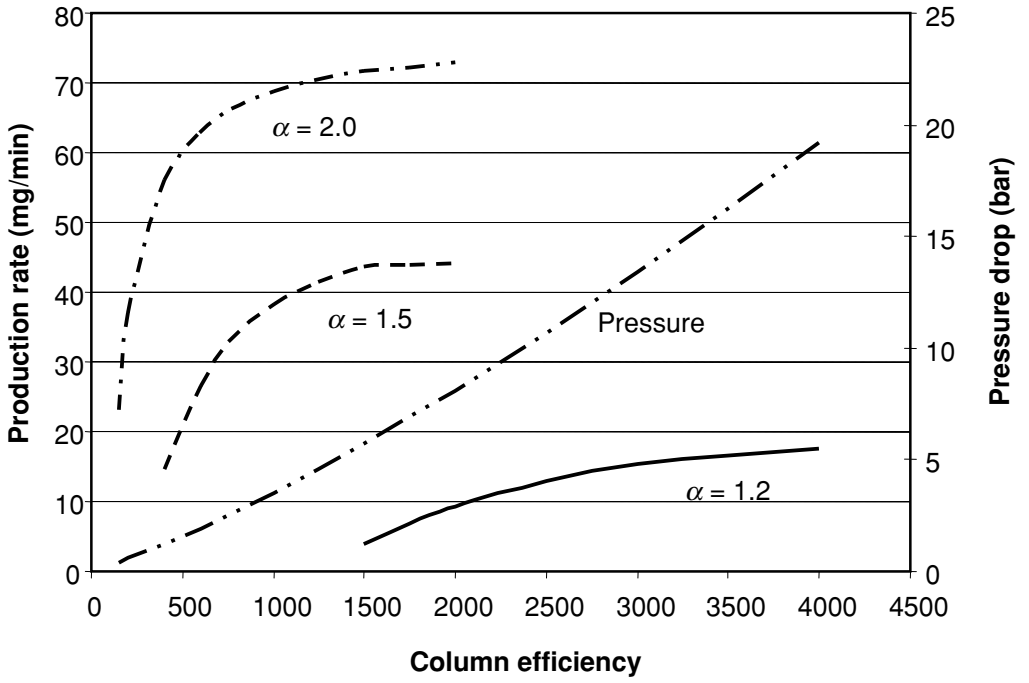


Figure 2.14 Effect of column efficiency on production rate at constant flow rate.

also doubled and this means that the production rate is constant. If the separation is being carried out on a column with barely enough plates, then increasing column length will allow a disproportionately larger load of sample and this will give a higher production rate.

This can be illustrated by considering the case of a moderate selectivity separation. In the usual case in the laboratory one has a single particle size (especially once one is using columns larger than around 5 cm diameter) and it is of interest to decide on the length of the column to use. Figure 2.15 shows the results of calculations of production rate as a function of efficiency for two column lengths, in this case 25 and 50 cm, choosing particles of 20 μm diameter. Ignoring for the first instance the pressure drop in these columns, it is clear that for the separation under consideration (selectivity = 1.5) the longer column always gives a higher production rate. At a given flow rate the longer column gives twice the efficiency of the shorter one and an identical production rate, thus the flow in the longer column can be increased, trading the extra efficiency for speed, and the production rate can thus be significantly increased. For the separation chosen here, the shorter column is limited in production rate by the required efficiency. The number of plates needed for the optimal production rate is somewhere around 650 plates, which corresponds to a flow rate of 43 mL/min at a pressure drop of 10 bar. Increasing the flow rate to use the maximum system pressure, in this case, will reduce the column efficiency to a value well below that which will allow resolution of the two components at the purity and recovery values desired. The column 50 cm in length requires a pressure of around 46 bar to reach the optimal flow rate of 101 mL/min. It should be noted that in terms of productivity, use of longer columns does not appear to be especially beneficial. In this case, at the optimal plate number, the 50 cm column has 2.29 times the production rate of the 25 cm column, but it contains twice

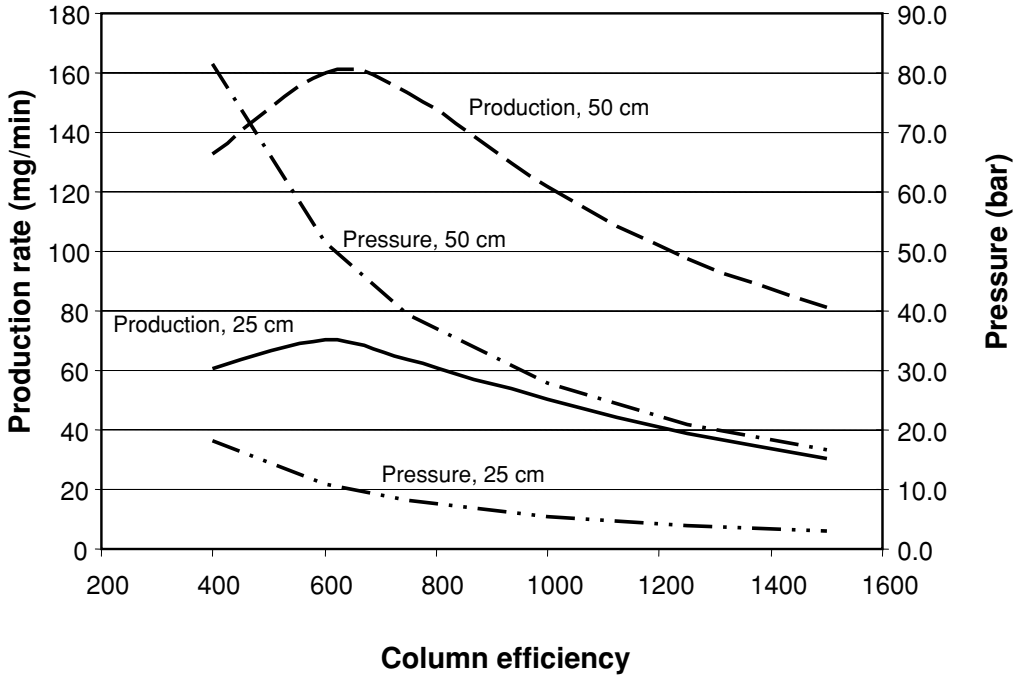


Figure 2.15 Effect of column efficiency and column length on production rate at constant particle size.

the quantity of stationary phase. This means that the productivity is only 1.15 times that of the shorter column. This is only important if the column has to be replaced frequently – if the column lasts a long time, the cost of the column will be a very small part of the cost of the production of the pure enantiomer and will disappear into insignificance.

2.5.5 *The effects of selectivity*

Some of the influence of selectivity on the production rate of a chromatographic system can be gleaned from the foregoing section on the effects of efficiency, since the two parameters have some interaction. In the laboratory, the rule is simple: a high-efficiency column can address all required separations while a low-efficiency column can address only the easy, low-selectivity separations. As one wishes to move to larger scale, selectivity becomes an important parameter because of its strong relation to the production rate. A relatively small increase in selectivity can give a large increase in production rate. Thus, most time spent on the development of large-scale separations is devoted to optimising the selectivity. The mechanism for such optimisation is left to a later chapter; here it is instructive to investigate how production rate is affected by the selectivity of the separation.

A simple relation can be derived from Guiochon's equation for the loading factor in terms of the ratio of capacity factor under load to the analytical value:

$$\frac{w_{x,2}}{w_{s,2}} = \left[1 - \left(\frac{k'_2}{k'_{o,2}}\right)^{1/2}\right]^2 \tag{2.8}$$

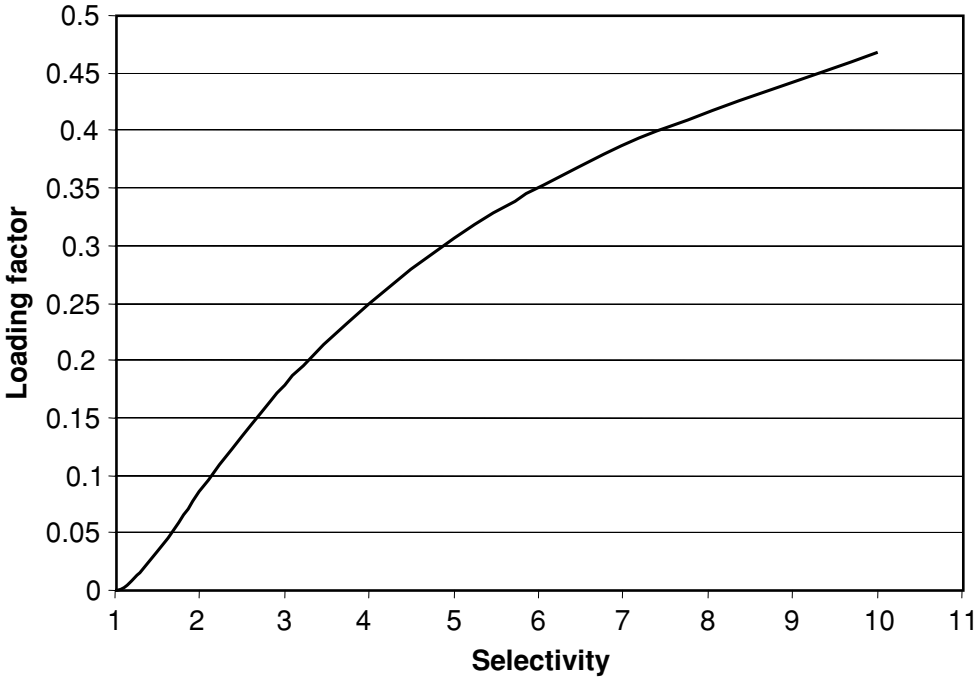


Figure 2.16 Loading factor vs selectivity.

where $w_{x,2}$ is the load of component 2 on the column which has a column saturation capacity $w_{s,2}$ (the ratio of the two is termed the loading factor), k'_2 is the capacity factor calculated from the peak maximum, while $k'_{o,2}$ is the capacity factor of that component at analytical load.

To a first approximation, if we ignore the interactions between the solutes and if the column efficiency is high, a touching band separation results when the capacity factor of the peak maximum of the second peak equals the capacity factor at analytical load of the first. Thus, we can substitute $k'_{o,1}$ for k'_2 in the above equation and obtain a value of the loading factor (w_x/w_s) that corresponds to a touching band separation:

$$\frac{w_{x,2}}{w_{s,2}} = \left[1 - \left(\frac{1}{\alpha}\right)^{1/2}\right]^2 \tag{2.9}$$

This relation is shown in Fig. 2.16 and illustrates, taking the loading factor to be directly related to production rate, that the production is strongly affected by the selectivity. It is also possible to obtain data relating production rate to selectivity from computer simulations, which include inter-solute interactions and can make allowance for the need to optimise the plate number and flow rates of the system as the selectivity is changed. The resulting data points, taken from the simulations carried out for the efficiency relationships above, are shown in Fig. 2.17 with the relevant curve calculated from Equation (2.8), and indicate that the influence of selectivity on the production rate is reasonably well described by the simple theory at the touching band level.

When overload beyond the touching band level is of interest, then other considerations come into play. As well as the required purity of the product, we must also define a recovery

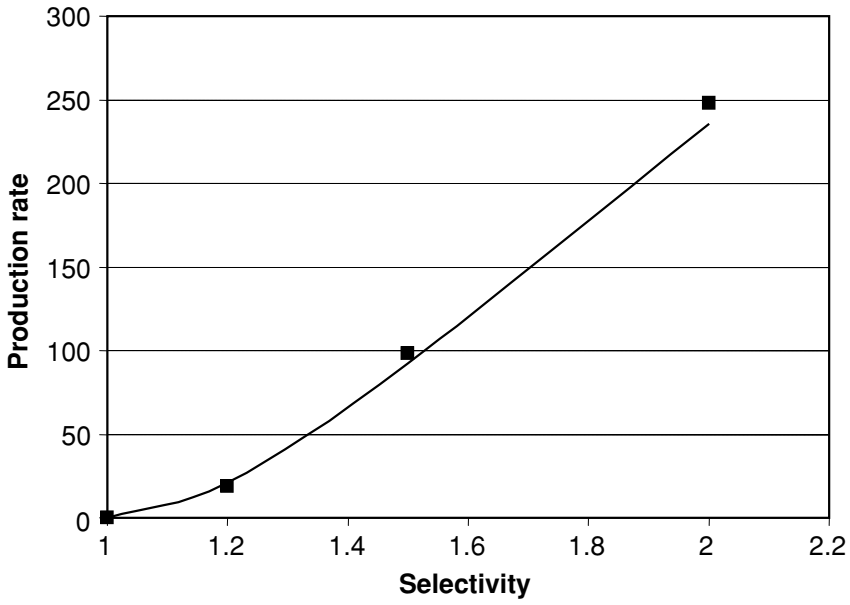


Figure 2.17 Effect of selectivity on production rate. Line = Equation (2.8). Points are optimised production rates from simulations.

yield for the enantiomer of interest. Because of the way in which the bands overlap in the chromatogram, the first eluting enantiomer is usually isolated with a higher rate of production than is the second. Figure 2.18 shows a typical case, where the load injected is increased with the collection of product with a constant purity. The production of each enantiomer reaches a maximum at a certain load, beyond which the loss in recovery is greater than the gain in sample load. The production rate of the two products is different, although in the case illustrated the maximum load of the racemate that gives the highest production rate is approximately the same. This does not mean, of course, that separations should always be run at very high sample load and low recovery. If the production rate is very important and the racemate is very cheap and plentiful, then there is a strong case for maximising the load and production rate in this manner. At high loads, the incremental production rate for larger injections is rather small and for any product that has at least a minimum value, the benefit in production rate will be offset by the cost of the loss of product. It is here that it becomes evident that optimisation in preparative chromatography at larger scale is not about production rate or sample recovery but is a function of the costs of the operation. In the laboratory environment, the product is usually valuable and reflects a significant effort on the part of the synthetic chemists. The usual target is to maximise the recovery to ensure minimal product losses. As the synthesis of the product is made more economic, then it is possible to envision losing some material to increase the production rate, and therefore the load per injection.

2.6 Economics of preparative separations

As noted earlier, at large scale the best metric for the optimisation of a preparative separation is the cost per kilogram of purified product. There are several cost contributions

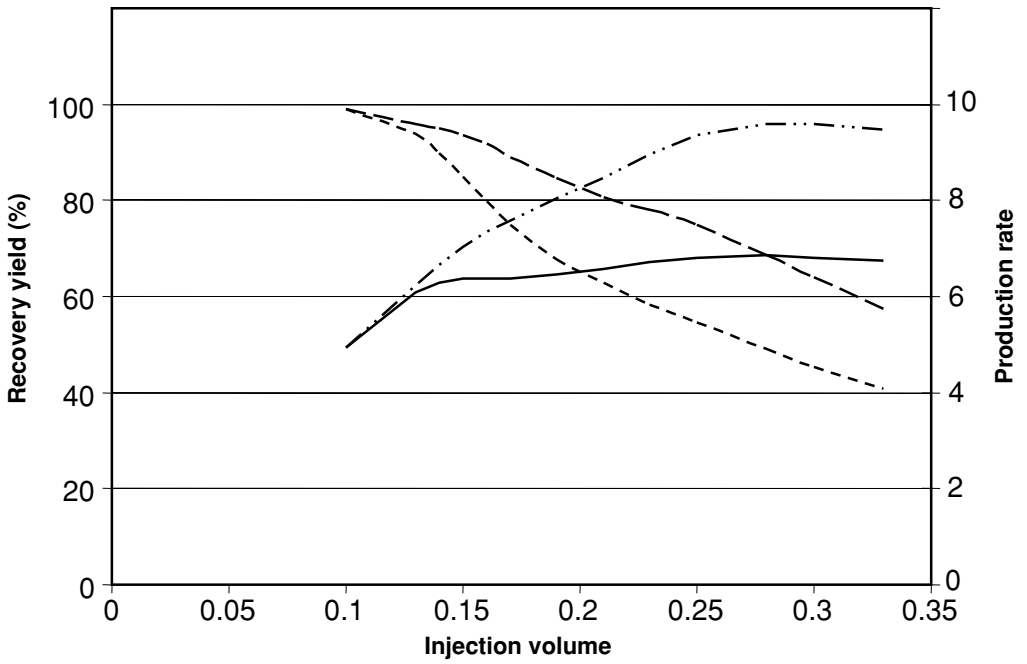


Figure 2.18 Production rate and recovery yield as a function of injected quantity. Long dashed line = recovery yield for 1st eluting component; short dashed line = recovery yield for 2nd eluting component; dashed-double dotted line = production rate for 1st eluting component; solid line = production rate for 2nd eluting component.

to a chromatographic process. The obvious ones are the cost of the equipment (amortised over a certain period), the cost of the packing material (although the lifetime of the packing material has to be factored into this cost), solvent and the cost of the labour involved. There are other costs such as the losses of product, the cost of recovery of the solvent (in most large-scale processes, the solvent is recovered and recycled, or at least used for some other purpose – even as a fuel for heating – so that its value is at least partially recovered), the cost of the site, of maintenance, of power and perhaps the cost of additional resources such as QC or site overheads. Some of the costs of operation are consistent with the scale of the separation, when calculated on the basis of the cost per kilogram of product. These are typically those associated with the solvent and the packing material; as the separation scale increases, these two items increase at the identical rate as the quantity of product. Other costs diminish with increasing production rate, since there are economies of scale to be taken into account. There are many opportunities for compromise in the optimisation of a separation. For example, there may be the option of choosing between a low viscosity, higher cost solvent such as acetonitrile and a higher viscosity, lower cost solvent such as ethanol. The lower viscosity of acetonitrile allows the faster operation of the separation at a given pressure and thus although the quantity of solvent used per kilogram of product will not change, the cost per kilogram per day of the other factors in the operation will drop significantly compared to the use of the more viscous ethanol. This reduction in other costs may well offset the higher cost of purchase of the solvent, especially when the different heats of evaporation are factored into the costs for the recovery of the solvent, where again, the more volatile acetonitrile will be favoured. There is indeed the possibility that the use of acetonitrile may give a lower cost per kilogram of product even though its use results in a lower selectivity

for the separation. This implies that the separation used at larger scale may not always be that used in the laboratory. Other examples of such a situation could be where the ease of recovery of a single solvent results in a lower cost than the case of where a mixed solvent is used, which gives a better selectivity but for which there are issues of solvent recovery.

It should be noted, however, that for the vast majority of preparative (rather than production scale) separations that are carried out, the fine-tuning of costs is not so very important since the prime need is to make some quantity of product for a specific purpose or test. When the separation is to be used for manufacture, the operating costs have to be optimised carefully and in many instances the early stage cost estimations for a large-scale chromatographic process determine whether or not such a process will be even considered as a means of production of a given product. Unfortunately, even favourable economics do not always ensure that a chromatographic process will be used, in part due to resistance to the adoption of a new technique into a manufacturing site, in part because other processes which, while less economically favoured, may use existing plant that is underutilised.

2.6.1 *Point of insertion of the chromatographic resolution in the synthetic route*

One aspect of enantioselective separations that has to be considered in any cost calculation is the fact that 50% of the starting material is to be discarded. Where the unwanted enantiomer cannot be used, either for another purpose, or after racemisation as fresh feed stock, there has to be a significant economic advantage in the process. This is sometimes the case, but in the design of any large-scale separation the possibility of racemisation has to be considered and may, in certain cases, define the point in the synthesis at which the chiral resolution is performed.

Another important aspect that has always to be considered is where in a synthesis the chirality should be established. There are a number of factors. All intermediates that contain the desired chiral centre(s) should be screened for chromatographic separation. Frequently one will be found that gives high selectivity and productivity; this is practically self-selecting. There are, however, other considerations. It is obviously necessary to check downstream of the proposed chromatographic step in the synthesis route to ensure that none of the following procedures induce racemisation. In certain cases, establishment of the chirality of the intermediate may precipitate a need to perform this and all following steps under cGMP, while postponing the resolution to a later stage may relax this need and allow greater flexibility in where and how to carry out the synthesis of the intermediates. Conversely, putting the resolution early in the synthesis reduces the chemical load on the subsequent steps, especially where re-racemisation of the unwanted enantiomer proceeds only at low yield or is not possible.

It is not the intent of this book to provide answers to all the problems of scaling to production. Nevertheless, the way that some of these factors are addressed in the industrial context can be found in later chapters that deal with larger scale operation.

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Preparative Enantioselective Chromatography
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3 Chiral stationary phases for preparative enantioselective chromatography

ERIC FRANCOTTE

3.1 Summary

The usefulness and broad applicability of enantioselective chromatography on chiral stationary phases (CSPs) for the preparative separation of enantiomers is now definitely established as a complementary approach to the more conventional methodologies such as enantioselective synthesis and enzymatically catalysed transformations. This development would not have been possible without the design and preparation of efficient CSPs, which have been specifically intended to fulfil the requirements of preparative purposes. The CSPs that are currently available for preparative separations are reviewed, with special emphasis on their application fields and limitations. Recent progress and future developments in this area are also discussed.

3.2 Introduction

Basically, there are two approaches, 'chiral and racemic', to prepare the single enantiomers of chiral compounds. The 'chiral approach' consists in designing a stereoselective synthesis of the desired single enantiomer either from chiral building blocks or using chiral auxiliaries or catalysts, while the 'racemic approach' involves the separation of the mixture of stereoisomers, which have been prepared by classical synthetic methodologies. This preparation is usually achieved by a reaction sequence that generally presents a much lower degree of difficulty than for the corresponding optically active forms. In the racemic approach, separation methods comprise the widely used technique of crystallisation of diastereoisomers, membrane systems and chromatographic methods. Among the chromatographic modes, the most used approach is undeniably LC separation on CSPs. This successful development has been made possible thanks to the simultaneous development of both new efficient CSPs and powerful separation techniques. In particular, the design of numerous CSPs has provided new tools suitable for preparative separations.

The enantioselective separation of enantiomers by chromatography on CSPs has gained increasing recognition over the last 15 years and the technique is now considered as a powerful approach for the preparation of optically pure compounds [1–5]. This trend is clearly demonstrated by the rapidly growing number of applications published in this area. In the field of preparative chromatography it has even been the driving force for the acceptance of the technology as an industrial option, in particular since the introduction of the simulated moving bed technology [6–9].

Although the enantioselective chromatographic technique is increasingly applied at a preparative scale, especially in the pharmaceutical industry, the situation must be regarded differently depending on the stage of development of the compound of interest, the goal

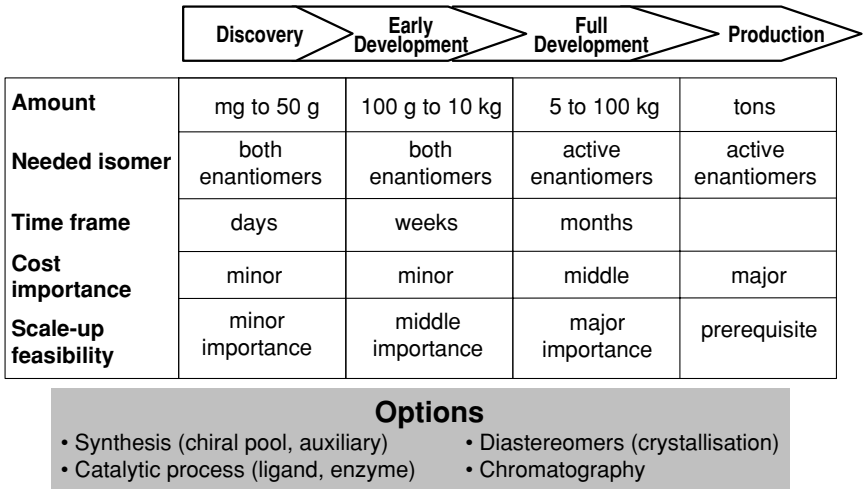

Figure 3.1 Requirements for the development of chiral drugs.

being to produce a certain amount of pure enantiomer in a certain time frame and under certain cost limitations (Fig. 3.1). Indeed, considering the current requirements for developing the single enantiomers of chiral substances with respect to amount, time frame, cost and scale-up feasibility, their respective importance considerably varies with the degree of development of the chiral drug or pesticide.

At the discovery stage, time is the most important factor. The process must be rapid and generally applicable. In addition, at this stage both enantiomers are generally needed for preliminary comparative biological tests and chromatography offers the advantage of furnishing both enantiomers in high optical purity. In early development, the time frame is still relatively short and scale-up feasibility should already be considered. At the stage of full development, the process must be established, it must be robust and cost becomes a significant parameter. At the production scale, cost is a major concern and scale-up feasibility is obviously a prerequisite.

CSPs are obviously a key element in preparative enantioselective chromatography. The ideal preparative CSP would exhibit the following features: (a) it should be universal (high and extended recognition ability); (b) it should have a high loading capacity; (c) it should be robust and durable (chemical and physical stability); (d) it should tolerate a wide range of mobile phases; (e) it should be available in large amounts and at a reasonable price. Even though the ideal CSP for preparative separations is not yet available and will probably never be available, a number of efficient CSPs have been designed over the last 20 years and allow most of the racemic compounds to be resolved on a preparative scale. This chapter describes the state-of-the-art regarding the currently available CSPs, emphasising the strengths and weaknesses of the different CSPs. Advances to be expected and future developments in this specific research area are also described.

3.3 Historical development of CSPs for preparative chromatography

CSPs were already applied as a preparative tool many years before their potential was recognised as a powerful technique for the analysis of chiral compounds.

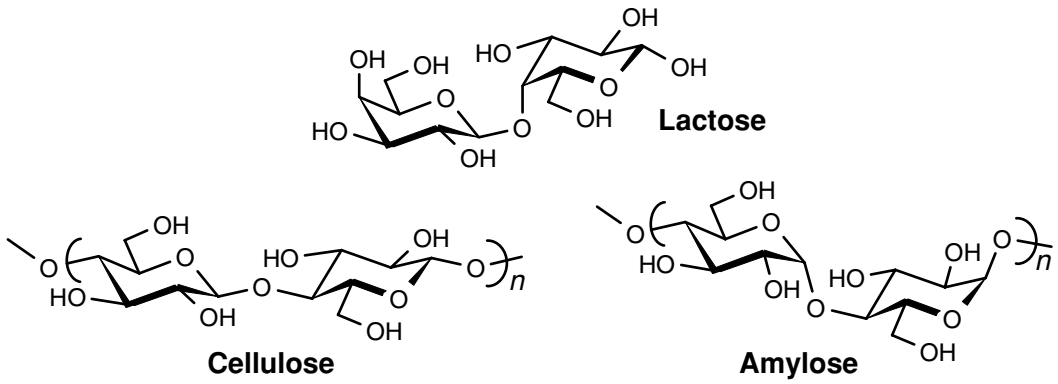

Figure 3.2 Structure of lactose, cellulose and amylose.

As early as 1904, Willstätter attempted to separate optical isomers on the optically active natural polymers wool and silk [10]. About 35 years later, lactose was used for the first partial chromatographic resolution of the enantiomers of *p*-phenylene-bis-imino-camphor by Henderson and Rule [11], and a few years later by Lecoq for the enantiomers of ephedrine [12], and by Prelog and Wieland for the enantiomers of Tröger's base [13].

Since 1948, polysaccharides such as cellulose and starch (see Fig.3.2) have been recognised as attractive CSPs for the chromatographic separation of enantiomers. Dent [14], Kotake *et al*. [15] and Dalgliesh [16] used paper cellulose for the preparative separation of the enantiomers of amino acids. In the 1950s, Krebs and co-workers intensively investigated the potential of cellulose and starch as CSPs [17–19], but in most instances, only partial resolution could be achieved. Musso and his group successfully applied starch for the preparative separation of atropisomers (Fig. 3.3) [20], which were difficult to separate by other methods. In 1966, however, Lüttringhaus and Peters [21] and Schlögl [22,23] pointed out that partially acetylated cellulose provided higher chiral recognition power

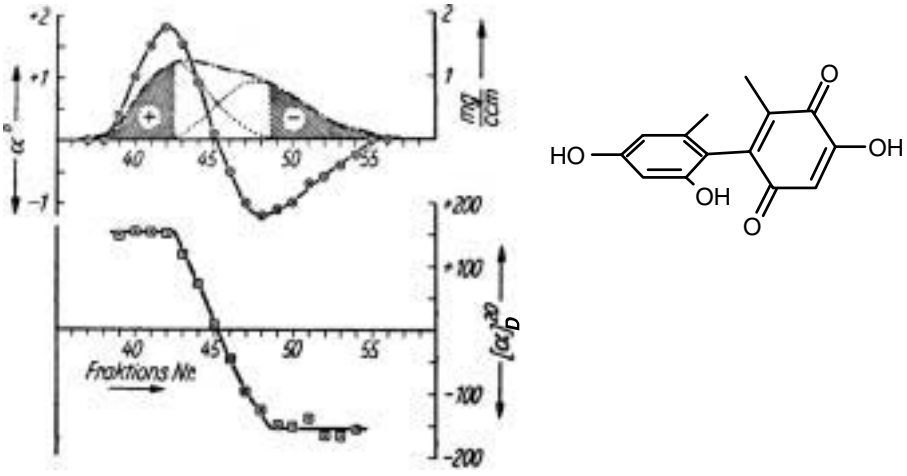

Figure 3.3 Preparative separation of atropisomers on starch. Reprinted from Ref. [20] with permission of Verlag Chemie, GmbH.

for the same purpose. All these earlier applications have been compiled in an exhaustive review [24].

The potential of cellulose acetate as a CSP was definitely established in 1973 by Hesse and Hagel who introduced fully acetylated cellulose (triacetylcellulose), which was shown to be a much more efficient material than was the partially acetylated organic material [25]. They successfully achieved the preparative separation of the enantiomers of various chiral compounds. One of the first successful applications on cellulose triacetate is shown in Fig. 3.4 for phenylcyclohexanone. For many years, triacetylcellulose was practically the only CSP available for preparative separations and it has been used for the chromatographic resolution of a broad variety of chiral structures [1–3,26,27].

As early as 1961, Helfferich introduced the principle of ligand exchange for the chromatographic separation of optical isomers [28], but it was only about 10 years later that independently, the group of Davankov in Russia [29] and the group of Bernauer in Switzerland [30] reported on the significant use of this type of chromatography for preparative purpose. A number of preparative applications using this kind of CSP have been reported between 1971 and 1987 [24,31–34], but since 1990 the significance of the ligand-exchange CSPs for preparative purpose has considerably decreased.

About 30 years ago, Blaschke designed purely synthetic CSPs obtained by emulsion polymerisation of acrylamides from amino acids [35]. These phases had been developed for preparative purposes and proved to be very efficient for the preparative resolution of various chiral drugs for which the enantiomers had been isolated for the first time. These earlier applications include the separation of the enantiomers of the sadly well-known drug thalidomide [36] (Fig. 3.5).

From the early 1980s, a growing number of analytical chiral columns became available and are now routinely used for the determination of the enantiomeric composition of mixtures of optical isomers from enantioselective syntheses, from biological investigations or from pharmacokinetic or toxicology studies. Some of these phases also became extremely useful for enantioselective preparative separations [1–4,24,27].

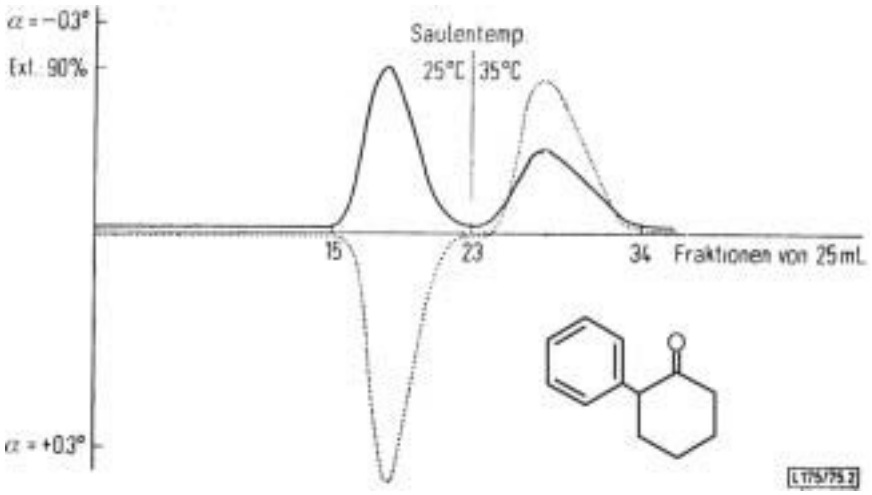

Figure 3.4 Preparative separation of the enantiomers of phenylcyclohexanone on cellulose triacetate. Reprinted from Ref. [26], with permission from Verlag Chemie, GmbH.

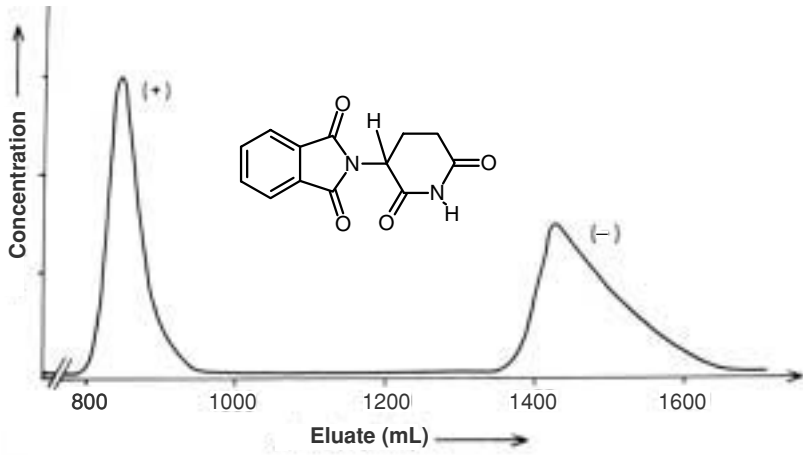

Figure 3.5 First preparative isolation of the enantiomers of thalidomide on cross-linked optically active polyacrylamide. Reproduced from Ref. [36] with permission of Marcel Dekker, Inc.

3.4 Preparative CSPs

Nowadays, numerous CSPs are available for the preparative separation of enantiomers and the section below summarises their properties.

3.4.1 *Classification of CSPs*

Fundamentally, one can distinguish three kinds of CSPs, chiral polymers (Type I), achiral matrices (mainly silica gel) modified with chiral moieties (Type II), and imprinted materials (Type III) (Fig. 3.6).

In the first group (Type I), which comprises most organic polymeric phases, the density of chiral information is generally high and a simultaneous participation of several chiral interaction sites or several polymeric chains has been proved in several instances. Even though it is not always easy to prove this mechanism, this was demonstrated in the case of some cellulose derivatives for which the influence of the supramolecular structure on enantioselectivity was definitely verified [25,26,37,38]. This mode of interaction can be defined as 'multimolecular or concerted'. The polymer can be in pure or diluted form when coated or grafted. Nevertheless, even in the 'diluted' form, the possibility for a multimolecular interaction is maintained. Oligo- and polysaccharides and their derivatives, polyacrylamides, polyacrylesters and the protein-based phases belong to this type of CSPs.

The second most used type of CSPs (Type II) are chiral sorbents obtained by attaching optically active molecular entities to achiral carriers (mainly silica gel) by means of ionic or covalent bonds. A wide range of optically active moieties have already been applied, including amino acid derivatives, crown ethers, cinchona alkaloids, carbohydrates, amines, tartaric acid derivatives, cyclodextrins and binaphthol. Although the silica carrier is also a polymer, in this class of CSPs the chiral interaction sites distributed at the surface or in the network of the achiral support are relatively far away from each other and essentially only a

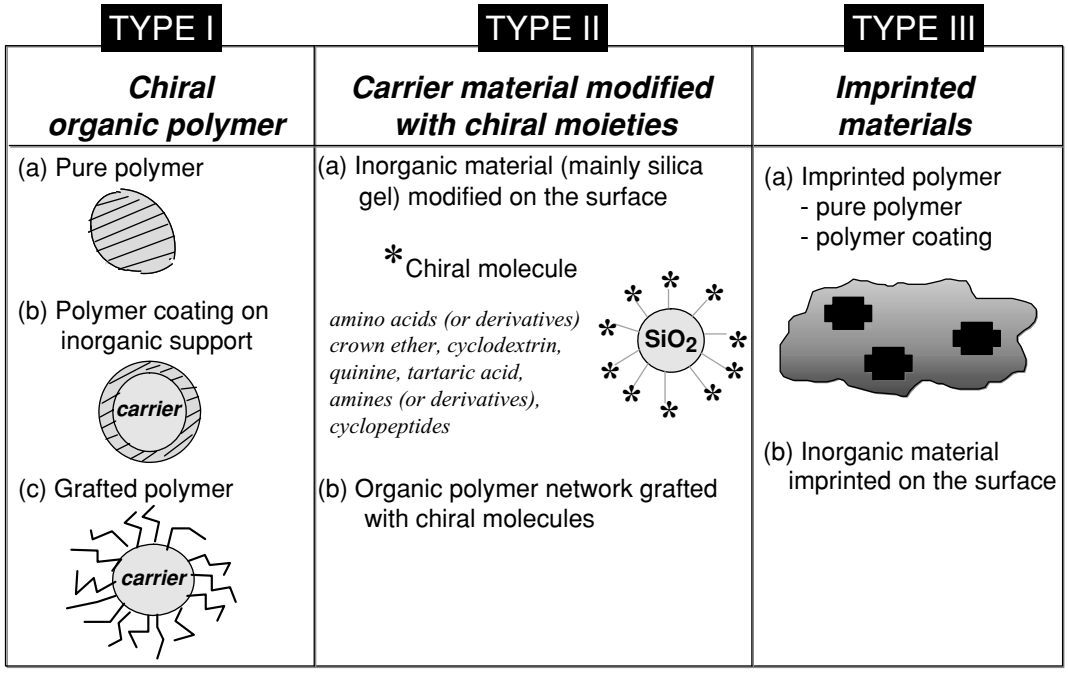

Figure 3.6 Classification of CSPs for liquid chromatography.

'bimolecular' stereoselective interaction is possible between the chiral solute and the chiral selector. However in one case, Pirkle was able to induce a double bimolecular interaction involving two chiral selectors simultaneously by using a dimeric solute bonded via a long spacer, resulting in very high enantioselectivity [39]. Cyclodextrins as chiral selectors constitute an intermediate case because the inclusion complexation with this macromolecule involves the interaction with several glucose residues, but nevertheless, a simultaneous interaction with two or more cyclodextrin molecules is very unlikely.

A third group of CSPs (Type III) includes those obtained by the imprinting technique initially developed by Wulff [40]. These phases do not contain chiral moieties but they consist of chiral cavities, whose chirality is only due to the tri-dimensional imprinting created in the polymer by the reversibly bonded template chiral molecule during the polymerisation process.

In all classes of CSPs the classical interaction forces such as ionic, dipolar, hydrophobic, hydrogen bonding and π–π interactions can be involved. Table 3.1 summarises the currently commercially available CSPs for preparative separations.

3.4.2 *Polymeric phases*

3.4.2.1 *Polysaccharide-based CSPs* A wide range of polysaccharide-based stationary phases (Fig. 3.7) have been developed during the last 20 years [41], and several of these CSPs are available for preparative purposes. These polymeric materials have been applied as pure polymers in a form adequate for chromatographic purposes or as a coating on an inert achiral support, conferring mechanical stability.

Table 3.1 Commercially available preparative chiral stationary phases

Packing name	Chiral selector (CSP type)	(CSP type)
Cellulose and amylose derivatives		
Chiralcel OB™	Cellulose tribenzoate	lb
Chiralcel OC™	Cellulose tris(phenylcarbamate)	lb
Chiralcel OD™	Cellulose tris(3,5-dimethylphenylcarbamate)	lb
Chiralcel OJ™	Cellulose tris(*para*-methylbenzoate)	lb
Chiralcel OF™	Cellulose	lb
Chiralcel OG™	Cellulose	lb
Chiralcel OK™	Cellulose tricinnamate	lb
CTA-I™	Cellulose triacetate	la
Chiralpak AD™	Amylose tris(3,5-dimethylphenylcarbamate)	lb
Chiralpak AS™	Amylose tris[(*S*)-phenylethylcarbamate]	lb
Synthetic polymers		
ChiraSpher™	Poly[(*S*), *N*-acryloylphenylalanine ethyl ester]	lc
CHI-DMB™	Cross-linked *O*-3,5-dimethylbenzoyl tartramide	lb
CHI-TTB™	Cross-linked *O*-4-tert-butylbenzoyl tartramide	lb
NEA™(R or S)	Poly(*N*-metacryloylnaphthylethylamine)	lb
Cyclodextrin selectors		
Cyclobond™	α-, β-, γ-Cyclodextrin	lla
ChirDex™	β-Cyclodextrin	lla
Chiral Prep CD ST/PM™	β-Cyclodextrin	lla
Chirobiotic		
Chirobiotic T™	Teicoplanin	lla
Chirobiotic V™	Vancomycin	lla
Chirobiotic R™	Ristocetin A	lla
Brush type		
DNBLeu™	3,5-Dinitrobenzoylleucine	lla
DNBPG™	3,5-Dinitrobenzoylphenylglycine	lla
Whelk-O 1™	3,5-Dinitrobenzoyl tetrahydrophenanthrene amine	lla
DACH-DNB™	Diaminocyclohexane 3,5-dinitrobenzamide	lla
ULMO™	Diphenylethylene diamine 3,5-dinitrobenzamide	lla
Chiris-QN™	Quinine	lla
Chiris-QD™	Quinidine	lla

3.4.2.1.1 *Pure cellulose derivatives* For many years, the most widely used cellulose derivative for separations on a preparative scale was cellulose triacetate (CTA) introduced in its fully acetylated form by Hesse and Hagel in 1973 [25]. This CSP is able to resolve a broad range of structurally different racemates [24]. The high versatility and the high loading capacity as well as the low preparation costs have certainly contributed to an extended use of this sorbent, even if there are some practical limitations. Microcrystalline CTA has been mostly used in the reversed-phase mode (usually methanol–water or ethanol–water mixtures). For CTA, it must be emphasised that the crystal structure of the polymeric material has a determining influence on the chromatographic properties and the chiral recognition ability [37]. Indeed, cellulose triacetate exists in at least two different crystal polymorphic forms that can readily be distinguished by X-ray diffraction [37,38]. Only the so-called CTA I structure (cellulose triacetate, crystal form I) shows a large spectrum of applications. This material is prepared under heterogeneous acetylation conditions that presumably preserve

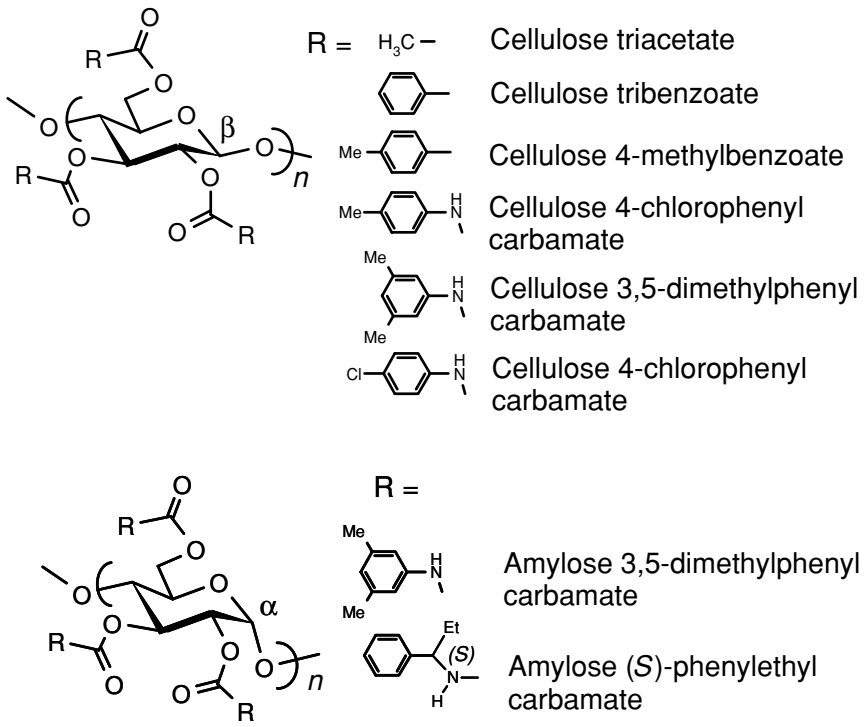

Figure 3.7 Chemical structure of cellulose- and amylose-based CSPs.

the original supramolecular structure of the starting cellulose [26]. Under these conditions, there is no dissolution either of the cellulose or the produced cellulose triacetate. If the cellulose ester is dissolved (during or after the acetylation), amorphous or another crystal form (called CTA II), thermodynamically more stable than CTA I, is obtained depending on the isolation conditions. CTA II shows completely different properties and generally shows a much poorer resolution power and in some cases even a reverse chiral selectivity relative to CTA I [24,26,37,38].

A broad variety of racemic structures have been resolved on CTA I on a preparative scale (1, 24), and it is actually somewhat astonishing that the significance of this CSP has considerably decreased over the last 10 years. Most of the chromatographic resolutions reported in the literature have been performed under medium pressure conditions. There have been different reasons for the popularity of this sorbent, but the easy and cheap preparation, its high versatility and its high loading capacity have certainly been the main advantages. These advantages compensated for the limitation imposed by the dissolution or strong swelling of the cellulose triacetate CSP in numerous solvents like chlorinated alkanes or tetrahydrofuran, dioxane, acetone and dimethylformamide. Usually mixtures of alcohol (preferentially ethanol or methanol) and water were used and gave very good results. In some cases it has been shown that mixtures of alkane and alcohol may yield a better selectivity [41–43]. Another drawback of CTA I is the relatively slow kinetics of the adsorption/desorption process, resulting in peak broadening and thereby decrease of resolution (efficiency). Data concerning the influence of the flow rate, temperature, eluent

composition, pressure and particle size on the enantioselectivity, efficiency and retention of optical isomers, as well the determination of the loading capacity of CTA I, have been reported by Mannschreck and his co-workers [44], by Rizzi [45] and by Isaksson *et al*. [46]. Although a number of theoretical investigations have been carried out on the interaction mechanism of CTA I with chiral molecules [47–49], the real mode of complexation is still not elucidated because of the complex structure of the polymer which seems to be characterised by the presence of multiple 'interaction sites' [50,51]. Moreover, the critical influence of the size of resolved structures [50,52] and the possibility to separate enantiomers of totally apolar molecules [53,54] point to the large contribution of a mechanism involving an intercalation of the solutes between the polymeric chains (shape-selective adsorption or inclusion chromatography). This explanation does not exclude the possibility that very large molecules that cannot penetrate the polymer matrix or that are very polar interact by simple adsorptive interaction on the surface. The multiple interaction possibilities render a prediction of the separation very difficult. The amounts generally injected ranged between 10 mg and 10 g but separation up to 200 g of racemate in one run has also been reported on a pilot scale [55], and simulated moving bed applications have also been reported [56].

Tribenzoylcellulose was also developed as a CSP in its pure polymeric form by Mannschreck *et al.*, who prepared benzoylcellulose by esterification of cellulose under heterogeneous conditions [57]. Independently, Francotte and Baisch [58] designed an emulsion process for the preparation of beads from various cellulose derivatives [59–61] (Fig. 3.8). As these phases consist of pure chiral material, they exhibit a high loading capacity. These CSPs

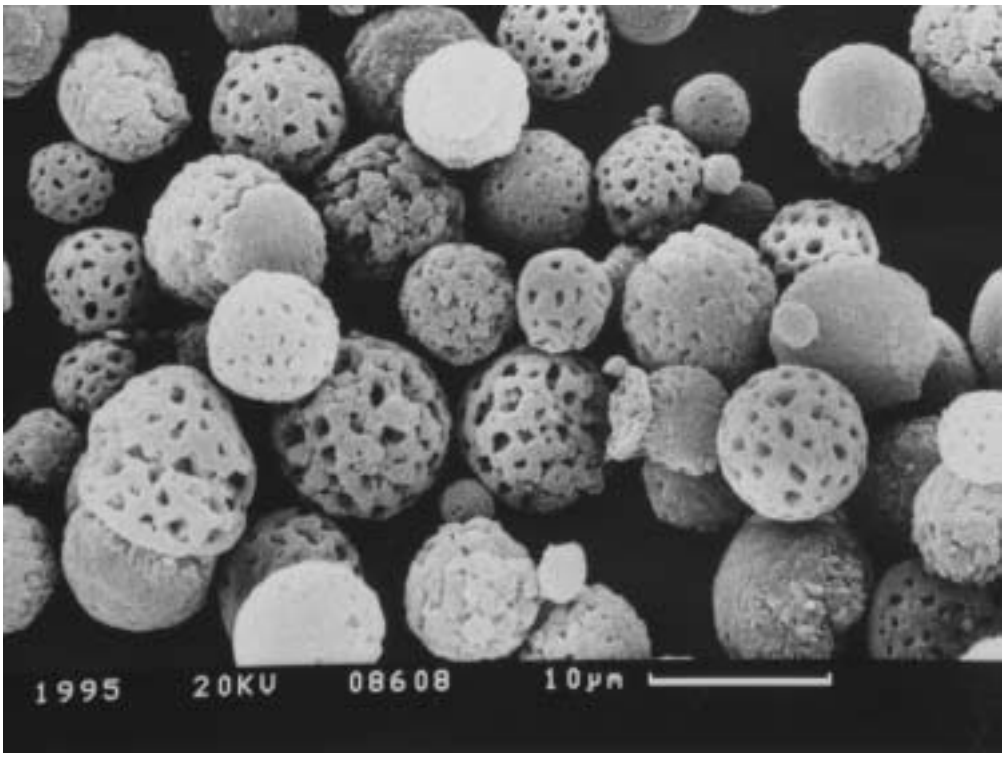

Figure 3.8 Scanning electron micrograph of 4-phenylbenzoylcellulose beads.

show interesting properties for preparative purposes, and their ability to resolve racemic compounds that are not separated on CTA has been demonstrated [59–62].

Beads prepared from 3,5-dimethylphenylcarbamate of cellulose have also been prepared according to the same emulsion process [63,64]. The resulting product also shows an exceptionally high loading capacity. However, owing to the compressibility of these beads, they can only be used under medium pressure chromatography and at moderate flow rates.

3.4.2.1.2 *Coated polysaccharide derivatives* The technology developed by Okamoto's group in Japan and which consists of coating silica gel with polysaccharide derivatives [41,65,66] has definitely changed the world of enantioselective chromatography and fundamentally contributed to establish the technique as an essential analytical and preparative tool. The immense contribution of Okamoto's group is documented in numerous papers and the CSPs derived from his work are now the most used phases for analytical and preparative applications.

These phases are prepared by coating macroporous silica gel with about 20% weight of the polysaccharide derivatives obtained by chemical derivatisation of the hydroxyl groups of the polysaccharides. Although a wide range of polysaccharide-based CSPs have been described, only a few derived from cellulose and amylose have been commercialised according to the list in Table 3.1. Among these phases, four have proved to be complementary and are capable together of covering about 90% of all analytical applications [67]. As all analytical separations can potentially be up-scaled, one might immediately realise the impact of Okamoto's work on the field of enantioselective chromatography. All analytical phases are also available in 20 μm particle size for preparative purposes. They are characterised by a good chemical and mechanical stability as long as they are used under defined conditions. The inert achiral support confers the suitable mechanical stability, but it considerably reduces the loading capacity owing to the presence of 75–80% of achiral material that does not contribute to chiral discrimination.

The coated polysaccharide-based phases have mostly been used under normal-phase conditions, but an increasing number of preparative applications have been reported under supercritical fluid chromatography [68] or reversed-phase mode [69]. However, these CSPs show a non-negligible drawback related to the relatively good solubility of most polysaccharide derivatives in many common organic solvents like chlorinated alkanes, ethyl acetate, toluene, acetone, tetrahydrofuran or dioxane. All mobile phases containing these solvents or mixtures of these solvents are excluded if one will avoid any irreversible damage of the columns. This is an important limitation for preparative applications, considering that the solubility of the racemates to be resolved is often a major issue when going from the analytical to the preparative scale.

The broad applicability of the coated polysaccharide-based CSPs has made them very popular; they are now widely used for preparative separation of enantiomers and large-scale applications up to metric tons per year have been reported [70,71].

3.4.2.2 *Polyacrylamide CSPs* Cross-linked, optically active polyacrylamides and polymethacrylamides constitute another class of polymeric CSPs. These CSPs were introduced by Blaschke and co-workers about 30 years ago and their usefulness for preparative applications was already demonstrated before the 1980s [72]. However, the gel structure of these cross-linked polymers prevents utilisation at high pressure, and only moderate

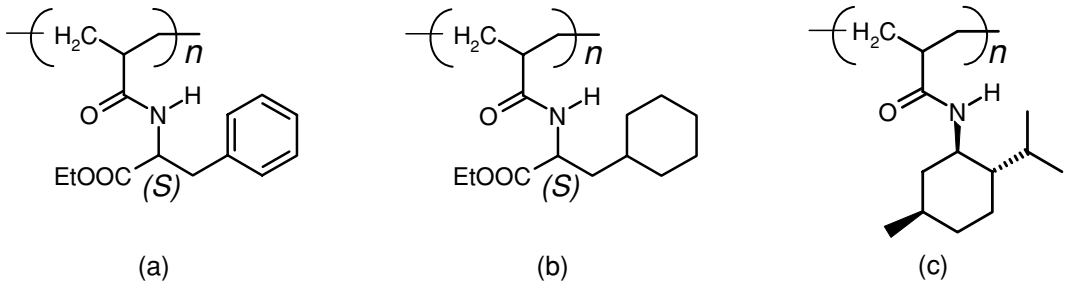

Figure 3.9 Structures of the polyacrylamide CSPs.

throughput could be obtained. Improvement of the mechanical performance of these CSPs was achieved by polymerisation of the acrylic monomer on the surface of silica gel, giving a grafted polymer [73,74]. The preparative separations reported in the literature have been carried out using (a) (*S*)-phenylalanine ethyl ester, (b) (*S*)-1-cyclohexylethylamine and (c) methyl amine [75] as the chiral selector (Fig. 3.9).

Although these materials are polymers, they probably interact in a 'bimolecular' way like Type II CSPs (Fig. 3.2), the polyacrylamide backbone playing the role of achiral matrix, and the amide part constituting the chiral unit. Indeed, DSC measurements and X-ray diffraction analysis suggest that these polymers are amorphous (E. Francotte, unpublished results, 1985). This lack of regularity of the polymeric structure does not allow an amplification of the chiral recognition, by participation of several chiral moieties in a concerted way, as has been speculated for most of the polysaccharide-based phases. Both modes, normal and reversed phase, have been applied for preparative separations on the silica-grafted polyacrylamide CSPs. Although the feasibility of achieving preparative separations on this last CSP has been demonstrated [74,76,77], only few preparative applications have been performed on these polyacrylamide CSPs over the last 10 years.

3.4.2.3 *Polymeric CSPs derived from tartaric acid* A new type of polymeric CSP has been introduced about 10 years ago by Allenmark *et al*. and has been commercialised under the trade names of Kromasil CHI-DMB™ and CHI-TTB™ [78–81]. These phases have been prepared by polymerisation of *N*,*N*′-diallyl derivatives of tartardiamide and grafted onto silica gel. Their structure is shown in Fig. 3.10. As these phases are cross-linked and bonded to silica gel, they are insoluble in organic solvents and there is no limitation regarding the choice of mobile phase. Normal-phase, reversed-phase and supercritical fluid conditions have been applied. They show a good mechanical stability, but the relatively high content of

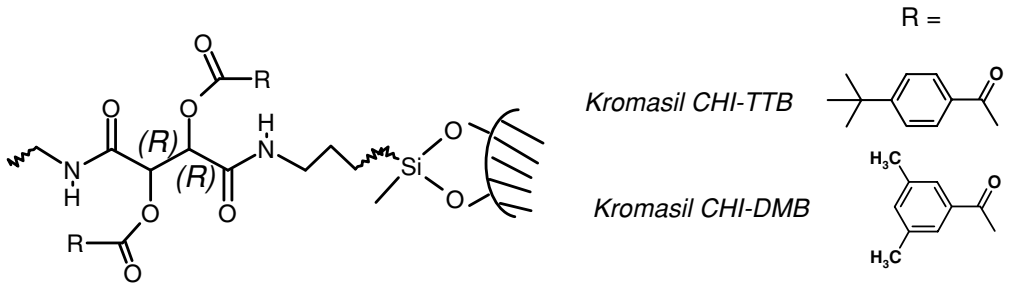

Figure 3.10 Structure of tartardiamide-based CSPs.

achiral silica gel in these CSPs reduces their loading capacity. A few preparative separations on these two CSPs have been reported [78–81], but so far they have not been widely used for preparative applications.

3.4.3 *Brush-type CSPs*

3.4.3.1 *π-acidic and π-basic phases* The brush type of CSP was introduced by Pirkle, who was one of the pioneers of modern enantioselective liquid chromatography [82]. The most frequently used π-acceptor phases are derived from the amino acids phenylglycine (DNBPG) (Fig. 3.11) or leucine (DNBLeu) covalently or ionically bonded to 3-aminopropyl silica gel [83,84]. These CSPs are commercially available for analytical or preparative separation of enantiomers. Further CSPs based on amino acid or amine chiral selectors such as valine, phenylalanine, tyrosine [85], 1,2-*trans*-diaminocyclohexane [86] and 1,2-*trans*-diphenylethylene diamine [87] were also developed (Fig. 3.11). These CSPs have been applied for the preparative separation of the enantiomers of a few racemic compounds, but the number of preparative applications remained very limited over the last 10 years. The application of the reciprocality concept has led to the design of various phases of the π-donor/acceptor type [88,89]. One very successful phase is the Whelk-O 1™ CSP developed by Pirkle and Welch [90–93].

These chemically modified silica gels are stable at high pressures and exhibit good chromatographic performance. Usually, these CSPs were used under normal-phase conditions, but chiral resolutions under reversed-phase mode or supercritical fluid chromatography have also been performed [92,94,95].

3.4.3.2 *Cyclodextrin-based CSPs* Cyclodextrins are cyclic oligosaccharides exhibiting the property of forming inclusion complexes in their highly hydrophobic cavity with a large

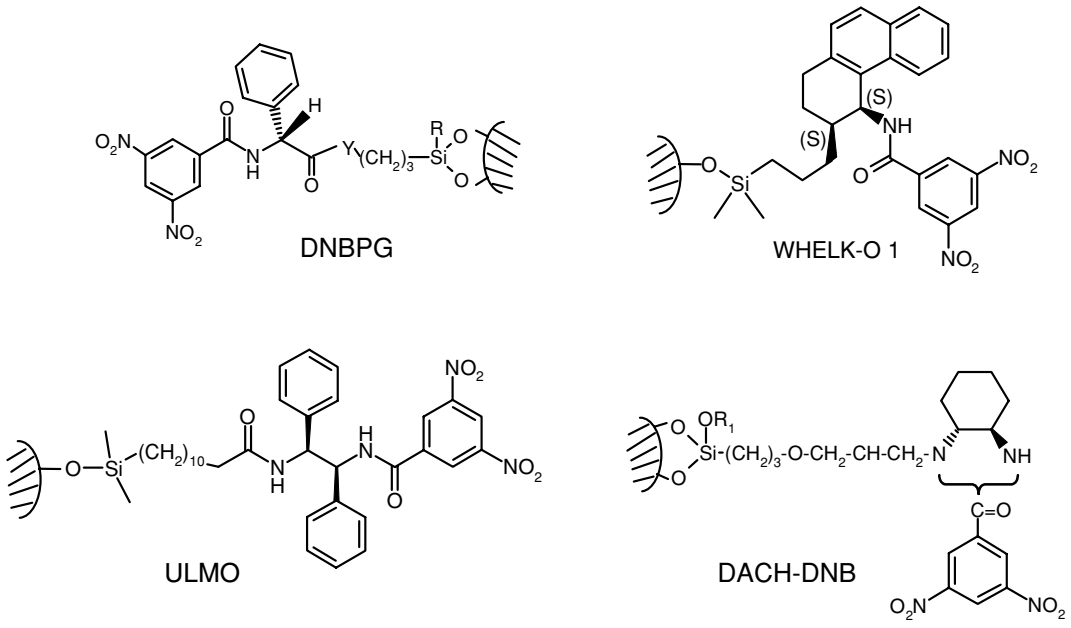

Figure 3.11 Structure of preparative π-acidic CSPs.

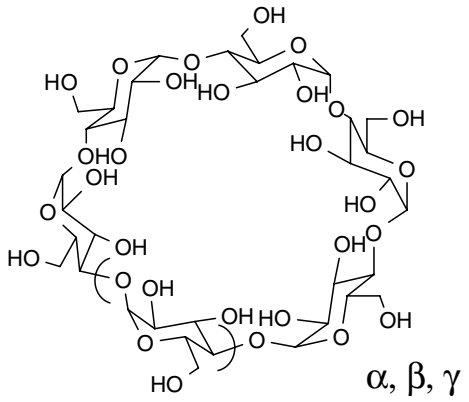

Figure 3.12 Structures of cyclodextrin selectors.

variety of molecules (Fig. 3.12). The size of the cavity, which differs for α-, β- and γ-cyclodextrins, and the substituent on the cyclodextrin play a determining role on the ability for complexing a defined molecule. Cyclodextrin CSPs were prepared by immobilising cyclodextrins in polymeric structures [96,97] or on silica gel [98,99], the latter CSPs showing good performance on an analytical scale. Preparative applications using cyclodextrins as chiral hosts were reported on polymers obtained by cross-linking of cyclodextrin with ethylene glycol-bis(epoxypropyl) [97] and on the silica-modified material Cyclobond I™ [100], ChiraDex™ [101].

Optimisation of the enantioselectivity can be achieved by modifying different factors, such as the concentration and nature of organic modifiers, pH, temperature and buffer concentration. Although reversed-phase conditions are usually applied with the cyclodextrin-based CSPs, analytical applications in the normal-phase mode have also been reported [102].

Further cyclodextrin derivatives have also been introduced and turned out to exhibit a better chiral recognition for some classes of racemates [103], but no preparative separations on these CSPs have been reported up till now. Recently, new cyclodextrin-based phases were also introduced by YMC under the trade name Chiral CD ST/PM™(www.ymc-europe.com/Ymc/Profamily.html) and it is suggested that these new CSPs have an improved loading capacity.

3.4.3.3 *Chirobiotic CSPs* A few years ago, Armstrong and his group developed a series of phases based on the macrocyclic glycopeptides vancomycin (Fig. 3.13), teicoplanin and ristocetin as chiral selectors [104,105]. These phases have been prepared by covalent immobilisation of the glycopeptides to silica gel according to standard procedures. The chiral selectors are characterised by the presence of several chiral cavities providing different environments for enantioselective interactions. The chirobiotic CSPs can be used under multi-modal conditions, including supercritical fluid chromatography [106]. Preparative applications at a scale of 20–40 mg have been reported [107,108].

3.4.4 *Chiral phases for ligand-exchange chromatography*

Ligand-exchange chromatography (LEC) is based on the reversible formation of complexes between metal ions (usually Cu^{2+} or Ni^{2+}) and chiral complexing agents carrying functional

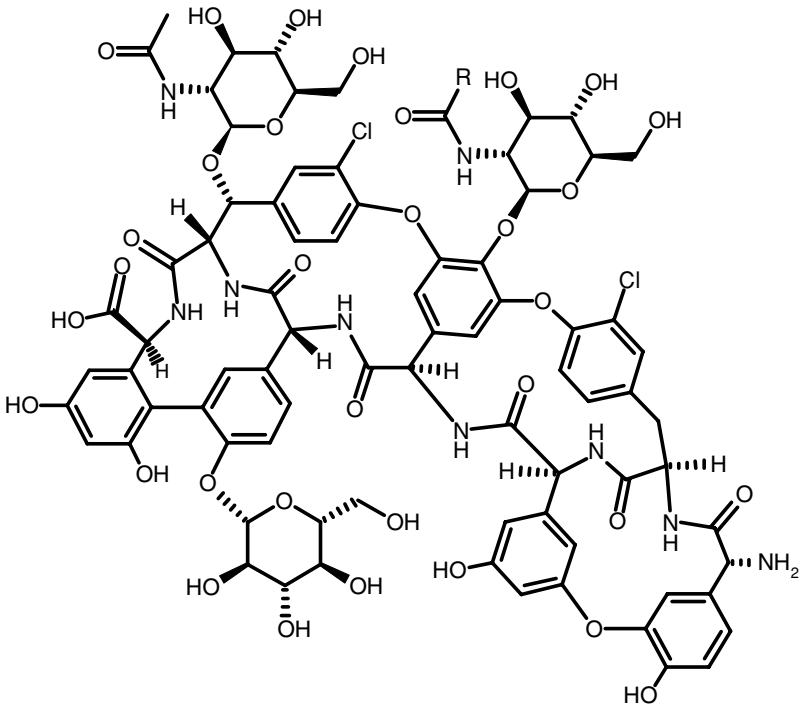

Figure 3.13 Structure of the antibiotic glycopeptide selector vancomycin.

groups capable of interacting as ligands. The chiral complexing agents mostly used are α-amino acids chelating through the carboxylic acid and the amino group.

CSPs of this type have been prepared by covalent bonding of the amino acid on organic polymeric and on inorganic silica supports. Most of the initial work was performed with cross-linked polystyrene as an inert-supporting phase. Further investigations in the field of LEC led to the introduction of an improved CSP using macronet isoporous styrene copolymers as supporting material [109–111]. A wide range of racemic amino acids can be analytically resolved on these CSPs, and even preparative resolutions (up to 20 g of racemic proline) have been performed on an L-hydroxyproline CSP of this type.

Later, other ligand-exchanging resins based on highly cross-linked polyacrylamide have been introduced on the market and especially recommended for medium-pressure LC preparative resolutions of amino acids [33]. Some preparative applications have been reported in the range of 200 mg to 10 g, but no practical application in the pharmaceutical field has been published. These applications have been summarised in an earlier review [24].

Owing to the nature of the interactions, this type of CSP is particularly appropriate for the resolution of racemic compounds carrying chelating functionalities such as amino acids, hydroxy acids and, in a few cases, amino alcohols. An advantage of the method is that derivatisation of the solute—even those as polar as amino acids—is usually not required prior to chromatography. However, the use of copper salt in the mobile phase, which has to be removed after chromatography, and the poor mass transfer, which leads to considerable peak broadening, probably explain the low interest for this approach on a preparative scale over the last 10 years.

3.4.5 *Imprinted phases*

As early as 1972, Wulff and Sarhan introduced the concept of using chirally imprinted polymers for the resolution of racemic compounds [40]. In their first attempt to prove the concept, the authors prepared copolymers from divinylbenzene and D-glyceric acid-(*p*-vinylanilide)-2,3-*O*-(*p*-vinyl-phenylboronate) as the chiral template. They showed that after removal of the chiral template, D-glyceric acid was preferentially taken up from a solution of the racemic mixture [112]. These phases do not contain chiral moieties but they consist of chiral cavities, whose chirality is only due to the asymmetrical spatial arrangement of the functional groups present in the polymer matrix. With this type of polymer, the separation does not result from an enantioselective interaction with a chiral molecule, but the chiral recognition takes place in chiral cavities which are created in the polymer by the reversibly bonded chiral template molecule during the polymerisation process. The imprinting technology was improved and broadly investigated by Mosbach [113], Sellergen [114] and Haginaka [115]. However, the poor mass transfer, resulting in peak broadening, and the low loading capacity of this type of CSP [116,117] considerably reduce their significance for preparative applications. In addition, preparation of larger amounts of CSP would require large quantities of the chiral template of the molecule to be resolved. For new chiral compounds, this availability is rather unlikely.

3.5 Chemical and physical properties of CSPs

3.5.1 *Loading capacity*

Obviously, the loading capacity of the stationary phase is a major consideration when preparative separations have to be performed. For the same reason, only a limited number of materials can be reasonably applied for preparative purposes among the broad range of available CSPs that have been developed for analytical separation of enantiomers. The estimated loading capacity of the most commonly used phases has already been previously discussed [3,24,79–81,118,119]. However, it must be emphasised that the optimal CSP in terms of loading capacity may vary from one racemate to another, depending on the structure of the racemic compound to be resolved, as it is a function of the number of accessible interaction sites per mass unit of the phase. The usual range of loading capacity of the most common preparative CSPs is shown in Fig. 3.14. All these phases are available in bulk quantities, but over the years, some of them have been recognised to be particularly effective and have been preferred for preparative purposes.

Cyclodextrin-based phases have been used for preparative applications [98,99,120], but because of their relatively poor saturation capacity, they never aroused a broad interest for preparative purposes. The poor saturation capacity is directly related to the structure of the phase and the nature of the interaction. The cyclodextrin-based phases have been obtained by bonding cyclodextrin to the surface of silica gel. Because cyclodextrin is a large molecule, however, the number of chiral selectors that can be bonded to the surface of silica is relatively low, leading to a low density of chiral interaction sites.

The loading capacity of the recently designed CSPs that have been prepared from the macrocycles vancomycin, teicoplanin and ristocetin [104,105] has been estimated on the basis of the few small-scale preparative resolutions that have been reported [107,108,121]. The real potential of these CSPs for large-scale separations still remains to be demonstrated.

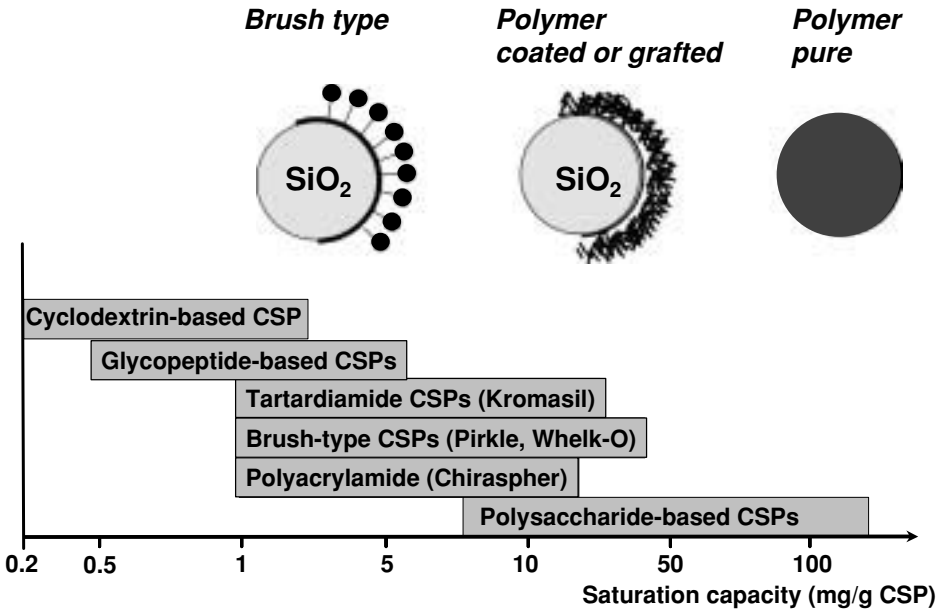

Figure 3.14 Typical saturation capacity of the most used commercially available CSPs.

The brush-type phases introduced by Pirkle and Welch [93] as well the polyacrylamide CSP Chiraspher [122] and the cross-linked di-allyltartaramide CSP Kromasil CHI and CHII [78–81] show similar loading capacity ranging between 1 and 30 mg racemate per gram of CSP and their potential as preparative CSPs has been clearly demonstrated. However, based on the applications published over the last 10 years and on our own experience, it appears that, for preparative applications, the most successfully and broadly applied phases comprise the cellulose- and amylose-based phases developed by Okamoto (Chiralcel™ and Chiralpak™) [66,123]. In our laboratory, these phases have been routinely used for the preparative isolation of the enantiomers or diastereoisomers of several hundreds of new drug candidates over the last 15 years. Their popularity is clearly demonstrated by the increasing number of published applications and is due to both their broad applicability and high loading capacity. The high saturation capacity can be attributed to the high density of chiral selectors, which constitute about 20% weight of the whole CSP. CSPs made of pure polysaccharide derivatives show even a substantially higher loading capacity but they suffer from their lower mechanical resistance to high pressure. The dependence of the loading capacity on the density of chiral material contained in polysaccharide-based CSP was investigated by Francotte *et al*., who designed a process to prepare beads made of pure cellulose derivatives [58–60,64] (Fig. 3.15).

If just a few micrograms of enantiomerically pure material are required, other commercially available CSPs may be used, and most of these microscale applications have been achieved on analytical columns. However, this type of application will not be discussed in this chapter, as it is unlikely that scale-up, even at the few gram scale, is feasible.

3.5.2 *Chemical and physical stability*

A series of chromatographic properties that are usually neglected for analytical applications becomes very important in preparative separations. An appreciable number of chiral phases

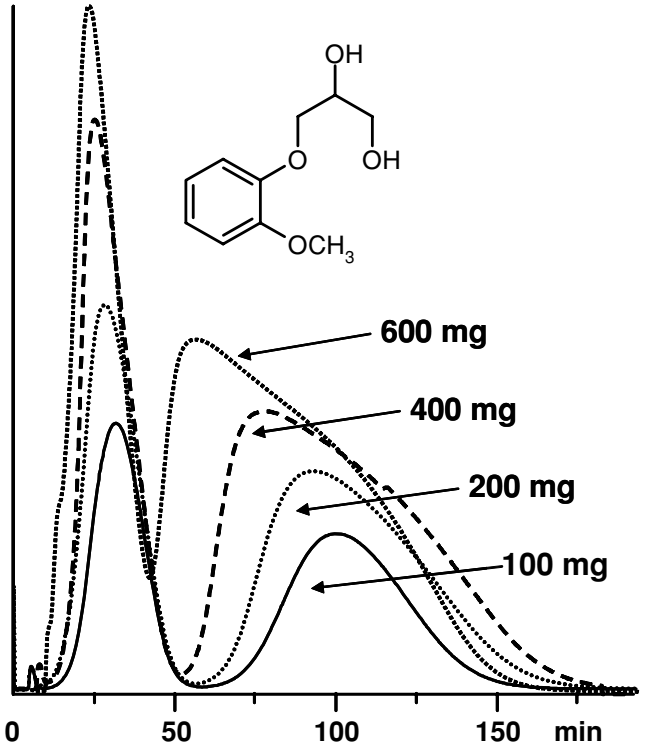

Figure 3.15 Loading capacity of cellulose 3,5-dimethylphenyl carbamate beads. Separation of the enantiomers of racemic guaifenesine. Injection, 100–600 mg; Column: 1.25 × 26.6 cm (32.6 mL), 5.4 g CSP; mobile phase, heptane/2-propanol 90/10; flow rate: 5 mL/min [64].

used for chromatographic resolutions are composed of organic polymeric materials that may exhibit some restrictions regarding their chemical, physical or mechanical properties. Most of these polymeric materials are not cross-linked or not immobilised and are therefore more or less soluble in numerous solvents. For example, cellulose triacetate, which has proved to be a very useful CSP for preparative purposes [24,42,50,124–127], cannot be used with chlorinated alkanes as mobile phases, because they dissolve the polymer. The same restriction applies to most of the other cellulose derivatives introduced for the chromatographic resolution of racemates as a coating on silica or in the pure polymeric form [24]. Nevertheless, CSPs of this type can be used under either normal- or reversed-phase conditions. Despite the above limitations, cellulose derivatives are currently the most used chiral phases for preparative separations, because of their broad applicability and/or their high loading capacity.

A strong swelling has also been reported for the polyacrylamide derivatives developed in the pure polymeric form some years ago by Blaschke and Donow [128]. Although these materials had been cross-linked, they could only be used under very low pressure conditions because of their gel structure in most organic solvents. In order to improve the mechanical properties, these materials have later been polymerised (graft polymerisation) on the surface of silica gel, but this treatment reduces the loading capacity, owing to the lower amount of chiral material per unit of mass. The CSPs obtained by immobilisation of chiral material on silica gel are generally stable and afford greater flexibility regarding the

chromatographic conditions, particularly concerning the choice of mobile phase. With these classes of sorbents, the type and composition of the mobile phase can be widely varied.

The CSPs developed for LEC are exclusively used under reversed-phase conditions, but in this case this limitation is imposed by the mechanism of interaction. A review devoted to the general features of LEC as well as to the recent developments in this field has been published by Davankov [129], who was a pioneer in developing this principle of chromatography.

The chemical stability of the stationary phase can also be a limiting factor. Indeed, phases bearing reactive chemical functions can be altered by injection of certain solutes. For example, stationary phases bearing labile ester functions such as cellulose acetate or benzoate and some polyacrylamides can easily react with nucleophilic amines. This was also the case for the first generation of Pirkle's CSPs obtained by ionic bonding. This aspect has to be considered before performing a separation on a defined CSP.

3.5.3 *Solubility of the chiral solute*

In preparative separations, the solubility of the solute is often the limiting factor in terms of throughput. The solubility mainly depends on the solvating properties of the mobile phase. However, for a number of CSPs, the choice of mobile phase is considerably restricted by the solubility of the CSP itself in the mobile phase. For the CSPs that are constituted of chiral selectors chemically bonded to an insoluble carrier (mostly silica gel), there is practically no limitation regarding the choice of mobile phase and selectivity is the factor governing the choice. Ideally, the best selectivity under high-solubility conditions should be applied. However, it is noteworthy that in many instances the highest selectivity is obtained with poorly solvating mobile phases while good solvents give lower selectivity. In practice, a good compromise between selectivity and solubility has to be found. By contrast, the most popular preparative CSPs (Table 3.1), those derived from the polysaccharides cellulose and amylose, and those that have now been used for large-scale separations up to tons of racemates, show one major drawback. They are more or less soluble in many organic solvents such as tetrahydrofuran, dioxane, toluene, chlorinated solvents and ethyl acetate. This property considerably reduces the choice of mobile phase, thus limiting the possibility of increasing selectivity, of varying retention time and of improving the solubility of the racemate. Improved forms of these CSPs have recently been achieved by immobilisation of the polysaccharide derivatives and are discussed in the next section.

3.6 New and future developments in the field of preparative CSPs

The successful application of enantioselective chromatography as a valuable approach to separate chiral isomers on a preparative and even production scale has considerably attracted the attention of most pharmaceutical companies. Even though the technology has been shown, in some instances, to be more competitive than the conventional approaches for the production of single enantiomers of chiral drugs, an intensification of its application will only occur through the optimisation of throughput and costs of the methodology. Various options are available to increase throughput: (1) further improvement of the chromatographic technique; (2) improvement of the loading capacity of the CSPs; (3) design of tailor-made CSPs, showing an optimal enantioselectivity for the enantiomeric mixture

to be separated; (4) design of CSPs with improved physical properties and allowing the utilisation of high flow rates (e.g. monolithic phases); (5) optimisation of mobile phase in order to increase solubility of the solute; (6) development of synthetic methodologies to racemise and recycle the undesired chiral isomers. Clearly, some of these options are directly or indirectly related to the design of new CSPs.

3.6.1 *CSPs with improved loading capacity*

The design of CSPs with enhanced loading capacity is not simple, as it implies the increase of the number of available or accessible chiral interaction sites per unit mass of the CSP. This approach was investigated by Francotte *et al*., who developed a process to prepare beads of polysaccharide derivatives without employing silica gel as a mechanical support [58–60,63–64]. These phases show a considerable enhancement of the loading capacity, but they have a limited mechanical stability. The same drawback was observed for the polyacrylamide phases developed by Blaschke [35,36] or for the cyclodextrin polymer phases prepared more than 20 years ago [96,97]. However, in the context of the growing interest for CSPs which afford higher productivity, the design of mechanically more stable phases of this type should probably be reconsidered.

3.6.2 *CSPs with improved selectivity*

To a great extent, productivity also depends on the enantioselectivity of the CSP toward the enantiomers to be separated. Indeed, higher selectivity allows the amount of solute to be loaded on the column to be increased. This means that, for each specific application, it is critical to identify a CSP exhibiting the highest enantioselectivity. Very high enantioselectivity may eventually lead to the utilisation of alternative separation processes such as simple filtration or extraction techniques.

There are several ways to generate high selectivity values: (1) design of a specific CSP based on molecular modelling; (2) screening of a large collection of CSPs; (3) design of a 'complementary' CSP by applying the reciprocality concept introduced by Pirkle; (4) identification and design of an appropriate CSP by applying a combinatorial approach.

3.6.2.1 *Designing CSPs by molecular modelling* The design of a specific CSP by molecular modelling requires a precise analysis and description of the interaction of the chiral solute with the chiral selector [130]. However, as very small differences in the energy of complexation with the respective enantiomers of a molecule are largely sufficient to obtain a good separation, most of the practical applications (selectivity < 7) fall out of the range of accuracy of molecular calculation. In addition, in many instances and especially for the very successful polysaccharide-based phases, the interaction sites are unknown. Therefore, design of a specific CSP for a particular racemate is currently hardly feasible. Moreover, it is known that the influence of the mobile phase on selectivity can be crucial, and molecular modelling does not generally account for this effect.

3.6.2.2 *Improving selectivity by screening CSPs* The most used strategy to identify the 'optimal' CSP for a particular chiral compound still consists in screening a series of available phases following a trial and error approach. This is the strategy which is applied by most

of the specialised laboratories over the world. However, in the context of the optimisation of productivity for large-scale separations, a slightly higher selectivity may have a considerable impact on the final production costs. Therefore, there is a growing interest in extending the number of phases screened. This approach is particularly easy to apply to the polysaccharide-based CSPs, for which the chiral recognition properties can be modulated by simple modification of the derivatising group of the hydroxyl function on the sugar moieties. We utilised this strategy for the separation of the enantiomers of the LTD4 antagonist iralukast and of the antimalarial agent benflumethol [131]. These two racemic drugs were only poorly resolved on the commercially available polysaccharide-based phases, whereas an excellent separation was obtained on the carbamate derivative of cellulose obtained from cellulose and 3-chloro-4-methylphenylisocyanate (Fig. 3.16). These examples show how it is possible to improve the separation by slight modification of the CSP.

In alignment with this strategy, Daicel and Chiral Technologies [132,133] recently launched a service for screening a wide range of polysaccharide-based phases which have been prepared according to the technique developed by Okamoto and his group but which are not yet available from the usual collection of Daicel CSPs. They are capable of testing more than 50 different columns within a short period of time. This approach should help in identifying the best appropriate polysaccharide-based CSP for the separation of the enantiomers of each individual chiral compound, with respect to enantioselectivity and productivity.

Recently, Lindner and co-workers developed a series of anion-exchange CSPs based on quinine and quinidine as chiral selectors [134–136] (Fig. 3.17). These phases are particularly appropriate for the separation of the enantiomers of chiral acidic compounds. Improvement of the chiral recognition power of these phases by rationally designed structural modifications has led to exceptionally high enantioselectivity, which, of course, are of great interest for preparative applications [136]. Screening these CSPs can also help in the identification of a suitable CSP for large-scale preparative separations.

Crown ether resins that are suitable for the enantiomeric separation of chiral amines and amino acids have also recently been prepared according to a proprietary process (D. Wellings, Avecia Inc., http://www.avecia.com/pharms/) (Fig. 3.18). The new chiral supports show particularly high enantioselectivity and exhibit high loading capacity. The

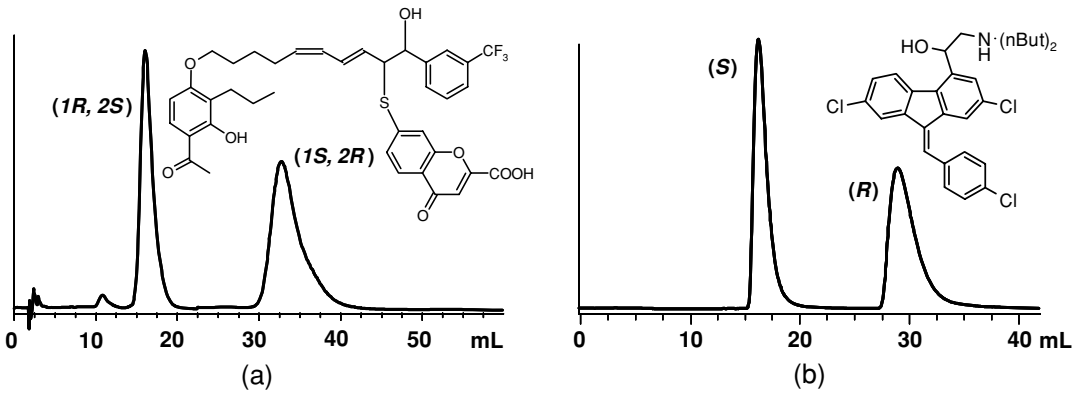

Figure 3.16 Separation of the enantiomers of (a) the LTD4 antagonist iralukast and (b) the antimalarial agent benflumethol on 3-chloro-4-methylphenylcarbamate of cellulose [131].

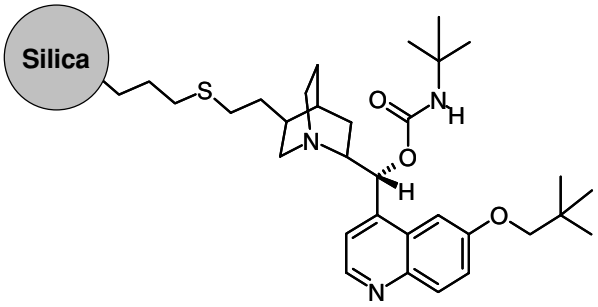

Figure 3.17 Structure of a quinine-based CSP.

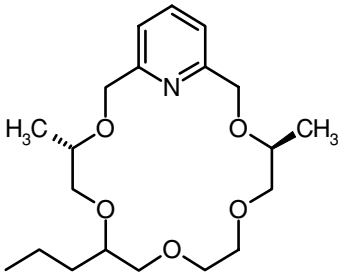

Figure 3.18 Example of chiral crown ether selector used for the preparation of crown ether resins (www.avecia.com).

separation process works simply in three steps: (1) loading of the sample; (2) washing the non- or weakly bonded stereoisomer; (3) desorption of the strongly bonded stereoisomer.

3.6.2.3 *Concept of reciprocality for designing CSPs* On a more rational basis (concept of reciprocality), Pirkle and Welch also developed a tailor-made CSP for the separation of the enantiomers of the analgesic agent naproxen and other non-steroidal anti-inflammatory drugs (NSAID) [137]. It is conceivable that this strategy will attract more attention in the future, specifically in the context of developing tailor-made CSPs with enhanced selectivity. On the basis of the same principle, Pirkle and Koscho prepared a proline-based CSP (Fig. 3.19), which was specifically designed for the preparative separation of the enantiomers of two chiral selectors [138].

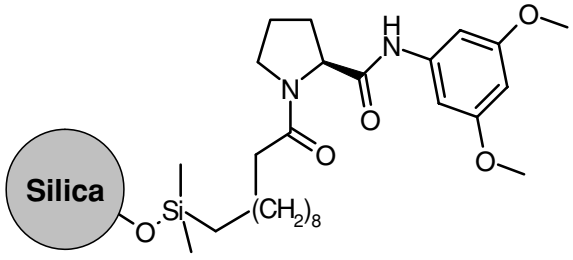

Figure 3.19 Structure of a proline-based CSP (π-basic) designed for preparative resolution on the basis of the reciprocality concept [138].

3.6.2.4 *Combinatorial approach to optimise CSPs* For the preparation of tailor-made CSPs, a new approach based on the combinatorial concept has recently been evaluated by different groups [139–144]. Although this approach is probably not very attractive for the development of specific CSPs for analytical purposes, it could be very valuable for preparative applications as optimised separation conditions may have a determining impact on cost. The approach consists in screening a great number of CSPs on a microscale in order to identify the CSP providing the best separation for a particular racemate. In different model experiments, this approach has been shown to be useful for the rapid development of highly selective CSPs, even on a preparative scale [139]. However, no practical preparative application of pharmaceutical relevance has been reported so far. The combinatorial approach has also been combined with the reciprocal concept of chromatographic separation [143,144], leading to the identification of improved CSPs for prostaglandin precursors, aryldihydropyrimidines and profen derivatives.

3.6.3 *Immobilised polysaccharide-based CSPs*

As discussed previously (Section 3.4.2), the silica-coated polysaccharide CSPs are currently the most widely applied phases for preparative purposes. However, as also noted, they have a major limitation owing to their good solubility in most organic solvents such as dichloromethane, chloroform, ethyl acetate, tetrahydrofuran or dioxane. This characteristic considerably reduces the possibility to optimise the enantioselectivity by modifying the composition of the mobile phase, which is known to play an essential role in the chiral interaction process. We often experienced that the enantiomers of a chiral substance could not be separated with a mixture of 10% ethanol in hexane, but could be completely resolved on the same polysaccharide-based CSP with a mixture of 10% 2-propanol in hexane. In some instances, the impact of the mobile phase is so dramatic that changing its composition causes an inversion of the elution order. Moreover, the restrictions regarding the mobile phase prevent the optimisation of the elution time and of the solubility of the chiral sample, two factors which have a considerable impact on productivity. To remove this drawback, various strategies have been developed by three different research groups, with the aim of making the polysaccharide-based phases insoluble in order to enlarge the choice of mobile phase. Okamoto and his group reported on a process to immobilise cellulose on silica gel through a dicarbamate linkage using diphenyl diisocyanate as a cross-linking agent [145,146]. However, this approach necessitates additional synthetic steps and appears to negatively affect selectivity as the number of linkages increases. Independently, the groups of Oliveros and Minguillon prepared immobilised polysaccharide CSPs by reacting allyl silica gel with the undecenoyl side chains introduced onto the polysaccharide [147–149]. Again, this process also necessitates additional synthetic steps and has the disadvantage of introducing a structural disturbing factor due to the presence of the undecenoyl ester moieties. Preparative separations have been reported on such a phase [150–151]. The third process, developed by Francotte and co-workers, simply consists in exposing the coated phases to a photochemical treatment or to heating in the presence of radical initiators like di-*tert*-butylperoxide [152–156]. By application of one of these processes, immobilisation occurs, presumably by cross-linking of the polysaccharide chains. Depending on the derivatising group and on the polysaccharide type, the thermal or photochemical process

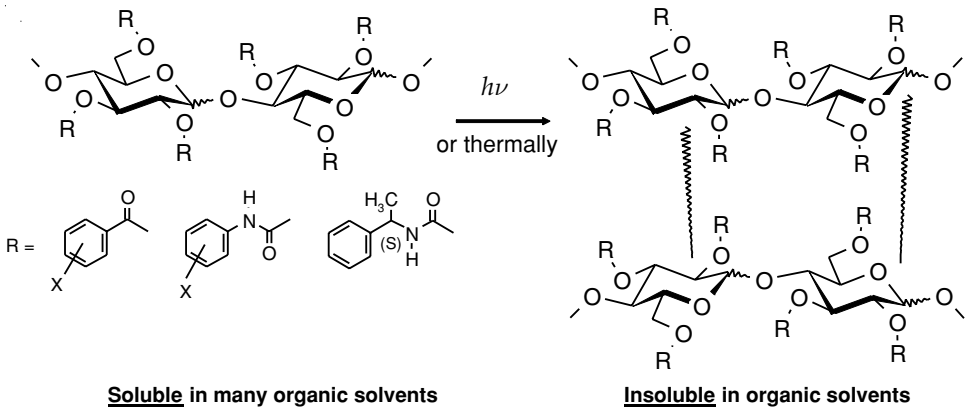

Figure 3.20 Process of immobilisation of polysaccharide-based CSPs.

is the most effective one (Fig. 3.20). Once immobilised, the CSPs can be used with a large variety of mobile phases without deterioration.

A broad range of immobilised polysaccharide derivatives have been prepared according to the new processes and their chiral recognition power has been investigated. The results show that by application of 'unconventional' mobile phases (1) significant improvement of selectivity can be achieved, (2) retention time can be better adjusted, and (3) considerable increase of solubility of the chiral solute can be obtained. All these parameters are extremely important for productivity. Practical applications, including simulated moving bed separations, which demonstrate the advantages of these new phases for preparative purposes, have already been reported [157]. Therefore, it can be anticipated that a rapidly increasing number of applications of these CSPs will be seen in the near future.

3.7 Conclusion

There is no doubt that enantioselective chromatography can now be considered as a powerful alternative for the preparation of optically pure compounds. The technology has been applied at all stages of development, from the laboratory to the production scale. This rapid and successful development is partly due to technological improvements but obviously, it would not have been possible without the design of efficient CSPs. Even though a number of preparative CSPs are already available, there is an increasing demand for CSPs showing higher loading capacity and allowing higher throughput to be achieved.

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Preparative Enantioselective Chromatography
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4 Method development for preparative enantioselective chromatography

CRISTINA SUTEU

4.1 Introduction

Preparative enantioselective chromatography became a real option for the production of chiral drugs in the last few years. At least four chiral drugs are currently produced by chiral chromatography, one such being the antidepressant Escitalopram or Cipralex from H Lundbeck A/S. The two main elements involved in the large-scale production process, the chiral stationary phase and the equipment, have made impressive progress in the last decade in order to meet the demands for very productive separations. The availability of the equipment for large-scale batch chromatography, simulated moving bed chromatography and supercritical fluid chromatography significantly increased the opportunity to find the most appropriate technique for a given enantioselective process; however, a preparative separation can be optimised by the means of good equipment only if the chiral stationary phase/solvent combination was previously optimised. Preparative chromatography at the laboratory scale also became increasingly used in the early stages of drug development, where small quantities of enantiomers are needed for the first biological activity tests. This huge increase in the number of preparative chromatography applications at any scale makes necessary a description of the present status of practical knowledge in this field, which may help chromatographers, beginners or already experienced, efficiently to use this technique. The aim of this chapter is to present an easy approach to method development which allows the choice of the right stationary phase–solvent combination applicable to small-scale or large-scale preparative enantioselective chromatography.

4.2 Chiral stationary phases for enantioselective chromatography

As mentioned in the introduction, the chiral stationary phase (CSP) is the most important part of the chromatographic process. The number of commercial CSPs has dramatically increased over the last ten years [1,2], even though only several CSPs are available for large-scale applications. Among them, the polysaccharide derivatives, manufactured by Daicel (Japan), stand out in the field because of their high selectivity and high loadability shown towards a large number of different molecules. Their applications go from high-performance analytical separations to the largest scale of preparative chromatography worldwide [3]. This is the reason why these phases serve to illustrate the examples of method development in this chapter. Nevertheless, the principles of preparative optimisation, detailed in Section 4.4, may be applied to any other CSP.

The excellent separative properties of polysaccharide derivatives are due to their structural complexity. To understand how they work, we should look deeper into their intrinsic polymeric structure. The chemical structure of these CSPs consists of a natural or synthetic

polysaccharide, cellulose or amylose, within which the hydroxyl groups are derivatised with acid chlorides or isocyanates to form esters and carbamates, respectively. Figure 4.1 shows the chemical structure of the available preparative stationary phases.

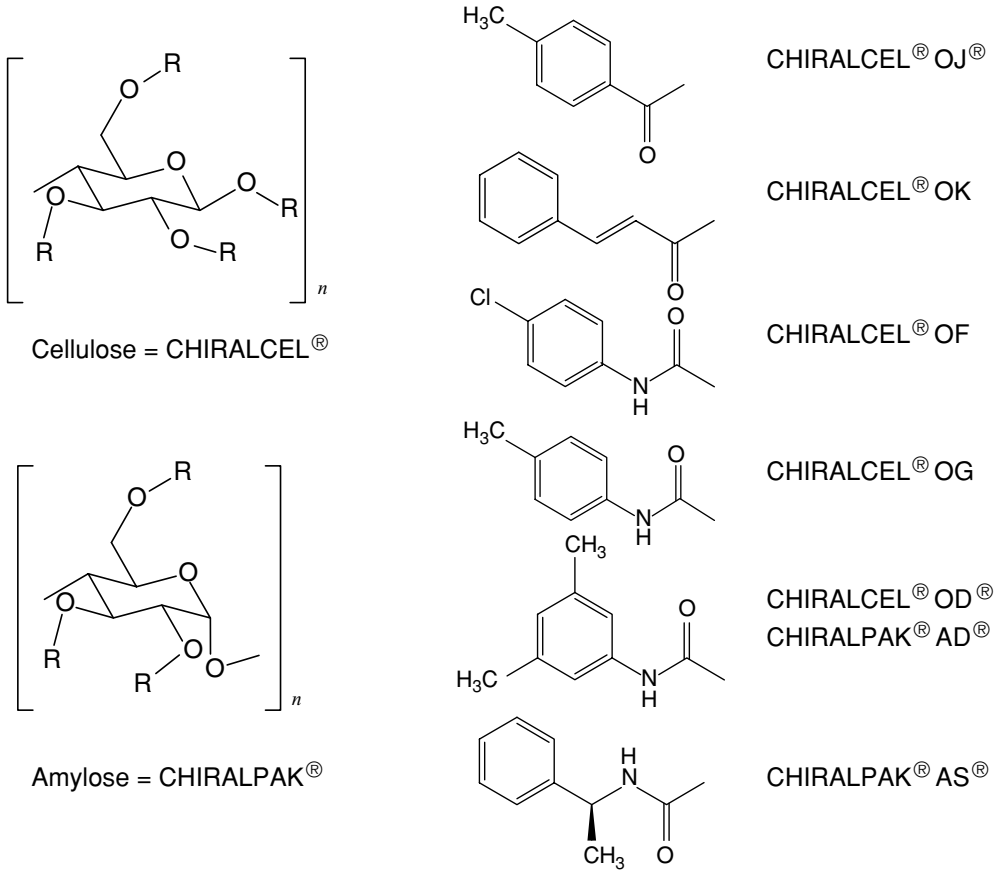

Figure 4.1 Structure of the polysaccharide derivatives available for preparative applications.

The chiral selector, i.e. the polysaccharide derivative having the structure shown in Fig. 4.1, is physically coated onto derivatised silica of which the spherical particles may be of 5, 10 or 20 μm in diameter. The silica particles are of wide pore diameter (over 1000 Å) to allow the coated polymer access to the internal surface area and thus to improve the mass transfer between solute and stationary phase. The coating process allows a high content of chiral selector (about 20% with respect to the stationary phase), which confers the chromatographic support with a high retentivity and loadability of solutes (see Section 4.4). These coated phases are stable to the most useful solvents; however, some limitations exist owing to the chiral selector solubility: solvents like tetrahydrofuran, chloroform, dichloromethane, acetone, ethyl acetate or dimethylsulfoxide are not permitted even as the sample dissolution solvent.

The polysaccharide derivatives possess a complex polymeric structure of which the chemically bonded groups (esters or carbamates) allow specific interaction with the solutes. The supramolecular structure, different for cellulose and amylose derivatives, regulates

the formation energy of temporary diastereomers between the stationary phase and solute enantiomers, making one enantiomer less retained than the other. This complex structure confers a wide applicability to these phases since many structurally different solutes may interact with them, although the chiral recognition mechanism is difficult to model and predict. Many researchers have attempted the understanding of the chiral interactions within the polysaccharide derivatives, the final aim being to predict which chiral phase would be the most appropriate for a given separation. Although it is not the purpose of the present chapter exhaustively to analyse their work, a short description of the current situation will be briefly presented in order to give the reader a basic view of the structural interactions involved in the recognition mechanism.

The mechanistic work reported in the literature may be divided into three categories: chromatographic studies, X-ray together with computational studies and NMR studies. The chromatographic studies involved either (a) the chromatography of series of related compounds to quantify their steric, electronic and lipophilic effects on the retention and separation with a given chiral phase [4–7], or (b) the chromatography of typical compounds using series of differently substituted stationary phases to pinpoint the possible interaction sites within the chiral phase [8]. These studies showed that the retention of solutes on these chiral phases is given by the hydrogen bonding (both donor and acceptor) capabilities of the carbamate groups of the CSP, the hydrogen bonding acceptor properties of ester groups of the CSP, the dipole–dipole interaction of the C=O groups in esters and carbamates with C=S or C=O dipoles in solutes, the π–π stacking of the phenyl groups in the esters and carbamates with aromatic groups of solutes and the lipophilicity of solutes that plays an important role in their steric inclusion within the chiral environment. The X-ray measurements together with the molecular mechanistic calculations of the CSPs [8] demonstrated their partial crystalline structure and indicated their helical structure, which results in the formation of chiral grooves with different dimensions in cellulose and amylose derivatives. The cellulose derivatives are left-handed 3/2 helices, whereas the amylose derivatives are left-handed 4/1 helices. The NMR studies [9] proved the existence of chiral cavities able to incorporate alcohols, which result in a higher degree of crystallinity of the chiral phase structure. We expect therefore in practice a different behaviour (sometimes complementary) of the cellulose and amylose derivatives because of the different inclusion mechanisms driven by the shape of the molecule. We can also expect adsorption of primary and secondary alcohols onto the chiral phase, which in practice is able to generate surprising effects on the enantiomer separation. Even though these studies are still unable to propose a way to predict unequivocally which phase will separate a given racemate, they are very helpful for the understanding of the chiral chromatographic behaviour. This knowledge of the solute–chiral phase interaction and of the solvent effects led us to develop an empirical approach based on the rational understanding of a great number of examples: a 'screening and optimisation strategy' able to cover all types of compounds to be separated. This approach may be easily applied in practice to choose the best phase for a given separation.

4.3 Screening and optimisation strategy for preparative chiral chromatography

The screening strategy presented below is based on the results from the development of over 1000 preparative methods performed in the last five years at Chiral Technologies. Its

aim is to define the right experiments to perform in order to choose the most appropriate CSP–solvent combination for a separation.

4.3.1 *Choice of the stationary phase*

The lack of *a priori* prediction of which phase will separate a given compound led us to develop full screening units, containing all available CSPs (see Fig. 4.1) packed in analytical columns of conventional size (250 × 4.6 mm). A switching valve and an automatic HPLC unit (comprising pump, autosampler, solvent switching unit and DAD detector) allow the rapid screening of different CSPs for a solute (Fig. 4.2). The statistical results obtained with this screening unit for over more than 200 compounds showed that almost 50% of them could be separated on CHIRALPAK AD, and approximately 15% could be separated on each of CHIRALPAK AS, CHIRALCEL OD and CHIRALCEL OJ. Because of their complementary behaviour, these four phases are able to separate almost 95% of all compounds tested. In those very few cases where no separation is obtained on these four phases, other CSPs (CHIRALCEL OF, OK, OG) might be tried as specific phases. To increase the chances rapidly to find the right stationary phase, screening units similar to that presented in Fig. 4.2 may be installed containing the four phases, CHIRALPAK AD, CHIRALPAK AS, CHIRALCEL OD and CHIRALCEL OJ, which have the widest range of application. Other CSPs can be added to this list; some chromatographers add one or two non-polysaccharide columns that have been found to be useful for their specific compound types. However, it is important to ensure that the columns used in the screening process are available for the scale of preparative chromatography to be performed. If the goal is to separate only a few 100 mg to 1 g of sample, there is a much greater choice available than for the situation where many kilograms of stationary phase will be needed for larger scale operation.

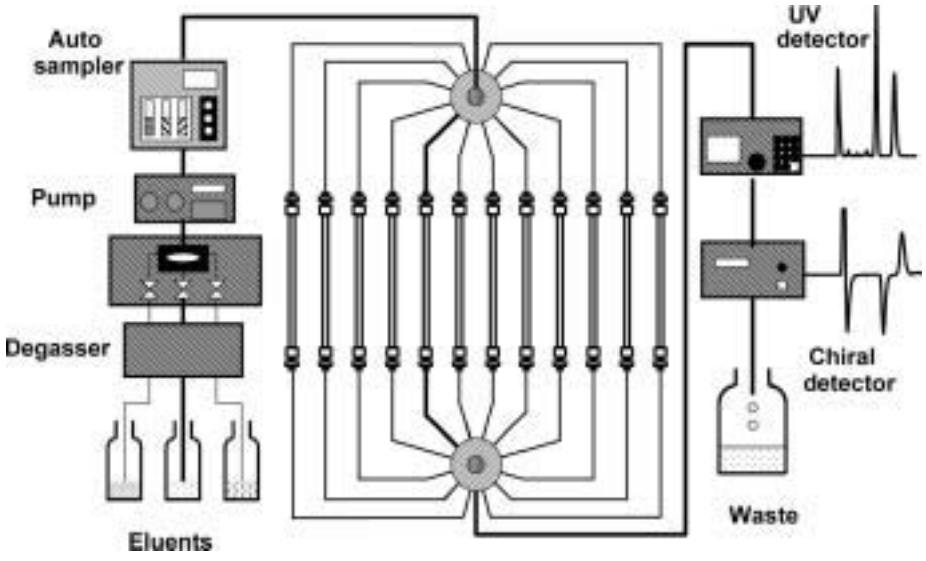

Figure 4.2 Typical laboratory screening unit for testing a large number of chiral columns.

4.3.2 *Choice of the mobile phase*

The esters and carbamates of cellulose and amylose (Fig. 4.1) can be used in a wide range of mobile phases because of their intermediate polarity; they are more polar than the normal phase solvents (alkane/alcohol mixtures) and they are less polar than reverse phase solvents (water/acetonitrile mixtures). The retention of a compound to be separated in most cases follows the polarity rule: the retention may increase (or decrease) by diminishing (or increasing, respectively) the modifier content, which is an alcohol in normal phase and acetonitrile in reverse phase. A third category of solvents, that of polar organic solvents, such as pure acetonitrile, pure alcohols and their mixtures may also be used. The retention of compounds in these polar solvents is mainly governed by the versatile solvation properties of the stationary phase correlated to the hydrogen bonding abilities of both compound and stationary phase, thus very often not following the simple predictive polarity rule (see examples given later). In our experience, three main categories of screening solvents may be defined for preparative applications: (1) pure acetonitrile, (2) a 50:50 mixture of ethanol and methanol and (3) 15% ethanol in hexane (or heptane). All four CSPs (CHIRALPAK AD, CHIRALPAK AS, CHIRALCEL OD and CHIRALCEL OJ) are compatible with these screening solvents. The most rapid way to test four phases with three screening solvents is to dedicate a set of columns to each solvent mode. Using solvent-dedicated columns will save the time needed for equilibration and transfer between solvents. More time can be saved by dedicating not only the columns but also the equipment to the screening solvents. In this way the experiments can be run in parallel so the entire screening process can be completed within 2–3 h. In case one needs to use the same set of columns for all modes, care must be taken during the transfer from one solvent to the other. The rule concerning this transfer is always to use 2-propanol when changing from alkane solvents to acetonitrile and vice versa, from ethanol/methanol to acetonitrile and vice versa.

Once one has obtained the screening results, one has to know how to optimise them. We have studied the effects of various solvent mixtures on the separation of numerous chiral compounds, and have defined a mobile phase optimisation strategy. This optimisation strategy includes all possible mobile phase combinations capable of leading to a separation or of adjusting the retention factors when necessary.

4.3.2.1 *Acetonitrile screening solvent* When a partial separation or no separation but at least some retention (capacity factor of 0.3–0.7) is obtained in pure acetonitrile, the addition of 5% of one of 2-propanol, methanol, ethanol, 1-propanol, 1-butanol, 2-butanol or 2-methyl-1-propanol may dramatically increase the separation. This alcohol effect in acetonitrile was observed almost exclusively on CHIRALPAK AD. In Table 4.1, the results of the optimisation by the alcohol effect of the separation of the enantiomers of two compounds (Compound 1 and Compound 2) are reported. The chromatography was performed on a column (250 $\times$ 4.6 mm) packed with 20 μm CHIRALPAK AD. Although the structures cannot be disclosed because of confidentiality agreements, they are not an issue for the present discussion because the procedures described here are generic in nature.

In Fig. 4.3, the variation of capacity factors of the two enantiomers of these two compounds, depending on the type of alcohol modifier, is graphically represented. We may observe that for Compound 1, the retention of the first eluted enantiomer is slightly affected by the change in alcohol, whereas the retention of the second eluted enantiomer is

Table 4.1 Optimisation of the separation of two compounds by the alcohol effect in acetonitrile on a CHIRALPAK AD column (250 × 4.6 mm, 20 μm), 1 mL/min, 25°C

	Compound 1			Compound 2		
Mobile phase	k'_1	α	Rs	k'_1	α	Rs
100% acetonitrile	0.31	1.69	1.3	1.28	2.92	4.34
Acetonitrile/methanol (95:5)	0.19	2.11	0.8	0.76	2.46	3.68
Acetonitrile/ethanol (95:5)	0.28	1.68	1.2	0.87	2.60	4.26
Acetonitrile/1-propanol (95:5)	0.24	2.32	1.9	1.68	1.86	3.20
Acetonitrile/2-propanol (95:5)	0.25	2.86	2.6	0.85	1.98	3.10
Acetonitrile/2-methyl-1 -propanol (95:5)	0.27	2.36	2.1	1.53	4.13	4.20
Acetonitrile/1-butanol (95:5)	0.24	2.10	1.7	1.82	1.99	3.71
Acetonitrile/2-butanol (95:5)	0.25	3.21	2.9	1.68	1.52	2.08

significantly increased or decreased independently of the polarity of the alcohol. The best separation was obtained with 2-butanol, which gave the biggest increase in the retention of the second eluted enantiomer and almost doubled the selectivity compared to pure acetonitrile. For Compound 2, the retention of both enantiomers is affected in a similar way by most of the alcohols, which has the effect of decreasing the selectivity compared to pure acetonitrile. The only exception is for 2-methyl-1-propanol which greatly increases the retention of the second eluted enantiomer and in consequence the selectivity. From these examples with many others from our database, we conclude that the alcohol type to use for a given compound cannot be predicted; consequently all these alcohols have to be systematically tested during optimisation.

In cases where the separation obtained in pure acetonitrile is too large or the retention times are too long, we may adjust these parameters by adding up to 20% methanol or ethanol to the mobile phase. For instance, in the separation of Compound 3 on a CHIRALPAK AS (250 × 4.6 mm, 20 μm) column using acetonitrile (1 mL/min, 25°C), the capacity factors were excessive ($k'_1 = 1.81$, $k'_2 = 2.95$). By adding 10% methanol to the mobile phase, the capacity factors were reduced by less than half ($k'_1 = 0.71$, $k'_2 = 1.17$) while the selectivity ($\alpha = 1.65$) and the loading capacity were unaffected. We will see in Section 4.4 that reduced $k\prime$ values combined with a good loading capacity may lead to a convenient productivity.

4.3.2.2 *Ethanol/methanol (50:50) screening solvent* In this case, the optimisation step involves the trial of pure ethanol and pure methanol if a partial separation is obtained with the 50% ethanol in methanol screening solvent. There are cases where the best separation is obtained with the 50:50 mixture (Compound 4, Fig. 4.4), and cases where pure ethanol gave the best result (Compound 5, Fig. 4.5).

Pure methanol is much more interesting as a preparative solvent because it has a lower viscosity than ethanol (see Section 4.4). When capacity factors that are too large are obtained in pure methanol, the retention may be reduced by adding up to 20% acetonitrile. The very high viscosity of pure ethanol or where retention times are long in 100% ethanol can be reduced in certain cases by adding up to 20% hexane or heptane. In such cases, the selectivity of the separation and/or the solute solubility in the mobile phase are not significantly affected. For instance, Compound 6 was separated on CHIRALPAK AD (250 × 4.6 mm, 20 μm) in pure ethanol with $k'_1 = 2.38$, $k'_2 = 5.33$, selectivity (α) = 2.2 and a resolution (Rs) = 2.7. By

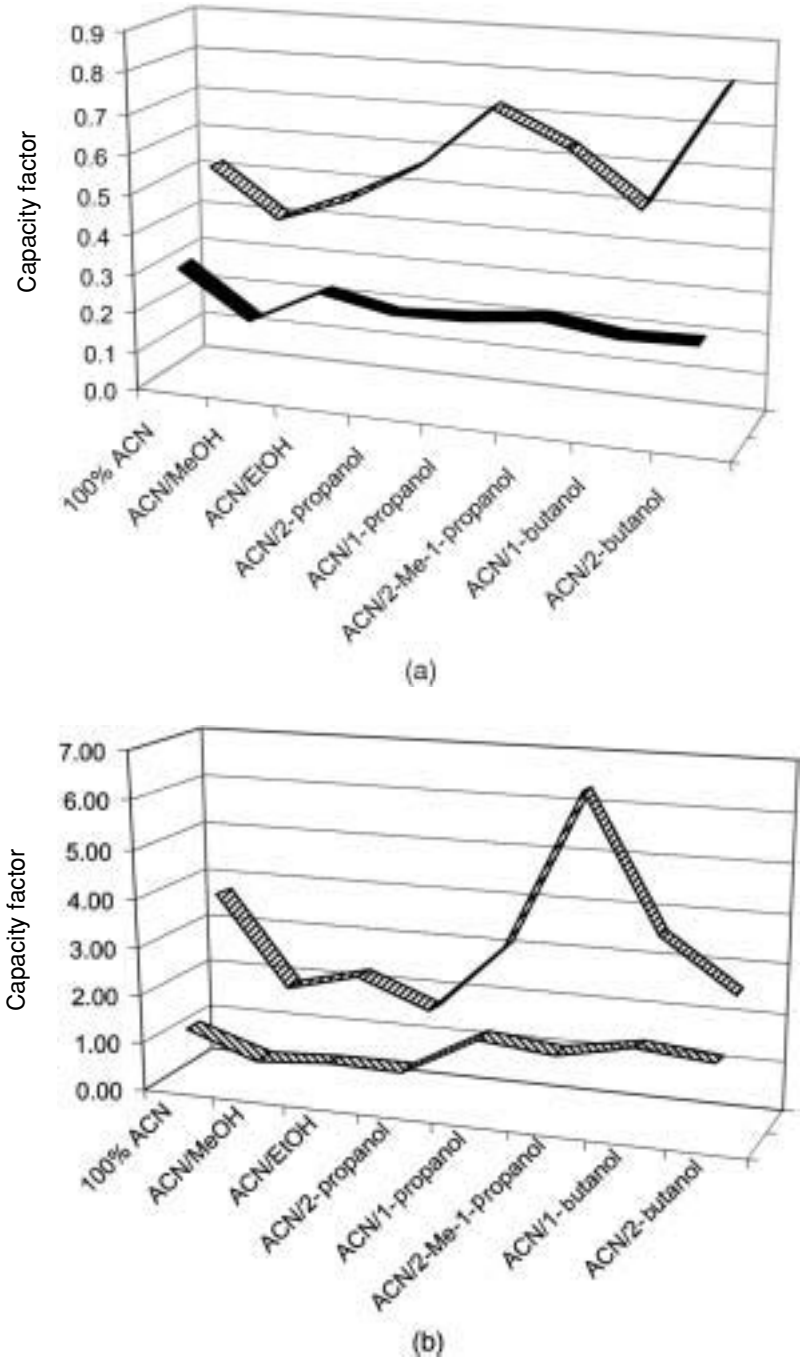

Figure 4.3 Variation of the capacity factors for two compounds depending on the alcohol type in acetonitrile mobile phase (composition 95:5 acetonitrile/alcohol, column CHIRALPAK AD (250 × 4.6 mm, 20 μm), 1 mL/min, 25°C). (a) Compound 1 and (b) Compound 2. Upper lines are for peak 2, lower lines for peak 1.

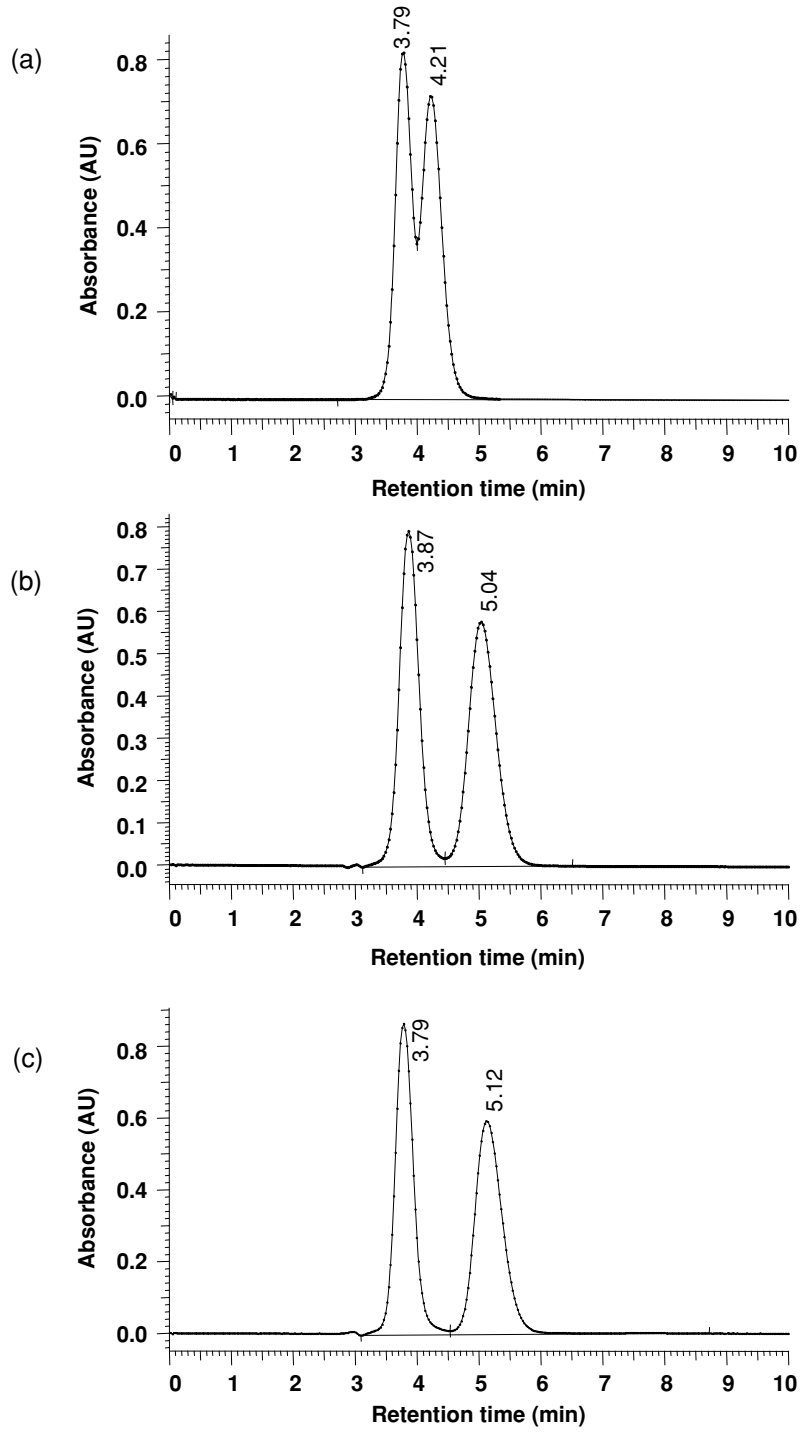

Figure 4.4 Comparison of the separation obtained in alcohols for Compound 4 on CHIRALPAK AD; column (250 × 4.6 mm, 20 μm), 1 mL/min, 25°C. (a) 100% Methanol: $k'_1 = 0.26$, $k'_2 = 0.40$, $\alpha = 1.54$, Rs = 0.8; (b) 100% ethanol: $k'_1 = 0.29$, $k'_2 = 0.67$, $\alpha = 2.31$, Rs = 1.7; (c) 50% ethanol/methanol: $k'_1 = 0.26$, $k'_2 = 0.70$, $\alpha = 2.7$, Rs = 2.1.

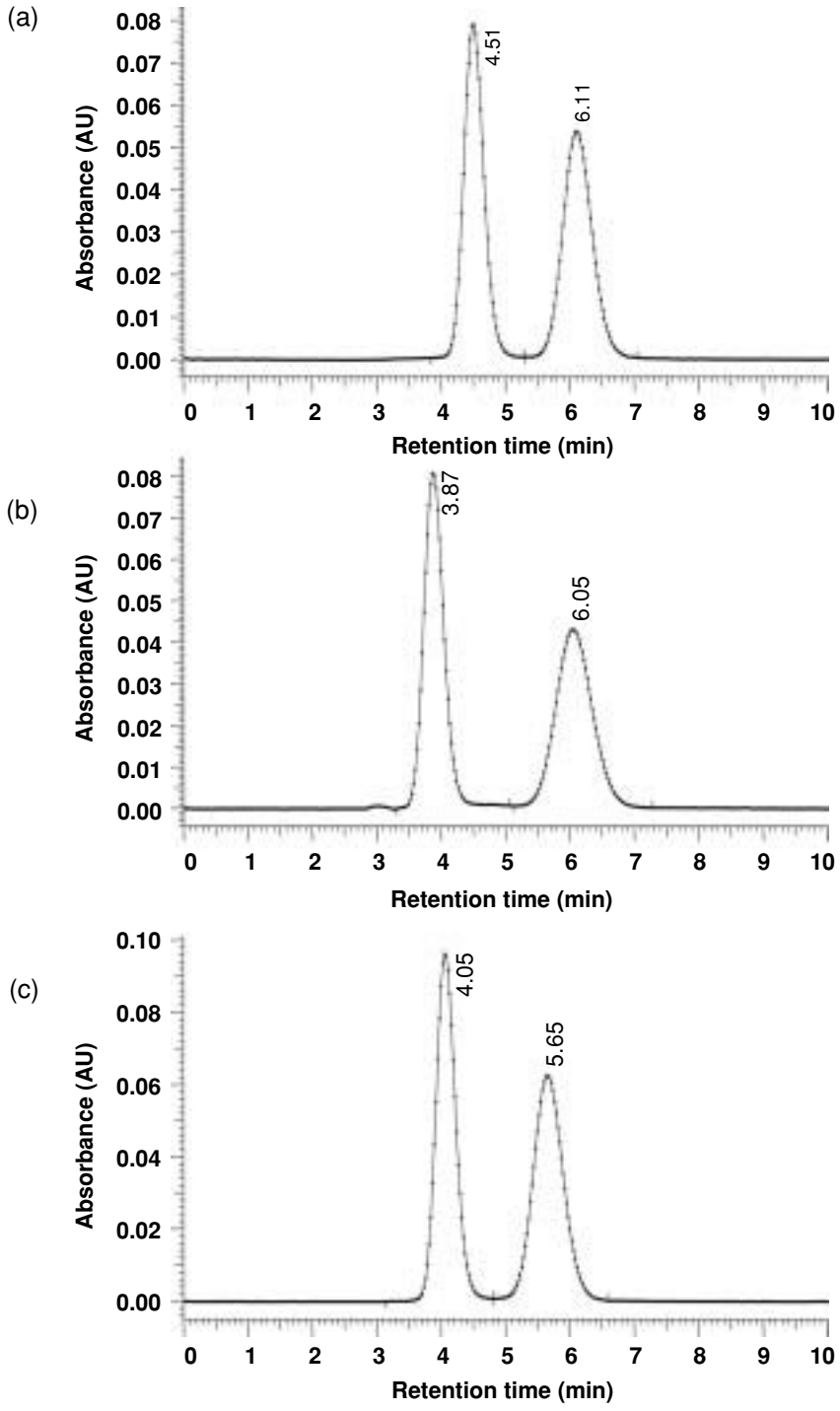

Figure 4.5 Comparison of the separation obtained in different alcohols for Compound 5 on CHIRALCEL OD; column (250 × 4.6 mm, 20 μm), 1 mL/min, 25°C. (a) 100% Methanol: $k'_1 = 0.50$, $k'_2 = 1.03$, $\alpha = 1.2$, Rs = 2.2; (b) 100% ethanol: $k'_1 = 0.29$, $k'_2 = 1.01$, $\alpha = 3.5$, Rs = 2.7; (c) 50% ethanol/methanol: $k'_1 = 0.35$, $k'_2 = 0.87$, $\alpha = 2.5$, Rs = 2.2.

adding 20% hexane, the viscosity of the mobile phase was reduced by approximately 15% and the capacity factors were reduced by 50% without significantly affecting the separation factor: $k'_1 = 1.20$, $k'_2 = 2.59$, $\alpha = 2.15$, Rs = 2.1. This illustrates that the solvent polarity is not important in retention in polar solvent mode; the addition of hexane is thought in this case to reduce the hydrogen bonding interactions occurring in the system, thus reducing the retention.

4.3.2.3 *15% Ethanol in hexane or heptane as screening solvent* If a partial separation or no separation is obtained with this screening solvent, then two cases are possible: either the retention is too low and it has first to be adjusted to a k' at least 0.3–0.7 by decreasing the ethanol percentage, or the retention is sufficiently high. Once the retention has the right value and the selectivity is increased, the best way is to replace ethanol by 2-propanol, or even higher carbon number alcohols like 1-butanol, 2-butanol or 2-methyl-1-propanol. If necessary their amount in the mobile phase is increased to keep similar retention values since these alcohols are less polar than ethanol. An example of the results obtained for the optimisation of the separation of Compound 7 is reported in Table 4.2. In experiments where the 5% ethanol in *n*-hexane was replaced with 10% of 1-propanol, 2-propanol, 1-butanol, 2-butanol and 2-methyl-1-propanol, the best separation was obtained in hexane/1-butanol, for which the α value was significantly increased compared to that using ethanol as modifier (2.02 compared to 1.42). There are few cases for which a mixture of two alcohols in heptane (or hexane) gives the best separation. For example, mixtures of heptane, ethanol and methanol (a minimum of 80% alkane and equal proportions of ethanol and methanol) have been used. It is worth mentioning that no preparatively significant differences in selectivity were observed when using hexane compared to heptane-based solvents. Heptane is often preferred as solvent, despite its higher cost and viscosity, because of the neurotoxicity of hexane.

4.3.3 *Screening and optimisation of specific phases*

It was mentioned above that CSPs like CHIRALCEL OK, OF and OG may be tried albeit with limited success to give separations in some specific cases. For CHIRALCEL OK, we may use the same screening and optimisation solvents as for the main phases (see above). CHIRALCEL OF may be used only in mixtures of 2-propanol in heptane or hexane

Table 4.2 Optimisation of the separation of Compound 7 by the alcohol effect in hexane on a CHIRALCEL OD column (250 × 4.6 mm, 20 μm), 1 mL/min, 25°C

	Compound 7		
Mobile phase	k'_1	α	Rs
Hexane/ethanol (95:5)	1.74	1.42	1.6
Hexane/1-propanol (90:10)	1.08	1.54	1.7
Hexane/2-propanol (90:10)	2.09	1.58	2.2
Hexane/1-butanol (90:10)	1.73	2.02	2.8
Hexane/2-butanol (90:10)	2.58	1.73	2.6
Hexane/2-methyl-1-propanol (90:10)	1.81	1.8	2.6

(up to 100% 2-propanol) and is usually tested in 20% 2-propanol in heptane (or hexane) with further optimisation by increasing or decreasing the alcohol content depending on the retention obtained in the screening. Mixtures of either ethanol or 2-propanol in heptane are allowed on CHIRALCEL OG, so that the screening may also be performed in 15% ethanol in heptane. Here the optimisation involves adjusting the retention and substituting 2-propanol for ethanol.

4.3.4 *Additives in the mobile phase*

For acidic or basic compounds, as well as for their salts, an acidic or basic additive in the mobile phase is in most cases necessary for the suppression of the unwanted interactions with the silica within the stationary phase, which can give rise to broad, deformed peaks. We cite here the additives most used in preparative chromatography; others are possible. Trifluoroacetic acid at 0.1% relative to the mobile phase is normally able to give good elution for carboxylic acids. When the compound has only weak acidic properties (phenols, for instance), the use of acetic or formic acid is possible and the content of additive decreases to 0.01%.

The most frequently used basic additive is diethylamine (DEA) at 0.1% relative to the mobile phase. Depending on the basicity of the solute, several possibilities may be encountered: if the compound is a very weak base (e.g. compounds possessing a tertiary nitrogen group), it can be separated in most cases without additive; compounds like secondary amines may need only previous conditioning of the stationary phase with the mobile phase containing 0.1% of DEA; primary amines usually need a basic additive in the mobile phase, which may be other than DEA. Additives like butylamine and ethanolamine may be used for primary amines and primary aminoalcohols, respectively, to improve peak shape.

The separation of acid salts may be obtained by adding 0.1% TFA to the mobile phase, which transforms *in situ* the salt into the free acid. However, the loading capacity in this case is limited and thus this technique is not useful in preparative separations. The acids should always be processed as free acids for preparative purposes. The salts of bases can be separated as the salt in case of tertiary amines (i.e. without additive), as a TFA salt by adding 0.1% TFA to the mobile phase or as a free base by adding 0.1% of the appropriate basic additive. As in the case of acids, the loading of the product as a salt or as a free base obtained *in situ* from a salt is limited to the analytical level. For preparative separation therefore, one has to convert salts to the free base prior to injection. Bifunctional compounds like the amino acids may be separated by adding 0.1% TFA in the mobile phase, although the preparative applications are extremely limited in load. In this latter case, either the acid is esterified and the compound is treated as a primary amine, or the amine group is protected as an amide and the compound is separated as a carboxylic acid.

When acidic and basic additives like TFA (b.p. 72°C), DEA (b.p. 55°C) or butylamine (b.p. 78°C) are used, the recovery of the product can be performed by evaporation, since these additives have a low boiling point, preventing their concentration in the product. Additives like ethanolamine (b.p. 171°C) are difficult to remove by evaporation; a supplementary washing step with water may be performed depending on the next step in the industrial – or laboratory – process. Care should be taken that the additive does not react with or modify the product. For example, TFA in presence of alcohols may esterify the acidic product, and secondary alcohols are preferred to primary alcohols because they may react more slowly.

In summary, the following screening and optimisation 'toolbox' should be considered in order to obtain the best separations:

(1) Four phases CHIRALPAK AD, CHIRALCEL OD, CHIRALCEL OJ, CHIRALPAK AS
(2) Three screening solvents: acetonitrile, ethanol/methanol (50:50) and 15% ethanol in *n*-hexane or *n*-heptane
(3) Four types of optimisation solvents: acetonitrile/alcohol mixtures, ethanol or ethanol/hexane, methanol or methanol/acetonitrile and hexane/alcohol mixtures
(4) Acidic or basic additives in the mobile phase for acidic and basic compounds, respectively, by taking into account that the compound has to be processed as free acid or base and not as a salt.

4.4 Preparative separations, criteria and objectives

The major difference between analytical and preparative method development lies in its objective. An analytical method has to provide the maximum information about the sample (purity, enantiomeric excess) whereas the preparative method has to provide the purification and isolation of the desired enantiomer.

In the preparative separation, the quantity of product injected onto the column (or the *load*) and that recovered from the separative process become important parameters to study. By applying the screening and optimisation strategy presented in the previous section, the user may identify one or several CSP–solvent combinations. All these conditions are obtained at analytical scale. Before going to production scale, it is most important to know and evaluate the parameters that have the greatest impact on the efficient recovery of the product. In our experience, the main parameters to evaluate are those related to the intrinsic properties of the CSP and those conferred by the solvent. The properties of the CSP relate to the *selectivity* (or separation factor) and the *loadability* (how much sample we can put onto the CSP), whereas the solvent properties give the sample *solubility* and also the working flow rate from its *viscosity*. These four main parameters are not independent and their optimisation to get the best production often results in making compromises between them. The following discussion details the role they play in preparative separations.

4.4.1 *Loadability and productivity*

The key experiment allowing the relation of the separation parameters to the sample quantity to be processed is the *loading study*. For a given set of analytical chromatographic conditions, a loading study may be performed by injecting increasing quantities of racemic onto the column until the bands are overlapped. Experimentally, it may be run on analytical size columns (250 × 4.6 mm) filled with the *same CSP* as that utilised at preparative scale.

At analytical scale, the injected sample volume or mass is so small (ideally 0.001–0.01% of the total mass of the stationary phase) that the peak width is not influenced by it. In other words, in analytical chromatography, the equilibrium concentrations of each component in the stationary and mobile phase are proportional and the equilibrium isotherm is linear [10]. At preparative scale, the process may only become productive if performed under overloaded

conditions, since the purpose is to inject as much as possible of the compound to be resolved to obtain pure enantiomers (more than 1% of sample with respect to the stationary phase). The overloaded conditions are ruled by a non-linear equilibrium, where the concentration of each component in the stationary phase at equilibrium is no longer proportional to its concentration in the mobile phase. The effect is that the chromatographic bands (or peaks) depend on the sample concentration and they are distorted by the effect of either mass or volume overload [11]. If the sample has sufficient solubility, mass overload is always the preferred mode because of minimum band spreading arising from the injection volume. That means in practice that the loading study has to be performed with a sample solution as concentrated as possible in (for preference) the same solvent as the elution solvent. The loading data may be used to estimate the productivity of a preparative process, by batch or simulated moving bed (SMB) chromatography. Let's take an example to illustrate how we may extract data from the loading study. In Fig. 4.6, a loading study for Tröger's base was performed on CHIRALPAK AD (250 × 4.6 mm, 20 μm) column in methanol using a solution at 20 g/L in the same solvent. Increasing volumes of sample solution corresponding to 5, 15 and 30 mg of sample were injected onto this column and the selectivity decreased from 2.6 in the analytical injection to 2.5, 2.4 and 2.1, respectively for the increasing load.

From the loading data, we may define the *loading capacity* of a given CSP towards a given compound expressed as grams of product per kilogram CSP, which can be calculated by the following ratio:

$$L(\text{g/kg CSP}) = \frac{\text{Qracemic}}{\text{QCSP}} \tag{4.1}$$

where Qracemic is the quantity of product (in grams) injected to obtain a touching band resolution, and QCSP (in kilograms) is the quantity of CSP contained in the column

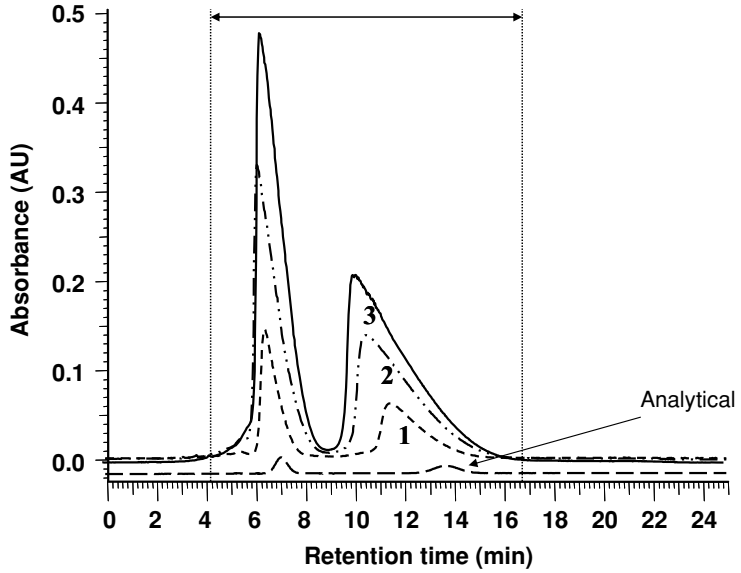

Figure 4.6 Loading study for Tröger's base on CHIRALPAK AD in methanol (MeOH); column (250 × 4.6 mm, 20 μm), 1 mL/min, 25°C. Dashed line = analytical load. Curve 1 = 5 mg load; curve 2 = 15 mg load; curve 3 = 30 mg load.

(approximately 0.0025 kg for packed polysaccharide derivatives in a 250 × 4.6 mm column). In batch chiral chromatography, touching band resolution provides good purity and recovery of the enantiomers in most cases. A touching band separation is achieved when the retention time of the front of the second enantiomer is equal to the retention of a near zero concentration of the first enantiomer (7). For example, the high load chromatogram corresponding to a 30 mg loading on a CHIRALPAK AD (250 × 4.6 mm, 20 μm) column is very close to a touching band condition. The calculated loading capacity of CHIRALPAK AD for this racemate is about 12 g/kg. This parameter may be useful to compare different CSP–solvent combinations. Taken together with the separation time in batch chromatography, it may serve to calculate the separation throughput:

$$\text{Throughput (batch)} = \frac{L}{\text{Time}} \left(\frac{\text{g}}{\text{kg(CSP)} \times \text{day}} \right) \tag{4.2}$$

where L is the loading capacity in g/kg CSP and Time is the separation time in days (24 h).

The separation time is estimated from the loading chromatogram at touching bands. We assume that overlapping or stacked injections are used. In this mode, the next injection is made during the current separation such that the first peak from this next injection elutes immediately after the second peak of the current chromatogram. For instance, the separation in Fig. 4.6 is of approximately 13 min duration as defined by the beginning and end of the peak pair, shown by the vertical lines; consequently the throughput in batch chromatography is estimated at:

$$12 \text{ g/kg/13 min} = 1329 \frac{\text{g}}{\text{kg (CSP)} \times \text{day}}$$

Productivity is expressed in g (en)/kg/day and is obtained from the throughput taking into account the recovery yield of the desired enantiomer.

The productivity in continuous chromatography by SMB may be also estimated by using the loading data, although it cannot be directly extracted from the loading chromatograms. The absolute values of the retention times measured at the apex or at the front of the peak together with the sample load values may be used to calculate the adsorption isotherm parameters, as well as feed, raffinate and extract flows for a defined SMB system. This calculation is usually performed using a computer-based simulation [12]. For instance, in Table 4.3, the loading data of Tröger's base on CHIRALPAK AD in acetonitrile at 30°C and 1 mL/min are reported.

The loading was performed at 30°C to account for small temperature variation at pilot scale. Loading points above the touching band resolution should be included in the

Table 4.3 Loading data for Tröger's base on CHIRALPAK AD (250 × 4.6 mm, 20 μm) in acetonitrile, 1 mL/min, 30°C

Concentration (g/L)	Injection volume (mL)	Load (mg)	Rt_1 (min)	Rt_2 (min)	k'_1	k'_2	N(1)	N(2)
0.5 (analytical)	0.05	0.025	4.19	5.23	0.4	0.74	1427	1040
25	0.02	0.5	4.13	5.07	0.38	0.69	1198	835
25	0.04	1	4.08	4.96	0.36	0.65	1070	694
25	0.08	2	4.05	4.83	0.35	0.61	927	525
25	0.10	4	4.03	4.75	0.34	0.58	710	372
25	0.24	6	4.03	4.69	0.34	0.56	574	262
25	0.28	7	4.03	4.69	0.34	0.56	506	–

simulation (in this case loads up to 7 mg were used; Fig. 4.7). By using the simulation software [12] for an SMB unit having eight columns, each 100 × 50 mm in size (8 × 50 SMB), the input data were the retention times, injection volumes and concentrations, and the output data after optimisation were the feed flow (22 mL/min), the feed concentration (40 g/L), the switching time (0.73 min), the extract flow (76 mL/min), the extract concentration (5.8 g/L at 99.8% ee), the raffinate flow (25 mL/min) and the raffinate concentration (17.6 g/L at 99.7% ee). From the feed flow and concentration, we may calculate the throughput and productivity for the 8 × 50 SMB system. The throughput (g racemic/day) is given by the product of the feed flow and feed concentration.

$$\text{Throughput (SMB)} = \text{Feed Flow (mL/min)} \times \text{Feed Concentration (g/L)} \times 60 \times 24 \tag{4.3}$$

Thus, in this case, the throughput is 1273 g racemic/day.

The production rate is the quantity of the desired enantiomer isolated per unit time taking into account the purity and recovery yield.

$$\text{Production Rate} = \text{Concentration} \times \text{Flow} \times \text{Recovery/Purity} \tag{4.4}$$

In this case the production rate for the extract product is given by the concentration (5.8 g/L) and flow (76 mL/min) of the extract stream corrected for the purity (here 99.9% pure) and the recovery (99.85%; 0.15% is lost to the raffinate stream). This is therefore

$$\text{Production Rate Extract} = (5.8 \times 76 \times 99.85)/99.9 = 440.6 \text{ mg/min} = 634.5 \text{ g/day}.$$

The productivity (g (en)/kg CSP/day) may be calculated by dividing the production rate by the quantity of CSP used for the separation. In the cited case, about 0.8 kg of CHIRALPAK

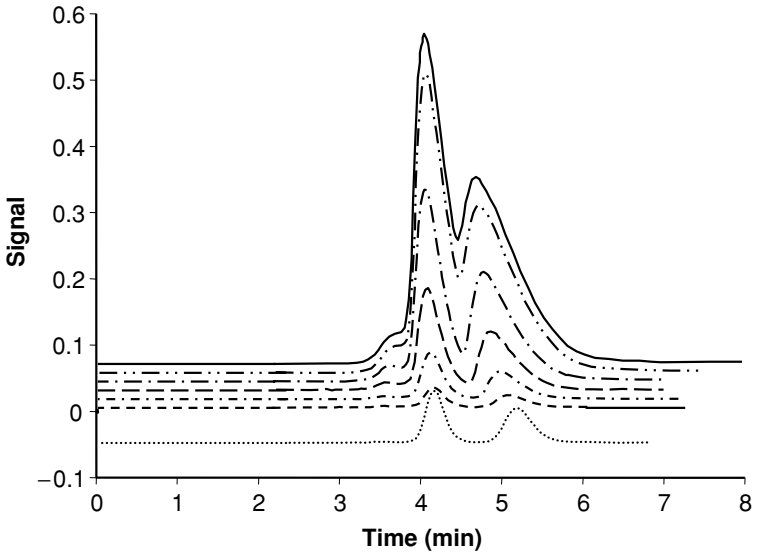

Figure 4.7 Loading study for Tröger's base on CHIRALPAK AD in pure acetonitrile (1 mL/min, 30°C, column (250 × 4.6 mm, 20 μm)).

AD phase would be used in the eight 50 mm diameter columns of the SMB unit; thus the simulated productivity was 793 g (en)/kg CSP/day.

We have seen how the loading data obtained on analytical size columns may be used to estimate the batch and SMB productivity. In this way, the loading data are very useful to compare different CSP–solvent combinations for a given racemic separation and to choose before starting a process the most appropriate conditions to obtain the best productivity. The more accurately the loading data are collected, the better is the estimation of productivity. Several experimental requirements may be mentioned in order to obtain more accurate loading data, such as

(a) the analytical column has to be correctly packed (efficiency $>$ 1000 plates per column for 20 μm particles)
(b) the stationary phase filling the column has to be as similar as possible to the preparative phase: same porosity, same use history
(c) the temperature variation at laboratory or production scale has to be considered
(d) the sample concentration has to be as high as possible to minimise sample volume effects
(e) the linearity of the detector used to run this study has to be verified to avoid saturation effects; a simple way to do it is to plot the peak areas against the quantity injected.

From the relationships (4.2), (4.3) and (4.4), we may easily observe that the productivity may be increased:

(a) in batch chromatography, by finding conditions where the loading capacity can be increased and the separation time reduced.
(b) in continuous chromatography, by finding conditions where the feed flow and concentration may be increased (equivalent to increasing the loading) or where the quantity of the stationary phase may be decreased.

In chiral chromatography, the loading capacity is specific to the solute–CSP–solvent combination and we cannot easily predict which would be the best before running a loading study. The only way to increase the loading capacity is to look for all possible combinations of solvent and CSP which give a maximum selectivity. So, another factor to be considered for its impact on the productivity is the selectivity.

4.4.2 *Selectivity and productivity*

The selectivity, also known as separation factor or α value, is controlled by the thermodynamics of the chiral interactions of the enantiomers within the sites of the CSP in a certain solvent environment. The selectivity is measured at the analytical scale and it is one of the parameters determined in the screening and optimisation method presented in the previous section. Under overloaded conditions, two other factors come into play: the saturation capacity (w_s) and the shape of the adsorption isotherm. The saturation capacity for a component, which is defined as the mass of monolayer of solute on the surface of the column, may be calculated from the loading data using the following equation [10]:

$$w_s = \sqrt{2N}\, w_x \frac{k'}{1+k'} \frac{k'_0}{k'_0 - k'} \tag{4.5}$$

where w_s is the column saturation capacity, N is the efficiency, w_x is the mass load and k' and k'_0 are the capacity factors at load and analytical, respectively. Saturation capacity may also be measured in terms of the solute concentration on the stationary phase (in g/L).

For solutes for which adsorption is governed by the Langmuir isotherm and for the same saturation capacity, the relationship between analytical selectivity and loading capacity is proportional: the higher the selectivity, the higher the loading capacity and the higher the productivity. In order to explore the relation between selectivity and productivity, computer simulations [12] were carried out. For these simulations, the capacity factor of the first eluted component was kept at 1, the saturation capacity was held constant at 100 g/L and the column efficiency was assumed to be 1500 plates. The productivity was calculated at touching band separation. Solubility was also varied (see Section 4.4.3). Plotting the productivity values for different solubilities against the selectivity of the separation (Fig. 4.8), we may observe that for a solubility of 500 g/L, the productivity increases by a factor of 3 when increasing the α value from 1.5 to 3.

The experimental results obtained over 15 preparative separations run on an 11 cm column at 500 mL/min flow rate are reported in Fig. 4.9. These separations were chosen from a set of over 40 following the criterion of similar saturation capacities (approximately 100 g/L), calculated from the loading data (with [12]). The productivity values plotted against the analytical selectivity gave a good fit, which illustrates the general tendency and supports the simulated data: doubling the selectivity may double the productivity.

The impact of selectivity on the SMB productivity may also be obtained by simulation [12] (Fig. 4.10a). To obtain the data, the capacity factor of the first eluted component was kept at 1 and the saturation capacity at 100 g/L. The adsorption behaviour was considered as following the Langmuir isotherm. With these assumptions, the plot of the productivity (in g (en)/kg/day), simulated for a 8 × 50 SMB, against the analytical α (Fig. 4.10a) shows a significant increase when increasing the separation factor: for instance, the productivity

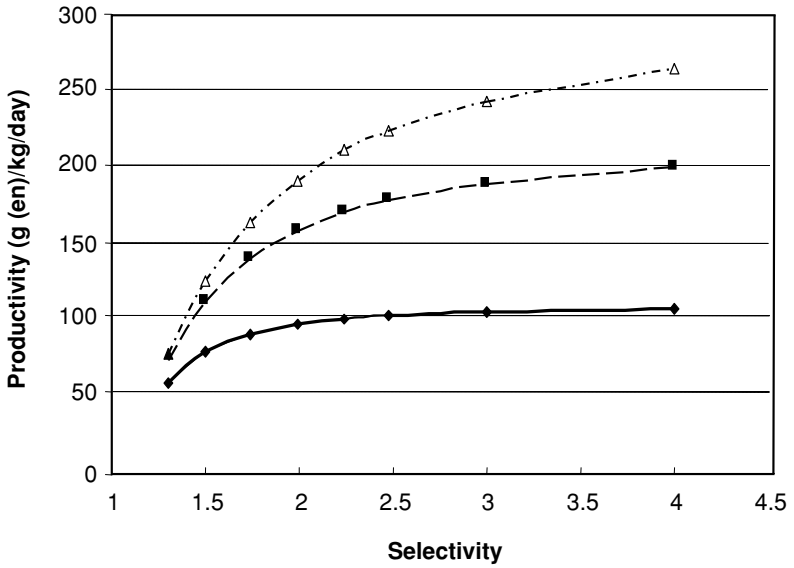

Figure 4.8 Variation of the productivity in batch chromatography (g (en)/kg/day) with the selectivity and solubility. Solid line = 30 g/L; dashed line = 100 g/L; dashed-dotted line = 500 g/L.

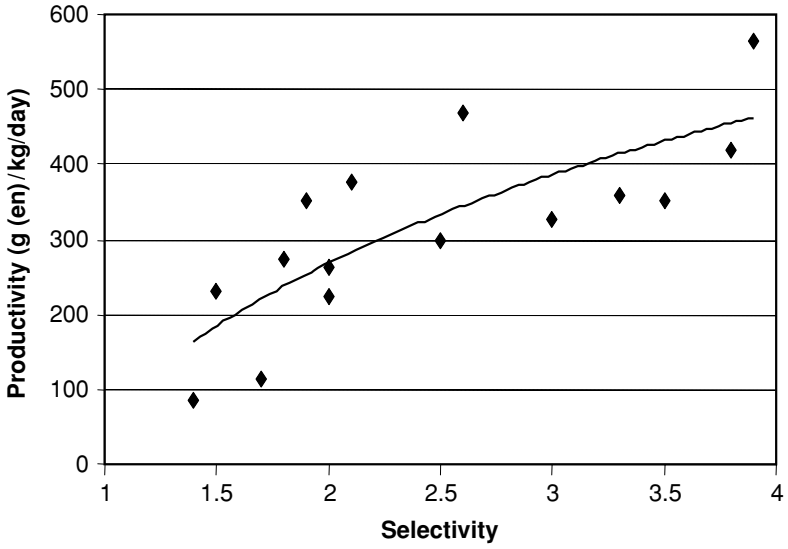

Figure 4.9 Experimental productivity obtained on an 11 cm column versus selectivity.

increased by two when increasing the separation factor from 2 to 3. In practice, the slope of this variation was found to be less steep. The experimental data using a 8×10 SMB system were obtained for 12 separations. From them, only those separations giving a similar capacity factor for the first eluted component and a similar saturation capacity were chosen and the graph of the productivity against α value was drawn (Fig. 4.10b). A good correlation similar to the simulated one was obtained, although the slope was significantly different: to increase the productivity by two (from 600 to 1200 for instance), the α value had to increase from 1.8 to 5.

For both batch and SMB, the concordance between the simulated and experimental productivity data depending on the selectivity was obtained only for the separations following the Langmuir isotherm. A significant proportion of chiral separations do not follow this type of isotherm.

The particularity of chiral adsorption often leads to enantiomer–CSP–solvent combination specific isotherms such that the isotherms for the individual enantiomers are different. It can occur that we may have smaller analytical α values giving a higher loadability/productivity than for a higher analytical selectivity. A loading study for Tröger's base in methanol mobile phase on two different columns, CHIRALPAK AD and CHIRALCEL OJ, is a typical case where the type of isotherm controls the loadability (Fig. 4.11). On CHIRALPAK AD, an analytical α value of 2.6 gives an estimated loading capacity at touching band of 12 g/kg, whereas an analytical α value of 5.2 on CHIRALCEL OJ leads to a lower loading capacity of only 6 g/kg. The latter separation is controlled by an adsorption isotherm of bi-Langmuir type.

There are cases where, despite a modest selectivity, the column has a high capacity for the product which allows a high productivity. An example of such a loading study is presented in Fig. 4.12. The analytical α value is 1.6, despite which we could load 10 mg with still close to baseline separation. This gives a calculated batch productivity of 1050 g (en)/kg/day and a calculated SMB productivity of 1610 g (en)/kg/day.

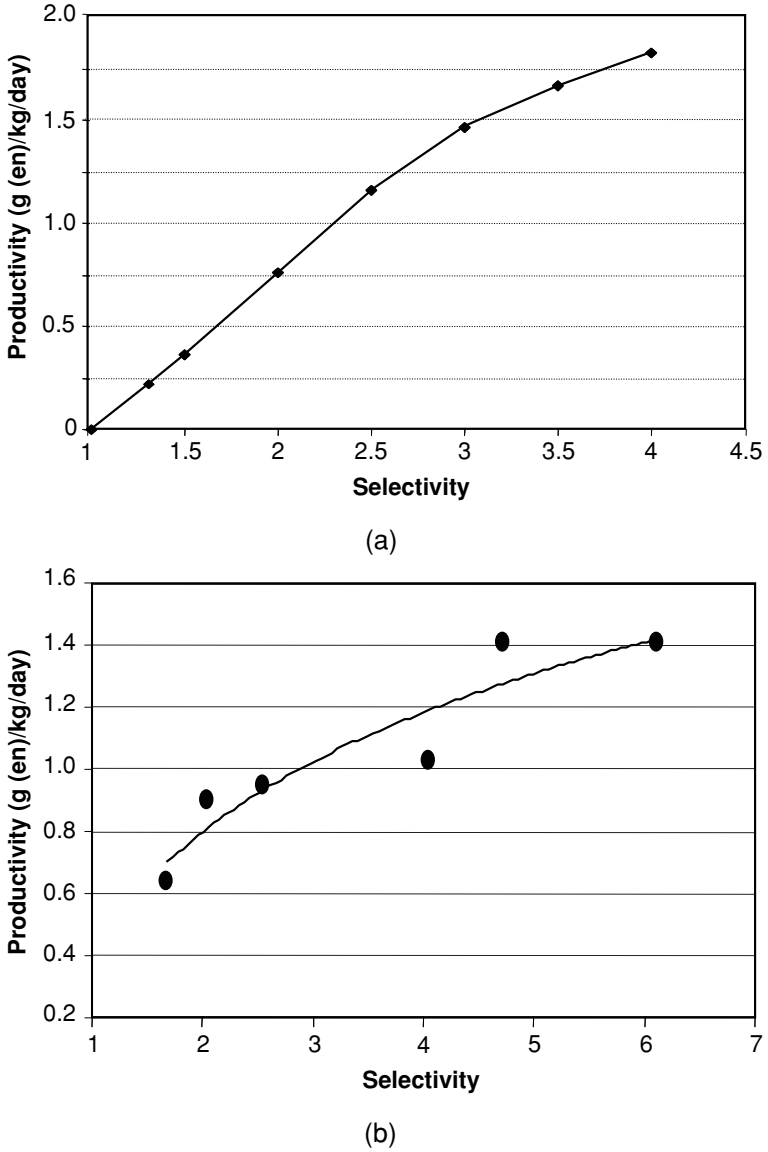

Figure 4.10 Variation of the productivity in SMB chromatography (g (en)/kg/day) with selectivity. (a) Calculated values from computer simulation; (b) Experimental values from a range of separations taken with similar saturation capacities but not necessarily similar isotherms.

It becomes clear from these examples that the selectivity optimisation for maximum productivity has to be considered together with the loading optimisation. The purpose of the selectivity optimisation is to find the maximum loading capacity in the shortest separation time. The separation time, or the time between the front of the first peak and the tail of the second peak, may be controlled by the type of adsorption isotherm (see Fig. 4.11 on CHIRALCEL OJ separation where a large tailing increased the separation time) and also by the retention factors. Peaks eluting with longer retention time elute in larger volume, thus increasing the separation time and decreasing the productivity. In some cases, the tailing may be significantly reduced by increasing the temperature, as in the example shown in

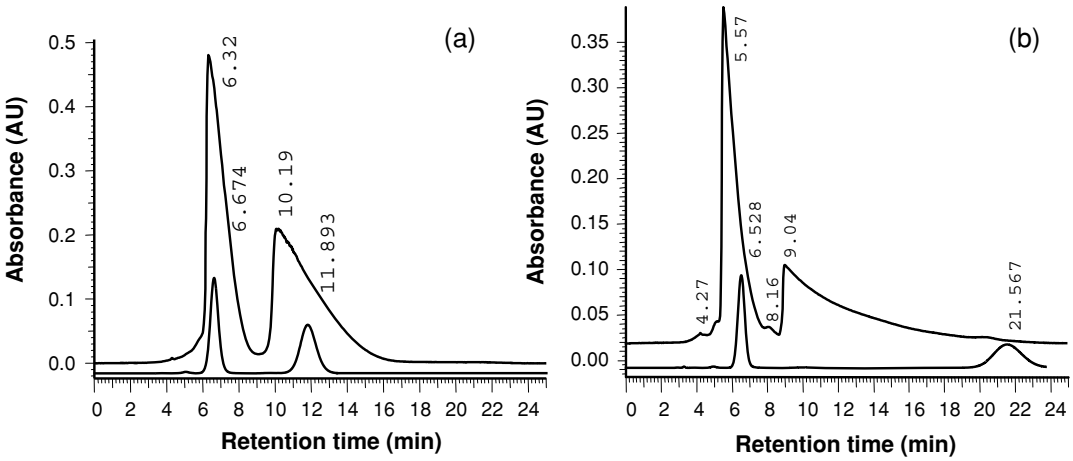

Figure 4.11 Analytical and overload chromatograms of Tröger's base on (a) CHIRALPAK AD (250 × 4.6 mm, 20 μm, load 30 mg) in acetonitrile and on (b) CHIRALCEL OJ (250 × 4.6 mm, 20 μm, load 15 mg) in methanol, both 1 mL/min, 25°C.

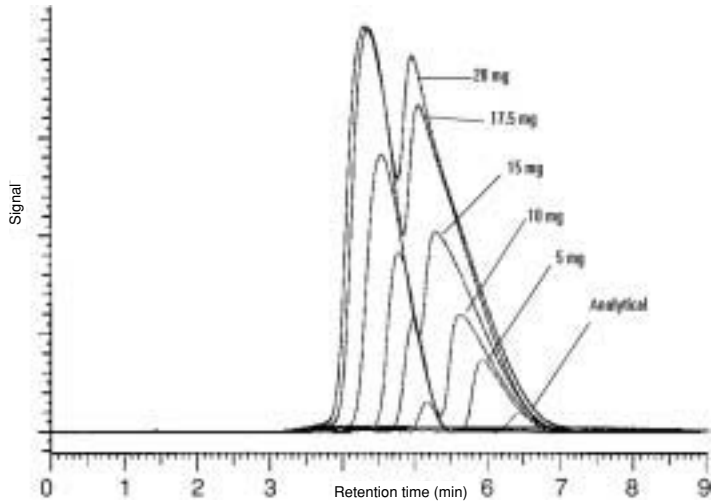

Figure 4.12 Loading study performed on a (250 × 4.6 mm, 20 μm) column.

Fig. 4.13. The chromatogram run at 25°C corresponds to peak elution between 3 and 25 min, whereas the chromatogram run at 40°C corresponds to peak elution between 3 and 17 min, so we expect a decrease by 30% of the separation time and consequently an increase by 1.4 of the productivity if the process is run at 40°C instead of 25°C. There are cases where changing the elution conditions may significantly shorten the retention times and at the same time increase the selectivity (see Case study 1).

4.4.3 *Solubility and productivity*

The solubility of the compound to be separated is often a productivity limiting factor. In many cases, a compromise should be made between high selectivity–low solubility and the contrary.

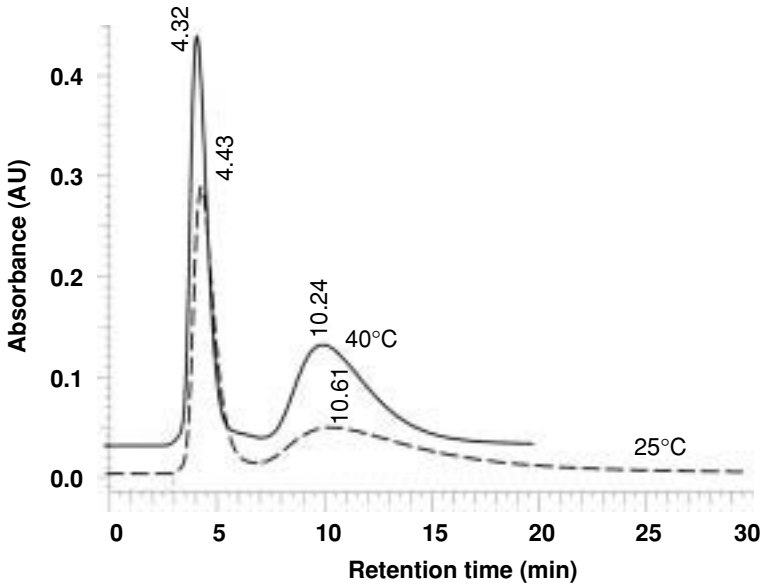

Figure 4.13 Effect of the temperature on the tailing and separation time of Compound 8 on CHIRALPAK AD. Experimental conditions: column (250 × 4.6 mm, 20 μm), eluent heptane/2-propanol (50:50), flow rate 1 mL/min, load 2 mg. Dashed line, 25°C; solid line, 40°C.

To illustrate the impact of the solute solubility on the process productivity, simulations were run for batch and SMB chromatography [12]. Figure 4.8 shows that for a selectivity of 2, an increase in solubility from 30 g/L to 500 g/L almost doubled the productivity in batch. If we look more closely at this simulation, we may also observe the interaction between the selectivity and the solubility effect to achieve productivity: for a low solubility (the 30 g/L curve), the productivity is almost not affected when selectivity is doubled from 2 to 4, whereas a high solubility (the 500 g/L curve) allows the productivity to increase by approximately 50% when selectivity is increased from 2 to 4. Moreover, this graph may help to understand why in practice we may choose a lower α combined with a higher solubility: a selectivity of 2 with a solubility of 100 g/L gives a productivity much higher than an α of 4 with solubility of 30 g/L.

The sample solubility also has a very important effect on the SMB productivity. In Fig. 4.14, the variation of the productivity with the solubility was simulated for a selectivity of 2 and value of capacity factor for the first eluted component of 1. The solubility of the sample in the mobile phase has to be at least 20 g/L to reach interesting productivity.

Solute solubility may be evaluated by (a) weighing a determined quantity of product and adding the mobile phase gradually to the point of complete dissolution, (b) preparing a saturated solution of the product in the chosen mobile phase, from which a determined volume of supernatant is removed, which is either dried and weighed or diluted and quantified by HPLC or UV measurement. The solubility is generally better in polar solvents (acetonitrile, methanol, ethanol) than in hexane or heptane mixtures. Consequently, a high priority should be given to screening in polar solvents. The solvent that gives the maximum compound solubility should be chosen when possible.

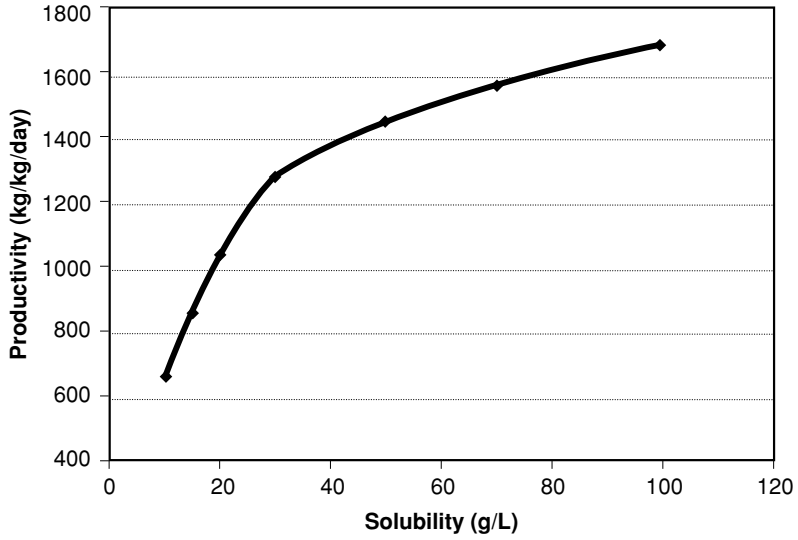

Figure 4.14 Simulation result for the effect of sample solubility on SMB productivity.

4.4.4 *Viscosity and productivity*

When the scale of the preparative process increases, the pressure in the system becomes a limiting factor. This is due both to limits in equipment as well as in the mechanical strength of the CSP. If we look at the Darcy equation (Equation (4.6)), we see that the pressure drop may be kept at a certain limit when increasing the flow rate if we may decrease the viscosity of the solvent, for a given column packing.

$$\Delta p = \frac{\Phi \times u \times L \times \eta}{{d_\mathrm{p}}^2} \tag{4.6}$$

where Φ is the resistance parameter (500 for spherical particles), u is the flow rate, L is the column length, d_p is the particle diameter. This means that decreasing the viscosity has a direct impact on the increase in productivity because we may run at higher flow rates.

Table 4.4 reports some experiments with a Licosep 8 × 50 SMB operated at 40 bar pressure, in three different solvents: ethanol, ethanol/methanol (50:50) and methanol. As we may clearly see in the graphs relating the productivity to the viscosity (Fig. 4.15), for almost similar selectivity and retention factors, the productivity is drastically diminished by the increase in solvent viscosity.

Table 4.4 Comparison of three solvents for a SMB separation (Licosep 8 × 50)

Solvent	k'	Selectivity	Viscosity (cPo)	Flow rate (mL/min)	Productivity (g (en)/kg/day)
Ethanol	0.36	2.26	1.19	127	1.02
Ethanol/methanol (50:50)	0.41	2.03	0.86	180	1.84
Methanol	0.46	1.95	0.55	280	2.87

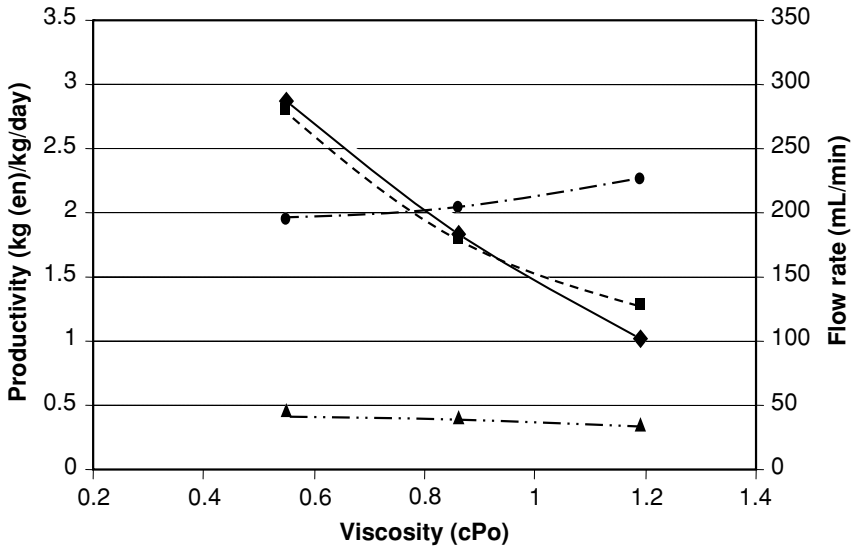

Figure 4.15 Variation of the experimental productivity by SMB (g (en)/kg/day) with the viscosity of the mobile phase. Solid line = productivity. Dashed line = solvent consumption. Dashed-dotted line = selectivity. Dashed-double dotted line = capacity factor for peak 1.

The properties of the typical solvents used on polysaccharide derivatives are summarised in Table 4.5: acetonitrile is very useful for its high sample solubility and its low viscosity, methanol would be preferred to ethanol owing to its low boiling point and viscosity, alkanes like *n*-hexane or heptane may be employed in those exceptional cases where the solubility is higher than 20 g/L because of their low viscosity.

4.4.5 *Chemical and enantiomeric stability*

Although not directly involved in the productivity value, the instability of the compound in the solvent may be a drastic shortcoming of a preparative separation. There are two possible types of compound instability: chemical and enantiomeric. Both may be encountered either during the chromatographic process or during product recovery. Both have to be investigated before going into production. The chemical stability in the solvent is the same for racemates

Table 4.5 Properties of the typical solvents for preparative chromatography on polysaccharide derivatives chiral phases

Solvent	Viscosity (CPo at T_{amb})	Sample solubility (g/L average range)	Boiling point (°C)
n-Hexane	0.30	0–10	68.7
n-Heptane	0.39	0–10	98.5
Acetonitrile	0.37	10–500	81.6
Methanol	0.55	10–300	64.6
Ethanol	1.07	10–200	78.3
2-Propanol	2.03	10–50	82.3

Note: Viscosity and boiling point from *Handbook of Chemistry and Physics*, 77th edn, 1996–1997.

and enantiomers, with the condition that both contain the same chemical impurities. It may be verified by heating the racemic solution at 40–50°C in the mobile phase at a concentration similar to the future preparative process. By analysing the sample before and after exposure to heat, we may quantify the loss in product and the presence of new impurities.

The enantiomeric stability (or the stability to racemisation) may be determined in the same way by using an enriched fraction or the pure enantiomer and following the enantiomeric excess by analysis to ensure it stays unchanged. When an enriched fraction is not available, it can be obtained by a small-scale preparative run on the analytical column used for the method development. A good analytical method should be developed in addition to allow the determination of eventual impurities and of enantiomeric excess.

In summary, the main parameters influencing the productivity of a preparative separation may be optimised as follows:

(a) optimise the selectivity to get the maximum loading capacity of the stationary phase by testing different CSP–solvent combinations
(b) minimise the retention times (or the capacity factors) and the separation time by testing different elution conditions
(c) optimise the solvent to provide the lowest viscosity and the highest sample solubility (20–40 g/L minimum).

4.5 Scale-up issues

A preparative enantioselective process may be run from milligrams to the production of kilograms and tons of enantiomers. Depending on the scale envisaged, one may adapt the complexity of the optimisation of the main parameters discussed in the previous sections.

In small-scale applications, up to several grams of enantiomers, the purpose is to obtain pure enantiomers for the first biological tests in a very short time and not necessarily to have the most productive separation. The screening and optimisation strategy presented in Section 4.3 is generally able to provide at least a separation that can be easily scaled up to columns up to 5 cm in diameter. In this case, optimisation of all parameters affecting the productivity may be too time-consuming.

4.5.1 *Laboratory-scale separations*

Because optimisation is not necessarily required for separations carried out at laboratory-scale and because usually the products from the resolution are required urgently for further reactions or for testing, the separations are often developed to touching band level in a standard analytical column. Scale-up is carried out by choosing a column of convenient dimensions, keeping the same linear velocity through the larger column as used in the smaller one and using a constant load per unit column volume. For columns of the same length, both load and flow rate are therefore increased by the ratio of the column cross-sectional areas. For instance, 2 mg of Tröger's base were injected onto a CHIRALPAK AD column (250 × 4.6 mm, 10 μm) in methanol at 1 mL/min with the following chromatographic parameters: $k'_1 = 0.95$, $k'_2 = 2.05$, $\alpha = 2.16$, resolution = 3.7 (Fig. 4.16a). For scale-up to a (250 × 20 mm, 10 μm) CHIRALPAK AD column, the load is multiplied by the ratio

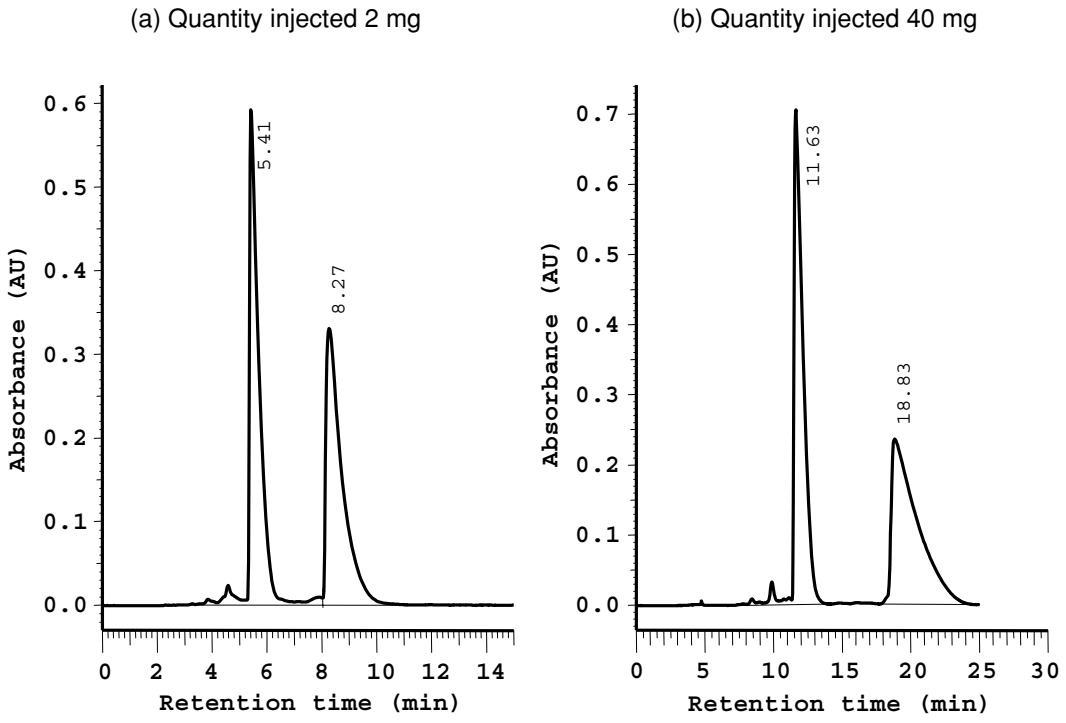

Figure 4.16 Load scale-up of Tröger's base in methanol from (a) CHIRALPAK AD (250 × 4.6 mm, 10 μm) at 1 mL/min to (b) CHIRALPAK AD (250 × 20 mm, 10 μm) at 9 mL/min (detection at 290 nm). Sample solution 20 g/L in methanol.

of the cross-sectional areas, i.e. $(20)^2/(4.6)^2 = 18.9$, which gives 37.8 mg. The flow rate in the semi-preparative column (250 × 20 mm, 10 μm) corresponds to 18.9 times that in the analytical column, in this case 18.9 mL/min. Normally, this would be the flow rate used, but in this case the separation had to be performed using an analytical HPLC and so the separation was performed using 9 mL/min (close to the maximum flow rate for a typical analytical HPLC unit). Thus, the retention times are doubled but the k' values remain constant. Consequently, the loaded chromatogram at 9 mL/min of 40 mg of product gives very similar chromatographic parameters to those found in the small column (Fig. 4.16b): $k'_1 = 0.94$, $k'_2 = 2.17$, $\alpha = 2.3$, resolution 3.2.

If the column length is changed, then the load is also varied according to the ratio of the column lengths; if the length is doubled, then so too is the load. This is because the column saturation capacity is a function of the quantity of CSP in the column, and as has been shown earlier, the overloaded behaviour of the sample is directly governed by the loading factor – the ratio of load to column saturation capacity.

4.5.1.1 *Case study: 1-naphthyl-1-(2-pyridyl)-methanol* A small quantity of the enantiomers of 1-naphthyl-1-(2-pyridyl)-methanol was required for a synthesis of a potential chiral ligand [13]. The standard screening procedure resulted in a series of possible separations as shown in Table 4.6. The best was obtained using a column packed with 20 μm CHIRALCEL OD and 15% 2-propanol in heptane. This chromatogram is shown in Fig. 4.17. Because only 1 g of racemate had to be separated, there was no need to optimise

Table 4.6 Selectivity values from standard screening for 1-naphthyl-(2-pyridino)-methanol

	Acetonitrile	50:50 EtOH/MeOH	15% IPA/heptane	15% EtOH/heptane
CHIRALPAK AD	1	1	1.43	1
CHIRALPAK L1[1]	1	1	1.33	1
CHIRALPAK AS	1	1	1.15	1
CHIRALPAK L2[1]	1	1	1.14	1
CHIRALCEL OD	1	1	1.51	1.29
CHIRALCEL OG	–	–	1.17	1.14
CHIRALCEL OJ	1	1	1.28	1.16
CHIRALCEL OK	1	1	1	1

[1] These phases are developmental Library CSPs, not commercially available, but used for scale-up of production-scale projects.

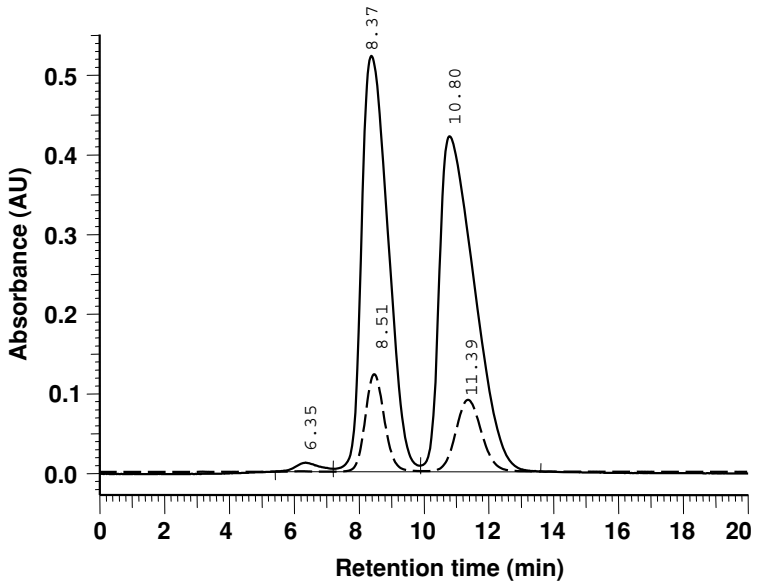

Figure 4.17 Load scale-up of 1-naphthyl-(2-pyridino)-methanol on a CHIRALCEL OD (250 × 4.6 mm, 20 μm) column. Dashed line = analytical load, solid line = 3 mg. Flow rate 1 mL/min, ambient temperature.

this separation beyond the simple conditions found in the primary screening. Despite the rather low solubility of 8 g/L, a loading experiment showed that a baseline separation could be obtained on the analytical column with 3 mg load, which would correspond to around 350 mg in a column 5 cm in diameter. Thus, the separation could be achieved within three injections at this scale. As was expected, the preparative separation followed the small-scale separation exactly (see Fig. 4.18) and the material was separated within 15 min under the conditions shown in Table 4.6.

4.5.2 *Large-scale separations*

The same scale-up principle may be applied to larger scale preparative chromatography, by taking into account the technical requirements connected to the large-scale equipment. For large production, the value of the productivity has to be as high as possible and the

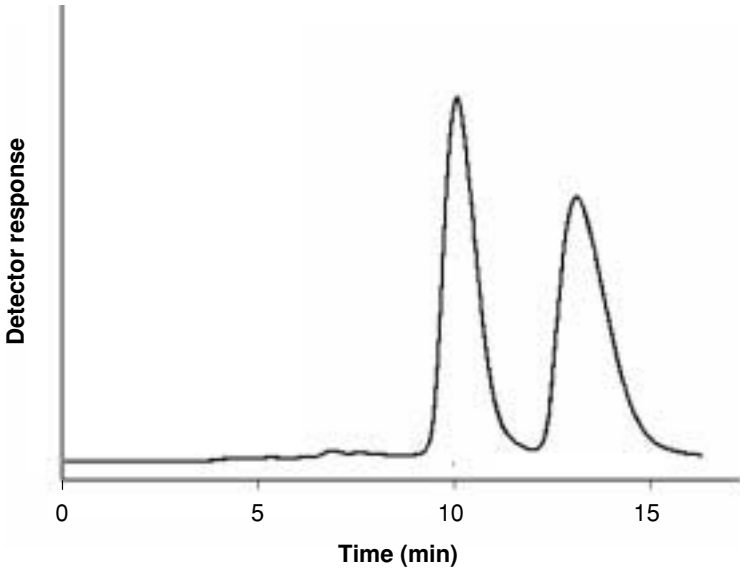

Figure 4.18 Preparative chromatogram of 333 mg 1-naphthyl-(2-pyridino)-methanol on a CHIRALCEL OD (250 × 50 mm, 20 μm) column. Flow rate = 100 mL/min.

optimisation should be carried out following all the considerations mentioned in Section 4.4. Not only has the CSP to generate a high loading capacity, but it also has to possess a high chemical and mechanical stability to allow long production campaigns, a high batch-to-batch reproducibility and it has to be available in large-scale produced under cGMP requirements. The solvent has to ensure not only the maximum solubility of the sample and the minimum viscosity to reach high flow rates but also a high volatility to facilitate the product recovery and ensure a low cost. The equipment has to be available at the right size and ideally all techniques, i.e. batch HPLC, SMB and SFC, should be evaluated to choose that most appropriate for the application.

If we take as example the SMB technique, the optimisation of all parameters discussed in Section 4.4 may lead to several chromatographic conditions that can only be evaluated for the productivity value either by simulation or by running the experiment. To illustrate the complexity of the optimisation involved in the estimation of the productivity by SMB, we present here three case studies.

4.5.2.1 *Case study 1* Compound 9 was screened following the method development treated in Section 4.2 and optimised following the recommendations from Section 4.3. In Table 4.7, the best results in terms of selectivity are shown. The first two entries report the results obtained after screening: the chosen CSP was CHIRALPAK AD, and two solvents, acetonitrile and 50% ethanol/methanol, were identified. Despite the good α values (3.3 and 4.4 respectively), the retention factors were very large and the optimisation involved trying to decrease them by using pure alcohols and acetonitrile/alcohol mixtures while maintaining or increasing if possible the α value. Pure ethanol gave smaller capacity factors ($k'_1 =$ 1.2) but with a lower α value (2.5), whereas a mixture of acetonitrile/ethanol (80:20) gave $k'_1 = 1.03$ with the highest α value (5.4). The highest solubility was obtained in acetonitrile and acetonitrile/ethanol (80:20): approximately 100 g/L. The viscosity decreases in

Table 4.7 Optimisation of compound 9 on CHIRALPAK AD (250 × 4.6 mm, 20 μm) (sample injected in the mobile phase 0.01 mg)

Solvent	Flow rate (mL/min)	Temperature (°C)	Rt_1	Rt_2	k'_1	α
Acetonitrile	2	40	4.35	11.01	1.90	3.3
Ethanol/methanol (50:50)	1	25	10.50	36.20	2.50	4.4
Ethanol	1	30	6.61	12.32	1.20	2.6
Methanol	1	30	18.61	46.24	5.20	2.7
Acetonitrile/2-propanol (95:5)	2	40	4.60	19.16	2.06	5.7
Acetonitrile/ethanol (95:5)	2	40	3.90	9.20	1.60	3.2
Acetonitrile/ethanol (90:10)	2	40	3.50	8.80	1.33	3.6
Acetonitrile/ethanol (80:20)	2	40	3.04	9.80	1.03	5.4

the following order: ethanol (1.07 cPo), acetonitrile/ethanol (80:20) (0.51 cPo), acetonitrile (0.37 cPo). Acetonitrile could be the best choice because of its low viscosity and high solubility, ethanol could be interesting owing to the shortest capacity factors, and the mixture of acetonitrile/ethanol (80:20) could possibly be the best choice because of the high α value with small k'. Loading studies were performed in all these three solvents and the following loading capacities were obtained: 8 g/kg in acetonitrile (20 mg load at touching band), 4 g/kg in ethanol (10 mg load at touching band) and 10 g/kg in acetonitrile/ethanol (80:20) (25 mg load at touching band). To decide which is the best compromise with these three solvents, simulations were run with [12] for 8 × 50 SMB and the highest productivity (680 g (en)/kg/day) was obtained for acetonitrile/ethanol (80:20), followed by pure acetonitrile (650 g (en)/kg/day) and pure ethanol (570 g (en)/kg/day). In this case, we may suppose that the productivity was mainly influenced by the loading capacity, which was the best in acetonitrile/ethanol (80:20). Even so, in practice, the best choice might be pure acetonitrile to ease the solvent recovery (pure solvent and high volatility).

4.5.2.2 *Case study 2* The separation of Compound 10 was optimised on CHIRALCEL OD in different solvents. The comparison between the simulated productivity [12] obtained with different parameters (selectivity, loadability determined from the loading study, solubility and viscosity) is reported in Table 4.8. By analysing the values of the parameters obtained in different solvents, we may observe that the best selectivity was obtained in ethanol (3.3) compared to the other four eluents in which the selectivity is about 2; the smallest capacity factor is obtained in ethanol (0.25) and the largest in heptane/ethanol

Table 4.8 Simulated productivity obtained with different chromatographic parameters in the optimisation of Compound 10 on CHIRALCEL OD (retention factor, selectivity and loading capacity at touching band obtained on (250 × 4.6 mm, 20 μm) column at 1 mL/min and 30°C)

Solvent	k'_1	α	Loading capacity (g/kg)	Solubility (g/L)	Viscosity (cPo)	Productivity (g (en)/kg/day)
Acetonitrile	0.61	1.86	2.0	400	0.37	1072
Methanol	0.46	2.01	1.8	400	0.55	614
Heptane/ethanol (90:10)	1.02	2.00	4.0	200	0.46	1097
Heptane/2-propanol (85:15)	0.96	2.21	4.8	200	0.64	980
Ethanol	0.25	3.32	2.4	200	1.07	860

and heptane/2-propanol solvents; the solubility is very high in all solvents; the loading capacity is limited in all solvents (values between 1.8 and 4.8 g/kg) with the highest in heptane/2-propanol; the viscosity is the lowest in acetonitrile and heptane/ethanol. In view of these considerations and before running the simulation, we may hypothesise that four of the five solvents, i.e. ethanol, acetonitrile, heptane/ethanol (90:10) and heptane/2-propanol (85:15) may give interesting productivities. The simulation confirms this hypothesis, since the highest productivity (around 1080 g (en)/kg/day) is obtained in acetonitrile or the heptane/ethanol mixture, followed by heptane/2-propanol and ethanol. In this case, we may suppose that the productivity was controlled mainly by the viscosity that was the lowest in acetonitrile and heptane/ethanol mixture.

Running the loading studies and the simulations for many elution conditions is certainly less time-consuming than running the experiment. But to what degree may we be confident with the simulation? Tröger's base was chosen as model compound to be run on a Licosep 8 × 50 SMB [14] to compare the experimental results with the simulation data.

4.5.2.3 *Case study 3* The optimisation results for Tröger's base on two columns and two solvents are presented in Table 4.9. The simulation results using the loading data under these two sets of conditions are reported in Table 4.10, and the experimental results obtained by running the separation on the 8 × 50 SMB are presented in Table 4.11.

By comparing these two series of simulated and experimental data, we may observe that the operating parameters are very close to each other resulting in similar simulated and experimental productivity. Both conditions, CHIRALPAK AD/acetonitrile and CHIRALPAK AS/hexane-2-propanol, lead to very similar values of 753 and 797 g (en)/kg/day, respectively. For the production of large quantities, we may decide to choose use of the single solvent mobile phase to ease the compound and solvent recovery. In this case, the simulation fitted well the experiment. The confidence on simulated data is given by the match of data using the simulation software and the experimental batch data. For instance, by using [12], we may represent a graph plotting the simulated and the experimental retention times

Table 4.9 Optimisation data for Tröger's base; column (250 × 4.6 mm, 20 μm), 1 mL/min, 30°C

Column	Solvent	k'_1	Selectivity	Viscosity (cPo)	Solubility (g/L)
CHIRALPAK AS-V	Hexane/2-PrOH (90:10)	0.39	2.20	0.47	30
CHIRALPAK AD	Acetonitrile	0.40	1.85	0.37	45

Table 4.10 Simulation data for Tröger's base (extract and raffinate purity >99.5% ee)

	Feed		Extract		Raffinate			
Column and solvent	Flow (mL/min)	Concn (g/L)	Flow (mL/min)	Concn (g/L)	Flow (mL/min)	Concn (g/L)	Cycle time (min)	Productivity (g (en)/kg/day)
CHIRALPAK AS-V and hexane/2-propanol	30	30	91	5.06	38	12.09	0.97	825
CHIRALPAK AD and acetonitrile	22	40	76	5.80	25	17.60	0.73	795

Table 4.11 Experimental data for Tröger's base (extract and raffinate purity >99.5% ee)

	Feed		Extract		Raffinate			
Column and solvent	Flow (mL/min)	Concn (g/L)	Flow (mL/min)	Concn (g/L)	Flow (mL/min)	Concn (g/L)	Cycle time (min)	Productivity (g (en)/kg/day)
CHIRALPAK AS-V and hexane/ 2-propanol	28	30.0	100	4.17	41	10.02	1.02	753
CHIRALPAK AD and acetonitrile	21	41.2	97	5.03	39	11.33	0.74	797

against the load. In Fig. 4.19, the retention times against the load for AS/hexane-2-propanol conditions are represented. A good fitting between the simulated curves (Sim Rt_1 and Sim Rt_2) with the experimental points (Rt_1 and Rt_2) is obtained. The same situation was observed for AD/acetonitrile data series.

In cases where this fitting is good, there is no need to run the experiment, while in those cases where the simulation data are a poor match for the batch loading study, it is expected that the SMB simulation will not be a good predictor for the experimental conditions. In the latter cases, only the experiment may give a realistic evaluation of the process productivity.

4.5.2.4 *Case study 4* To exemplify such a case, we discuss below the separation of an industrial compound (Compound 11) (CTE internal data and A. Depta, Bayer Chemicals, unpublished data, 2004) which was obtained on CHIRALPAK AD using acetonitrile as solvent with $k'_1 = 0.72$ and a selectivity of 3. The solubility of the compound in the mobile phase is high (>100 g/L). The simulation was run [12] with the loading data obtained

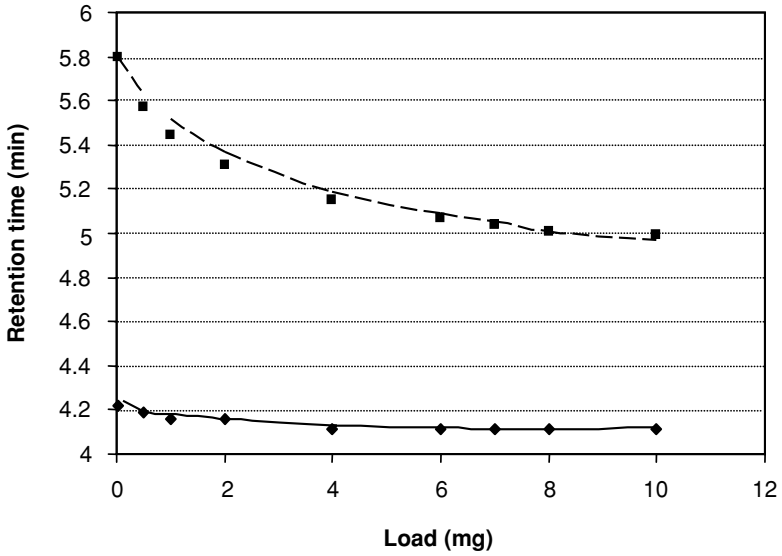

Figure 4.19 Simulated (curves) and experimental (points) variation of retention times with load for Tröger's base on CHIRALPAK AS in hexane-2-propanol. *Key*: solid line: simulation, peak 1 (Sim Rt_1), dashed line: simulation, peak 2 (Sim Rt_2); diamonds: experimental, peak 1 (Rt_1); squares: experimental, peak 2 (Rt_2).

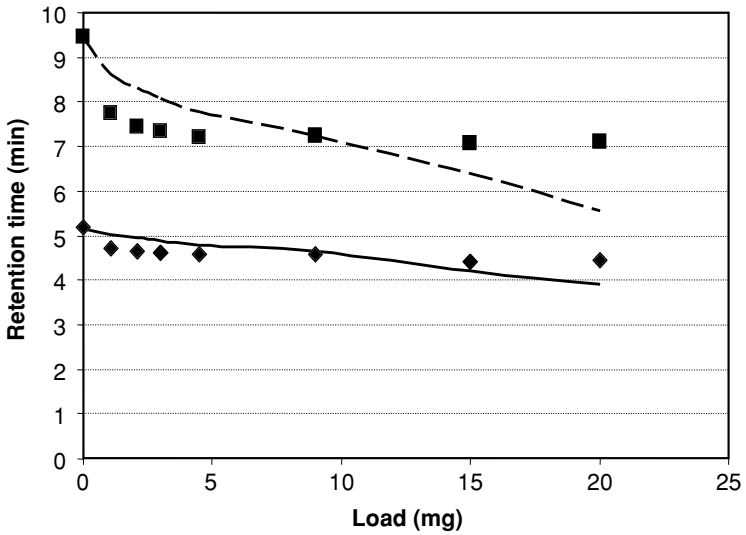

Figure 4.20 Simulated (the curves) and experimental (the points) variation of retention times with load for Compound 11 on CHIRALPAK AD in acetonitrile. *Key*: solid line: simulation, peak 1 (Sim Rt_1), dashed line: simulation, peak 2 (Sim Rt_2); diamonds: experimental, peak 1 (Rt_1); squares: experimental, peak 2 (Rt_2).

on a (250 × 4.6 mm, 20 μm) column. Figure 4.20 shows a comparison of the retention times calculated from the simulations (Sim Rt_1 and Sim Rt_2) with the experimental data (Rt_1 and Rt_2). The fitting of these data for the second eluted peak is poor and one cannot expect that the simulation of the process will give good predictions. Table 4.12 presents the experimental results from the SMB separation of the enantiomers of Compound 11 using a laboratory-scale (8 × 50) unit for comparison with the results of the computer simulations [12]. There are significant differences between the simulated and experimental raffinate flow and the productivity. In the case of the productivity, the experimental value was 22% greater than that predicted by simulation. It is clear that when the fit of the isotherm parameters is not good, the simulation result is suspect and only experimental data should be relied on.

4.6 Conclusion

Preparative enantioselective chromatography is a very useful tool to obtain pure enantiomers at small and large scales. Method development for this type of chromatography aims to optimise the separation conditions such that the chiral stationary phase together with solvent

Table 4.12 Comparison of experimental and calculated operating conditions for Compound 11 (extract and raffinate purity >99.5% ee)

	Feed		Extract		Raffinate			
CHIRALPAK AD and acetonitrile	Flow (mL/min)	Concn (g/L)	Flow (mL/min)	Concn (g/L)	Flow (mL/min)	Concn (g/L)	Cycle time (min)	Productivity (g (en)/kg/day)
Simulated	28	94	255	5.2	34	39.2	0.94	2300
Experimental	17	180	259	5.9	56	27.0	0.73	2800

and equipment give the appropriate process productivity for a given enantiomeric target. Polysaccharide derivatives are used here as examples of CSPs because they are the most widely used world over, although the general principles for optimisation may be applied to any other support. Polysaccharide derivatives also generate the most complex interactions with the solutes, and the general resolution rules presented here may facilitate their use by chromatographers at all levels of experience. The optimisation strategy presented in this chapter illustrates the main parameters influencing the chromatographic process and how to optimise them experimentally by the means of the best chiral support–solvent combination.

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Preparative Enantioselective Chromatography
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5 Scaling-up of preparative chromatographic enantiomer separations

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5.1 Introduction

Over the last few years, preparative chromatographic separation of racemic compounds has progressively become the method of choice to rapidly prepare pure enantiomers. This has come about through improved understanding of the separation mechanisms, together with the commercial availability of larger amounts of universally applicable chiral stationary phases (CSPs). Furthermore, the introduction of continuous processes in this particular field of application has opened the gateway to use chromatographic techniques as a tool for real production scale enantiomer separations.

Because of the ease of the approach and execution of chromatographic enantiomer separations, many pharmaceutical companies nowadays consider this technology as the first approach for their process development work before exploring the possibilities of stereospecific synthesis methods. Chromatography certainly appears to be a good strategy when compared with chemical processes since its scale-up is straightforward if some simple basic rules are taken into account. Especially when the unwanted enantiomer can be easily racemised, it is absolutely necessary at the beginning of a project to make an economic comparison between the expected cost of developing a stereospecific synthesis method and the optimisation and scaling-up of a batch or continuous chromatography process to determine the best process for supply of larger amounts of a promising new product. Unfortunately, even to the present date, many scientists involved in discovery research and process development do not accept preparative chromatography as a tool to produce economically metric ton quantities of a product. Often, when a chromatographic method has been used to prepare the first amounts of a new promising product, one of the items that certainly will come up during meetings about the development strategy is how to eliminate chromatography from the process. Furthermore, in many cases, it needs even stronger persuasive powers to convince production engineers that preparative chromatographic techniques are sufficiently developed that they can be considered as standard unit operations like distillation and crystallisation and certainly do not have to be seen any longer as some kind of black magic. It is quite clear that the history of the company and the overall experience with chromatographic techniques of the people involved will determine to a large extent whether chromatographic techniques are acceptable as a standard methodology for production purposes or not.

The goal of this contribution is to give an insight into the approach we are using to fulfil the requirements of discovery research, pilot chemistry and chemical development. Each department has its own specific demands and requirements. Discovery research, for example, generally needs separation of the final product into both enantiomers with high enantiomeric excess in order to be able to generate relevant pharmacological data. Preparative chromatographers know well that it is a lot easier to isolate the first eluting product with high purity, but that isolation of the second eluting component is far more difficult.

When the biological activity of a certain substance has been established and several hundred gram or kilogram quantities of product are required, it is usually not straightforward to perform the enantiomer separation on the final product as has been done for discovery research. The reason may be found in the fact that the production capacity of a chromatography process is strongly influenced by the sample solubility. The knowledge that intermediates are often more soluble than the final product itself makes it necessary that the separation and solubility characteristics of several chiral intermediates in the synthetic route are investigated. However, before starting these experiments, a few important questions have first to be addressed:

- Will the product racemise in one of the following reaction steps?
- Is it better to separate a larger amount of an intermediate rather than a smaller amount of the final product?

Once these questions have been answered, analytical screening experiments on different types of stationary phases of the selected intermediate(s) are started and the solubility of the product(s) determined in the solvents that are commonly used on the available CSPs. When good separation conditions have been found, a small quantity of intermediate is separated in order to give the synthetic chemist both enantiomers for further synthetic work in order to determine which of the two enantiomers of the intermediate results in the desired final product. If the first eluting peak leads to the desired enantiomer, no further work has to be done. Otherwise, it is worthwhile to investigate other stationary phases or experimental conditions in order to reverse the elution order.

If larger quantities have to be separated, combining a chromatographic separation process with crystallisation often allows one to isolate the desired enantiomer via chromatography with a lower enantiomeric excess and to use a subsequent crystallisation step to obtain a product that fulfils the optical purity requirements. It is therefore beneficial to determine a melting point diagram by means of differential scanning calorimetry. The knowledge of the location of the eutectic point can determine if this approach will be successful and what purity of the enantiomer will be required from the chromatography.

When a product moves into full development, the overall economy of the process together with cGMP issues come into the picture, and therefore an in-depth investigation of all process parameters (e.g. the daily throughput per kilogram of stationary phase, the possibility to racemise the unwanted enantiomer, recovery of solvent, the validation of process parameters and the robustness of the process) is carried out.

5.2 Analytical screening models

5.2.1 *Standard procedure*

Prior to preparative work, suitable chromatographic conditions have to be established by means of analytical experiments. With current fully automated chromatography systems, it is very easy to pre-programme instruments to perform a series of standard experiments on different columns. In order to simplify the transfer from analytical experiments to preparative chromatography work, columns of 250 mm $\times$ 4.6 mm are filled with bulk (20 μm) packing material. Furthermore, technical grade solvents filtered through a 1 μm filter are used. This

work is typically carried out in our laboratories using equipment from Waters Associates. The columns are installed in a column oven/column switching module (Spark-Mistral) and kept at a constant temperature of 25°C.

In our laboratories, the first set of experiments is always performed on the four well-known physically coated cellulose and amylose CSPs from Daicel (Chiralpak AD, Chiralcel OD, Chiralcel OJ and Chiralpak AS). In conjunction with these phases, we use five different solvent combinations (see Table 5.1) in the basic screening model. The chromatographic profiles obtained with this set of solvents in general allow the choice of a suitable stationary phase for further investigation. This sequence of the solvents (see Table 5.1) has specifically been chosen in order to avoid damage to the columns. The whole solvent set is used sequentially on each column before being repeated on the next. The process is continued until the four columns have been examined.

Table 5.1 Solvents used in the analytical screening model

(1) Methanol/ethanol (50:50, v/v)
(2) Ethanol/acetonitrile (95:5, v/v)
(3) Acetonitrile/ethanol (80:20, v/v)
(4) Ethanol
(5) *n*-Heptane/ethanol (70:30, v/v)
(6) Ethanol (column storage)

Columns are conditioned with about 4.5 times the column dead volume using a flow rate of 1.5 mL/min. After this conditioning step, the product is analysed using a flow rate of 1.2 mL/min and a total run time of 20 min. The sequence of conditioning and analysis is repeated until all solvent combinations have been tested on each stationary phase. The last step in the screening process is a conditioning period using pure ethanol as the eluent. This process assures a safe storage and enhanced lifetime of the columns.

In general, unless on the basis of historical data the necessity to use a basic or acidic mobile phase is expected, the set of experiments is always done under neutral elution conditions on a set of columns reserved for such solvents. Two additional sets of columns are available to be used respectively under basic or acidic conditions, depending on the structure of the molecules to be separated. When basic or acidic conditions have been chosen, respectively 0.1% of triethylamine (TEA) or 0.1% of trifluoroacetic acid (TFA) is added to the screening solvents prior to the start of the experiment.

For one product the whole screening process on the four selected columns takes about 11 h. Thus, it is possible only to screen on one instrument two different products in 24 h. Therefore, unless there is no sample backlog, this extensive procedure is used only when amounts above 50–100 g have to be separated. In many cases the screening process provides a stationary–mobile phase combination that can be used for the preparative chromatographic work. If fine-tuning of the operational conditions is required to improve the separation, all further experiments are done manually.

A graphical illustration of the final result of a complete screening experiment is given in Fig. 5.1. For this particular example, it is not very difficult to decide which type of stationary phase has to be chosen to select the conditions for a preparative chromatographic separation. On each type of column, resolution between the two enantiomers can be observed. This

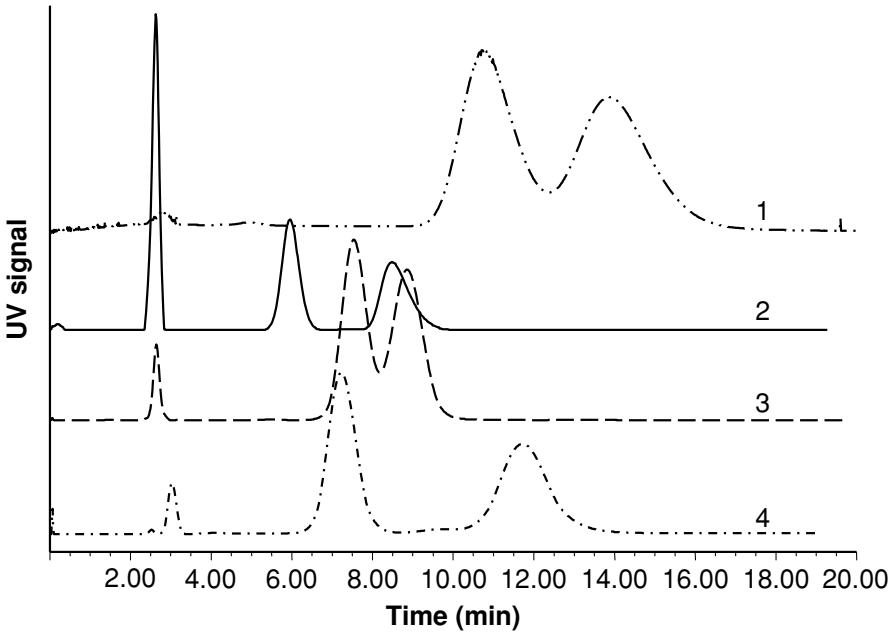

Figure 5.1 Results of screening experiments. (1) Chiralpak AD (20 μm): 250 mm × 4.6 mm. Mobile phase: 50% methanol in ethanol; (2) Chiralcel OD (20 μm): 250 mm × 4.6 mm. Mobile phase: 30% ethanol in *n*-heptane; (3) Chiralpak AS (20 μm): 250 mm × 4.6 mm. Mobile phase: 30% ethanol in *n*-heptane; (4) Chiralcel OJ (20 μm): 250 mm × 4.6 mm. Mobile phase: 5% acetonitrile in ethanol. Flow rate: 1.2 mL/min. Detection: UV photodiode array.

certainly has to be considered as a luxurious situation. More difficult problems are usually to be expected where only partial separation can be observed on the columns tested.

The correct interpretation of the screening experiments is important. Even experienced laboratory technicians sometimes get trapped when they examine the information of an analytical screening experiment. Very often insufficient attention is given to the value of the optical density (especially using the auto-scaled plots of most modern HPLC instruments) of the chromatograms and mistakes are made owing to the presence of small racemic impurities in the sample; the major components do not elute and a method is developed for the separation of the impurities.

5.2.2 *Fast analytical screening process*

As noted above, the fully automated general screening model is slow and therefore alternative methods have to be explored when a large number of small-scale chiral samples have to be separated within a certain time window. It is then necessary to limit the number of analytical experiments used to establish suitable conditions for preparative work. Sometimes some analytical work has already been performed by the synthetic chemists to test their products and these data are often sufficient for the small-scale preparative separation process. However, if these data are not available, another approach is required.

Through experience we have learned that 50 × 4.6 mm guard columns filled with 10 μm cellulose- and amylose-based CSPs are very suitable for this purpose. These have small dead

volume and so equilibration and analysis times are short; run times of 5 min are generally more than sufficient. Experiments on these columns are always done manually and at first only Chiralpak AD and Chiralcel OD are tested using pure ethanol as the mobile phase. This solvent immediately gives an indication about the further strategy that has to be followed. Depending on the chromatographic profile observed, other experimental conditions are chosen:

- Little or no retention: the eluent strength is reduced by means of the addition of *n*-heptane.
- Retention but insufficient separation: ethanol is replaced by methanol; mixtures of methanol and ethanol; acetonitrile or mixtures of acetonitrile with ethanol or methanol.
- If a separation is observed with *n*-heptane/ethanol mixtures, the effect of 2-propanol is also investigated.

An example of this type of analytical approach is illustrated in Fig. 5.2. Using pure ethanol as the eluent, the product eluted at the dead volume of the column and it was clear that some *n*-heptane had to be added to adjust the elution power of the solvent. The next experiment was done with a mixture of 20% ethanol in *n*-heptane. Although a separation was observed that could have been used for small-scale preparative work, the effect of changing the type of polar modifier was studied. Ethanol was replaced by the same concentration of 2-propanol, but the separation deteriorated. When the ethanol was replaced by a 50% mixture of methanol and ethanol, the resolution did not improve but the products eluted

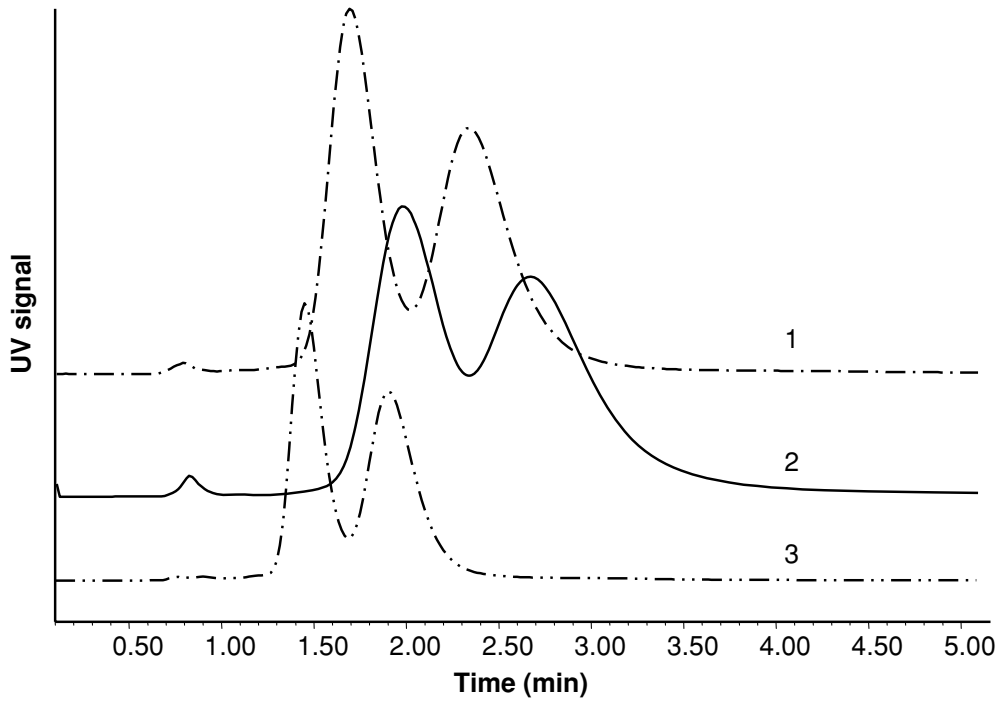

Figure 5.2 Rapid screening using short columns. Chiralpak AD (10 μm): 50 mm × 4.6 mm. Mobile phases: (1) 20% ethanol in *n*-heptane, (2) 20% 2-propanol in *n*-heptane, and (3) 10% ethanol and 10% methanol in *n*-heptane. Flow rate: 1.0 mL/min. Detection: UV photodiode array.

faster. This mobile phase composition was therefore used to perform the preparative chromatography. In total, this analytical screening experiment took only about 40 min.

To the present day, about 85–90% of all our chiral separation problems have been solved (the majority easily, a few others with some difficulty) on our four 'standard' Daicel-type phases. If no satisfactory results are obtained on one of the four standard phases, some additional experiments are done on a few other types of phases:

- Some other types of Daicel phases (Chiralcel OB, Chiralcel OC, etc.)
- Poly-acryloyl amide (Chiraspher; Merck)
- Tartardiamide phases (Kromasil; Akzo Nobel)
- Cation-exchange phases based on quinine and quinidine derivatives
- Chemically bonded macrocyclic glycopeptide phases (Vancomicin or Teicoplanin; Astec)

5.3 Scaling-up from milligram to kilogram quantities

5.3.1 *Introduction*

For pilot laboratory scale (a few hundred grams) and smaller scale development work (a few kilograms) in general, preparative chromatographic separations have to be done within very strict timelines. The major task for this type of work is to produce the desired products within the requested time window to assure that further experimental work is not delayed. Therefore, it is usually not possible fully to explore the many chromatographic parameters that have an influence on the throughput. This is one of the reasons that our analytical screening model is based on the use of technical grade solvents in combination with columns filled with the 20 μm bulk material. A suitable separation observed in the screening experiments mostly guarantees success on the preparative columns filled with the same material. Instead of optimising the amount of product that can be injected on an analytical column, loading experiments are always directly performed on the preparative column. At first, an adequate amount of product is dissolved in the chosen eluent. Thereafter, 10 mL of sample solution is injected by means of a loop. The resultant chromatogram not only allows a check on the packed-bed quality but also directly indicates whether the eluent composition results in an acceptable chromatographic profile on the preparative column. The first example to illustrate our scaling-up strategy for this type of application is the separation of a xanthone derivative.

5.3.2 *Separation of a xanthone derivative*

For medicinal chemistry, at first a few small-scale batches of the free base were chromatographed but the recovery yield after chromatography was disappointing. Therefore, in consultation with the medicinal chemists involved, it was decided to prepare the *N*-BOC derivative of the secondary amino function that was present in the molecule. At first a small-scale experiment had to be performed to access both enantiomers of the *N*-BOC protected product for further synthesis work. Compared to the unprotected derivative, the preparative chromatographic separation of the *N*-BOC component using a Chiralpak AD column was

easier and the recovery yields were excellent. Furthermore, conditions to de-protect the purified enantiomers without racemisation were found.

For the larger amount of product originating from the pilot chemistry laboratory, it was decided to execute a general analytical screening to evaluate the behaviour of the larger scale sample on our standard CSPs. As illustrated in Fig. 5.3, the combination of a Chiralpak AS column with 20% ethanol in acetonitrile as the mobile phase resulted in a good separation of the enantiomers. The product was fairly well soluble in the mobile phase and a solution containing 12 g of product per litre of eluent was prepared. The result from a small-scale injection is given in Fig. 5.4 and was comparable to the analytical one. Therefore, in the learning mode of the chromatography system a volume of 200 mL of sample solution (2.4 g) was injected. A better than baseline separation was obtained and so the injection volume was increased to 400 mL (4.8 g). Because the total amount of product that had to be separated was situated in the range between 100 and 500 g, the chromatogram obtained for an injection of about 5 g was certainly acceptable and no further loading or optimisation experiments were done.

The learning mode possibility of the software is a wonderful tool that allows rapid establishment of all parameters required to execute a fully automated process. When the learning mode is active, the operator can manually control the injection volume, the solvent composition, the flow rate, the wavelength of the UV detector, the temperature of the column and the eluent, an introduction of a plug of stronger solvent, recycle, the determination of the fractionation points [trigger type (valley, peak, slope, etc.), UV and time window] and

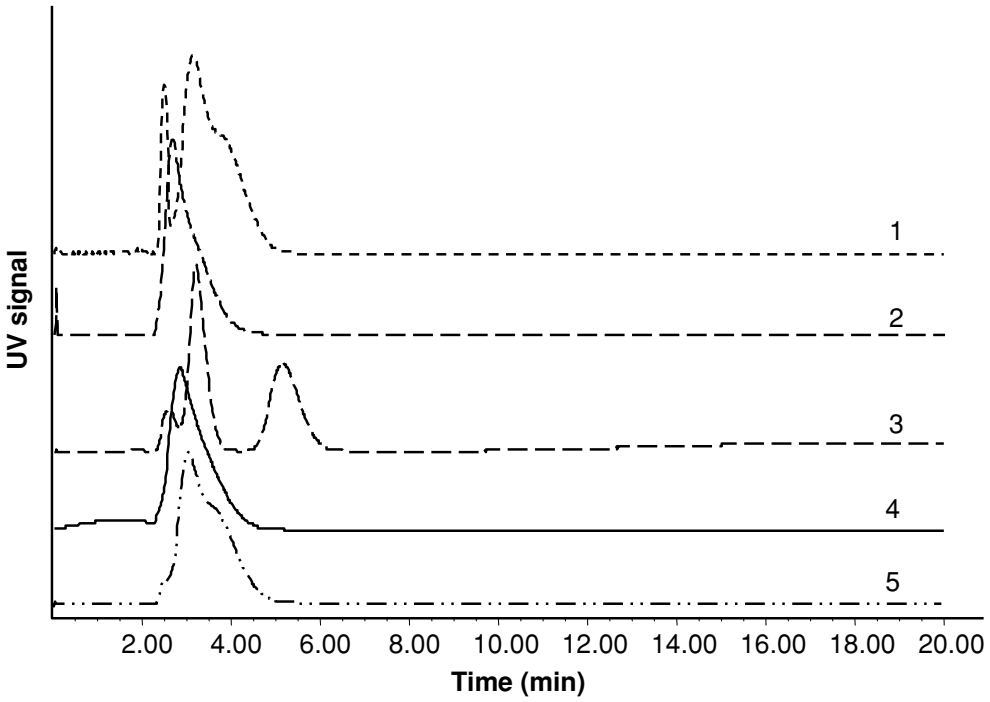

Figure 5.3 Analytical screening for a BOC xanthone. Chiralpak AS (20 μm): 250 mm × 4.6 mm. Mobile phases: (1) 50% methanol in ethanol, (2) 5% acetonitrile in ethanol, (3) 20% ethanol in acetonitrile, (4) ethanol, and (5) 30% ethanol in *n*-heptane. Flow rate: 1.2 mL/min. Detection: UV photodiode array.

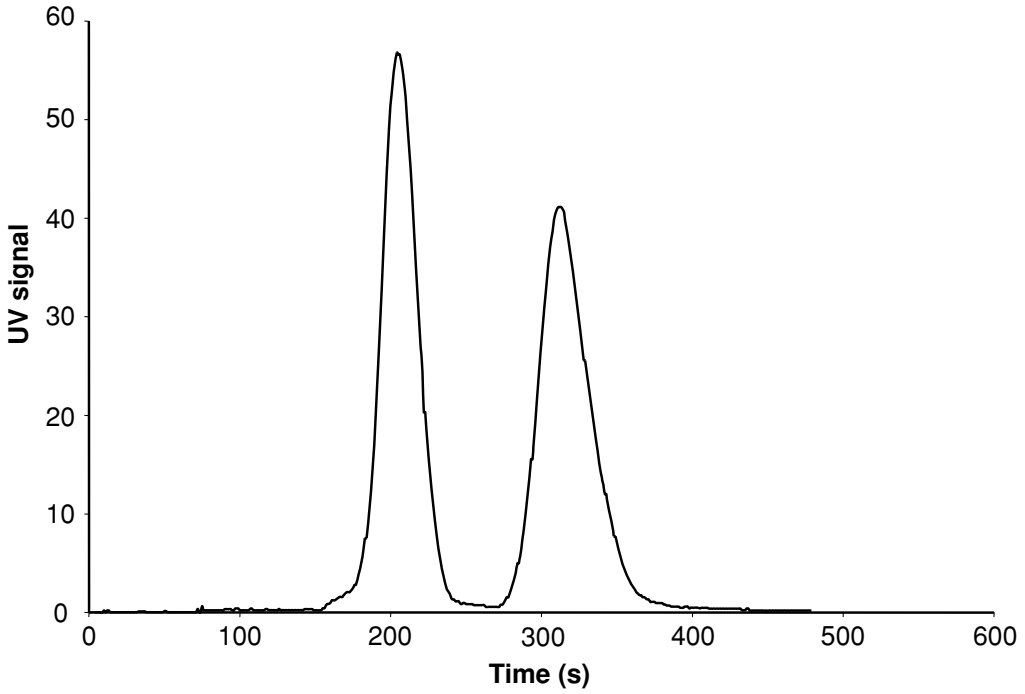

Figure 5.4 Small-scale injection of a BOC xanthone on an 11 cm i.d. column. Chiralpak AS 2000 g (20 μm) – dynamic axial compression: 110 mm i.d. Mobile phase: 20% ethanol in acetonitrile. Flow rate: 750 mL/min. Detection: UV 254 nm. Sample amount: 12 g/L in eluent. Injection volume: 10 mL (loop) = 120 mg of product = 0.06 mg/g of CSP. Eluent temperature: 35°C. Column temperature: 37°C.

the injection point for injection within a run for overlapping injection mode. Once all parameters have been fixed, the data are downloaded into the PLC controlling the unit and it is possible to start within learning mode a test run (executing a predetermined number of injections) to evaluate the fractionation points, the point of injection and of course the quality of the isolated fractions. If the process is successful, the learning mode is stopped and the total amount of material is chromatographed using the method thus established. [Note: This is a 'home-built' system with customised operating software; not all preparative liquid chromatography systems (unfortunately) have such a facility.] For the separation of the xanthone derivative a relatively simple separation table could be used, as shown in Table 5.2. A chromatogram of the chromatography process executed according to the instructions of Table 5.2 is depicted in Fig. 5.5. Only a few hours of continuous operation were required to obtain both enantiomers with a high optical purity (>99% ee) and a very good yield (above 93%).

5.3.3 *Separation of a 'dibenzocycloheptanol' derivative*

When the second component in a preparative chromatography process elutes as a relatively broad peak, a common practice in our laboratories is to use a plug of stronger eluent to compress this elution band in order to reduce the run time and the volume of solvent that has to be removed to recover the product. The general analytical screening model is an

Table 5.2 Separation conditions for the xanthone derivative separation

Action number	Time (s)	Time window		Action	Trigger Type	UV detection window		
		Before	After			Value	−	+
1	180	30	30	Fraction 1	+ Slope	30	0	40
2	90	30	30	Fraction 10	Valley	–	–	–
3	10	1	1	Fraction 2	Time	–	–	–
4	5	1	1	Sample	Time	–	–	–
5	125	30	30	Fraction 10	−Slope	10	10	0
6	5	1	1	End run	Time	–	–	–
Total time	415							

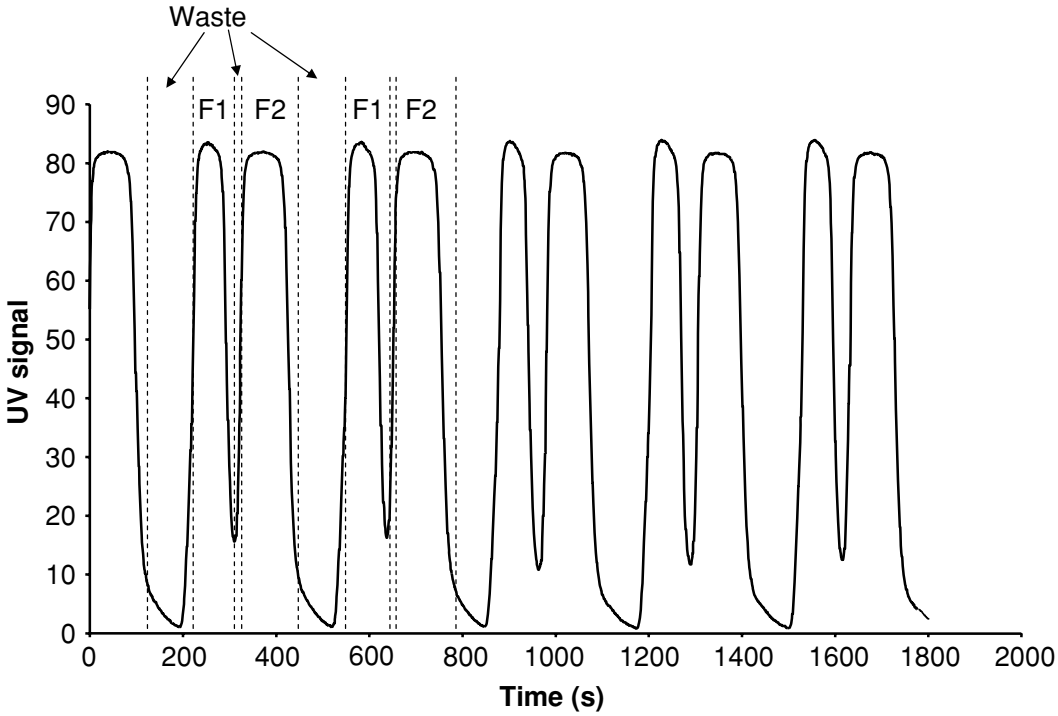

Figure 5.5 Preparative separation of a BOC xanthone. Conditions as Fig. 5.4, except – injection volume: 400 mL (=4.8 g = 2.4 mg/g of CSP); time between two injections: 327 s = 11 injections/h = 52.8 g/h = 1.267 kg/24 h; eluent temperature: 35°C; column temperature: 37°C.

excellent tool to determine which solvent or solvent combinations are suitable to perform this operation. In our equipment, solvent mixtures are made by means of switching valves installed in a mixing loop–heat exchanger combination. As can be expected, this principle results in a relatively large system volume and if the solvent switch were made via this mixing unit, the peak compression effect will not be observed because of the long delay time. Therefore, the stronger solvent is directly introduced on top of the column through a bypass, which also ensures that the solvent composition in the mixing loop is not disturbed, which allows very rapid return to the original eluent.

An example of this peak compression principle is illustrated for the enantiomer separation of a 'dibenzocycloheptanol' derivative. A test chromatogram of a small-scale injection is illustrated in Fig. 5.6. As this clearly indicates, the second peak is relatively broad compared to the first eluting enantiomer. From the analytical screening experiments it was known that with pure ethanol as the eluent, both enantiomers eluted quickly and so a plug of pure ethanol was introduced to speed up the elution of the second enantiomer. A chromatogram of the continuous process (1 h) with an indication of the different sample injections and the location of the compression plug are graphically illustrated in Fig. 5.7. The separation table that has been used to execute this process is given in Table 5.3.

In this particular example, the plug of stronger solvent has been introduced somewhat too early in the chromatogram. To take full benefit of the peak compression action, the point of introduction of the stronger eluent has to be chosen in such a way that the second eluting peak is swept completely from the column by the plug of stronger solvent. Therefore, in this example it would have been better to start the introduction of the pure ethanol (action number 2) about 60–70 s later in the chromatogram.

Although an interesting tool, this principle is not always applicable. It works only when one solvent in the eluent mixture has a strong influence on the retention time or if another solvent or solvent combination can be found which has a similar effect on the retention behaviour of the components. Specifically for enantiomer separations on the physically coated cellulose and amylose derivatives, this solvent or solvent mixture furthermore needs to be fully compatible with the stationary phase–eluent combination. A disadvantage of this

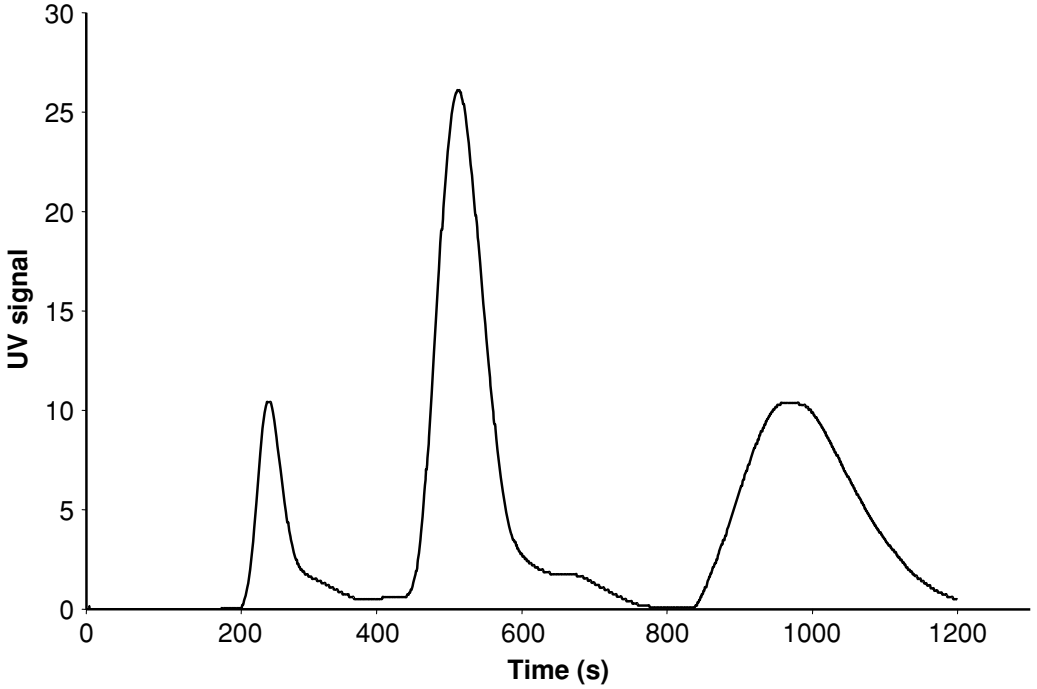

Figure 5.6 Test chromatogram of a dibenzocycloheptanol derivative. Chiralpak AD 6000 g (20 μm) – Dynamic axial compression: 200 mm i.d. Mobile phase: 20% ethanol in *n*-heptane. Flow rate: 2000 mL/min. Detection: UV 254 nm. Injection volume: 50 mL (loop) = 4.22 g of product (0.7 mg/g of CSP). Eluent temperature: 30°C. Column temperature: 33°C.

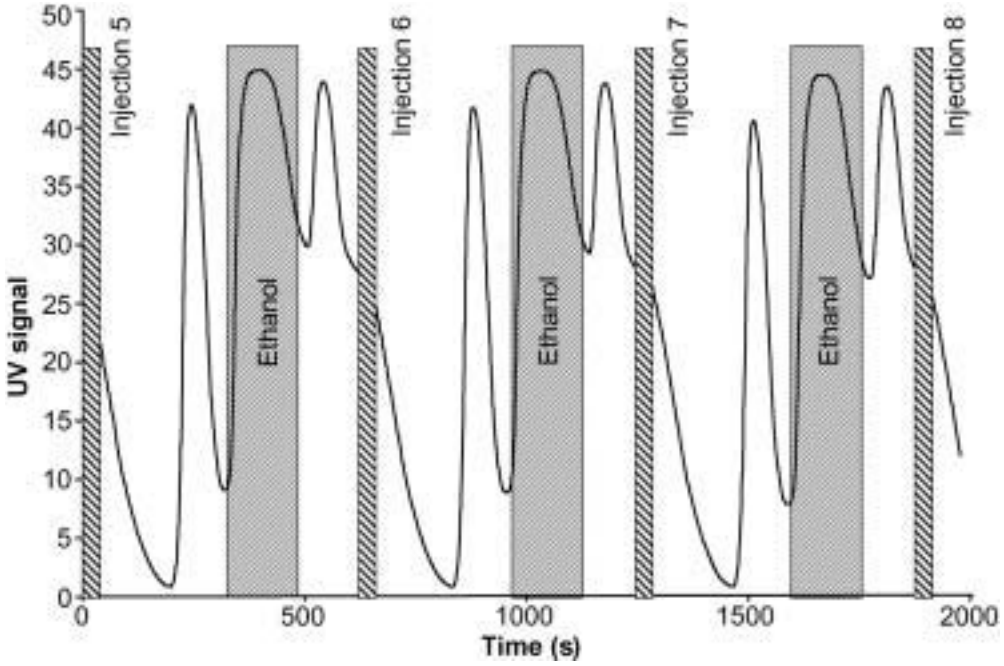

Figure 5.7 Preparative separation of a dibenzocycloheptanol derivative. Conditions as Fig. 5.6, except – injection volume: 800 mL (= 84.4 g = 14.07 mg/g of CSP); time between two injections: 632 s = 5.69 injections/h = 480.23 g/h = 11.526 kg/24 h. Solvent 1: 20% ethanol in *n*-heptane; Solvent 2: ethanol.

Table 5.3 Separation conditions for the dibenzocycloheptanol derivative separation[1]

Action number	Time (s)	Time window		Action	Trigger type	UV detection window		
		Before	After			Value	−	+
1	210	30	40	Fraction 10	+ Slope	25	5	5
2	110	30	40	Solvent 2	Valley	–	–	–
3	2	1	40	Fraction 1	+ Slope	25	5	5
4	120	20	50	Fraction 10	– Slope	31	3	1
5	2	1	1	Solvent 1	Time	–	–	–
6	150	1	1	Sample	Time	–	–	–
7	150	1	1	End run	Time	–	–	–
Total time	744							

[1] Description of the action numbers – 1: Detection of the impurity in front of the first eluting enantiomer by means of a positive slope recognition. 2: Start of the compression plug of pure ethanol based on the detection of a valley between the impurity and the first eluting enantiomer. Volume of the plug of ethanol: 4033 mL [(121 s) = 0.53 times the column dead volume]. 3: Detection of the peak of interest by means of a positive slope. 4: Detection of the down going slope of the first eluting enantiomer. 5: Restart of the elution with the *n*-heptane/ethanol mixture (time based). 6: Injection of the next sample (time based). Time between restart of the elution with the *n*-heptane/ethanol mixture and the sample injection: 149 s equivalent to a volume of 4967 mL (0.66 times the column dead volume). 7: End of the run (time based).

approach is certainly the more complicated recovery of the solvent. When the stronger component of the eluent mixture is used, it is very easy to readjust the composition of the combined distillates of both peaks by adding the required amount of the weakest solvent. If a completely different solvent is used, the distillate resulting from the evaporation of the second eluting product is a combination of the constituents of the eluent together with the stronger solvent, and therefore, this solvent stream has to be treated separately by means of fractional distillation.

Besides reduction of the elution volume of the second eluting enantiomer, a plug of stronger solvent can also be very helpful if, owing to impurities in the sample, the separation deteriorates after a certain number of injections. Instead of thoroughly rinsing the column after a particular number of runs, the introduction of a small plug of stronger solvent in each run is often as effective to remove the disturbing impurities and takes less time to execute.

5.3.4 *Separation of a 'pyrido-pyrimidin-4-one' derivative*

Even for separations in the range of a few hundred grams, it is sometimes required to investigate more extensively the separation parameters before starting a preparative chromatographic process. Often, solubility problems are the primary cause of this additional work. When small quantities have to be separated, it is not dramatic to be forced to inject small amounts, resulting in very diluted solutions of the separated enantiomers. Here, the final volume remains, in general, limited to an acceptable amount of solvent that easily can be handled by means of laboratory scale rotary evaporators. It is, however, very easy to conclude from the data generated if it would not be feasible to use the same process on a larger scale.

For a product with poor solubility characteristics, one first looks for stationary–mobile phase combinations resulting in a separation of the two enantiomers by means of the general analytical screening model. Once this information is available, solubility data is collected for the solvents. Where solvent mixtures are used, measurement of the product solubility in the pure solvents that make up the mixture can be very helpful to determine how to modify the mobile phase to improve the separation without the risk of substantially reducing the solubility of the product. A convenient way to determine the solubility of a compound is to shake an excess of the product at 20°C for a time period of 24 h with the solvent of choice. After filtration, the concentration of the substance in the filtrate is determined by means of UV spectroscopy.

The chromatographic profile, together with the solubility data, allows the selection of the most suitable conditions to perform the separation. In addition, the influence of temperature on the resolution is often examined as the final task before starting the preparative chromatographic process. For the preparation of the sample solution we always choose a product concentration below the solubility value, which has been determined by means of UV measurements to be sure that we are not going to be confronted with partial crystallisation of the product during the execution of the continuous process.

An illustration of this type of problem approach is given for the separation of a 'pyrido-pyrimidin-4-one' derivative. The general analytical screening model indicated that the racemate could be separated as well on a Chiralpak AD as a Chiralcel OJ column. On both columns a separation could be observed using methanol/ethanol or acetonitrile/ethanol as the mobile phase, although the best peak shape and the highest resolution were observed on

the Chiralcel OJ column. The result of the analytical screening experiment on this column type is depicted in Fig. 5.8. Using 5% acetonitrile in ethanol, an acceptable separation was observed within a relatively short elution time. Before starting the preparative chromatography work, the solubility of the product was determined as described above. As can be seen in Table 5.4, the solubility is rather limited at room temperature. Therefore, an additional test was done to determine the solubility of the product in the ethanol/acetonitrile mixture at a temperature of 40°C. The observed solubility of more than 4 g/L of eluent was considered to be more than sufficient for the separation of a 100 g quantity, and so the total amount of 100 g was dissolved in 25 L of the eluent and filtered through a 1 μm filter into a 50 L stirred feed tank maintained at 40°C.

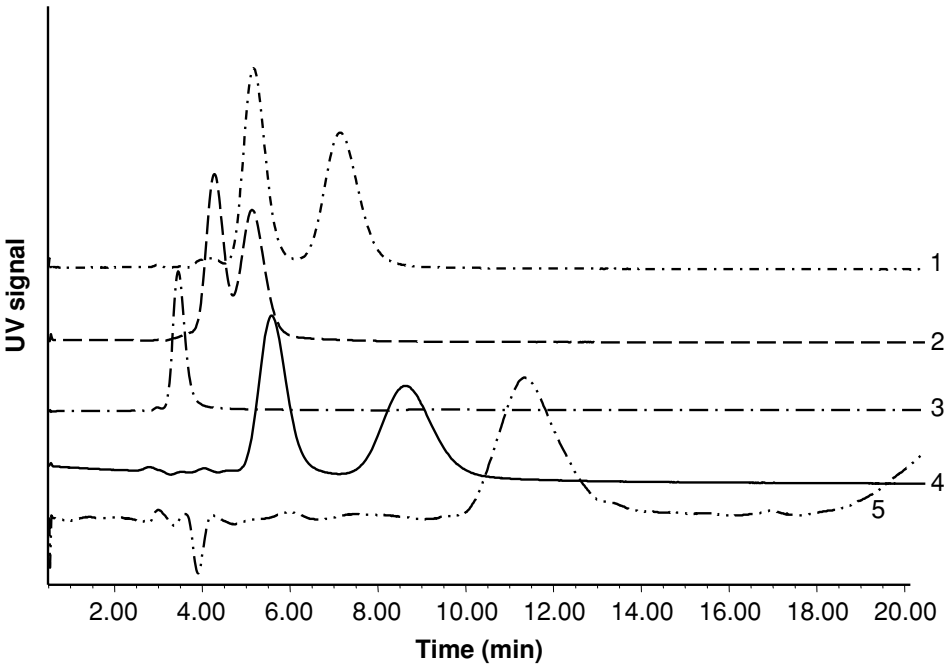

Figure 5.8 Analytical screening of a pyrido-pyrimidin-4-one. Chiralcel OJ (20 μm): 250 mm × 4.6 mm. Mobile phases: (1) 50% methanol in ethanol, (2) 5% acetonitrile in ethanol, (3) 20% ethanol in acetonitrile, (4) ethanol, and (5) 30% ethanol in *n*-heptane. Flow rate: 1.2 mL/min. Detection: UV photodiode array.

Table 5.4 Solubility of the 'pyrido-pyrimidin-4-one' derivative in different solvents

	Solubility (g/100 mL)	
Solvent	At 20°C	At 40°C
Ethanol	0.14	–
Methanol	0.29	–
Acetonitrile	0.04	–
Ethanol/acetonitrile (95:5, v/v)	0.12	0.45
Ethanol/acetonitrile (90:10, v/v)	0.16	–
Ethanol/acetonitrile (80:20, v/v)	0.22	–
Ethanol/methanol (50:50, v/v)	0.14	–

As can be seen from the preparative chromatogram for the injection of 3 g (Fig. 5.9), it was not possible to obtain a full baseline resolution and so a recycling–peak shaving technique had to be used. Using this technique, it was possible to obtain the second eluting enantiomer with the desired optical purity in an acceptable yield (90.6%) but, unusually for such an experiment, the first eluting peak had to be re-chromatographed using the same chromatographic conditions to reach the required enantiomeric excess of 99%. Even with this additional purification step, it was possible to recover the first eluting enantiomer with a yield of 94.4%. A chromatogram of the further purification of the first eluting peak is illustrated in Fig. 5.10.

After this separation had been performed, there was an urgent need for a larger quantity of both enantiomers. As we were not completely satisfied with the result obtained on the Chiralcel OJ column because of the limited production rate and the additional chromatography step, some additional experiments were done on the Chiralpak AD column. In the analytical screening experiments, a nice separation was observed on this column using 20% ethanol in acetonitrile. Additional experiments resulted in finding the best resolution using 25% methanol in acetonitrile and the effect of temperature on the resolution was investigated. The results of these experiments are graphically illustrated in Fig. 5.11. As can be seen, the resolution remains approximately constant over a temperature range of 29–35°C. At a temperature of 20°C, the standard solubility test indicated that it was

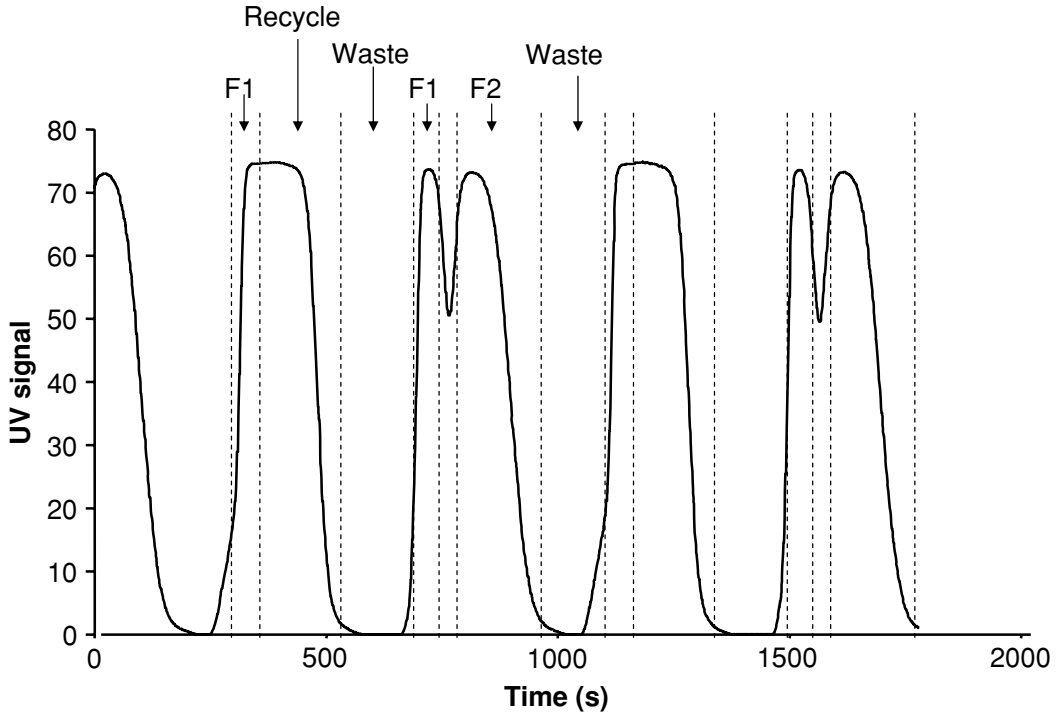

Figure 5.9 Preparative separation of a pyrido-pyrimidin-4-one. Chiralcel OJ 2000 g (20 μm) – Dynamic axial compression: 110 mm i.d. Mobile phase: 5% acetonitrile in ethanol. Flow rate: 750 mL/min. Detection: UV 254 nm. Injection volume: 750 mL (= 3 g = 1.5 mg/g of CSP). Eluent temperature: 38°C. Column temperature: 40°C. Time between two injections: 801 s = 4.49 injections/h = 13.47 g/h = 7.42 h of continuous chromatography to separate a 100 g amount of product.

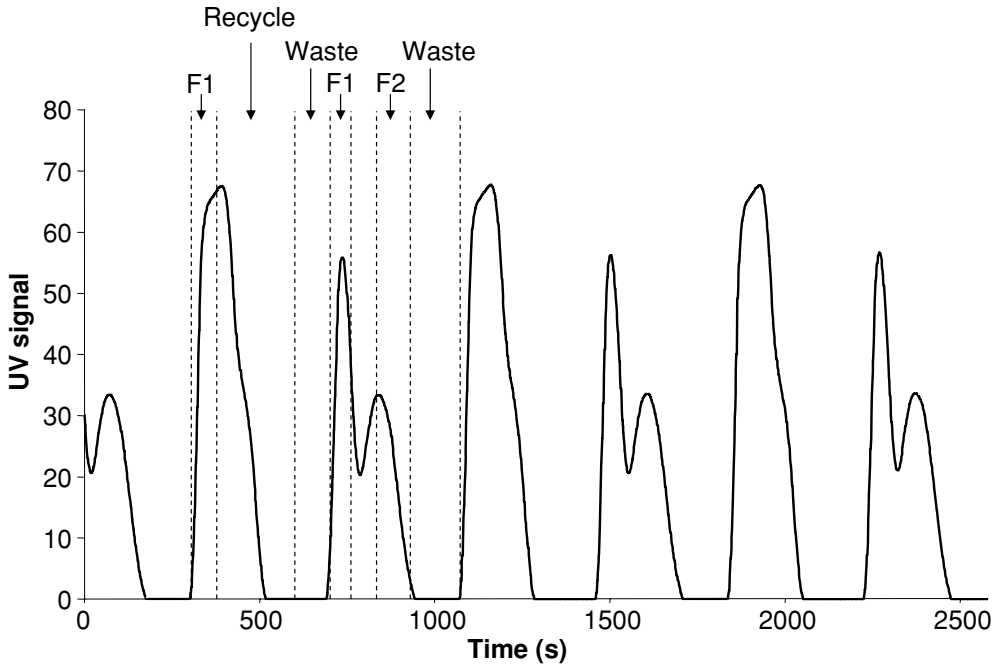

Figure 5.10 Re-chromatography of the pyrido-pyrimidin-4-one Fraction 1. Conditions as Fig. 5.9, except – injection volume: 1000 mL (= 3.13 g = 1.5 mg/g of CSP); total amount re-chromatographed: 50.08 g; eluent temperature: 38°C; column temperature: 40°C.

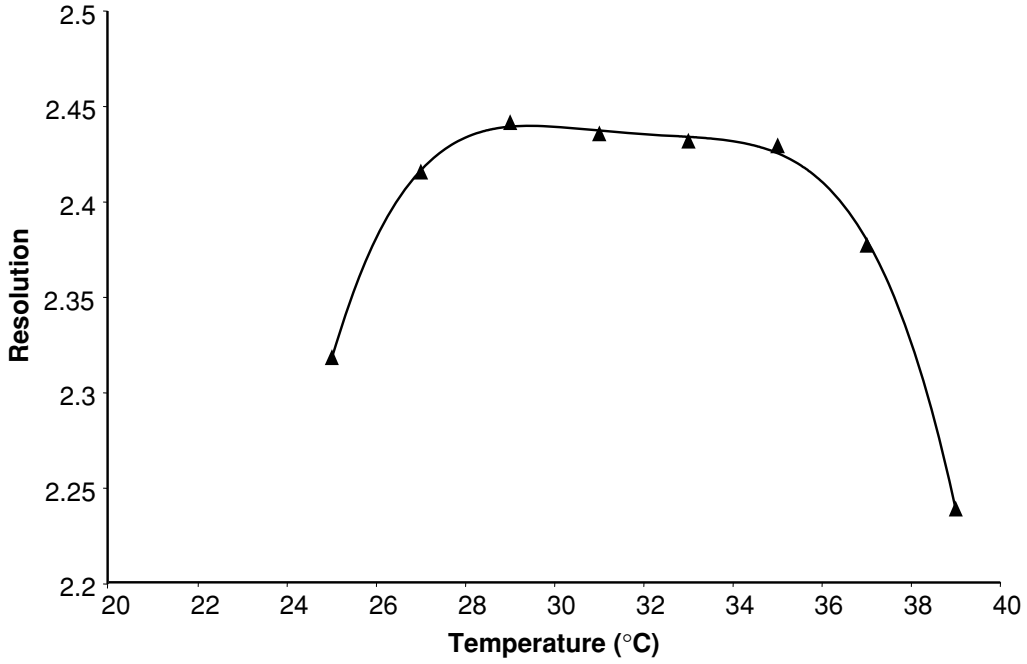

Figure 5.11 Resolution of pyrido-pyrimidin-4-one enantiomers as a function of temperature. Chiralpak AD (20 μm): 250 mm × 4.6 mm i.d. Mobile phase: 25% methanol in acetonitrile. Flow rate: 1.2 mL/min. Detection: UV photodiode array.

possible to dissolve about 1.9 g/L in the mobile phase. Because the largest available stirred feed tank only had a volume of 50 L, it was decided to dissolve the total amount of product at a concentration of 1.6 g/L so as to store the feed solution in a standard 200 L stainless steel drum without the need to maintain it at an elevated temperature. A chromatogram of the preparative chromatography process is illustrated in Fig. 5.12. Using this method it was possible to separate 3.2 g of product in one cycle on a 110 mm i.d. column filled with 2 kg of stationary phase. The cycle time between two consecutive injections was 643 s, equivalent to about 5.6 injections/h, a throughput of 17.9 g of racemate/h. Compared to the method on the Chiralcel OJ column a capacity improvement of 4.4 g/h could be realised. The actual productivity increase is higher when we take into account that on the Chiralcel OJ column the first eluting enantiomer had to be further purified.

5.3.5 *Separation of a piperazinyl–piperidine derivative*

It is important to note that the cellulose and amylose derivatives are also useful not only for the resolution of enantiomers but also for the separation of spatial isomers. When we have to deal with a difficult separation problem of this kind, our strategy is to analyse the product according to our standard chiral screening model. Very often acceptable separations are observed. The use of Chiralcel OD to separate a *cis–trans* mixture is illustrated in the following example.

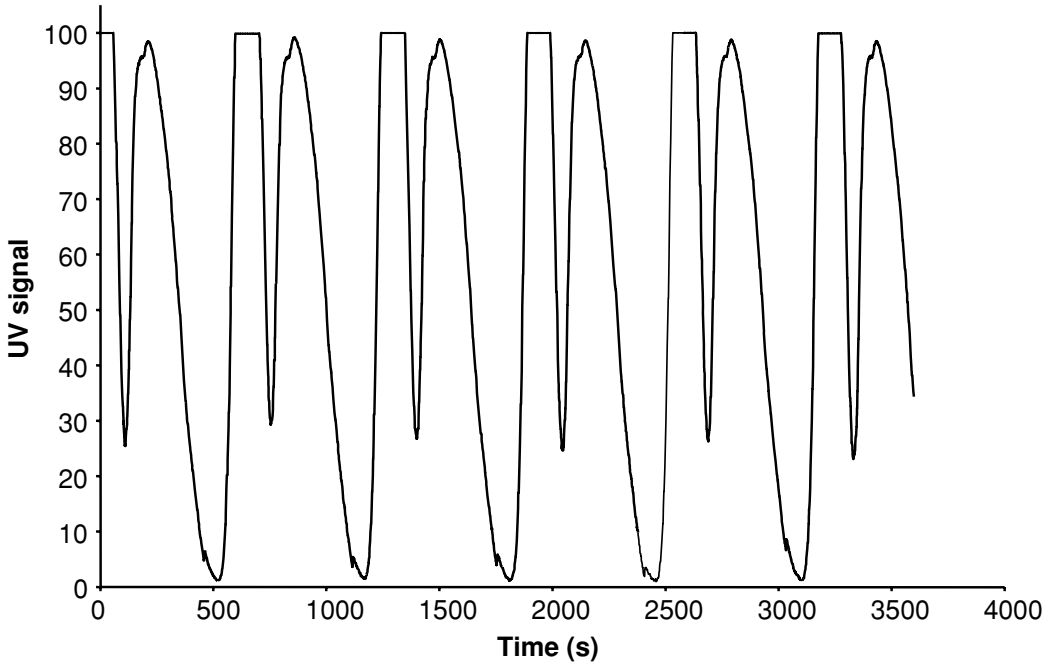

Figure 5.12 Preparative separation of a pyrido-pyrimidin-4-one. Chiralpak AD 2000 g (20 μm) – Dynamic axial compression: 110 mm i.d. Mobile phase: 25% methanol in acetonitrile. Flow rate: 750 mL/min. Detection: UV 254 nm. Injection volume: 2000 mL (= 3.2 g = 1.6 mg/g of CSP). Time between two injections: 643 s = 5.6 injections/h = 17.92 g/h. Eluent temperature: 35°C. Column temperature: 37°C.

It was possible to separate this product on silica, but we had to deal with significant tailing of both peaks, which resulted in a limited throughput. Since a Chiralcel OD column using 100% ethanol as the mobile phase gave a nice separation and good peak shapes, we decided to separate the total amount of the isomer mixture on a 200 mm I.D column filled with 6 kg of Chiralcel OD.

A few injections performed in the learning mode indicated that it was possible to inject 1 L of a sample solution containing product at 100 g/L. In this way, approximately 8 injections/h could be made, corresponding to a throughput of 805 g/h or 19.3 kg/day. To separate the total amount of 125 kg of product, 6.5 days were required. During the chromatography process the solvent from the isolated fractions was continuously removed by means of distillation and the distillate was immediately reused in the process. The preparative chromatograms are shown in Fig. 5.13.

5.3.6 *Summary – scale-up*

For samples resulting from pilot chemistry laboratories and products from early chemical development work, it is usually not possible to optimise the separation method fully owing to time constraints. However, experience has taught us that a well-thought-out analytical screening model based on the use of columns filled with the same batch of packing material

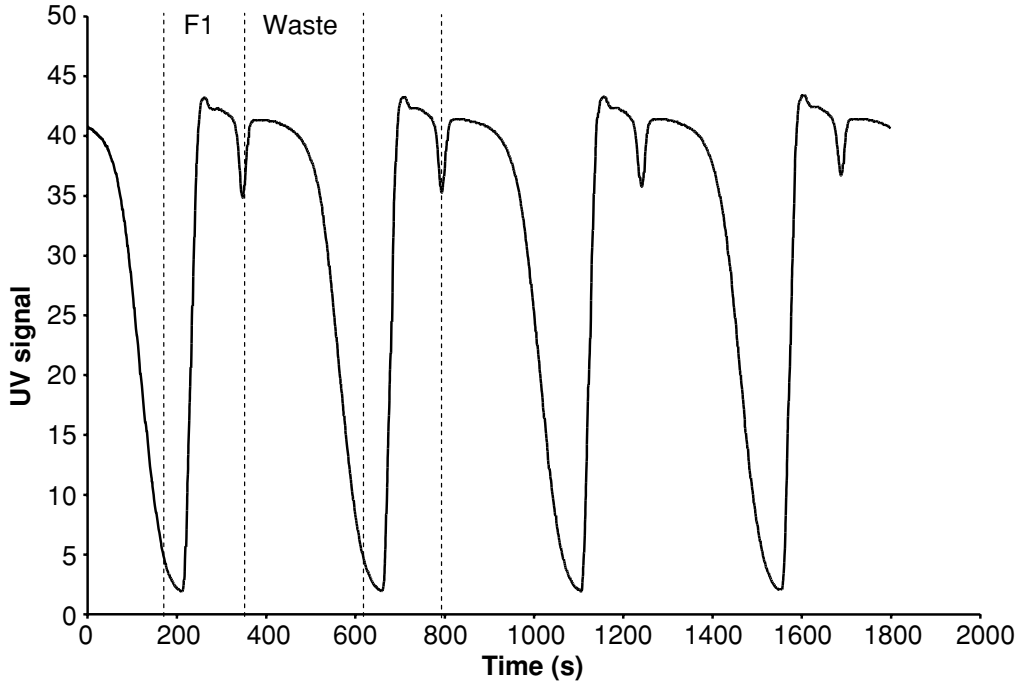

Figure 5.13 Preparative separation of *cis–trans* isomers. Chiralcel OD 6000 g (20 μm) – Dynamic axial compression: 200 mm i.d. Mobile phase: ethanol (denatured with 2% of methanol). Flow rate: 2200 mL/min. Detection: UV 254 nm. Injection volume: 1000 mL (= 100 g = 16.67 mg/g of CSP). Time between two injections: 447 s = 8.05 injections/h = 805.37 g/h = 19.33 kg/24 h. Eluent temperature: 33°C. Column temperature: 35°C.

as the preparative columns, combined with standard grade solvents as the eluent, often give acceptable methods without further optimisation. Sometimes it can be beneficial to perform a few additional tests by replacing partially or completely one type of alcohol by another one to improve the separation observed in the general analytical screening model.

For products with poor solubility characteristics, it is important, even for separations of only a few hundred grams, to investigate in more detail the parameters that can have an effect on the product solubility. Solubility tests in the solvents that result in a separation as well as tests in the neat solvents that make up the eluent mixture are crucial to select the most suitable chromatographic conditions. As solubility increases with increasing temperature, the effect of temperature on the resolution should also be investigated prior to starting the experiments on the preparative column.

5.4 Larger scale separations

5.4.1 *Introduction*

The ultimate task of development work is to develop a reproducible and robust production process, which from an economic point of view is at least competitive with the other routes that have been considered to reach the same goal. Thus, to make a proper judgement about the feasibility of preparative chromatography to separate larger amounts of a racemate, it is very important to have a thorough understanding of the many parameters influencing the separation process. To start extensive chromatographic process research, a number of points of interest can be immediately formulated:

- Purity requirements of the separated enantiomers (possibility to combine chromatography with crystallisation)
- Solvent recovery and adjustment (influence of small composition changes of the eluent)
- Possibility to racemise the unwanted enantiomer
- Influence of water
- Necessity to use additives to reduce tailing
- Batch-to-batch reproducibility of the input quality (chemical purity–product content)
- Long-term stability of the process

The following example is an illustration of the experiments that have been performed to investigate the separation of an intermediate that had to be done on a 350–400 kg scale shortly after the chromatography of the first kilogram batches was finished. The investigational efforts were considered sensible because it was expected that the product demand would move very quickly to even larger quantities.

5.4.2 *Separation of a 'pyrolidino-quinolinone' derivative*

In this particular case the chiral separation was done relatively early in the synthesis route. The chromatography step was followed by five synthesis steps, some of which had a relatively low yield. This automatically resulted in a demand for larger quantities of the chromatographically separated intermediate.

The racemate of interest could be easily separated on a Chiralpak AD column using a mixture of acetonitrile and ethanol. Analytical optimisation experiments indicated that the highest resolution was observed for this solvent combination in a 50:50 volume ratio. As indicated in Table 5.5, the solubility of the product in this mixture was relatively high (up to 78 g/L of the eluent). An analytical chromatogram using the optimised chromatographic conditions is given in Fig. 5.14. Using this method several tens of kilograms had been separated on a 110 mm i.d. column filled with 2 kg of stationary phase.

After these initial separation experiments, small amounts of the chromatographically separated enantiomers were crystallised to get both products optically and chemically pure. These products were then used to draw up a melting point diagram by means of differential scanning calorimetry measurements. The melting point diagram allows the identification of

Table 5.5 Solubility of the 'pyrolidino-quinolinone' derivative in different solvents

Solvent	Solubility (g/100 mL) at 20°C
Ethanol	2.5
Methanol	4.8
Acetonitrile	1.6
Ethanol/acetonitrile (50:50, v/v)	7.1–7.8

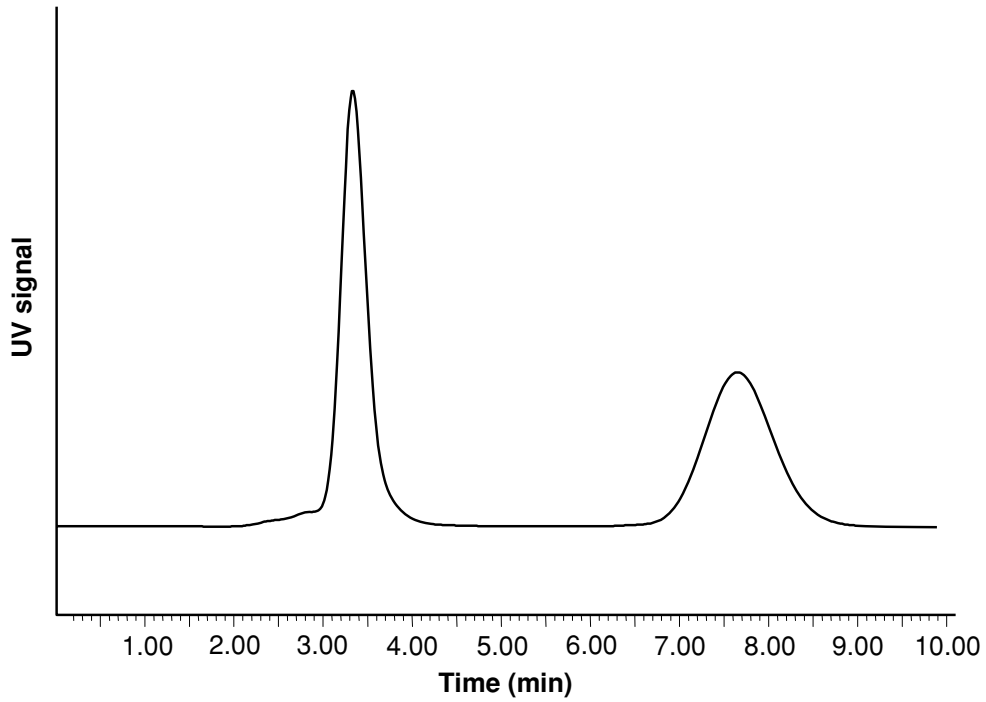

Figure 5.14 Analytical chromatogram of a pyrolidino-quinolinone derivative. Chiralpak AD (20 μm): 250 mm × 4.6 mm. Mobile phase: 50% acetonitrile in ethanol. Flow rate: 1.2 mL/min. Detection: UV photodiode array.

the type of racemate [conglomerate, racemic compound or pseudoracemate (solid solution)] and this information can also be helpful to optimise the resolution method by combining a partial chromatographic resolution with a simple crystallisation process. In a conglomerate for example, the racemate has about twice the solubility of the pure enantiomers. Therefore, to obtain a pure enantiomer from a partially resolved conglomerate, it would be sufficient to adjust the concentration such that it corresponds to the racemate solubility. For a racemic compound (more than 90% of all racemates) it is important to know the position of the eutectic in the phase diagram, because the most soluble part of the mixture has the eutectic composition. Depending on the composition of the enriched sample with respect to the eutectic, recrystallisation under equilibrium conditions will result either in a pure enantiomer or in the racemate. The melting point diagram illustrated in Fig. 5.15 indicates that for this product we have to deal with a conglomerate.

The liquidus curve in this phase diagram has been calculated by using the Schröder–Van Laar equation, which describes solid–liquid equilibria:

$$\ln x = \frac{\Delta H_f}{R}\left(\frac{1}{T_0} - \frac{1}{T_s}\right)$$

where x is the mole fraction of one enantiomer, ΔH_f is the heat of fusion of the pure enantiomer (J/mol), T_s is the melting point of the mixture with composition x (K), T_0 is the melting point of the pure enantiomer (K) and R is the gas constant [= 8.314 J/(K mol)]. In a conglomerate, the racemate is nothing but a eutectic, and therefore, its melting point is necessarily lower than that of the pure enantiomers. The melting point depression going from

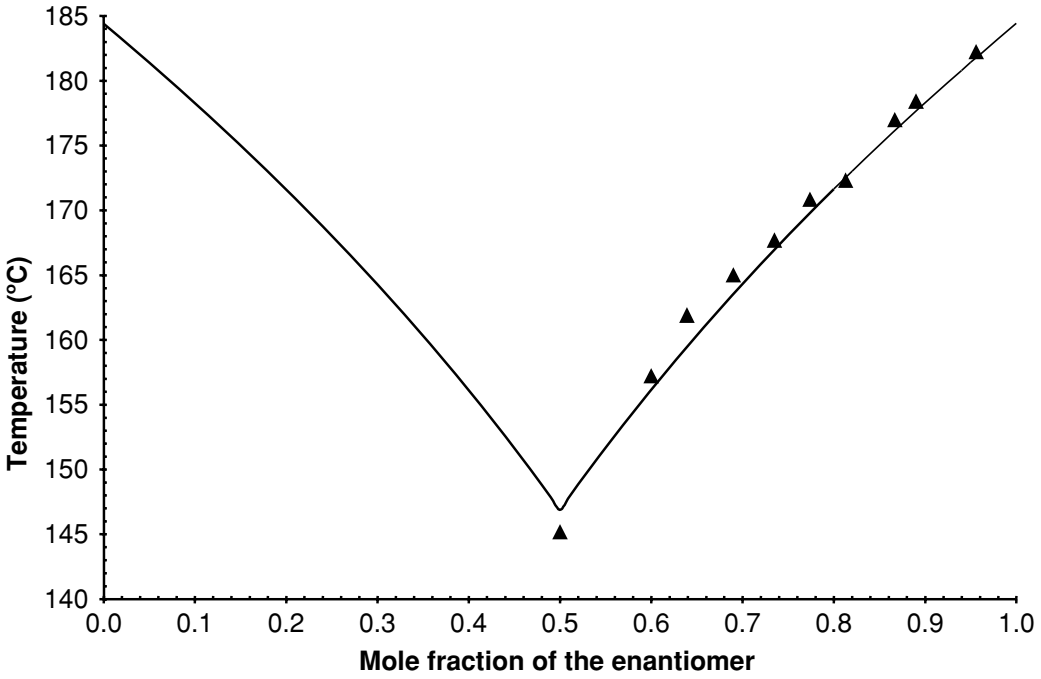

Figure 5.15 Melting point diagram of the pyrolidino-quinolinone conglomerate. Line: calculated liquidus curve. Points: experimental.

the pure enantiomers to the conglomerate is usually in the range of 25–35°C. A purification process by means of crystallisation of partially resolved materials can be designed based on the phase diagram properties. Having to deal with a conglomerate is advantageous because even weakly resolved samples can be purified.

Therefore, we started some experiments to investigate the crystallisation behaviour of mixtures having different enantiomeric excess ratios. As both enantiomers were available in a sufficiently large quantity after the first separation experiments, it was very easy to make samples of known composition. As crystallisation solvent we selected ethanol denatured with 2% of methanol and all experiments were done using automated synthesis equipment [MultiMax RB2-250 (Mettler Toledo)]. Using this type of instrument guarantees dependable results, because one can be sure that all tests are done exactly under the same experimental conditions (stirring speed, heating rate, cooling rate, time, etc.).

The compositions of the different mixtures used in the crystallisation tests are summarised in Table 5.6. The results of the crystallisation experiments are summarised in Table 5.7 and graphically illustrated in Fig. 5.16. These data clearly demonstrate that even for a sample that has been enriched only to an enantiomeric excess of about 40%, it is possible in one crystallisation step to reach optical purities above 96%. However, under the experimental

Table 5.6 Crystallisation conditions[1]

Composition (%)			
R enantiomer	S enantiomer	Enantiomeric excess (%)	Total weight (g)
60.41	39.59	20.82	10.01
66.96	33.04	33.92	10.00
70.14	29.86	40.28	10.00
74.84	25.16	49.68	10.00
79.48	20.52	58.96	10.02
85.06	14.94	70.12	10.00
89.23	10.77	78.46	10.02
95.22	4.78	90.44	10.03

[1] Experimental conditions: (1) Reactor size: 250 mL. (2) Stirring speed: 400 rpm. (3) Temperature profile: 0–420 s – 25°C; 440–2220 s (heating) – 25 → 80°C; 2240–4760 s – 80°C; 4780–6120 s – 85°C; 6140–15140 s (cooling) – 85 → 25°C. (4) Solvent volume: 175 mL, except for the experiments with the highest R enantiomer content. For these samples respectively 180, 182 and 195 mL (85, 89 and 95%) had to be used to completely dissolve the mixture.

Table 5.7 Crystallisation results

Enantiomeric excess of the mixture (%)	Yield after crystallisation (g)	Optical purity (%)	Enantiomeric excess (%)
20.82	No crystallisation	–	–
33.92	No crystallisation	–	–
40.28	4.01	96.40	92.80
49.68	4.98	96.61	93.23
58.96	5.90	96.34	92.67
70.12	6.40	97.37	94.74
78.46	6.52	97.86	95.72
90.44	7.28	98.79	97.59

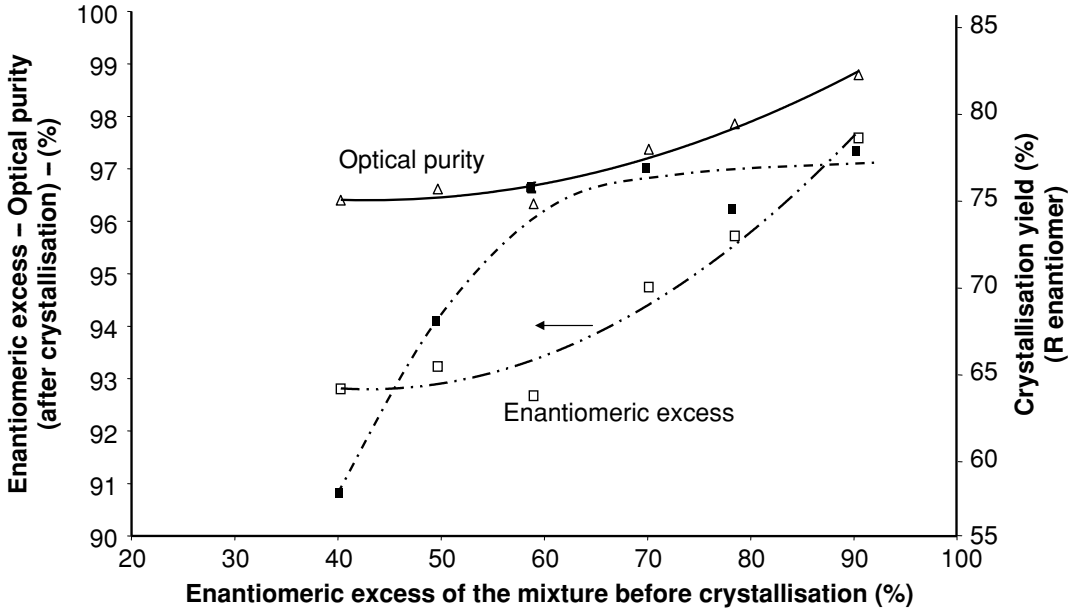

Figure 5.16 Comparison between optical purity and crystallisation yield with enantiomeric excess of the starting material. Solid line: optical purity; dashed-single dotted line: enantiomeric excess (% ee); dashed-double dotted line: crystallisation yield.

conditions applied, the recovery yield for starting materials having an enantiomeric excess below 50% is significantly lower than for the mixtures with higher ee values. The yield is, however, effectively constant for samples enriched to an enantiomeric excess between 60 and 90%. From this, we concluded that for this intermediate the chromatography process certainly did not have to be optimised to reach high optical purities. Since the chromatographic separation process was followed by a number of reaction–crystallisation steps to get the final product, we considered the combination of a chromatography process having as target an optical purity between 90 and 95% with a subsequent crystallisation step as the obvious way to proceed. Were the project to enter into full development, it would of course be necessary to optimise the crystallisation parameters. Furthermore, it would be required to find the optical quality from the chromatography step that in combination with the optimised crystallisation process has the highest impact on the overall economy of the process.

An important cost factor in a chromatography process is certainly the solvent. Therefore, solvent recovery and reuse is an important topic in the optimisation of a chromatographic separation. To separate the intermediate into its two enantiomers, a mixture of acetonitrile and ethanol in a 50:50 volume ratio was used. Because of the mixing principle, some small difference will always be observed between the set point and the actual composition of the mixture generated by the preparative chromatography unit. Quantitative capillary gas chromatography measurements indicated that the mixture coming from the chromatography system had a volume ratio of 51% of ethanol and 49% of acetonitrile. Evaporation of ethanol/acetonitrile mixtures under atmospheric pressure results in an azeotrope with a boiling point of 72.9°C and a composition of 43% acetonitrile in ethanol. However, the usual evaporation processes we consider for evaporation of larger amounts of solvent are operated under vacuum. Therefore, it was important to check the composition of the distillate

obtained under such conditions. The second eluting enantiomer was evaporated in the pilot plant in a reactor while the much smaller volume of the first eluting peak was evaporated in our department in a fully automated thin film evaporator. These gave different compositions; the reactor evaporation yielded 49% acetonitrile while the rotary evaporator gave a solvent composition of 53.3% acetonitrile. Combination of the outputs of these evaporation processes resulted in an average composition of the recovered solvent of exactly 50% of ethanol and 50% of acetonitrile. To judge whether this small difference should have an influence on the chromatography process, a broad range of eluent composition was tested in an automated sequence on an analytical column. The results of this test are graphically illustrated in Fig. 5.17. It is clear that within the investigated range (40–60% of acetonitrile), the resolution decreases significantly. However, even with 60% of acetonitrile in the eluent, the resolution value still remains above 3. Since the composition of the combined distillates will be situated around a 50:50% ratio, the resolution difference for the 1% change in acetonitrile concentration turned out to be 0.016, or 0.45%. This guaranteed that the recovered solvent could be used as such without any problems.

In general, solvents contain some water. Therefore, it is of utmost importance to investigate the effect of water on the chromatographic behaviour, because during the recovery process of the eluent by means of distillation, the water content can change. The specification for the water content in technical grade ethanol is less than 0.3% and for acetonitrile is less than 0.1%. In order to measure the influence of water on the separation, a large volume of eluent was prepared. Thereafter, 250 mL portions of this eluent were spiked with the desired amount of water and were used as mobile phase for the separation on an analytical column. The results of the experiments performed are summarised in Table 5.8. Because

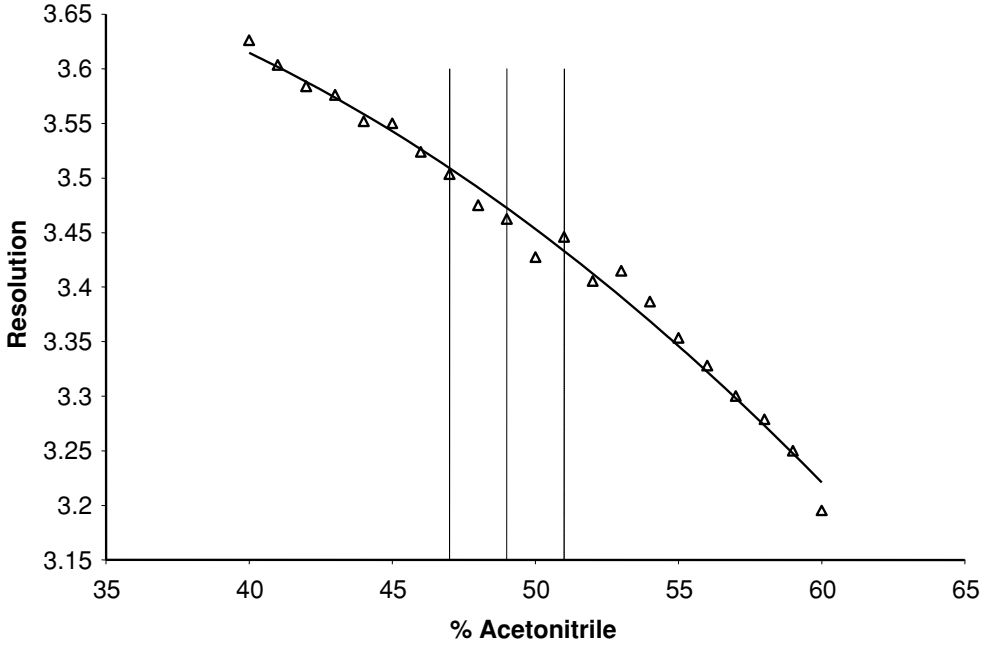

Figure 5.17 Resolution versus acetonitrile content. Conditions as Fig. 5.14, except the mobile phase composition was varied from 40 to 60% acetonitrile in ethanol.

Table 5.8 Influence of water concentration on the resolution

% of water added to the eluent mixture	Resolution value
0	3.46
0.05	3.48
0.1	3.46
0.2	3.47
0.5	3.48
1.0	3.45

no trends could be observed for the resolution values with variations in the water content between 0 and 1%, it is clear that in this case the water concentration is not process critical.

The product that had to be separated was a relatively strong base. Therefore, it was also interesting to study the effect of the addition of TEA as tailing reducer. Although the additive was tested at concentrations between 0.05 and 0.15%, no significant difference was observed in comparison with the pure eluent. Because use of additives complicates the solvent recovery process, we prefer to work without them.

Another important topic that impacts the final production cost is the racemisation of the unwanted enantiomer. Several approaches were explored and the best results were obtained with TFA in acetonitrile/ethanol as the solvent. Using this procedure, it was possible to easily racemise the unwanted enantiomer with an overall yield of about 80%.

The major task of development work is the maximisation of the productivity, whether this is for batch or for continuous chromatography. The preferred instrument to reach this goal is the use of powerful mathematical optimisation tools. For our simulated moving bed (SMB) unit (eight columns of 110 mm i.d. each filled with 500 g of packing material), the parameters to obtain an optical quality of 93–95% for both enantiomers were calculated. Using these parameters it was possible to separate 1.26 kg of product (18 L of sample/h equivalent to 30 kg of racemate/24 h) on a total weight of 4 kg of stationary phase. The eluent volume required to perform the separation of a 30 kg amount was 1152 L.

Because the SMB unit was occupied by another project, the total amount of 350 kg of the intermediate had to be separated by means of batch chromatography using a 20 cm i.d. column filled with 6 kg of Chiralpak AD. With a capacity of 18.75 kg/24 h, about 19 days of continuous chromatography were required to separate the total amount of product. The solvent volume required for the batch chromatography was about 4.4 times higher than for the SMB process. The batch process resulted in an average optical purity of 94–95% for the desired product (second eluting enantiomer). A chromatogram of the batch process is illustrated in Fig. 5.18. A comparison of the operating parameters for the HPLC and SMB experiments is shown in Table 5.9.

5.5 Scale-up problems in early development

5.5.1 *Introduction*

Through experience we have learned that scaling-up of preparative chromatographic separations from the milligram to the multigram scale is not always as straightforward as

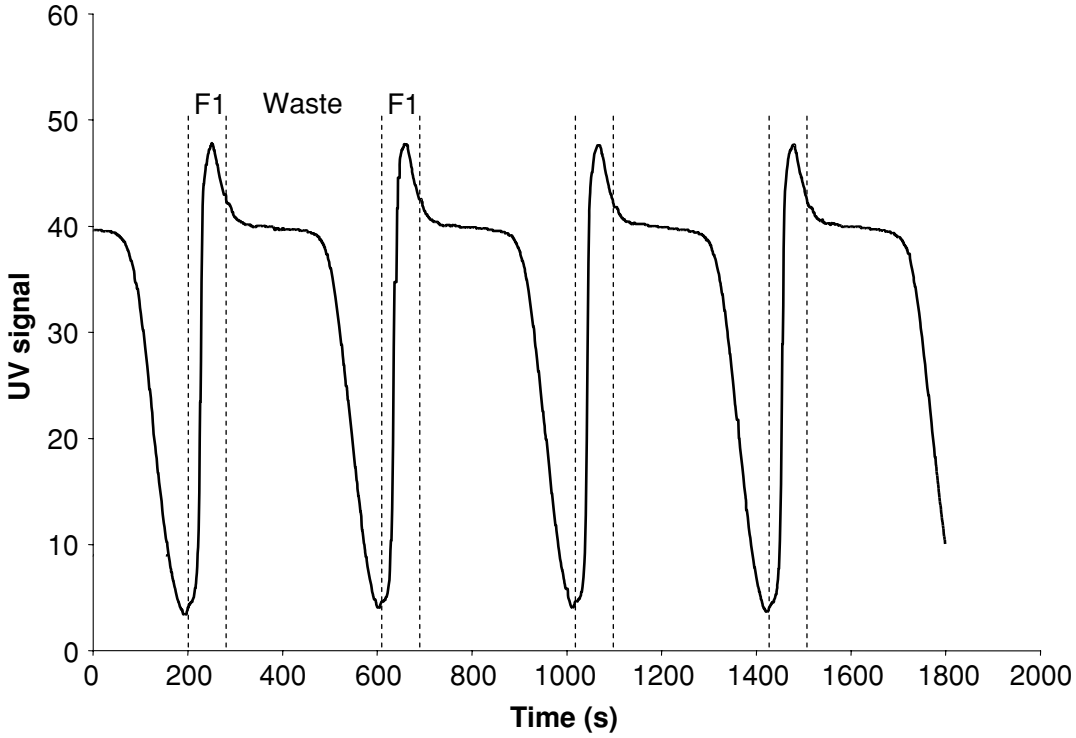

Figure 5.18 Preparative separation of the pyrolidino-quinolinone derivative. Chiralpak AD 6000 g (20 μm) – Dynamic axial compression: 200 mm i.d. Mobile phase: 50% acetonitrile in ethanol. Flow rate: 2200 mL/min. Detection: UV 254 nm. Injection volume: 1150 mL (= 88.5 g = 14.75 mg/g of CSP). Time between two injections: 408 s = 8.8 injections/h = 781.39 g/h = 18.75 kg/24 h. Eluent temperature: 38°C. Column temperature: 40°C.

Table 5.9 Comparison of SMB and HPLC

Technique	Production rate (kg/day)	CSP quantity (kg)	Solvent use (L/day)	Productivity (kg(en)/kg/day)	Solvent consumption (L/kg(en))
SMB	30	4	1152	3.75	76.8
HPLC	18.75	6	3168	1.56	337.9

generally might be expected. In the early stage of product development, a synthesis step will not always yield products with a high chemical purity. In general, pilot chemistry laboratory experiments will most frequently result in a chemical purity of products around 90–95%. Quality differences between different lots of product can often cause scale-up problems due to a difference in solubility characteristics. Most preparative chromatographers have been confronted more than once with the phenomenon that further purification of a crude product that has been pre-purified by means of chromatography is not that easy anymore. In many cases, it is often no longer possible to dissolve the evaporation residue of the pre-purification step in the solvent combination that was used before and often other chromatographic conditions have to be applied.

5.5.2 *Separation of a 'tetracyclic' compound*

After the initial small-scale separation for medicinal chemistry, a 0.55 kg amount of this product had to be separated and both enantiomers were required for further pharmacological studies. The small amount of product could be easily separated on a Chiralpak AD column using 25% of ethanol in *n*-heptane as the mobile phase. To separate the larger quantity, a test run was made on a 110 mm diameter preparative column filled with 2 kg of packing material using the identical experimental conditions that were used to separate a few hundred milligrams for discovery research. A comparable separation was observed and the total amount of 550 g was dissolved in 55 L of the eluent (10 g of product/L). The loading experiments performed on the preparative column indicated that it was possible to inject 700 mL of the sample solution corresponding to an amount of 7 g of the crude product. The chemical purity of this product was about 93.5%. Therefore, the 7 g of crude sample corresponds to about 6.5 g of racemate. A chromatogram of this separation is depicted in Fig. 5.19.

A few weeks later, a second amount of about 900 g had to be chromatographed. Because the separation of the first amount went smoothly, there was no need further to optimise the method. However, the chemical purity of this batch was about 3% higher than that of the first batch, which immediately manifested itself in a decreased solubility. Instead of being able to dissolve 10 g/L only 8 g of product could be dissolved. Fortunately, it was possible to inject 880 mL of this solution, resulting in a daily capacity of about 477 g on a 110 mm

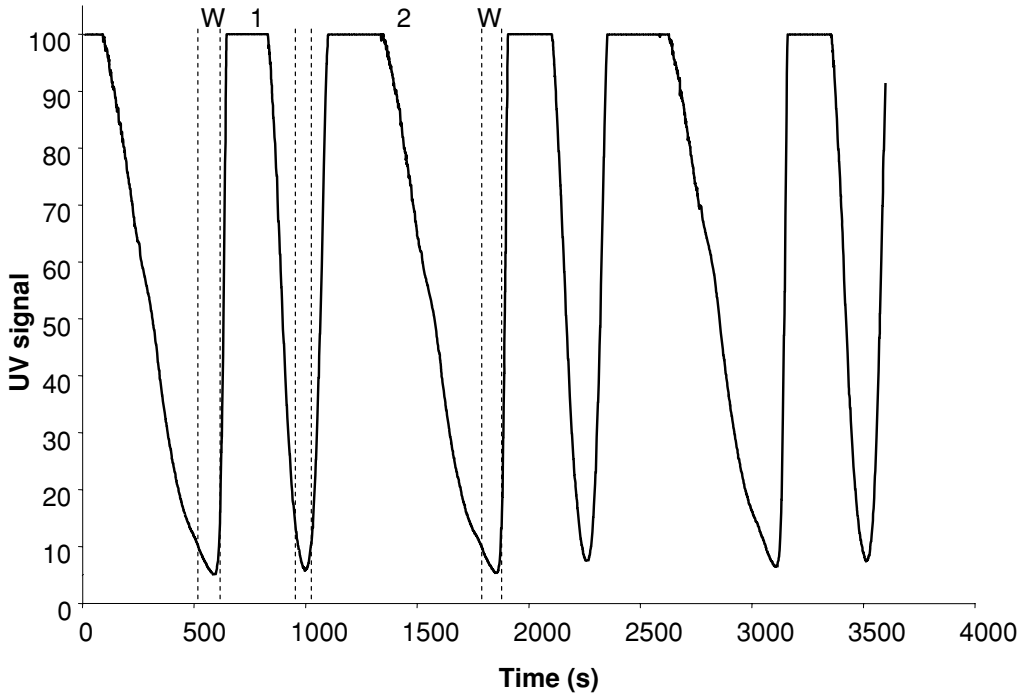

Figure 5.19 Preparative separation of a tetracyclic compound. Chiralpak AD 2000 g (20 μm) – dynamic axial compression: 110 mm i.d. Mobile phase: 25% *n*-heptane in ethanol. Flow rate: 700 mL/min. Injection volume: 700 mL (7.0 g of product = 6.5 g of racemate = 3.25 mg/g of CSP). Time between two successive injections: 1259 s = 2.86 runs/h = 18.59 g/h = 446 g of pure racemate/24 h. Eluent temperature: 38°C. Column temperature: 40°C.

i.d. column, which was comparable with the result of the first batch. The chromatograms of these separations were effectively identical.

The next task was the separation of a 14 kg amount of racemate with a chemical purity above 99.5%. As we already had been confronted with a difference in solubility characteristics between the first and the second batch due to differences in chemical purity, it was necessary to perform some solubility tests before starting the preparative chromatographic separation of this larger batch, which had to be performed under cGMP conditions. The results of these tests are summarised in Table 5.10 and graphically illustrated in Fig. 5.20.

Table 5.10 Solubility of the different synthesis batches

	Solubility (g/100 mL)				
Solvent or solvent mixture	20°C	30°C	35°C	40°C	60°C
n-Heptane/ethanol	0.15	0.26	–	0.42	–
(25:75, v/v)	0.17	–	–	0.45	1.00
	0.15	–	–	0.43	0.75
Ethanol	–	0.17	0.20	0.25	–
	0.10	–	–	0.25	0.48
	0.092	–	–	0.23	0.46
Acetonitrile	–	0.94	1.18	1.35	–
	0.55	–	–	1.3	2.4
	0.55	–	–	1.3	2.5

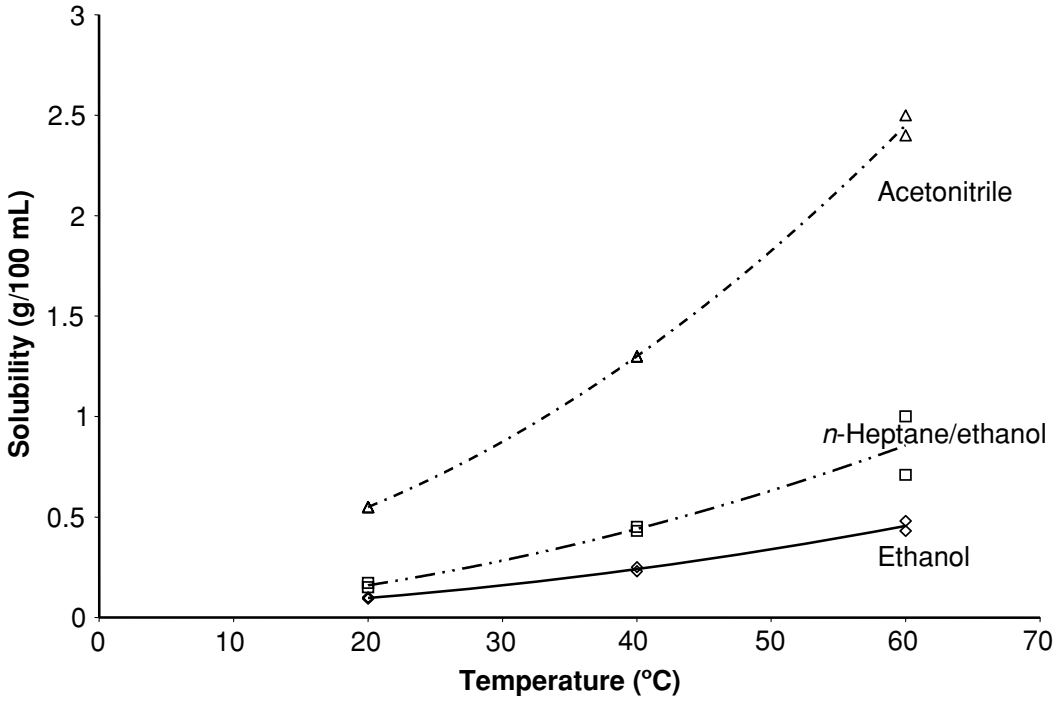

Figure 5.20 Graphical illustration of the solubility experiments summarised in Table 5.10. Solid line: ethanol; dashed-double dotted line: 25% *n*-heptane in ethanol; dashed-dotted line: acetonitrile.

The graphical representation of the solubility behaviour of the tetracyclic component in the three solvents investigated clearly indicates that even at lower temperatures the best solubility was observed in acetonitrile. But even for this solvent the solubility was rather limited at a temperature of 40°C (13.5 g/L). This means that about 1037 L of solvent are required to dissolve 14 kg of product. Taking into account that we never go to the limit of solubility to prepare the sample solution, an even larger sample volume can be expected.

Unfortunately, it was not possible to resolve both enantiomers on Chiralpak AD with acetonitrile-based eluents. However, the general analytical screening model indicated some separation on Chiralcel OD using 20% ethanol in acetonitrile. Therefore, additional experiments were done on this column. Using pure acetonitrile as the eluent, a close to baseline separation was obtained. Because the solubility of the product strongly diminished with decreasing temperature, no further experiments were done to investigate the effect of temperature variations on the resolution.

For larger scale separations, our department always works together with the pilot plant to prepare the sample solution or to evaporate, if necessary, the solvent from the isolated chromatography fractions. In this particular case, the plant agreed to supply sample solution at a temperature of 35–40°C that immediately could be transferred into a stirred and heated tank in our department. To make this operating model practically feasible, the total amount of 14 kg was treated in four portions of 3.5 kg. The warm sample solution coming from the pilot plant was immediately pumped into a 500 L stirred tank that was maintained at a temperature of 35°C. Furthermore, the transfer line from the storage tank to the chromatography unit was also heated to a temperature of 35°C. The result of a test run on a 20 cm i.d. column filled with 6 kg of Chiralcel OD using pure acetonitrile as the eluent is given in Fig. 5.21.

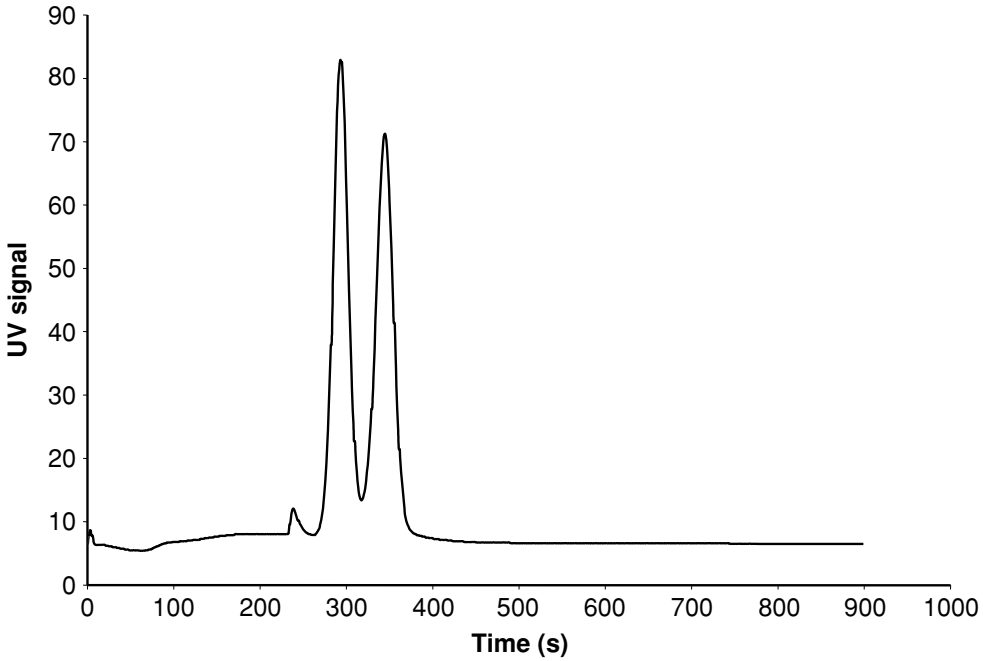

Figure 5.21 Test chromatogram of the tetracyclic compound. Chiralcel OD 6000 g (20 μm) – Dynamic axial compression: 200 mm i.d. Column length: 31.8 cm. Mobile phase: acetonitrile. Flow rate: 2000 mL/min. Detection: UV 270 nm. Injection volume: 10 mL loop (= 143 mg of product = 0.024 mg/g of CSP). Eluent temperature: 35°C. Column temperature: 38°C.

Using the learning mode facilities of the preparative chromatography unit, three experiments were done injecting respectively 10, 12.5 and 15 g of product. From the beginning it was clear that the recycling–peak shaving technique had to be used. With this technology, it was possible to inject about 15 g of product in two cycles to get both enantiomers with an optical purity above 98.5%. Because in this example we had to deal with a conglomerate, this purity was sufficiently high to obtain optically pure products after crystallisation. A chromatogram of the separation of a 14.3 g load is depicted in Fig. 5.22 while the separation table used to perform this separation is illustrated in Table 5.11.

During the execution of the chromatography process, the solvent from both enantiomer fractions was evaporated and the distillate was directly reused in the chromatography process, illustrating the benefit of being able to use a single solvent to perform a separation.

5.5.3 *Non-natural amino acids*

We recently had to separate somewhat larger quantities of three promising components within a series of non-natural amino acid derivatives containing one or more heterocyclic base functions. All of the products had been separated before on the 100–500-mg scale for medicinal chemistry purposes. Therefore, as part of a standard practice, the separation of each new batch was first checked using an analytical column filled with 20 μm bulk material with the identical mobile phase composition as that used for the small-scale preparative chromatographic separation. Surprisingly, from the three compounds, only one showed the same chromatographic profile as before. For the two others, hardly any separation could

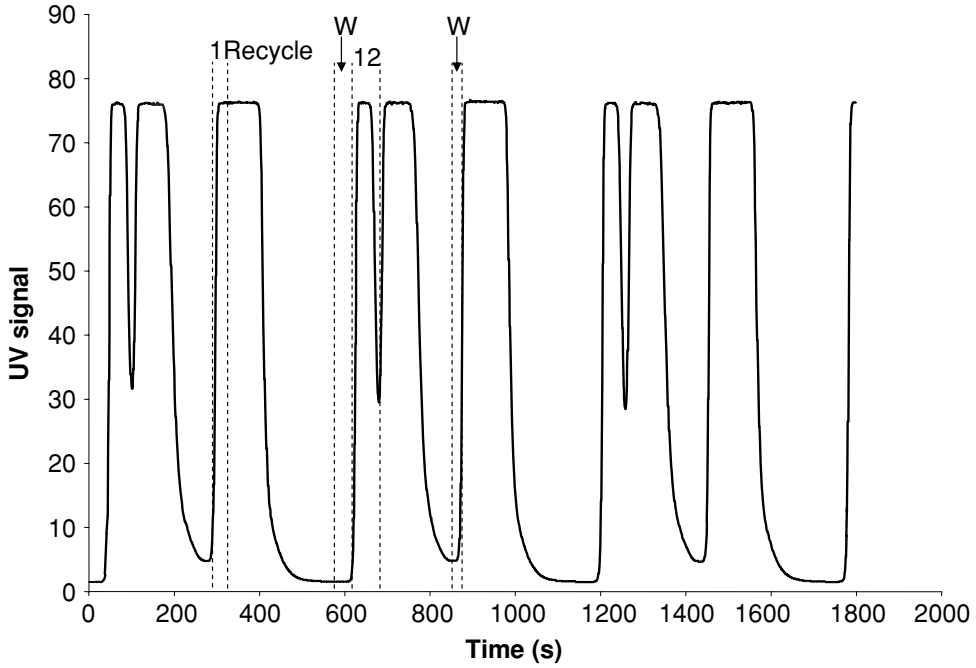

Figure 5.22 Recycle chromatography of a tetracyclic compound. Conditions as Fig. 5.21, except – Detection: UV 254 nm; injection volume: 1500 mL (=14.36 g of product = 2.39 mg/g of CSP); two cycles per injection; eluent temperature: 35°C; column temperature: 38°C.

Table 5.11 Separation conditions for the 'tetracyclic' component

Action number	Time (s)	Time window		Action	Trigger Type	UV detection window		
		Before	After			Value	−	+
1	240	60	60	Fraction 1	+ Slope	20	10	10
2	34	1	1	Recycle	Time	–	–	–
3	100	60	60	Fraction 10	– Slope	8	5	0
4	65	1	1	Recycle	Time	–	–	–
5	246	1	1	Fraction 10	Time	–	–	–
6	2	1	1	Sample	Time	–	–	–
7	30	30	60	Fraction 1	+ Slope	20	10	5
8	53	30	30	Fraction 1	Valley	–	–	–
9	5	1	1	Fraction 2	Time	–	–	–
10	163	1	1	Fraction 10	Time	–	–	–
11	5	1	1	End run	Time	–	–	–
Total time	943							

be observed. This problem was shown not to be due to the known memory effects of the polysaccharide phases, and so further experiments were carried out.

Meanwhile, the preparative separation of the product that was successful was begun. Chromatograms of the analytical and the small-scale preparative chromatographic separations are depicted in Fig. 5.23. As can be seen in the figure, the eluent composition (45% of 2-propanol in heptane) used previously to separate 200 mg of product for the medicinal chemists did not result in a complete baseline separation. Therefore, some fine-tuning experiments were carried out. The 2-propanol concentration in the eluent mixture was changed and the alcohol systematically replaced by ethanol, methanol or a mixture of both. Changes in the TFA concentration in the eluent were also investigated. As can be seen in Table 5.12, however, no significant improvement of the resolution value was observed. It was finally decided to start the separation using a small modification of the original mobile phase composition, which enhanced the product solubility.

Fortunately, the first experiment on the larger diameter column demonstrated a fairly good separation of the enantiomers. In the small-scale experiment, a loading of 0.04 mg of product/g CSP over two cycles was used. In the present case it was possible to separate 2 g of product in one single run on the 110 mm i.d. column filled with 2000 g of stationary phase (1 mg of product/g of CSP). This is still a very low loading capacity, but given the amount that had to be chromatographed, it was certainly feasible to perform the separation. A chromatogram of this separation is given in Fig. 5.24.

To be able to dissolve the product in the eluent used, it was necessary to add an additional amount of TFA. Unfortunately, the product was very unstable in acidic medium. Therefore, it was decided to perform the separation of the total amount of 60 g in batches of 10 g. In order to avoid decomposition, after each run the collected fractions were immediately neutralised using triethylamine. Work-up of the purified fractions was continued by evaporation of the eluent to low volume, dissolution in dichloromethane and back-extraction of the salts with water acidified with acetic acid to a pH value of 6.5. To assure that no salts were left in the product, both enantiomers were further purified on silica. Despite the tedious work-up procedure and additional chromatography step, we were able to isolate respectively 24.4 g of peak 1 (81.3%) and 22.2 g of peak 2 (74%), which was more than sufficient for the intended purpose.

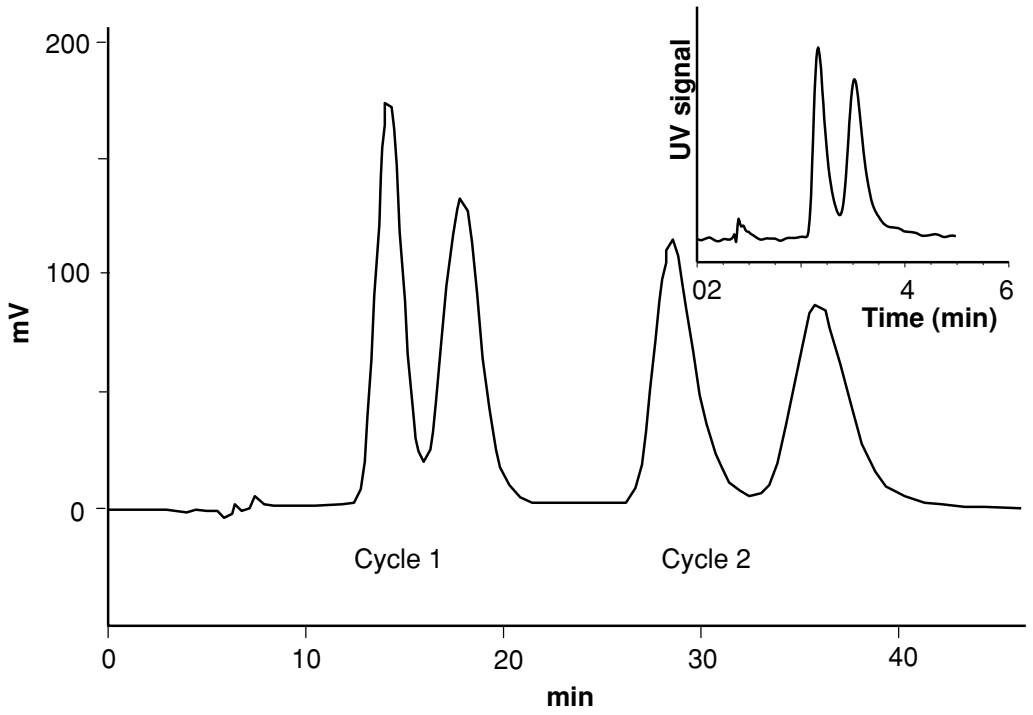

Figure 5.23 Recycle chromatography of a non-natural amino acid. Chiralpak AD 500 g (20 μm) – dynamic axial compression: 50 mm i.d. × 410 mm. Mobile phase: 45% 2-propanol in *n*-heptane containing 0.5% trifluoroacetic acid. Flow rate: 80 mL/min. Detection: UV 225 nm. Sample amount: 0.55 g/L in eluent (= 0.044 mg/g of CSP). Two cycles per injection. *Inset*: Analytical chromatogram. Chiralpak AD (10 μm): 4.6 mm i.d. × 50 mm. Mobile phase: 45% 2-propanol in *n*-heptane containing 0.5% trifluoroacetic acid. Flow rate: 1.0 mL/min. Detection: UV 225 nm.

As already mentioned, hardly any separation was found for the larger scale batch of the second amino acid derivative of the series of three when it was checked on an analytical column using the same experimental conditions [acetonitrile/methanol (85:15, v/v)] as used earlier. Because no improvement could be observed on investigation of the influence of variations in the acetonitrile/methanol ratio, the product was tested using the standard screening model under neutral, acidic and basic conditions. In addition, elevated additive

Table 5.12 Effect of mobile phase composition on resolution

n-Heptane	2-Propanol	Ethanol	Methanol	Trifluoroacetic acid	Valley point resolution
50	50	0	–	0.5	0.872
50	45	5	–	0.5	0.921
50	40	10	–	0.5	0.947
50	10	40	–	0.5	0.851
50	–	50	–	0.5	0.407
50	–	45	5	0.5	0.855
50	45	–	5	0.5	0.941
50	50	–	–	0.5[1]	0.814

[1]0.5% trifluoroacetic acid + 0.47% triethylamine.

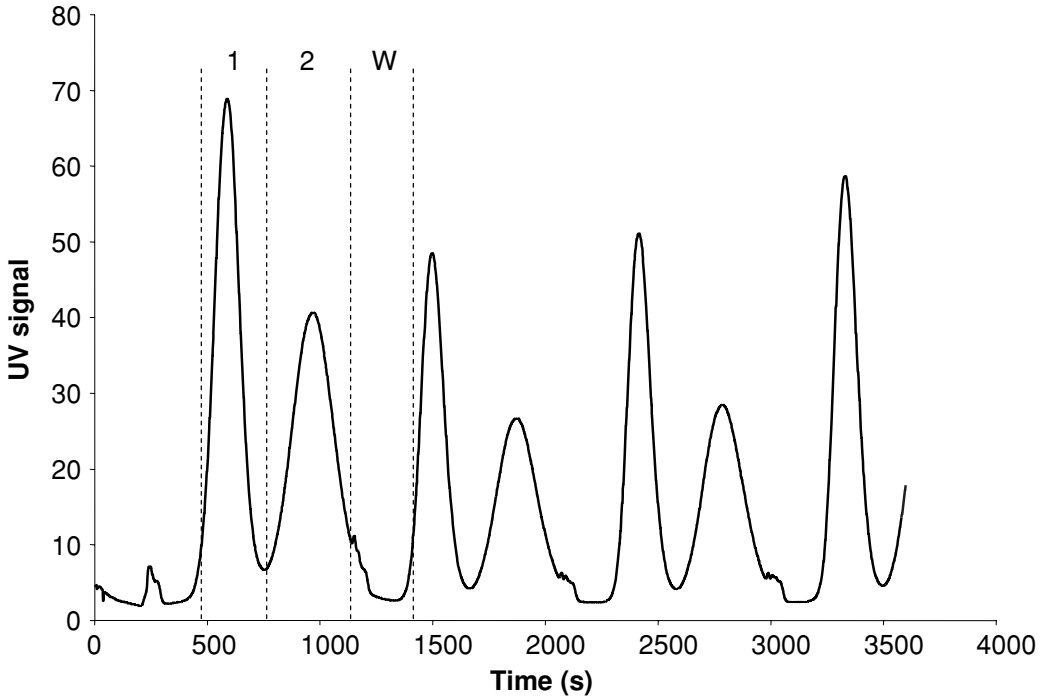

Figure 5.24 Preparative separation of a non-natural amino acid. Chiralpak AD 2000 g (20 μm) – Dynamic axial compression: 110 mm i.d. Mobile phase: 50% 2-propanol in *n*-heptane containing 0.5% trifluoroacetic acid. Flow rate: 750 mL/min. Detection: UV 310 nm. Sample amount: 10 g dissolved in 1000 mL of 2-propanol/70 mL of water/10 mL of TFA and diluted with 420 mL of *n*-heptane. Injection volume: 300 mL (= 2 g of product = 1 mg of product/g of CSP). Column temperature: 30°C. Eluent temperature: 32°C.

levels (up to 1%) were investigated. None of the experiments resulted in a profile similar to that obtained for the medicinal chemistry batch. Because there was an urgent need for the pure enantiomers, some experiments were done on other types of stationary phases. Although two peaks could be observed (valley point resolution: 0.17) on the Kromasil CHI-TBB phase (Akzo Nobel) using a mixture of 5% ethanol in methyl-*t*-butyl ether containing 0.2% TFA as the mobile phase, getting sufficient bulk material off this stationary phase within a reasonable period of time was rather difficult. Therefore, all further efforts were concentrated on the Chiralpak AD column.

As the product was an ionisable substance, we decided to add an electrolyte to the mobile phase. Therefore, 10 mM of ammonium acetate was added to the methanol component of the mobile phase. This succeeded in getting an acceptable separation on the analytical column, as shown in Fig. 5.25. The effect of changes in the volumetric ratio between acetonitrile and methanol/ammonium acetate, together with the influence of a column temperature increase, is depicted in Fig. 5.26. This clearly indicates that the retention behaviour of the second eluting enantiomer is strongly influenced by the temperature while this is not the case for the first eluting product. Although a concentration of 10% of methanol/10 mM ammonium acetate in acetonitrile gave the best separation, it was decided for preparative work to use the 15% methanol/ammonium acetate, which resulted in less dilution of the separated products to facilitate work-up of the fractions.

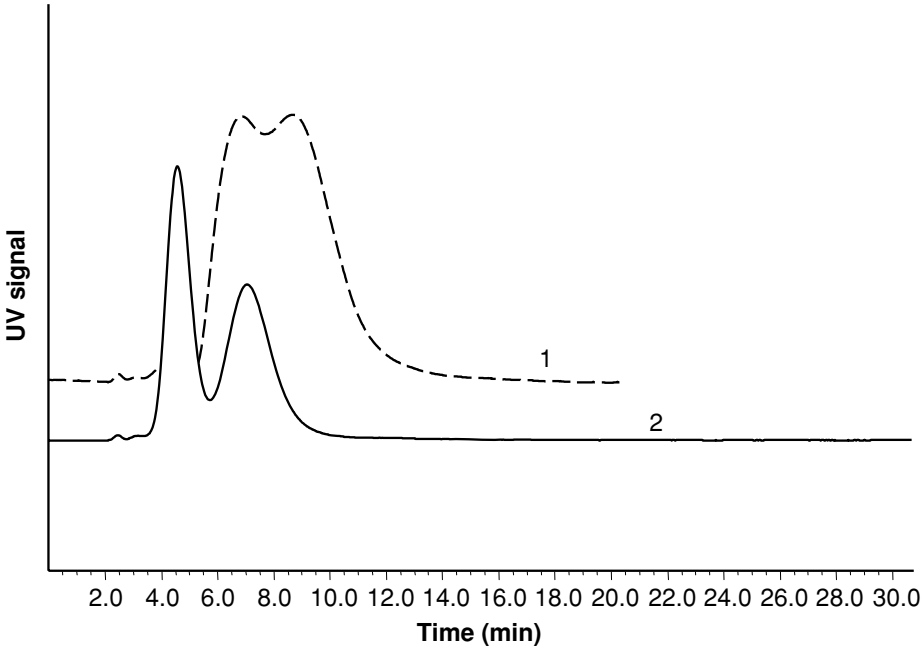

Figure 5.25 Analytical chromatography of a non-natural amino acid. Chiralpak AD (20 μm): 250 mm × 4.6 mm i.d. Mobile phases: (1) 15% methanol in acetonitrile, and (2) 15% methanol containing 10 mM ammonium acetate in acetonitrile. Flow rate: 1.2 mL/min. Detection: UV photodiode array. Column temperature: 25°C.

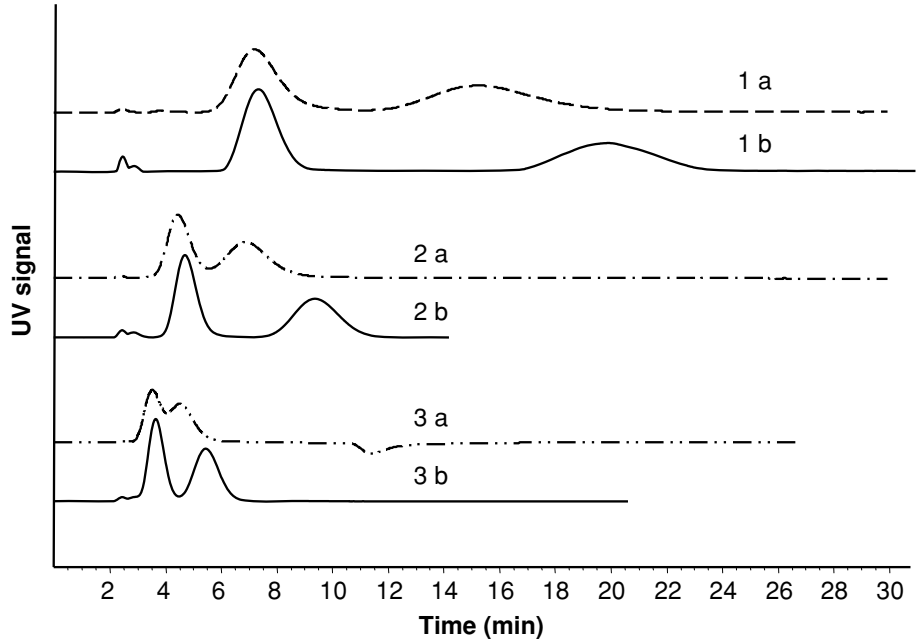

Figure 5.26 Influence of mobile phase composition and temperature on the analytical chromatography of a non-natural amino acid. Chiralpak AD (20 μm): 250 mm × 4.6 mm i.d. Mobile phases (column temperature: a = 25°C; b = 40°C): (1) 10% methanol containing 10 mM ammonium acetate in acetonitrile, (2) 15% methanol containing 10 mM ammonium acetate in acetonitrile, and (3) 20% methanol containing 10 mM ammonium acetate in acetonitrile. Flow rate: 1.2 mL/min. Detection: UV photodiode array.

Preparative chromatographic separation of the total amount of 150 g of product was done using feed batches of 20 g. To dissolve this amount of product, a mixture of 4 L of the eluent, 240 mL of water and 60 mL of TEA were needed and the solution had to be heated to get a homogeneous solution. Because water was needed to dissolve the product, the composition of the eluent was also slightly modified by adding some water (400 L of acetonitrile was mixed with 70 L of methanol and 1.5 L of water containing 250 g of ammonium acetate). The results of a test run via a 10 mL injection loop on the preparative column filled with 2 kg of Chiralpak AD confirmed the data obtained on the analytical column.

For the first injection via the elution pump, a volume of 400 mL (corresponding to a product amount of 1.86 g) was chosen. Probably because of solubility problems caused by differences in composition of the sample solution and the eluent, a distorted peak shape was observed for the first eluting peak. To circumvent this problem, the injection volume had to be reduced to 200 mL (0.93 g of product). Although the first peak eluted as a relatively narrow band, it was required to use the recycling–peak shaving technique to get both enantiomers optically pure (99% ee). A chromatogram of the automated process is given in Fig. 5.27. Working this way, it was possible to perform 3.44 injections/h corresponding to a product amount of 3.2 g of racemate/h (76.8 g/24 h). To purify a 20 g portion, it takes more than 6 h of continuous chromatography. Combined with the necessity to evaporate a large volume of solvent to recover both enantiomers, this process is certainly not suitable to separate larger quantities and another approach has to be considered. Fortunately, the stability of this amino acid was good compared to the other products within the series of three. Therefore,

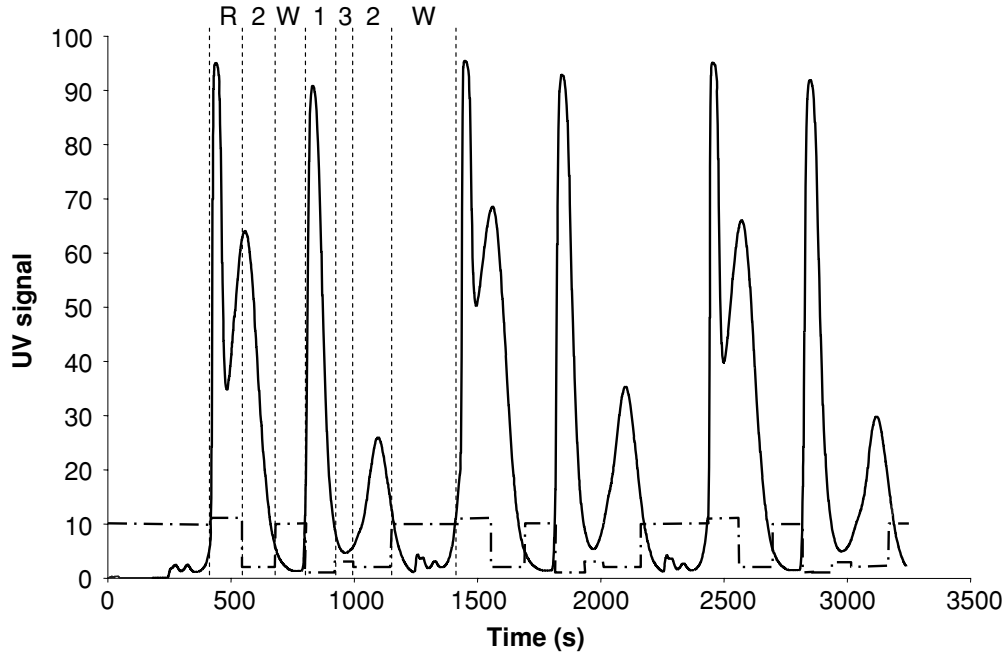

Figure 5.27 Preparative separation of a non-natural amino acid. Chiralpak AD 2000 g (20 μm) – Dynamic axial compression: 110 mm i.d. Mobile phase: 84.79% acetonitrile/14.84% methanol/0.32% water/6.9 mM ammonium acetate. Flow rate: 750 mL/min. Detection: UV 270 nm. Sample amount: 20 g dissolved in 4000 mL of the eluent, 240 mL of water and 60 mL of triethylamine (4.65 g/L). Injection volume: 200 mL (= 930 mg of product = 0.465 mg of product/g of CSP). Column temperature: 40°C. Eluent temperature: 38°C.

fewer precautions had to be taken to avoid decomposition. It was very easy to recover the product as a chemically pure solid by stirring the evaporation residue in water.

The third amino acid derivative from the series contained the largest number of heterocyclic base functions of the three investigated compounds. The analytical and preparative chromatograms of the small-scale separation are respectively depicted in Fig. 5.28. These clearly show that in both cases very nice separations had been obtained. However, for the new larger batch of product it was not possible to separate the product on an analytical column. Because there was an urgent need for an additional amount of a few hundred milligrams of both enantiomers, a small amount of product was transferred to the laboratory where the first experiments were done. An analytical chromatogram of this batch is illustrated in Fig. 5.29, together with a small-scale preparative separation. Figures 5.28 and 5.29 clearly indicate the strong difference in behaviour between the two different lots of product. For the new batch, even after recycling it was not possible to get a baseline resolution for a 100 mg amount, while for the first batch of the same compound there was an easy baseline separation. After this experiment, the original sample was rechecked by means of analytical reversed-phase chromatography but we were not able to detect high levels of impurities, which could have been the reason of our chiral separation problem. Nevertheless, based on knowledge acquired with a comparable kind of problem we had been confronted with some time before, we decided to purify 200 mg of product by

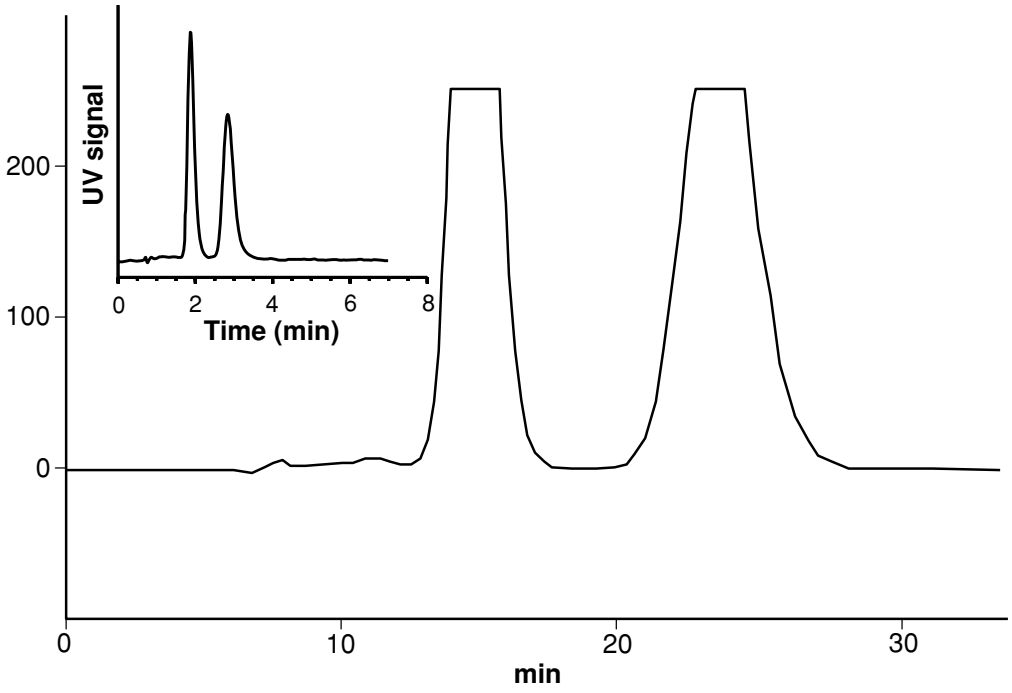

Figure 5.28 Separation of the enantiomers of a first batch of a non-natural amino acid. Chiralpak AD 500 g (20 μm) – Dynamic axial compression: 50 mm i.d. × 410 mm. Mobile phase: 50% 2-propanol in *n*-heptane containing 0.5% trifluoroacetic acid. Flow rate: 80 mL/min. Detection: UV 225 nm. Sample amount: 2.5 g/L in eluent (= 0.2 mg of product/g of CSP). *Inset*: Analytical separation. Chiralpak AD (10 μm): 50 mm × 4.6 mm i.d. Mobile phase: 50% 2-propanol in *n*-heptane containing 0.5% trifluoroacetic acid. Flow rate: 1.0 mL/min. Detection: UV 225 nm.

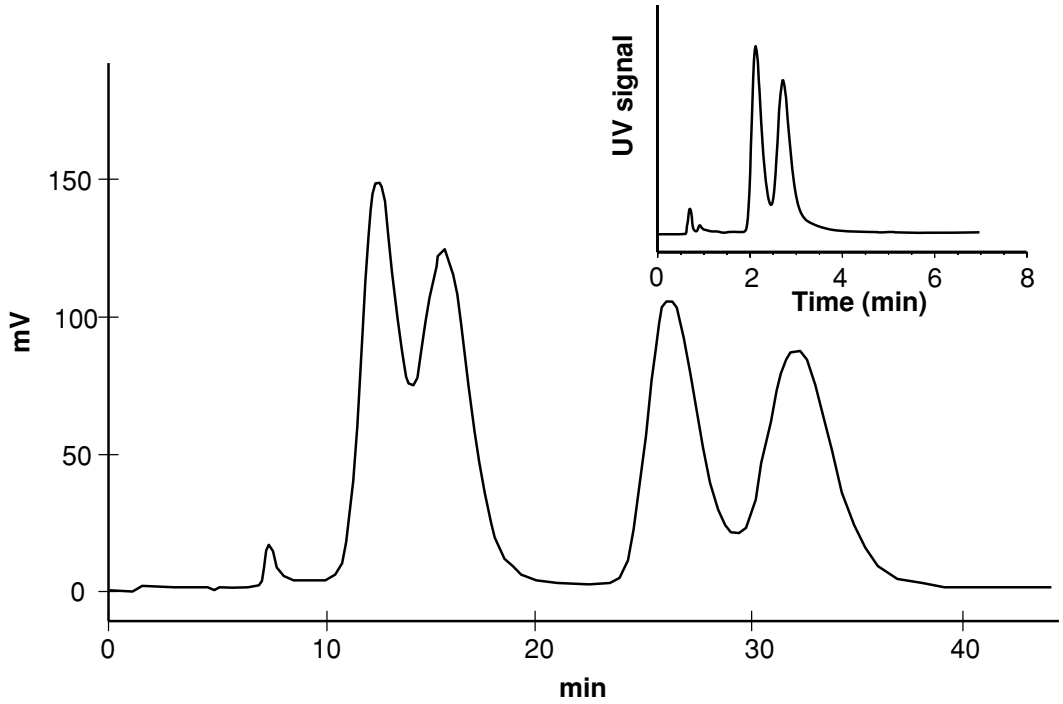

Figure 5.29 Separation of the enantiomers of a second batch of a non-natural amino acid. Chiralpak AD 500 g (20 μm) – Dynamic axial compression: 50 mm i.d. × 410 mm. Mobile phase: 50% 2-propanol in *n*-heptane containing 0.5% trifluoroacetic acid. Flow rate: 80 mL/min. Detection: UV 225 nm. Sample amount: 100 mg dissolved in 40 mL of the eluent (= 0.2 mg of product/g of CSP). *Inset*: Analytical chromatogram. Chiralpak AD (10 μm): 50 mm × 4.6 mm i.d. Mobile phase: 50% 2-propanol in *n*-heptane containing 0.5% trifluoroacetic acid. Flow rate: 1.0 mL/min. Detection: UV 225 nm.

reversed-phase chromatography. The earlier problem had arisen on preparative chromatography of a second, apparently chemically pure synthesis batch of a product previously easily purified by chromatography. After two or three injections, the separation had been completely lost and could only be recovered by rinsing the column with water/ethanol, although it had disappeared again in the next few injections. Purification using silica gel did not help, but after a reversed-phase purification step the total amount of product had been easily separated using a fully automated process. The problem was finally tracked to the presence of a catalyst in the product. Unfortunately, in the present case no difference in chromatographic profile could be observed after purification with reversed-phase chromatography.

Because the result on the amylose derivative was not that excellent to separate a larger amount of product, some additional analytical experiments were done using a Chirobiotic column (Astec) and a quinine derivative grafted onto an experimental monolithic silica (Merck, Darmstadt). The best resolution that could be achieved on the Chirobiotic column was certainly not sufficiently promising to perform a larger scale preparative chromatographic separation. However, a much better separation was observed on the quinine derivative.

Therefore, some preparative separation experiments were done on an identical 20 mm i.d. monolithic column. Unfortunately, even for relative small injection amounts, a distorted peak shape resulting in only a partial resolution of the enantiomers was observed on this

larger column. This phenomenon was probably due to the bad solubility characteristics of the product (relatively large injection volumes had to be used and it was necessary to dissolve the sample in a solvent mixture which was completely different from the eluent composition). After these experiments, we finished the analytical investigations on this product and it was decided in consultation with the chemists to separate only the amount of product (a few grams) that was absolutely necessary to finish the planned pharmacological experiments by means of 100 mg injections (two cycles) on the 50 mm i.d. column filled with 500 g of Chiralpak AD material.

5.5.4 *Separation of an 'indole' derivative*

In this example we had to separate a second 0.4 kg batch of a product that had already been done before on a similar scale without any problems. Therefore, the separation of the second batch was started using the same experimental conditions as before. In this separation, we had to deal with the problem that after a certain number of injections, the process was interrupted because the system could not detect any longer the peak recognition points within the initially established UV windows. A preparative chromatogram originating from the separation of the first batch is depicted in Fig. 5.30.

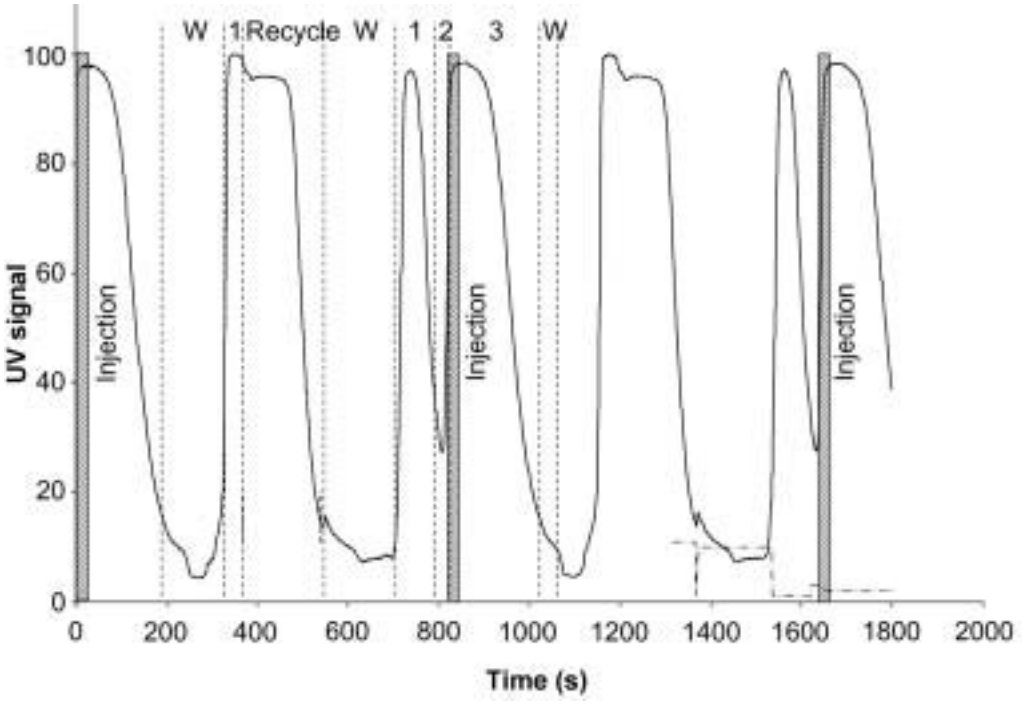

Figure 5.30 Preparative separation of an indole derivative. Chiralcel OJ 2000 g (20 μm) – Dynamic axial compression: 110 mm i.d. Mobile phase: 30% ethanol in *n*-heptane. Flow rate: 750 mL/min. Detection: UV 254 nm. Eluent temperature: 30°C. Column temperature: 32°C. Sample amount: 14.87 g/L in eluent. Injection volume: 400 mL (= 5.95 g of product = 2.97 mg/gram of CSP). Time between two successive injections: 824 s = 4.37 injections/h = 26 g/h = about 624 g/24 h.

To separate a 6 g amount on the 110 mm i.d. column, it was required to use the recycling–peak shaving technique to recover both enantiomers with an enantiomeric excess larger than 98%. As can be seen in Fig. 5.30, the second peak eluted in a relatively large volume and it certainly should have been possible to reduce this volume by using a plug of stronger solvent as described before (see Section 5.3.3). Because the second batch had the same size as the first and the separation had to be done within a very narrow time window, no further optimisation experiments could be done and the separation was immediately started with the chromatographic parameters used to separate the first larger amount of product.

After the first shut-down of the process due to the 'Out of UV window' alarm, the product was eluted from the column and the stationary phase was rinsed using our standard procedure (ethanol–water/ethanol re-equilibration with the eluent). The process was restarted and worked well for a number of injections. Therefore, we had to find an alternative to keep the process running. The two options considered were either to rinse thoroughly the column after a certain number of runs or to use a plug of pure ethanol in each run. The second solution seemed to be very successful. Besides the effect of being able to run the process continuously, the volume of the second eluting enantiomer could be reduced to less than half the original. The only disadvantage was that compared to the separation of the first batch, the time between two successive injections increased, resulting in a smaller daily capacity (513 g instead of 624 g). However, by optimising the point of injection, we are quite sure that it is possible to compensate for this capacity loss. An overlay chromatogram of both batches, together with the location of the rinsing plug, which clearly indicates the difference in elution volume of the second peak, is given in Fig. 5.31.

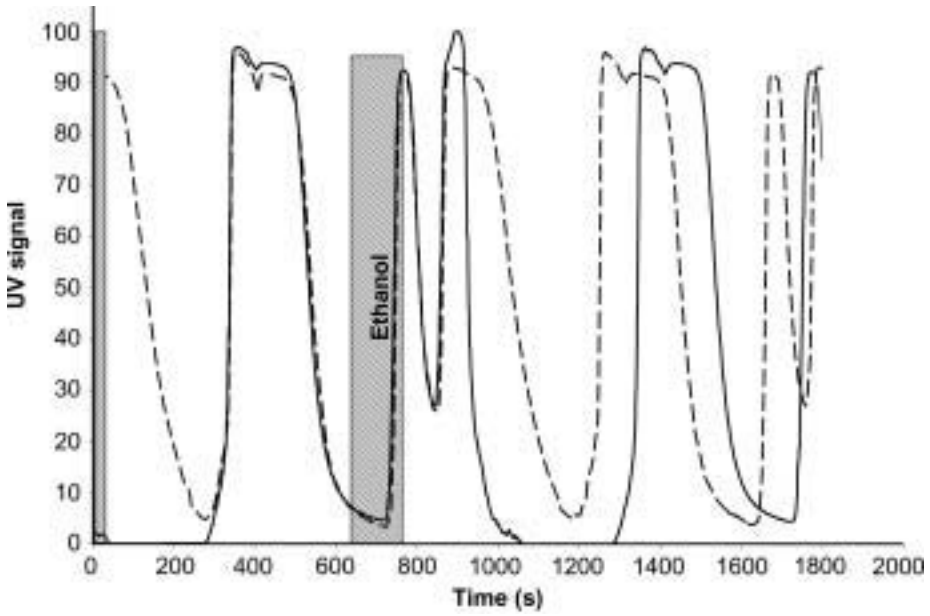

Figure 5.31 Preparative separation of an indole derivative using an intermediate ethanol solvent plug. Conditions as Fig. 5.30, except for the introduction of an ethanol flush 630 s after injection. Sample amount: 15 g/L in eluent. Injection volume: 400 mL (= 6 g of product = 3.0 mg/g of CSP). Time between two successive injections: 1010 s = 3.56 injections/h = 21.39 g/h = about 513 g/24 h.

5.5.5 *Summary*

The examples above clearly illustrate that one needs to be very careful when answering the question 'How much time is required to separate a certain amount of product?' based on the information gathered during the separation of a few hundred milligrams for medicinal chemistry purposes.

The phenomenon that it is no longer possible to separate a product using the initial separation parameters is very annoying in an analytical environment but will certainly be dramatic if it occurs when there is an urgent need to use preparative chromatography to make a certain amount of enantiomerically pure product for further pharmacological or clinical studies. Small amounts of certain impurities can have a strong influence on the separation. Furthermore, for polysaccharide derivatives, the history of the packing material can play an important role because of the supramolecular structure of the polymer which can adopt different molecular conformations in environments with different solvating properties. Therefore, the packing material has to be treated carefully to guarantee a consistent performance and a long lifetime. For the physically coated phases, rinsing the column on a regular basis with a water/ethanol mixture, followed by a rinsing step with pure ethanol has proven to be a very effective tool to keep the packing in a good condition.

Improvement of the product quality due to process optimisation can have a strong effect on the solubility characteristics of the substance. Working at higher temperatures resulting in better solubility can be helpful but often other solvents or solvent–column combinations will have to be used to be able to perform the chromatography.

Besides reducing the volume of the second eluting enantiomer the use of a plug of stronger solvent can also be very helpful to remove some interfering impurities during the execution of a continuous process.

5.6 General conclusions

A reliable analytical screening model is the basis for an efficient approach to a preparative chromatography problem. For small-scale separations an extended screening procedure takes in many cases too much time and a simplified model based on the use of a limited number of columns and mobile phase combinations has to be used. For pilot laboratory and smaller scale development work, it is in general not possible to thoroughly investigate the different chromatographic parameters that have an influence on the separation. For this type of work, the scaling-up approach is mainly driven by time constraints. To separate the desired amount of racemate within the expected time window, it is in most cases acceptable to use not fully optimised processes.

Components resulting from early development experiments can often cause some problems due to strong differences in solubility characteristics between batches. Also, small differences in chemical composition of the sample can strongly influence the column performance. For this type of product, it is certainly advisable to determine for each new batch the solubility and to test the behaviour of the product by means of some analytical experiments prior to starting preparative chromatography.

For larger scale preparative chromatography work, a thorough knowledge of the different chromatographic parameters is required. From an economic point of view, the possibility to combine preparative chromatographic enrichment with a crystallisation step has to be considered and furthermore, solvent recovery and reuse also becomes a very important issue.

Preparative Enantioselective Chromatography
Edited by Geoffrey B. Cox

6 Steady-state recycling and its use in chiral separations

CHARLES M. GRILL and LAURENCE M. MILLER

6.1 Overview

Steady-state recycling (SSR) is a chromatographic process similar to simulated moving bed (SMB) chromatography, but much simpler in design. In this chapter, we explain the ideas behind SSR and discuss briefly the operation of an SSR system. We then discuss three case studies in which SSR, SMB and conventional (batch) chromatographic techniques are compared using real-world separations. It is shown that SSR frequently occupies a middle ground between batch preparative chromatography and SMB as regards production rates and solvent usage. In general it is found that for binary separations at moderate scales (up to several hundred kilograms), SSR competes well with SMB, and is usually superior to conventional chromatographic processes.

6.2 Introduction

The necessity to generate individual enantiomers has become a growing priority in pharmaceutical R&D and manufacturing. This necessity is directly related to the increased knowledge of the effect differing enantiomers have in biological systems. There are two approaches to obtaining enantiomerically pure chemicals. These are asymmetric synthesis of the desired enantiomer and resolution of a racemic mixture into individual enantiomers. While asymmetric synthesis is useful when larger quantities of enantiomers are required, the time required to develop the synthesis can make this approach impractical when small quantities (1–10 kg) are needed. Asymmetric synthesis can also be impractical in the early stages of pharmaceutical R&D when time is of the utmost priority. An additional disadvantage of asymmetric synthesis is the generation of only one of the enantiomers. Resolution of a racemic mixture has the advantage of producing both enantiomers, essential for testing. Resolution methods include recrystallization, enzymatic resolution, indirect chromatographic resolution and direct chromatographic resolution. The time required to develop a recrystallization method or an enzymatic resolution can also make this an inefficient approach for the generation of enantiomers during early pharmaceutical R&D. Indirect chromatographic resolution, involving derivatization of the enantiomers to form a pair of diastereomers, followed by separation on an achiral stationary phase is useful for the generation of individual enantiomers [1]. While the separation of diastereomers is usually easier than the separation of enantiomers, and has the advantage of being easier to scale up, there are limitations to this approach. First, high enantiomeric purity of the derivatizing agent is essential. Also, removal of the derivative after chromatographic separation should not cause racemization of the desired enantiomer. Finally, the use of indirect chromatographic separation adds two steps to the synthesis, the reaction to form the derivatives prior to chromatography and the reaction to remove the derivatives after chromatography. The Separations Group at

Pharmacia has found the use of preparative chromatography using chiral stationary phases (CSPs) to be the most efficient approach for generation of small (1–10 kg) quantities of enantiomers during early pharmaceutical R&D as well as later in development and manufacturing when metric tons of enantiomers are required [2–6]. In past years the preparative resolution of racemic mixtures has rapidly become a standard approach for the generation of enantiomers in pharmaceutical R&D [7–12].

SMB chromatography has been utilized in the food and petrochemical areas for over thirty years. SMB is often less expensive than batch chromatography. Higher productivities and decreased solvent consumption have been observed for SMB vs batch operations. Only in the past seven years has the use of SMB for enantiomeric separations become a routine operation [12–22]. Recently, SMB for chromatographic resolution of enantiomers has been used on a manufacturing scale by numerous pharmaceutical companies [23]. Closed-loop recycling has been shown to be a useful technique in chiral separations in which resolution is poor [24]. SSR has shown promise in approaching the performance of SMB [25–28,29].

6.3 SSR – concept and operation

6.3.1 *Concept*

SSR is a binary chromatographic technique similar in some respects to SMB, although, in contrast to SMB, the feed supply is discontinuous through its cycle. A binary chromatographic process is one in which only two fractions (or product streams) are collected. In the case where the feed has more than two components, only one fraction or product stream can contain a pure component; the other would contain a mixture of the remaining components. When the feed contains only two components, as in a racemic mixture, each fraction or product stream can in principle contain a pure component.

The reason SSR and SMB are binary techniques is that feed is injected into the interior of the circulating chromatographic profile. Thus, the only possible locations on the profile where purified components could exist are at the leading and trailing edges. The interior of the profile, in relative close proximity to the feed injection point, will consist of only partially resolved components. In SMB, the raffinate is collected from the leading edge of the profile, and the extract is collected from the trailing edge. In SSR, Fraction 1 is collected from the leading edge; Fraction 2, from the trailing edge.

SMB is a continuous process in which feed is continuously injected into the system, and products (extract and raffinate) are continuously collected. To accomplish this, at least four columns, and frequently more, are required. SSR, on the other hand, uses only one column. Therefore, SSR cannot be run as a truly continuous process in the manner of SMB. Each event – collection of Fraction 1, injection of feed, collection of Fraction 2, etc. – occurs sequentially. Although not continuous, SSR is a repetitive process that is readily automated.

Both SMB and SSR reach what can be described as a periodic or cyclic steady state [27]. At steady state the amount of material collected (feed components plus mobile phase) is equal to the amount of material injected, and the profiles, when measured by a detector, are virtually identical from cycle to cycle. SSR and SMB processes will always reach steady state, but attainment of steady state does not guarantee that the purity requirements will

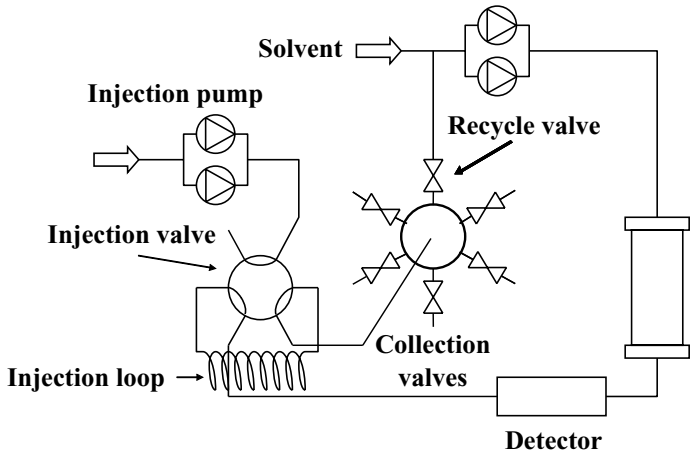

Figure 6.1 Schematic diagram of a typical SSR system.

be achieved. The art and science of the two techniques is to assure that at steady state the purity, recovery and productivity requirements of the project are met.

6.3.2 *Operation*

Figure 6.1 shows a schematic diagram of a typical SSR system. It is basically a closed-loop recycling system to which has been added an automated injection pump/loop. An SSR process consists of the following steps:

(1) As the leading edge elutes from the column it is collected through one of the collection valves as Fraction 1.
(2) At the appropriate time, the Fraction 1 collection valve closes and the recycle valve opens. This ends the collection of Fraction 1 and sends the unresolved part of the profile through the mobile phase pump and back onto the column.
(3) At the appropriate time, the profile is diverted through the injection loop (which has previously been filled with feed) by switching the injection valve to the inject position. This has the effect of putting a pulse of feed into the interior of the profile.
(4) As the profile continues to elute from the column, Fraction 2 is collected at the appropriate time by closing the recycle valve and opening the Fraction 2 collection valve. The column's effluent continues to flow through the injection loop until all of the profile has eluted from the column and the detector registers a baseline situation.
(5) When the detector registers baseline, the injection loop is switched to the load position, and the loop is refilled with feed. (If the loop had been switched to the load position prior to the baseline situation, some of the profile would have been captured by the loop and lost to loop waste when the loop was filled with feed.)
(6) The next cycle begins with step 1 above.

Figure 6.2 shows a typical situation in which an SSR separation is evolving toward steady state. The separation is a reverse phase separation of methyl and propyl *p*-hydroxybenzoate, but the appearance of the profiles is similar to those seen in chiral separations. Methyl

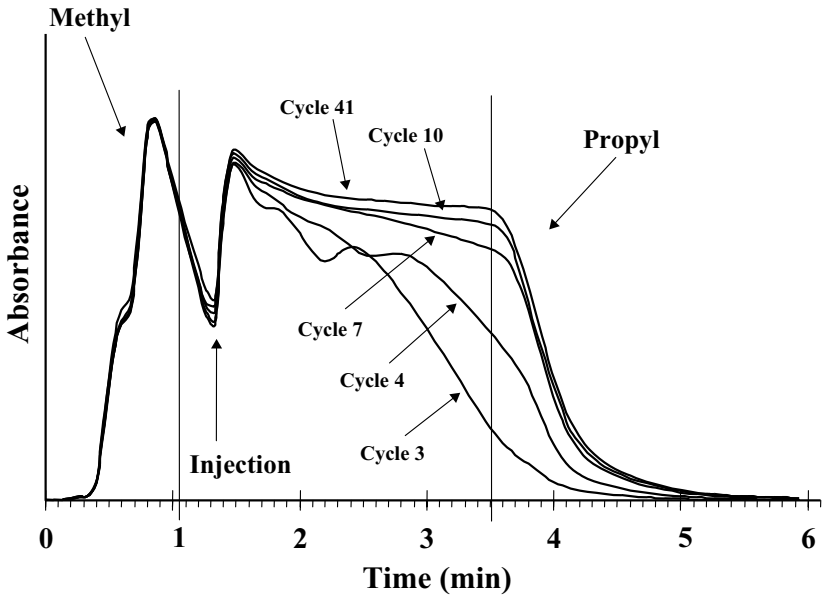

Figure 6.2 SSR separation of methyl and propyl *p*-hydroxybenzoates. Superimposed SSR chromatograms for Cycles 3, 4, 7, 10 and 41, illustrating the evolution toward steady state. Sample: methyl and propyl *p*-hydroxybenzoates, each 30 mg/mL, dissolved in methanol/water (80:20). Injection volume, 5.0 mL containing 150 mg of each component. Mobile phase, methanol/water (90:10); flow rate, 20 mL/min; cycle time, 6.1 min.

p-hydroxybenzoate elutes first and is collected first. Feed is then injected into the profile, as the profile is diverted through the injection loop. At the appropriate time propyl *p*-hydroxybenzoate is collected. When the detector registers baseline, the injection valve is returned to the load position, and the loop is filled with feed.

It can be seen that more than 10 cycles were required to reach steady state, illustrated by the trace for cycle 41. During this time the concentration of propyl *p*-hydroxybenzoate increases until at steady state the amount of propyl *p*-hydroxybenzoate collected equals the amount injected. Methyl *p*-hydroxybenzoate, on the other hand, reaches steady state at a much earlier cycle. The superposed chromatograms of cycles 36–41 (not shown) are virtually identical, indicating that steady state has occurred. Details of this separation, including method development, and further general information on the operation of the SSR process are included in Ref. [25].

6.3.3 *Role of the injection loop*

To understand why the injection loop is needed, consider what would happen if the loop were not present and we injected feed into the interior of the profile. When we inject feed in this manner, we add a volume of new fluid to the system. Because liquids are incompressible, we must open a collection or waste valve to allow fluid to leave the system to make room for the new fluid that is being injected. All extra-column components – pumps, detector, collection valves and the valve by which the leaving fluid exits the system – must be located somewhere between the outlet of the column and the inlet of the column. Since during injection the mid-portion of the chromatographic profile would be eluting from the outlet

of the column and would be recycled through the inlet of the column, the part of the system between the outlet and the inlet of the column would be filled with the unresolved (middle) portion of the chromatographic profile. Thus, when we open a valve during injection, part of this unresolved portion of the chromatographic profile would be lost through the valve and cannot be recycled.

An injection loop solves this problem by adding volume to the system at the time of injection to compensate for the volume of feed injected. In the interval between the elution of the tail of the profile and the elution of the front of the recirculated profile, only pure mobile phase is eluting from the outlet of the column. The injection valve is still in the inject position because feed had been injected earlier in the cycle. Thus the loop fills with pure mobile phase. During this interval, the injection valve is switched to the load position, and the injection pump turns on, filling the loop with feed. The pure mobile phase that had been in the loop is sent to waste, displaced by the incoming feed. Therefore, only pure mobile phase leaves the system as a result of filling the loop – no part of the chromatographic profile is lost.

The actual injection of the feed occurs later when the mid-portion of the chromatographic profile elutes from the column. During injection, the injection valve is switched to the inject position, and the chromatographic profile is diverted through the injection loop. This has the effect of placing a fresh slug of feed precisely at the correct spot in the chromatographic profile. During injection, the volume of the injection loop is actually added to the system. Thus, during injection no fluid has to leave the system to make room for the new fluid that is being injected, and none of the unresolved portion of the chromatographic profile is lost. All of the unresolved part of the profile is recycled.

The injection valve stays in the inject position from the time of injection until the entire profile has eluted from the outlet of the column and recycled through the inlet of the column. At this point all of the chromatographic profile is on the column and the detector records a baseline condition. If the injection valve were switched to the load position while the chromatographic profile was still eluting from the column, some of the chromatographic profile would be captured by the loop and would be sent to waste during the filling of the loop with feed.

6.4 Case studies

We will discuss three case studies that have been published in the scientific literature or have been presented at symposia. In these studies SSR results will be compared to those of SMB and other chromatographic techniques.

6.4.1 *Case study 1*

A detailed account of this study can be found in Ref. [26]. In this study the separation of a pharmaceutical intermediate was performed by SSR, SMB and by batch chromatography. The main purpose of the study was to determine the underlying mechanism of the SSR process. Another purpose was to compare the solvent usage and recoveries achieved with the three techniques under conditions in which the production rates were similar. The purity specification of the project was $\geq$98% ee for both enantiomers.

For all techniques the stationary phase was Chiralpak AS. Acetonitrile was the mobile phase for the SMB and batch chromatography runs. For the SMB run, the particle size of the CSP was 20 μm. Two batch chromatographic runs were performed. In one the CSP particle size was 10 μm, and in the second run the particle size was 20 μm.

For the SSR separation, two CSP–solvent systems were used: (1) 10 μm Chiralpak AS and 90:10 acetonitrile/methanol and (2) 20 μm Chiralpak AS and acetonitrile. The SSR control parameters used in the runs of this case study are shown in Table 6.1. In all the case studies reported here, the relative time method [25] was used to determine the initiation times of collection and injection events in the SSR runs. In this method, the leading edge of the profile was detected by setting a time window in which the control software looked for a detector response (e.g. ascending height or ascending slope) of a certain value selected and set by the operator. When this condition was met, the collection valve for Fraction 1 was opened and a timer was started; the times of all other collection and injection events were then defined relative to the opening of the Fraction 1 collection valve (hence the term 'relative time').

6.4.1.1 *SSR separation on 10 μm Chiralpak AS* For these experiments, the CSP was 10 μm Chiralpak AS, the column dimensions were 20 mm i.d. × 250 mm, and the mobile phase was 90:10 acetonitrile/methanol. The feed solution concentration was 12 g/L of racemate, and a feed volume of 5.0 mL, i.e. 60 mg of racemate, was injected through the injection valve each cycle.

Using the methods development technique described previously [25], we obtained the SSR conditions (shown in Table 6.1) used in the initial attempt. Under these conditions, Enantiomer 1 was isolated at >99.9% ee and Enantiomer 2 at 96.5% ee. Thus Enantiomer 1 met the purity specification, but Enantiomer 2 did not. Figure 6.3 shows a steady-state UV chromatogram (Cycle 20) of this initial run. Superimposed on the UV chromatogram in Fig. 6.3 is a graph called the profile analysis. The profile analysis, produced by slicing Cycle 21 into equal fractions taken every 10 s and analyzing them by HPLC, shows the distribution of the enantiomers throughout the profile.

In an attempt to improve the purity of Enantiomer 2, the injection point was moved 0.3 min to the left (toward Fraction 1) to 1.1 min after the start of Fraction 1. All other parameters remained unchanged. It was hypothesized that by providing more sorbent between the injection point and Fraction 2, resolution would increase in this region, thus decreasing the amount of Enantiomer 1 bleeding into Fraction 2. The resulting steady-state UV chromatogram (Cycle 20) and profile analysis (Cycle 21) are shown in Fig. 6.4. The purity of Enantiomer 1 has decreased somewhat, but at 99.0% ee still exceeds the requirement of 98% ee. The purity of Enantiomer 2 has increased to 97.2% ee but was still below the specified purity limit.

Comparison of the profile analysis data for the initial and second experiments (Figs. 6.3 and 6.4) shows that for the second run the profile of Enantiomer 1 appears to be sharper, and in the region of Fraction 1 it appears to be more concentrated. However, the slope of the Enantiomer 2 concentration profile at the boundary of Fraction 1 appears to be steeper in the second experiment and elutes earlier (1.6 min instead of 1.9 min). Thus, the concentration of Enantiomer 2 is higher at the Fraction 1 boundary, and this is probably the cause of the slight decrease in purity of Enantiomer 1.

Table 6.1 Conditions and event times for the SSR processes of Case study 1

		Run 1[1], Fig. 6.3		Run 3[1], Fig. 6.5		Run 1[2], Fig. 6.6		Run 2[3], Fig. 6.8	
Event	Description	Absolute time (min)	Relative time (min)	Absolute time (min)	Relative time (min)	Absolute time (min)	Relative time (min)	Absolute time (min)	Relative time (min)
1	Inject valve to load position, valve W opened	0.0	–	0.0	–	0.0	–	0.0	–
2	Load injection loop	0.0	–	0.0	–	0.0	–	0.0	–
3	Collection of Fraction 1, valve F1 opened	0.35	0.0	0.43	0.0	0.23	0.0	0.27	0.0
4	End of Fraction 1	0.85	0.5	1.03	0.6	0.73	0.5	0.87	0.6
5	Injection of sample	1.75	1.4	1.53	1.1	1.33	1.1	1.87	1.6
6	Collection of Fraction 2, valve F2 opened	3.85[4]	3.5[4]	4.03[4]	3.6[4]	4.43[4]	4.2[4]	4.27[4]	4.0[4]
7	Collection of Waste 2, valve W2 opened	4.85[4]	4.5[4]	5.03[4]	4.6[4]	5.63[4]	5.4[4]	5.47[4]	5.2[4]
8	Mobile phase pump switched off	5.35	5.0	5.43	5.0	5.83	5.6	5.67	5.4

[1] CSP, 10 μm Chiralpak AS; mobile phase, acetonitrile/methanol (90:10); flow rate, 16.9 mL/min; cycle time, 5.5 min.
[2] CSP, 20 μm Chiralpak AS; mobile phase, acetonitrile; flow rate, 21.2 mL/min; cycle time, 6.0 min.
[3] CSP, 20 μm Chiralpak AS; mobile phase, acetonitrile; flow rate, 21.4 mL/min; cycle time, 5.8 min.
[4] These are the values entered into the TurboPrep control program. To determine the actual cut points of Fraction 2 and Waste 2 on the chromatograms in Figs. 6.2–6.4, subtract 0.3 min. This correction is necessary because for these events, the volume of the injection loop (5.0 mL) separates the detector and the collection valves. At a flow rate of 16.9 mL/min, this represents a lag time of 0.30 min between passage of the cut point through the detector and its arrival at the collection valve manifold. Similarly, for Fig. 6.7 (flow rate, 21.2 mL/min), subtract 0.24 min, and for Fig. 6.9 (21.4 mL/min), subtract 0.23 min.

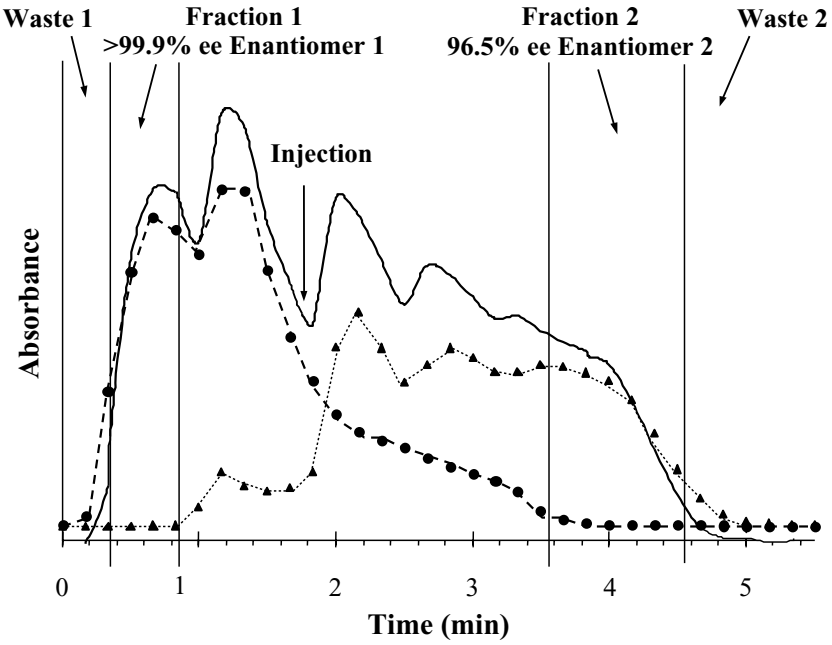

Figure 6.3 Steady-state chromatogram and profile analysis for first SSR experiment on 10 μm Chiralpak AS. Column dimensions, 20 mm i.d. × 250 mm; mobile phase, acetonitrile/MeOH (90:10); flow rate, 16.9 mL/min; cycle time, 5.5 min; injection, 60 mg racemate/cycle. See Table 6.2 for further operating parameters.

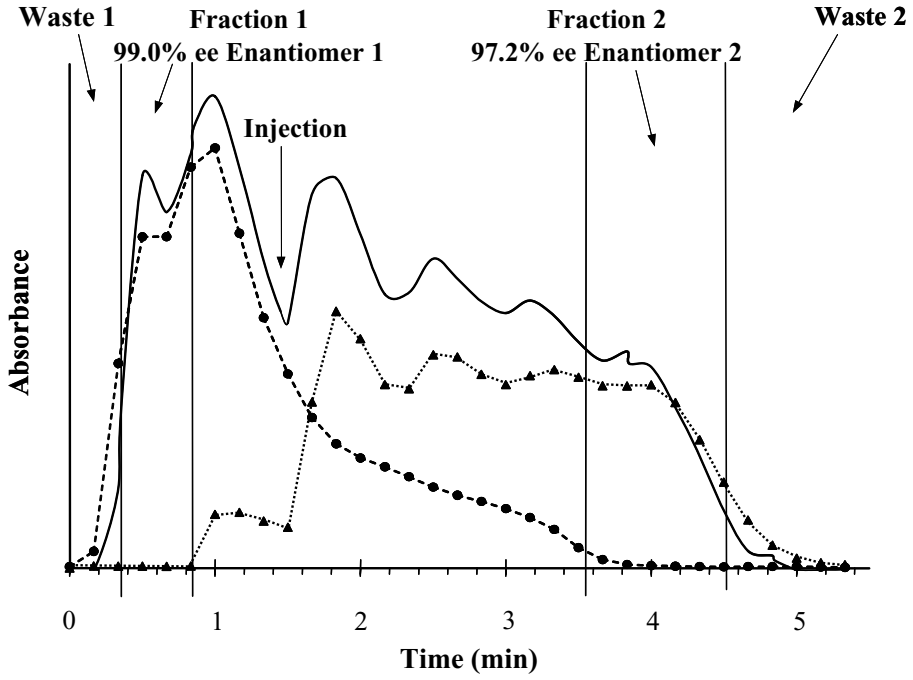

Figure 6.4 Steady-state chromatogram and profile analysis for second SSR experiment on 10 μm Chiralpak AS. Column dimensions, 20 mm i.d. × 250 mm; mobile phase, acetonitrile/MeOH (90:10); flow rate, 16.9 mL/min; cycle time, 5.5 min; injection, 60 mg racemate/cycle. See Table 6.2 for further operating parameters.

It appeared that the major cause of Enantiomer 1 contamination in Fraction 2 was that the steady-state inventory of Enantiomer 1 on the column was too large. In both previous experiments, an appreciable tail of Enantiomer 1 extended into Fraction 2. Therefore, the logical next step was to decrease this steady-state inventory of Enantiomer 1. Accordingly, the collection time of Fraction 1 was increased to 0.6 min from 0.5 min (Table 6.1, 10 μm, Final Run). This decreased the buildup of Enantiomer 1 in the early cycles so that when steady state was reached, the total amount of Enantiomer 1 on the column (the inventory of Enantiomer 1) was decreased.

Figure 6.5 shows the resulting steady-state chromatogram (Cycle 20) and profile analysis (Cycle 21). The strategy was successful: the purities of both enantiomers (Enantiomer 1, 99.8% ee; Enantiomer 2, 99.5% ee) exceed the purity requirements. Comparison of the profile analysis plots in Figs. 6.4 and 6.5 shows the reasons for this improvement in purities. The steady-state inventory of Enantiomer 1 has been substantially reduced in the final experiment, which has resulted in less Enantiomer 1 tailing into Fraction 2. Also the concentration of Enantiomer 2 at later times (including in the region of Fraction 2) has increased significantly in the final experiment.

With the benefit of several years' experience since this study was published, we can say that actions such as those performed in the second run to increase the purity of Fraction 2, i.e. moving the injection point closer to Fraction 1, will be counterproductive. Such actions will decrease the number of adsorption sites in this area and will often result in overloading this area with Enantiomer 2, thus contaminating Fraction 1 with Enantiomer 2 (see Section 6.4.1.2 for an example). In general, the first thing to try to increase Fraction 2 purity is to increase the collection time of Fraction 1. This will decrease the inventory of Enantiomer 1

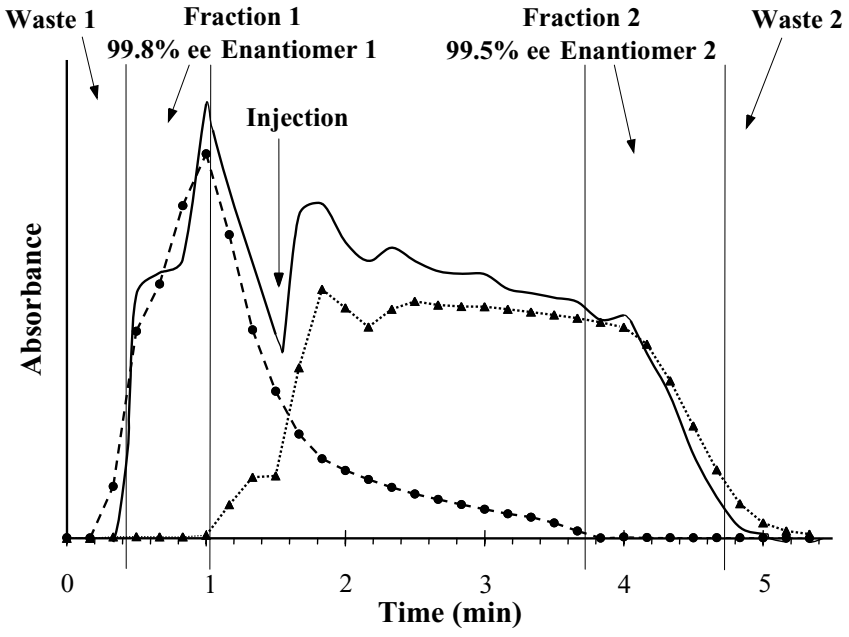

Figure 6.5 Steady-state chromatogram and profile analysis for final SSR experiment on 10 μm Chiralpak AS. Column dimensions, 20 mm i.d. × 250 mm; mobile phase, acetonitrile/MeOH (90:10); flow rate, 16.9 mL/min; cycle time, 5.5 min; injection, 60 mg racemate/cycle. See Table 6.2 for further operating parameters.

on the column and should result in less tailing of Enantiomer 1 into Fraction 2. If this fails to give the desired purity of Enantiomer 2, one must decrease the amount of feed injected.

The production rate, solvent usage, purity and recovery results for the final experiment (Fig. 6.5) are shown in Table 6.2. Discussion of these results is deferred to a later section.

6.4.1.2 *SSR separation on 20 μm Chiralpak AS* The next set of experiments was undertaken to compare the performance of the SSR process to SMB. The racemic pharmaceutical intermediate had previously been separated by SMB using 20 μm Chiralpak AS and acetonitrile. Thus, in the experiments discussed here, SSR used the same CSP and mobile phase. The approach was to approximate with SSR the production rate and purities obtained with SMB and to compare solvent usage and recoveries for the two techniques. The SSR column had dimensions of 20 mm i.d. × 250 mm. The feed solution concentration was 10 g/L of racemate in acetonitrile. Once each cycle, 5.0 mL of the feed solution (50 mg of racemate) were injected through the injection valve. The mobile phase flow rate was 21.2 mL/min, and the cycle time was 5.8 min.

The initial event times are shown in Table 6.1 (20 μm, Initial Run) and were again determined using the methods development technique reported previously [25]. Figure 6.6 shows a steady-state UV chromatogram (Cycle 30) and profile analysis (Cycle 31) for the initial run. The purity of Enantiomer 1 (92.4% ee) is lower than our specification; however, the purity of Enantiomer 2 (98.8% ee) has exceeded our requirement. As seen in Fig. 6.6, the inventory of Enantiomer 2 has built up to a high level near 1.1 min, and a significant amount of Enantiomer 2 has spilled over into Fraction 1.

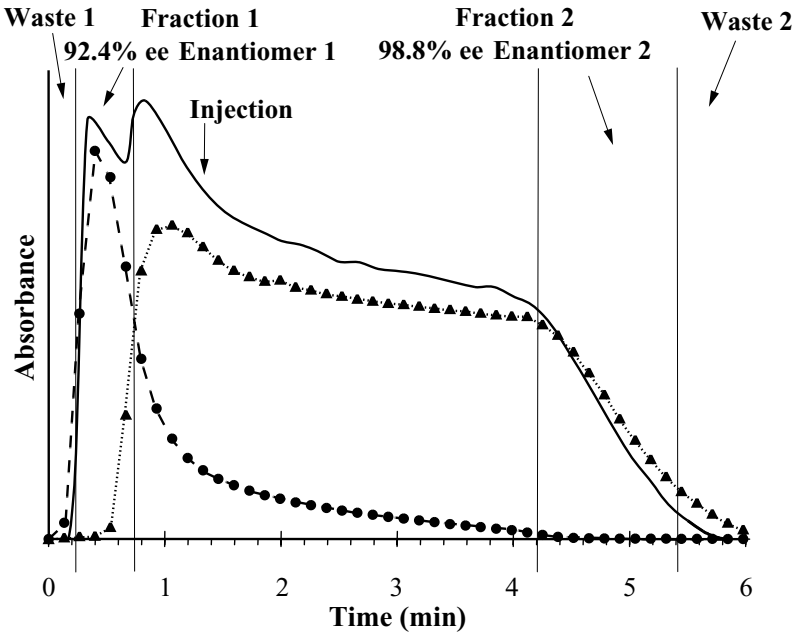

Figure 6.6 Steady-state chromatogram and profile analysis for first SSR experiment on 20 μm Chiralpak AS. Column dimensions, 20 mm i.d. × 250 mm; mobile phase, acetonitrile; flow rate, 21.2 mL/min; cycle time, 5.8 min; injection, 50 mg racemate/cycle. See Table 6.2 for further operating parameters.

The reason for this buildup in Enantiomer 2 inventory is shown in Fig. 6.7, where the chromatograms of Cycles 2–7 are plotted together. The second maximum in the chromatograms is due to Enantiomer 2 (as can be verified by comparison to the profile analysis shown in Fig. 6.6). The second maximum increases in height and moves to shorter retention times with each succeeding cycle. This behavior is indicative of Enantiomer 2 overloading in this region of the profile.

In order to improve the purity of Enantiomer 1, the overloading of Enantiomer 2 in the region between the injection point and Fraction 1 must be decreased or eliminated. One option is to decrease the quantity of racemate injected, but this would lower the production rate. Another option is to increase the number of adsorption sites in this region by moving the injection point to the right to a higher retention time. The injection point was moved 0.5 min to the right to 1.6 min. In addition, the size of Fraction 1 was increased to 0.6 min from 0.5 min (see Table 6.1). With these changes the steady-state purities of both enantiomers (Enantiomer 1, 99.2% ee; Enantiomer 2, 99.6% ee) exceeded our requirements. Figure 6.8 shows the steady-state UV chromatogram (Cycle 20) and the profile analysis (Cycle 21).

6.4.1.3 *The SMB separation* In previous work performed for Pharmacia at Chiral Technologies Europe in Strasbourg, France, SMB was used to separate the racemic pharmaceutical intermediate. The SMB system was a Licosep 12-26 (NovaSep, Nancy, France). The CSP was 20 μm Chiralpak AS (Daicel Industries, Tokyo, Japan) and was packed into 12 Superformance® glass columns (Merck KGaA, Darmstadt, Germany). The i.d. of the

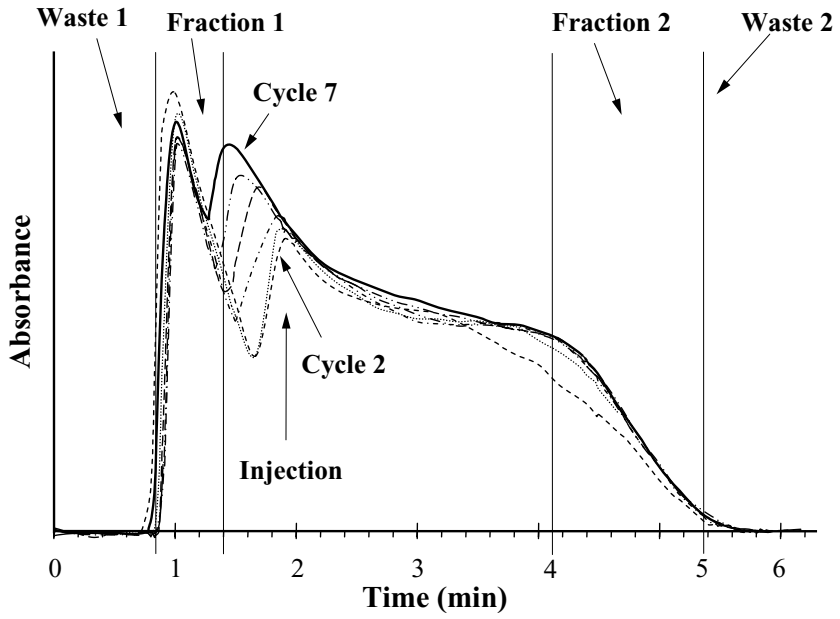

Figure 6.7 Chromatograms of Cycles 2–7 for the first SSR experiment on 20 μm Chiralpak AS, showing the evolution toward steady state in which the Enantiomer 2 inventory is increasing and shifting to lower retention times. Note that these are early cycles in the method development process, and that the Waste 1 fraction is larger than in Fig. 6.7. In later cycles, the distance between the injection point and Fraction 2 (SMB Zone II) was increased, which expanded the length of the chromatographic profile and decreased the size of the Waste 1 fraction.

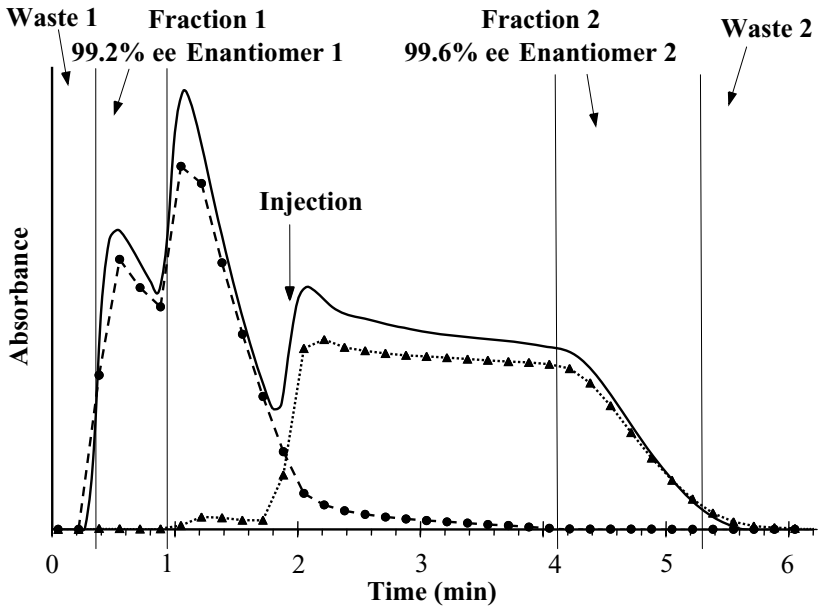

Figure 6.8 Steady-state chromatogram and profile analysis for final SSR experiment on 20 μm Chiralpak AS. Column dimensions, 20 mm i.d. × 250 mm; mobile phase, acetonitrile; flow rate, 21.2 mL/min; cycle time, 5.8 min; injection, 50 mg racemate/cycle. See Table 6.2 for further operating parameters.

columns was 26 mm and the bed length of each column was 9.5 cm. The mobile phase was HPLC-grade acetonitrile. The following flow rates were used: feed, 4.5 mL/min; eluent, 17.9 mL/min; extract, 14 mL/min; raffinate, 8.4 mL/min; and recycle, 54.0 mL/min. The concentration of racemate in the feed was 1.3 g/L. The resulting production rate and solvent usage (see Table 6.2) were not optimized, but serve as useful benchmarks for this study.

6.4.1.4 *The batch chromatography separations* Two sets of preparative HPLC experiments were performed at Pharmacia. For the first set of experiments, the preparative chromatograph consisted of two Rainin (Woburn, MA) SD-1 pumps, a Model UV-M variable-wavelength detector (set at 225 nm) and a Kipp and Zonen (Delft, the Netherlands) BD41 two-channel recorder. A Rheodyne (Cotati, CA) Model 7125 syringe loading sample injector equipped with a 10 mL loop (Valco, Houston, TX) or a Gilson Model 401 Dilutor (Middleton, WI) in combination with a Rheodyne electrically actuated Model 7010 injector was used for sample injection. The column effluent was fractionated using a Gilson Model 02 fraction collector. The column was obtained from Chiral Technologies (Exton, PA) and was prepacked with 10 μm Chiralpak AS (Daicel Industries, Tokyo, Japan). The column dimensions were 20 mm i.d. × 250 mm. The mobile phase was HPLC-grade acetonitrile, and the flow rate was 20 mL/min. The sample load was 40 mg of racemate per injection.

For the second set of preparative HPLC experiments, the preparative chromatograph consisted of two Rainin SD-1 pumps, a Model UV-1 variable-wavelength detector (set at 225 nm) and a Kipp and Zonen BD41 two-channel recorder. A separate Rainin SD-1 pump was used for sample injection. The CSP was 20 μm Chiralpak AS and was obtained in bulk form from Chiral Technologies. The CSP (1.5 kg) was packed into an 8 cm i.d. Prochrom

Table 6.2 Summary of SSR, SMB and HPLC results for Case study 1

Technique	Solvent	Particle size (μm)	Figure	Productivity (g racemate/kg CSP/day)	Solvent usage (L solvent/g racemate)	Purities (% ee)		Recoveries (%)	
						Enantiomer 1	Enantiomer 2	Enantiomer 1	Enantiomer 2
SSR	Acetonitrile/MeOH (90:10)	10	6.5	334	0.77	>99	>99	99	99
SSR	Acetonitrile	20	6.9	255	1.07	>98	>98	99	99
SMB	Acetonitrile	20	–	240	0.85	>98	>98	99	99
HPLC	Acetonitrile	10	–	327	2.0	>98	>98	93	93
HPLC	Acetonitrile	20	–	192	1.5	>98	>98	98	93

(Indianapolis, IN) dynamic axial compression column, and the final bed length was 53 cm. The mobile phase was HPLC-grade acetonitrile, and the flow rate was 300 mL/min. The sample load was 2.0 g of racemate per injection.

6.4.1.5 *Comparison of the techniques* The production rate, solvent usage, purity, and recovery results for both SSR methods (Figs. 6.5 and 6.8) are shown in Table 6.2. Also shown are the results of the SMB and preparative HPLC experiments. The SSR results compare well with the other techniques. The production rate for the first SSR method was 334 g racemate/kg CSP/day, and is higher than the production rates of any of the other procedures reported in Table 6.3. It should be emphasized that none of the SSR, the SMB or the HPLC procedures were fully optimized as to production rate. Nevertheless, an optimized SSR method using 90:10 acetonitrile/methanol should have a higher production rate than one using 100% acetonitrile as the mobile phase. The acetonitrile/methanol mixture is a stronger solvent for this separation, which implies that a higher inventory of each enantiomer can be tolerated before the column becomes overloaded. This should result in a higher production rate.

However, because none of the production rates were optimized and because all the purities obtained with each method were greater than our target of 98% ee, the most meaningful results to compare for the various techniques are solvent usage and recoveries.

SSR and SMB procedures had lower solvent usages than the HPLC methods. This is due (1) to the large amount of solvent sent to waste in the HPLC procedures, and (2) to the fact preparative HPLC fractions are more dilute than SSR fractions and SMB product streams. In SMB, there are no waste streams, only the two product streams. In SSR, the waste

Table 6.3 Conditions and event times for the SSR processes used in Case study 2

		Fig. 6.14[1]		Fig. 6.15[2]	
Event	Description	Absolute time (min)	Relative time (min)	Absolute time (min)	Relative time (min)
1	Inject valve to load position, valve W opened	0.0	–	0.0	–
2	Load injection loop	0.17	–	0.17	–
3	Collection of Fraction 1, valve F1 opened	0.18	0.0	0.25	0.0
4	End of Fraction 1	0.78	0.60	0.90	0.65
5	Injection of sample	1.18	1.00	1.25	1.00
6	Collection of Fraction 2, valve F2 opened	2.26[3]	2.08[3]	2.33[3]	2.08[3]
8	Mobile phase pump switched off	3.10	2.92	3.17	2.92

[1] Flow rate, 125 mL/min; cycle time, 3.25 min.
[2] Flow rate, 125 mL/min; cycle time, 3.50 min.
[3] These are the values entered into the LC ReSponder control program. To determine the actual cut points of Fraction 2 on the chromatogram as shown in Fig. 6.3, subtract 0.24 min. This correction is necessary because for these events, the volume of the injection loop (30.0 mL) separates the detector and the collection valves. At a flow rate of 125 mL/min, this represents a lag time of 0.24 min between passage of the cut point through the detector and its arrival at the collection valve manifold. Similarly, for Fig. 6.4 (loop size 40 mL), subtract 0.32 min.

fractions can usually be made small by judicial choice of the Fraction 2 collection point to adjust the size of the chromatographic profile. Note that the first SSR method listed in Table 6.2 had the lowest solvent usage, 0.77 L solvent/g racemate. As discussed in Ref. [26], the lower capacity factor (k') for this separation means that the profile is traveling through the column faster relative to the mobile phase than is the case when 100% acetonitrile is the mobile phase. Thus for a given cycle time (as is approximately the case for the two SSR methods discussed in this section), each collection valve (waste 1, Fraction 1, Fraction 2, waste 2) will be opened for approximately the same amount of time for the two methods. However, the flow rate will be lower for the method with the lower k'; thus, this method will use less solvent per cycle. If the production rate for the lower k' method is greater than or equal to that of the method with higher k', then the fractions of the lower k' method will be more concentrated. From another perspective, if the flow rates were the same for each method, all collection valves would be opened for less time for the method of lower k', resulting in less collection (and thus usage) of mobile phase. The cycle time would be shorter, and thus the productivity would be further enhanced.

The most interesting comparison of solvent usage is between the second SSR method listed in Table 6.2 and the SMB method, both of which used acetonitrile as the mobile phase and 20 μm Chiralpak AS as the CSP. As stated earlier, an objective in developing this SSR method was to duplicate as nearly as possible the SMB production rate and enantiomeric purities. As seen in Table 6.2, this goal was achieved, and we can therefore make a valid comparison of solvent usage and recoveries between the two techniques for this separation. At 0.85 L solvent/g racemate, SMB had a lower solvent usage than SSR (1.07 L solvent/g racemate). As we will see, it seems to be a general rule that for a given production rate, SMB will use the least amount of solvent, SSR will use somewhat more solvent than SMB and batch chromatography will use the most solvent. SSR thus appears to occupy the middle ground between SMB and batch chromatography in this regard.

The recoveries of both enantiomers were 99% for the SMB method and for both SSR methods. The recoveries are high because SMB and SSR are steady-state processes: for each cycle, everything that is injected is collected. In a two-component feed, if each fraction is highly pure, then the recovery of each component will be close to 100%. Note that the recoveries obtained with the non-steady state, batch chromatography methods were lower than those obtained with SSR and SMB.

6.4.2 *Case study 2*

Significant quantities of another pharmaceutical intermediate had to be separated using preparative chromatography, laboratory-scale SMB and process-scale SMB. Proof-of-principle experiments using SSR and closed-loop recycling were performed to determine at what scale these techniques could most effectively be employed. Acetonitrile, from various sources, was the mobile phase in each of the chromatographic techniques. In each case, the CSP was Chiralpak AS, 20 μm particle size (Daicel Industries, Tokyo, Japan). This work is reported in more detail in Ref. [28].

6.4.2.1 *Preparative chromatography* The preparative HPLC run was performed at Pharmacia (Skokie, IL) using a prepacked column (10 cm i.d. × 50 cm, 2.35 kg CSP) obtained from Chiral Technologies (Exton, PA). The preparative chromatograph was a SepTech

ST/1000 XPS system (Varian, Wakefield, RI). The flow rate was 500 mL/min. The feed solution was made by dissolving a sufficient quantity of racemate or enriched enantiomeric mixture in acetonitrile to give a final concentration of 30 g/L.

The preparative HPLC separation was carried out in two steps as shown in Fig. 6.9. In the first step 7.0 g of racemate were injected onto the column. Three fractions were collected in the first step: most of the undesired, first-eluting enantiomer; a small overlap fraction; and the rest of the profile consisting of a small amount of Enantiomer 1 and all of the desired, second-eluting enantiomer.

Fraction 3 was dried using rotary evaporation and redissolved in acetonitrile at a concentration of 30 g/L. In the second step, 4.5 g of the dried product, consisting of Enantiomer 1 and Enantiomer 2 in the weight ratio of 3:97, were reinjected onto the column. The stacked injection technique was used in each step to maximize throughput. In the first step, the cycle time was 5.5 min; in the second step, 4.5 min.

As shown in Table 6.4 the purity and recovery of Enantiomer 2 were 98% ee and 93%, respectively. The solvent usage was 0.71 L/g of racemate and the overall production rate

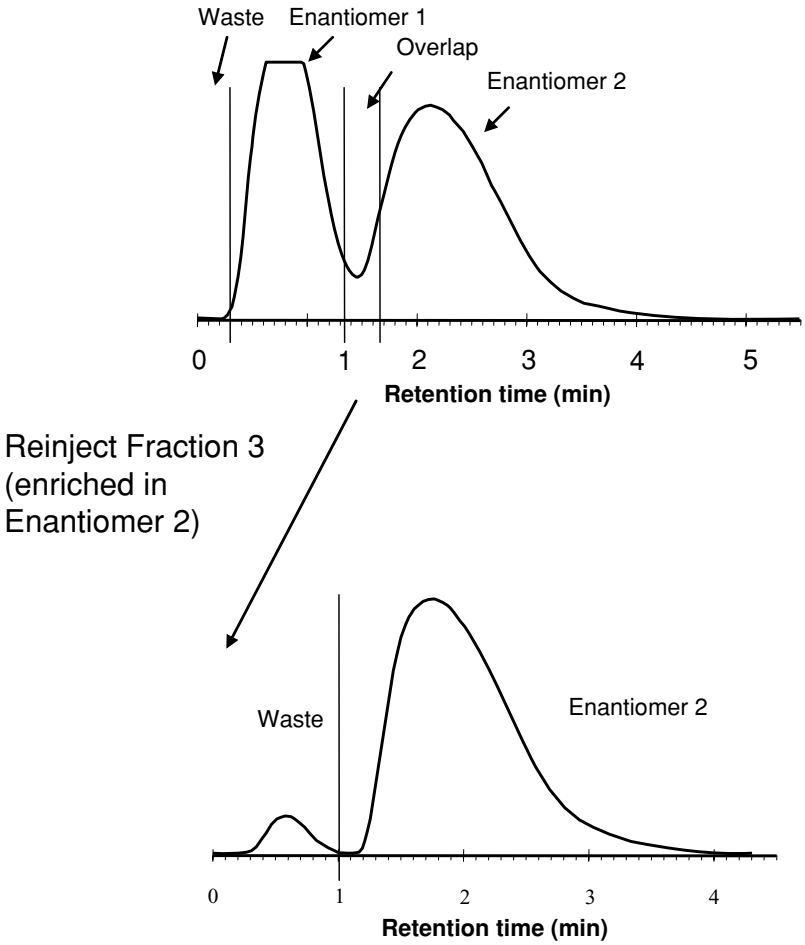

Figure 6.9 The two-step preparative process. Column dimensions were 10 cm i.d. × 50 cm. In the first step, 7.0 g of racemate were injected. In the second step, 4.5 g of Fraction 3 from step 1, which were enriched in Enantiomer 2 (93% Enantiomer 2), were reinjected after an evaporation step.

Table 6.4 Comparison of results for Case study 2

Technique	Number of columns	Column size (cm i.d. × cm)	Amount processed (kg racemate)	Production rate (g racemate/kg CSP/day)	Purity of Enantiomer 2 (% ee)	Recovery of Enantiomer 2 (%)	Solvent usage (L/g racemate)
Batch chromatography (two-step process)	1	10 × 50	1.05	435	98.0	93.0	0.71
Closed-loop recycling	1	5 × 20	0.001[1]	514	98.4	>90	0.94
SSR (Method #1)	1	5 × 20	0.135[2]	1662	98.3	94.8	0.26
SSR (Method #2)[3]	1	5 × 20	0.036[4]	2057	97.8	89.6	0.21
SMB (lab-scale, Contractor #1)	8	4.8 × 10.7	33	1454	99.5	91	0.17
SMB (lab-scale, Contractor #2)	8	4.8 × 10.7	19	1105	>98	98.4	0.16
SMB (process-scale, Contractor #3)	6	20.2 × 10.9[5] 20.2 × 8.6[6]	247	4100	98.4	93	0.11

[1] Proof-of-principle study. Only one 1200 mg injection was performed. Production rate assumes a 14 min stacked injection cycle time.
[2] Proof-of-principle study. 150 cycles performed. Steady state reached after about 10 cycles.
[3] Purity and recovery values are slightly out of specification.
[4] Proof-of-principle study. 30 cycles performed. Steady state reached after about 10 cycles.
[5] Average length of columns 1–5.
[6] Length of column 6.

for the two-step process was 435 g racemate/day/kg CSP. Because the evaporation process was faster than the chromatography process, the chromatography process never had to shut down to wait for product from the evaporator. Thus the evaporation step did not adversely affect the production rate.

6.4.2.2 *Closed-loop recycling* The SSR and closed-loop recycling experiments were performed at the Pharmacia laboratories in Skokie, IL, using a NovaPrep® 200 preparative chromatograph (manufactured by Varian, Inc., Wakefield, RI) modified to perform the SSR process. This instrument has been described elsewhere [25]. The 10 mL injection loops used in the SSR processes were made of 1/8 in. o.d. stainless steel tubing, three or four of which were connected in series to give the desired injection volume.

LC ReSponder control software (Varian, Wakefield, RI) was used to automate the system in both the SSR and closed-loop recycling modes. The preparative column used for the SSR and the closed-loop recycling runs was a Prochrom 50 mm i.d. × 500 mm column (NovaSep, Nancy, France), packed with 240 g of CSP. The bed length was 20 cm. The feed solution was made by dissolving a sufficient quantity of racemate in acetonitrile to give a final concentration of 30 g/L.

The two-step preparative process described in the last section is labor-intensive in that two automated methods, one each for steps 1 and 2, must be developed. Also, from a chromatographic perspective, this process is an inefficient method of recycling because the partial separation accomplished in the first step is lost when the profile is collected and evaporated to dryness. Closed-loop recycling with peak shaving is generally considered to be a more efficient method of recycling [24].

This separation was performed using closed-loop recycling. As shown in Fig. 6.10, 1200 mg of racemate were injected onto the 50 mm × 200 mm column. The resolution of the enantiomers increased each cycle, and the leading and trailing edges of the profile were shaved as needed to achieve the desired purity and recovery of Enantiomer 2. As shown

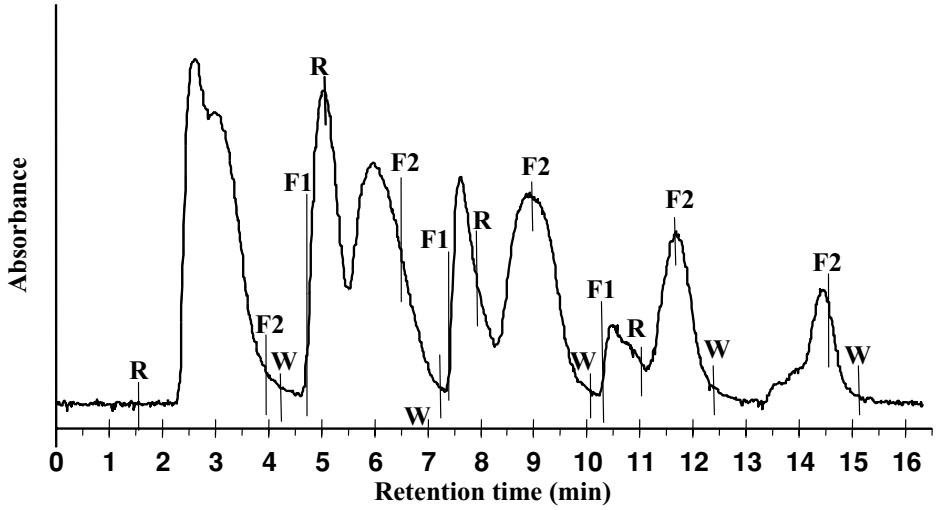

Figure 6.10 Closed-loop recycling with peak shaving in which 1200 mg of racemate were injected. Column dimensions were 5.0 cm i.d. × 20 cm. R = recycling, W = waste, F1 = Fraction 1, F2 = Fraction 2.

in Table 6.4, the purity (98.4% ee) and recovery (>90%) of Enantiomer 2 were within the project specifications. The solvent usage was 0.94 L/g of racemate and was higher than that obtained using the two-step preparative process. The productivity (514 g racemate/day/kg CSP) was calculated assuming a stacked injection cycle time of 14 min and was somewhat higher than the two-step result.

6.4.2.3 *Steady-state recycling* Two SSR methods, summarized in Table 6.3, were developed to compare SSR to the other chromatography techniques. The purpose was not to produce significant quantities of the desired enantiomer, but to determine the relative usefulness of SSR and where it might be useful for future projects.

In SSR Method #1, 900 mg of racemate were injected in each cycle (see Fig. 6.11). With a cycle time of 3.25 min, this gave a production rate of 1662 g racemate/kg CSP/day. The purity and recovery of Enantiomer 2 were 98.3% ee and 94.8%, respectively. This SSR production rate represents an increase by a factor of 3.2 relative to closed-loop recycling and an increase by a factor of 3.8 relative to the two-step preparative process. Also, at 0.26 L/g of racemate, the SSR solvent usage was significantly lower than those of the recycling techniques. These results are summarized in Table 6.4.

To ascertain the distribution of the enantiomers within the steady-state profile, fractions were collected across the profile every 0.08 min. Each fraction was then analyzed by HPLC. The results are also shown in Fig. 6.11. As shown, Fraction 1 contains some Enantiomer 2, but the recovery of Enantiomer 2 in Fraction 2 is greater than the required 90%. Enantiomer 2 is essentially pure.

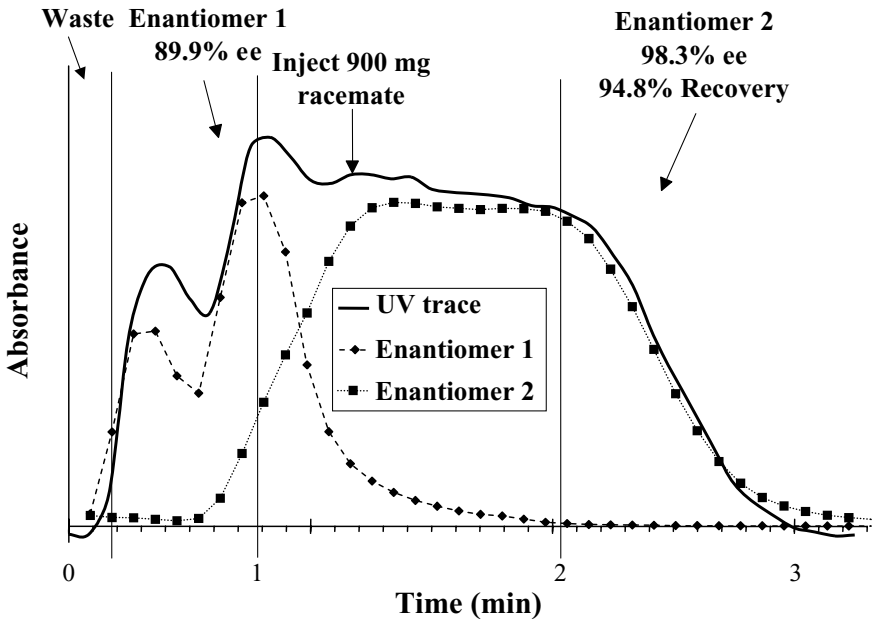

Figure 6.11 SSR Method #1 in which 900 mg of racemate were injected in each cycle. Column dimensions were 5.0 cm i.d. × 20 cm. The UV trace is indicated by the line with no symbols, the ♦ symbols [diamonds] indicate the concentration profile of Enantiomer 1 and the ■ symbols [squares] indicate the concentration profile of Enantiomer 2.

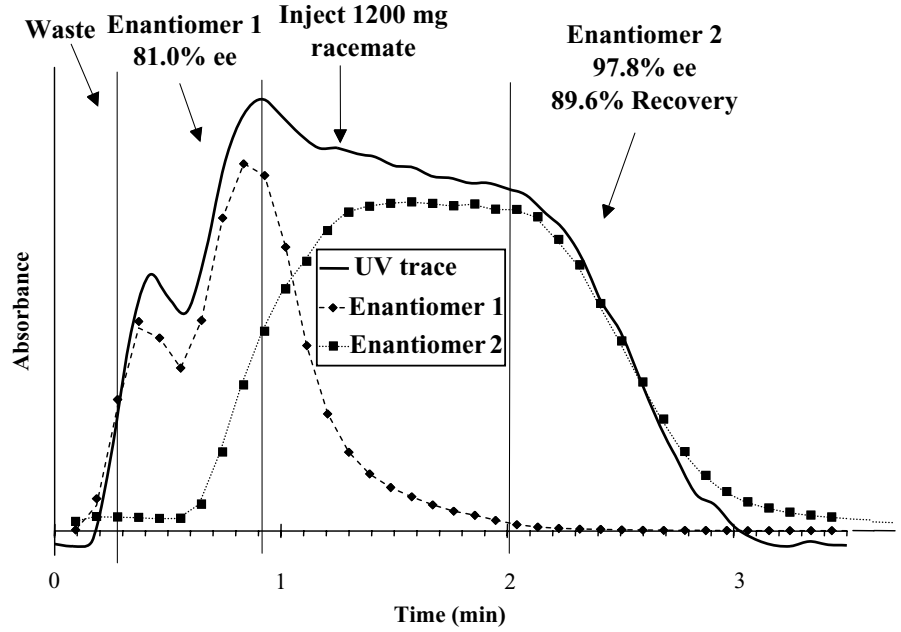

Figure 6.12 SSR Method #2 in which 1200 mg of racemate were injected in each cycle. Column dimensions were 5.0 cm i.d. × 20 cm. Note that the purity and recovery of Enantiomer 2 are slightly outside project specification of >98% ee and 90%, respectively. The UV trace is indicated by the line with no symbols, the ♦ symbols [diamonds] indicate the concentration profile of Enantiomer 1 and the ■ symbols [squares] indicate the concentration profile of Enantiomer 2.

In SSR Method #2 (Fig. 6.12), 1200 mg were injected every 3.5 min to give a production rate of 2057 g racemate/kg CSP/day. As summarized in Table 6.4, solvent usage decreased to 0.21 L/g of racemate. The purity and recovery of Enantiomer 2 (97.8% ee and 89.6%) were slightly outside project specifications. It is likely that minor adjustments, such as shifting the injection point slightly to the right and taking a slightly larger first fraction [26], would have improved the yield and purity of Enantiomer 2. Time constraints precluded further optimization. However, assuming that such minor adjustments would have been successful, the SSR Method #2 gave a production rate 4.0 times greater than closed-loop recycling and 4.7 times greater than the two-step preparative process.

6.4.2.4 *Laboratory-scale SMB separation* The laboratory-scale SMB work was performed by contractors at two different sites (Contractor #1 and Contractor #2). Both groups used Licosep Lab 8-50 SMB systems (NovaSep, Nancy, France). The CSP was 20 μm Chiralpak AS (Daicel Industries, Tokyo, Japan) and was packed into eight Self Packer columns, each with 50 mm i.d. (Merck KGaA, Darmstadt, Germany). Each column was packed with 110 g of CSP to give an average bed length of 10.7 cm. The operating conditions used by each group are summarized in Table 6.5.

The laboratory-scale SMB techniques used in this study can be described as conventional SMB in that in each case all eight columns had the same length. As summarized in Table 6.4, the laboratory-scale SMB procedure used at Contractor #1 gave a production rate of 1454 g racemate/kg CSP/day, a purity and recovery of the desired enantiomer of 99.5% ee

Table 6.5 SMB operating conditions for Case study 2

Group	SMB system	Feed concentration (mg/mL)	Temperature (°C)	Flow rates[1]					Switch time (min)
				Feed	Eluent	Extract	Raffinate	Recycle	
Contractor #1	Licosep 8-50	36.1	30	24.67	128	98.38	54.29	374.19	0.62
Contractor #2	Licosep 8-50	30	30	22.5	84.75	66.0	41.25	376.5	0.51
Contractor #3	Licosep 6-200	40	25	55	182.2	129.4	107.8	480.3	0.66

[1] Flow rates expressed in mL/min for Licosep 8-50 systems and in L/h for Licosep 6-200 system.

and 91%, respectively, and a solvent usage of 0.17 L/g racemate. The laboratory-scale SMB procedure used at Contractor #2 gave a production rate of 1105 g racemate/kg CSP/day, a purity and recovery of the desired enantiomer of >98% ee and 98.4%, respectively, and a solvent usage of 0.16 L/g racemate. As shown in Table 6.4, these results are comparable to those obtained with SSR Method #1. The solvent usage obtained with SSR Method # 1 is higher than those obtained with the laboratory-scale SMB methods.

6.4.2.5 *Process-scale SMB separation* The process-scale SMB work was performed at a third location by Contractor #3 using a Licosep 6-200 SMB system (NovaSep, Nancy, France). The CSP was packed into six dynamic axial compression columns, each with 202 mm i.d. (Merck KGaA, Darmstadt, Germany). Five of the columns were packed with 2.2 kg of CSP to give an average bed length of 10.9 cm; the sixth column was packed with 1.8 kg of CSP to give a bed length of 8.6 cm. The operating conditions are summarized in Table 6.5. The collected products were analyzed by chiral analytical HPLC.

The process-scale SMB procedure used in this study is unconventional in that one column (column 6) is shorter than the other five. This shorter column corrects for dead volume in the system caused by the recycle pump and other components [30]. As fewer columns and less CSP are required, this SMB technique frequently results in higher production rates relative to conventional SMB.

The process-scale SMB results are shown in Table 6.4. At 4100 g racemate/kg CSP/day, the process-scale SMB separation had the largest production rate. The solvent usage, 0.11 L/g racemate, was also the lowest of the techniques. The purity and recovery of the desired enantiomer, 98.4% ee and 93%, were comparable to other techniques.

6.4.2.6 *Discussion of Case study 2 results* As a general principle it should be possible to obtain the required purity and recovery of the desired components for a given separation regardless of which chromatographic technique is chosen. This is reflected in Table 6.4 in which all of the techniques, with the exception of SSR Method #2, meet the purity and recovery specifications of the separation. Thus, the most meaningful way to compare techniques is with production rate and solvent consumption results. These quantities are the most important factors in determining operating costs.

Using the criteria of production rate and solvent usage, the six-column SMB technique used in the process-scale separation is clearly the superior technique. Relative to the scaled-up eight-column SMB method used in the laboratory-scale work, the six-column method would require significantly less CSP, processing time and solvent recovery for a given throughput. Recently a new enhanced SMB process, the Varicol process, has been reported [31–33]. Preliminary modeling suggests that if the Varicol process were used, the productivity could be increased by 10%, and the solvent consumption could be reduced 7% relative to the six-column SMB process.

A comparison of the SSR and laboratory-scale SMB results is interesting. As shown in Table 6.4, the two techniques had comparable production rates. Also, the SSR solvent usage was higher than those of the laboratory-scale SMB runs, but was significantly lower than the two-step preparative process and the closed-loop recycling process. Thus the operating costs of a suitably scaled SSR system would have been somewhat higher to those encountered with the laboratory-scale SMB system, but significantly lower than those of the two-step preparative and the closed-loop recycling processes. Modeling work performed

by Contractor #3 suggests that a fully optimized eight-column SMB process would give a production rate of 2500 g racemate/kg CSP/day with a solvent usage of 0.17 L/g of racemate (comparable to, but in both respects better than, SSR Method #2). It appears at the scale of tens of kilograms of racemate where it might not be worth the effort to fully optimize an SMB separation, SSR could be a viable choice. Other factors are that for systems of comparable capacity, the complexity and capital cost of an SSR system are less than those of an SMB system.

Finally, as shown in Table 6.4, the production rate for closed-loop recycling was somewhat higher than that obtained with the two-step preparative process. However, the two-step preparative process used less solvent than the closed-loop recycling method. Thus, the results of this study show no clear advantage of closed-loop recycling over the two-step preparative process.

6.4.3 *Case study 3: collection of three SSR fractions*

As explained in Section 6.2, under normal circumstances, both SSR and SMB are binary chromatographic processes. That is, only two fractions can generally be collected: one at the leading edge of the profile and the other at the trailing edge.

Under special conditions, however, three pure or enriched fractions can be collected in an SSR process [29]. Figure 6.13 shows three cycles of the closed-loop recycling separation of the enantiomers of warfarin (no fractions were collected in this experiment). As expected, the resolution of the two enantiomers increases with each cycle. However, note the smaller peaks/shoulders at 9.5, 14 and 19 min. These peaks are due to an impurity that has a retention time of about 4.7 min. It is not seen on the first cycle, presumably because it is not resolved from the profile containing the two enantiomers. However, it can be seen on the second cycle at about 9.5 min ($\sim 2 \times 4.7$ min), on the third cycle at about 14 min ($\sim 3 \times 4.7$ min) and on the fourth cycle at about 19 min ($\sim 4 \times 4.7$ min).

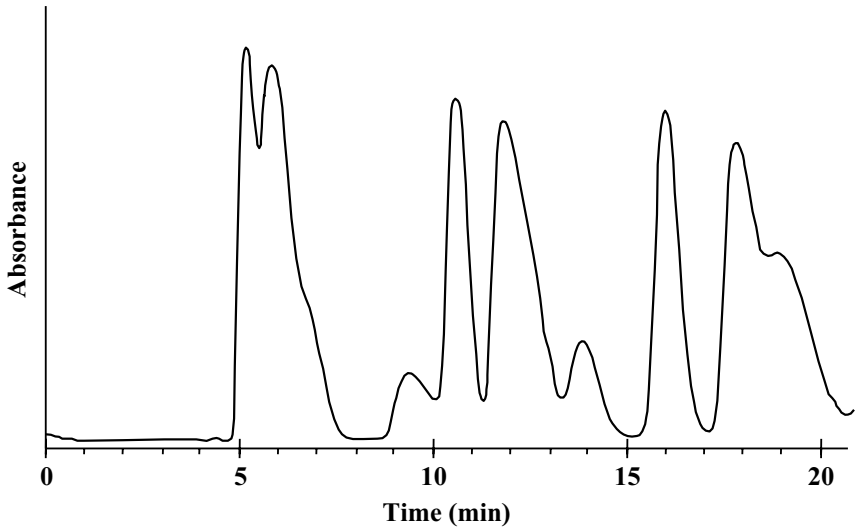

Figure 6.13 Closed-loop recycling separation of racemic warfarin. CSP: (R,R) Whelk-O 1. Column dimensions: 50 mm i.d. × 250 mm. Mobile phase, methanol; flow rate, 120 mL/min; sample load, 600 mg.

Figure 6.14 shows two consecutive cycles in the SSR separation of warfarin. Both enantiomers are collected at greater than 99% ee, and both enantiomer fractions are free of the impurity. The impurity was collected in a highly enriched form in the waste fraction. Cycle 19 begins with the leading edge of the impurity. The impurity is collected in the waste fraction, Enantiomer 1 is collected in Fraction 1, injection occurs at about 2.7 min and Enantiomer 2 is collected in Fraction 2. On this timescale, Cycle 20 begins at 6.3 min, and the next impurity peak occurs at about 7 min: about 4.5 min after the injection in Cycle 19. The impurity, having a lower retention time than Enantiomer 1, elutes between the profiles of Cycles 19 and 20 before Enantiomer 1 starts to elute in Cycle 20. The elution time of 4.5 min is slightly less than was seen in the closed-loop recycling experiment (4.7 min), probably because the column is more overloaded in the SSR run, resulting in a slight displacement of the impurity.

Another example is shown in Fig. 6.15, which shows the SSR separation of the enantiomers of a pharmaceutical intermediate. Again an impurity is seen to elute between the enantiomeric profiles. In this real-world example, each enantiomer was collected with a purity of greater than 99% ee, and both enantiomer fractions were free of the impurity.

Two aspects of the SSR technique make the collection of a third pure or enriched fraction possible. The first is that injections are pulsed in SSR. This allows impurities to travel through the column as peaks. If they happen to elute between the profiles, they can be collected in a third fraction. By contrast, in SMB systems the injection of feed is continuous. Thus, the impurity is spread out over the entire profile, and the impurity generally is collected in both fractions.

It is possible to introduce pulsed injections of feed into an SMB system. For example, one could use injection loops for each column as is done in SSR for its single column.

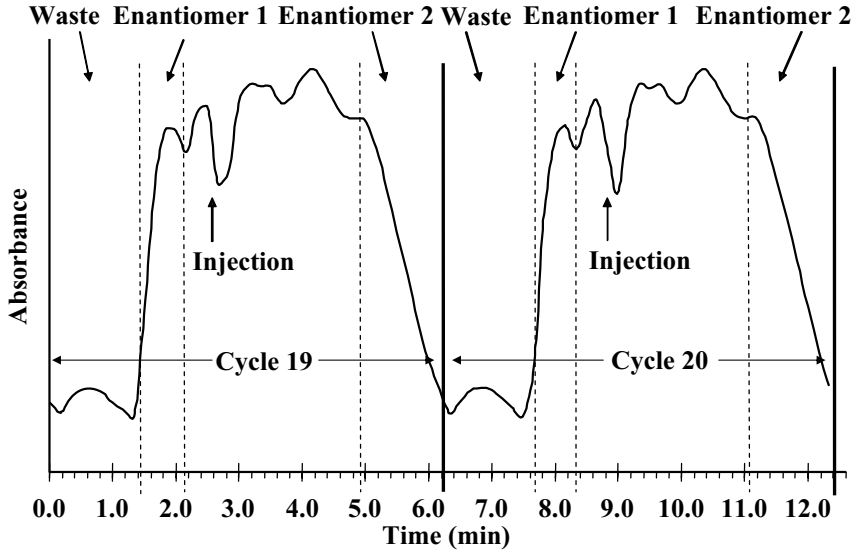

Figure 6.14 SSR separation of racemic warfarin, illustrating collection of impurity in the waste fraction. CSP: (R,R) Whelk-O 1. Column dimensions: 50 mm i.d. × 250 mm. Mobile phase, methanol; flow rate, 120 mL/min; cycle time, 6.3 min; sample load, 600 mg.

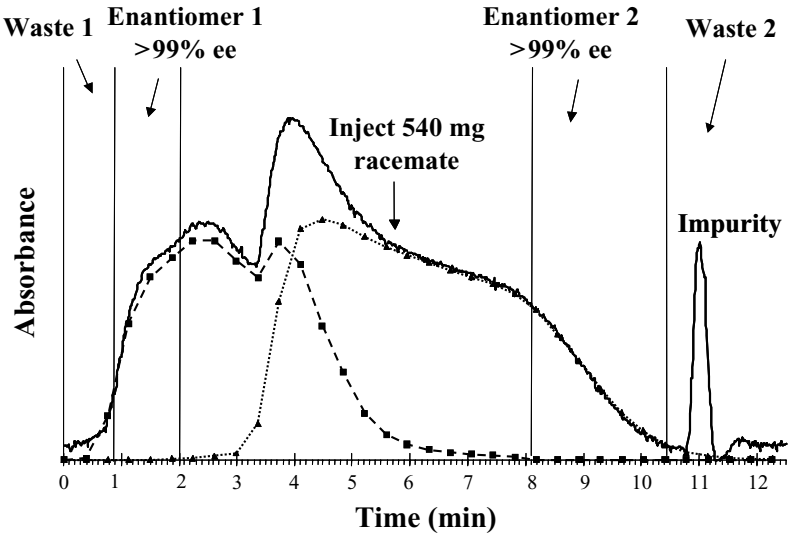

Figure 6.15 SSR separation of a racemic pharmaceutical intermediate. Note the collection of the impurity in the Waste 2 fraction, which occurs between the profiles. CSP: Chiralpak AD 20 μm. Column dimensions: 50 mm i.d. × 500 mm. Mobile phase, ethanol/diethylamine (100/0.1); flow rate, 120 mL/min; cycle time, 12.5 min; sample load, 540 mg.

Another approach is to isolate the column receiving the feed [34]. This allows the feed to form a narrow band at the top of the column. At the start of the next period, this column is reinserted into the column array, and the next column is isolated and injected. This is in effect pulse injection, and any impurity present would propagate through the column system as a peak. However, in such an SMB system, the impurity peaks cannot be collected in pure form because the raffinate stream is collected continuously. Thus, the impurity peak cannot reach the region in between the profiles, but would be swept out in the raffinate. It should be possible, however, to use a detector to monitor the raffinate stream and to divert the stream to a third fraction when the impurity peak is detected. This diverted stream would be a mixture of raffinate and impurity and thus cannot be considered a pure fraction. However, this would be a way to keep the collected raffinate stream free of the impurity.

In the SSR technique it is possible to collect a pure third fraction. This is because the fractions are collected discreetly rather than continuously. This allows the impurity peak to reach the region between the profiles and to be collected in pure or enriched form.

In order to collect a third fraction, the retention time of the impurity must be such that it will elute between the profiles. Actually, what usually happens is that the impurity peak is observed close to this region, either at the end of Fraction 2 or at the beginning of Fraction 1. One then tries to move the peak to the desired location by moving slightly the injection point. Another approach that is useful when the peak is at the end of Fraction 2 is to start the collection of Fraction 2 at an earlier time. This makes the length of the profile shorter and thus enlarges the region between the band tail of the current profile and the band front in the next. The required conditions for collecting a third peak are not usually met. However, they do occur often enough to be useful, and chromatographers using the technique should be alert to the possibility.

6.5 Conclusions

SSR is a production tool that seems ideally suited to those laboratories or pilot plants that perform many different binary separations per year. SSR compares favorably with SMB at moderate scales (up to several hundred kilograms) as to production rates, purities and recoveries. Solvent usage is somewhat higher with SSR compared to SMB, but SSR solvent usage is generally significantly lower than that obtained with conventional chromatographic methods.

Another factor that favors SSR at this scale of operation is the turnaround time between projects. SMB requires considerable effort to clean the system, and to unpack, repack and test the columns. In our laboratory, it takes about 3 person-weeks to turn around a laboratory-scale SMB system. An SSR system by contrast takes no more time to turn around than a conventional preparative chromatography system, typically 1 or 2 person-days.

Finally, there is the issue of capital costs. The SSR process is patented; thus a licensing fee must be paid. However, an SSR system (including license fee) costs about five times less than an SMB system of comparable productive scale. At large scales where an SMB system can be dedicated for months or years to a specific separation, the lower operating costs of SMB will compensate for any difference in capital costs. However, for the facility that performs many different separations per year, the lower capital cost and shorter turnaround times favor SSR.

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7 Simulated moving bed and related techniques

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7.1 Overview

In recent years, Simulated Moving Bed (SMB) technology has received considerable attention in both academic and industrial fields. The objective of this chapter is to introduce the SMB concept and to present the modeling strategies to simulate and to implement the design of SMB processes. Results will be shown, illustrating the influence of equilibrium adsorption isotherms and mass transfer resistances on SMB performance. At the end of the chapter, two examples of SMB-related techniques will be discussed: the Varicol process, to be used in SMB units with a low number of chromatographic columns; and the pseudo-SMB process, a modification of classic SMB operation to perform ternary separations.

7.2 The SMB concept

Adsorption and chromatographic processes are widely used in chemical industry for separation, purification and recovery. Extensive reviews concerning adsorption techniques with applications in the field of preparative and production scale chromatography can be found in literature [1–7]. Adsorption processes are generally more complex than the more conventional separation techniques such as fractional distillation, solvent-aided separations, or crystallization. However, separation by adsorption can be achieved for some systems, which are either difficult or impossible to obtain by other simple methods [8,9].

SMB is one of the most powerful techniques for preparative scale chromatography. The concept of SMB chromatography has been known since 1961 when the first patent by UOP (Universal Oil Products, Illinois, USA) appeared [10]. This technology was originally developed in the areas of petroleum refining and petrochemicals, and became generally known as the Sorbex process. Recently, SMB technology has found new successful applications in the areas of biotechnology, pharmaceuticals and fine chemistry. Following the increasing interest in preparative chromatographic separations, other companies developed alternative SMB schemes and applications. For example, several pharmaceutical companies and custom chemical manufacturers are installing commercial-scale SMB units for producing pure enantiomeric compounds. Large-scale chromatographic separations were in the past limited mainly because of the high cost of the adsorbent, the high dilution of products and the large amount of mobile phase needed. With the introduction of the SMB technology, large-scale separations can now be carried out under cost-effective conditions. Concerning the pharmaceutical industry and the production of enantiomeric pure products, preparative enantioselective chromatography by SMB technology is now considered as an alternative to the enantioselective synthesis or diastereoisomeric crystallization.

The concept of SMB operation is based on the countercurrent contact between the liquid and the solid phases so that the mass transfer driving force can be maximized, leading to a significant reduction in mobile and stationary phase consumption when compared to classic elution chromatography. The principle of SMB operation can, in this way, be best understood by reference to the equivalent true moving bed (TMB) process. In the ideal TMB operation (see Fig. 7.1), liquid and solid flow in opposite directions, and are continuously recycled: the liquid flowing out of section 4 is recycled to section 1, while the solid coming out of section 1 is recycled to section 4. The feed is continuously injected in the middle of the system, between sections 2 and 3, and two product lines can be continuously collected: the extract, rich in the compounds that are more retained and so preferentially carried out with the solid phase; and the raffinate, rich in the less retained species that move upwards with the liquid phase. Pure eluent is continuously injected at the beginning of section 1, with the liquid recycled from the end of section 4.

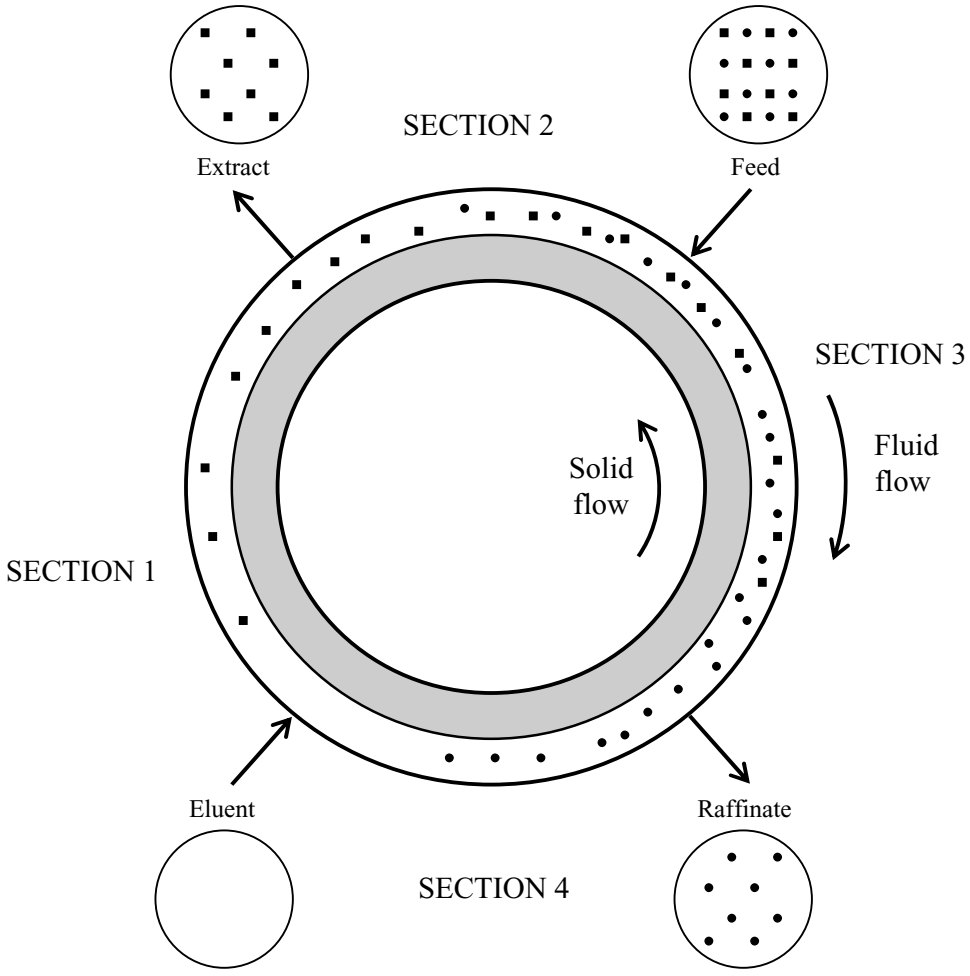


Figure 7.1 Schematic diagram for TMB operation.

Because of the addition and withdrawal of the four streams (eluent, extract, feed and raffinate), the TMB unit is divided into four sections: section 1, begins with the eluent inlet and ends in the extract withdrawal point; section 2, begins after the extract withdrawal point and ends just before the feed inlet; section 3, begins with the feed inlet and ends at the raffinate withdrawal point; and section 4, begins after the withdrawal point and ends just before the eluent inlet.

In the TMB operation, the solid flow-rate is constant all over the unit. However, because of the injection and withdrawal points, the liquid flow-rates differ from section to section. This fact enables the four sections of the unit to perform different functions. To simplify the description of the function of each section, let us consider a feed mixture containing only two components: component A, the less adsorbed species and preferentially recovered in the raffinate, and component B, the more retained species and desirably recovered in the extract (see Fig. 7.2). In sections 2 and 3, the two components must move in opposite directions. The less retained component A must be desorbed and carried with the liquid phase, while the more retained species B must be adsorbed and carried with the solid phase. Considering that in these zones the objective is to prevent the contamination of the extract and raffinate streams by the undesired component, we can summarize saying that section 2 is the zone of desorption of the less retained species A, while section 3 is the zone of adsorption of the more retained component B. In section 4 both components must be adsorbed in order to regenerate the eluent that will be recycled to the first zone. Since the component A is the less retained species, the conditions for adsorption of this component will also allow the adsorption of the more retained species. On the other hand, section 1 is the zone of solid regeneration. In this section, both components must be desorbed in order to obtain a solid phase free from both components at the beginning of this zone. Since component B is the more retained species, the conditions for desorption of this component will also allow the desorption of the less retained component.

Unfortunately, the operation of this ideal TMB unit introduces problems concerning the movement of the solid phase. A uniform flow of both solid and liquid is difficult to obtain and also mechanical erosion of the adsorbent phase will occur. In view of these difficulties, an SMB technique was developed in order to retain the process advantages of continuous and countercurrent flow without introducing the problems associated with the actual movement of the solid phase (Fig. 7.3). In the SMB system the solid phase is fixed and the

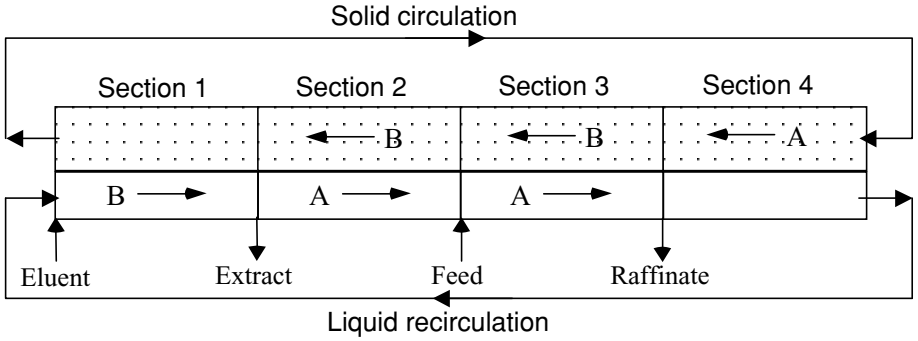


Figure 7.2 Desired net fluxes of a two species feed in each section of a TMB unit.

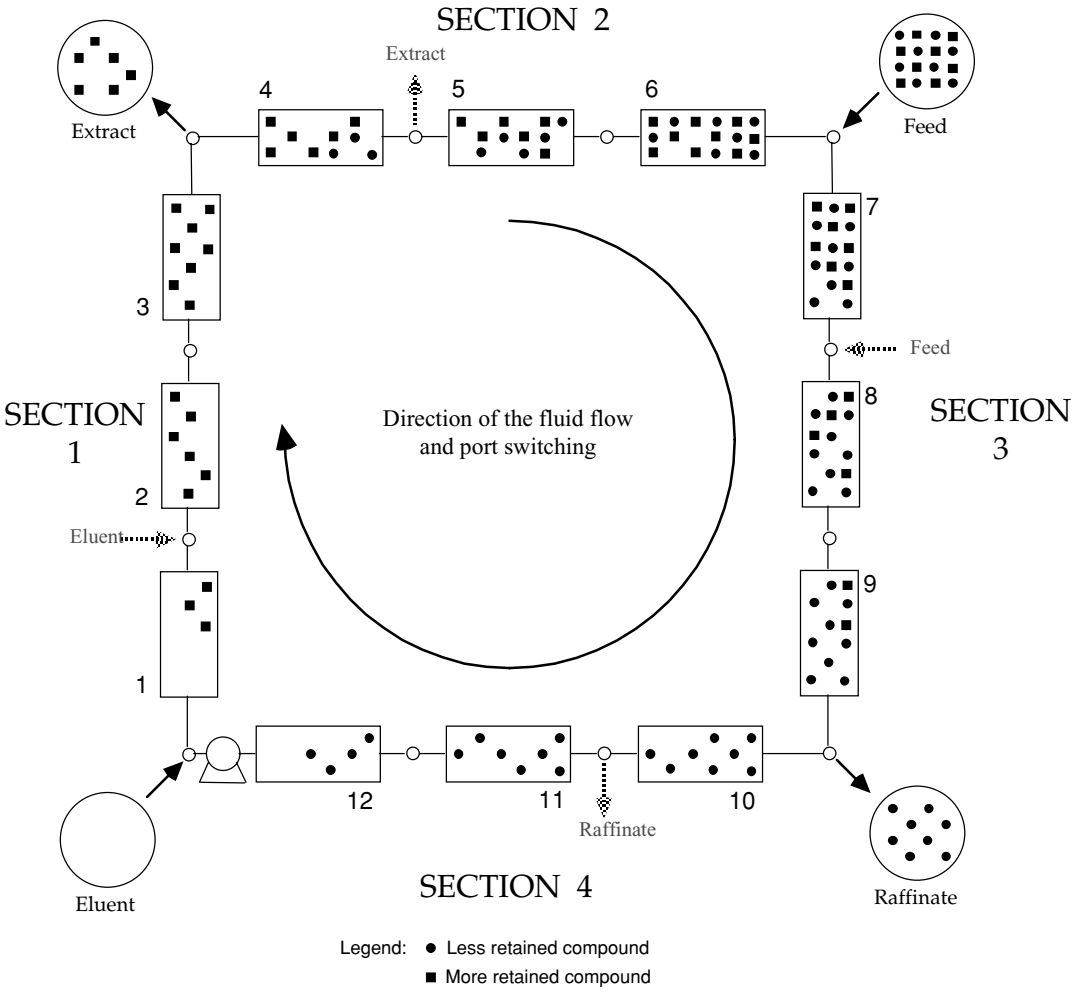


Figure 7.3 Schematic diagram for SMB operation.

positions of the inlet and outlet streams move periodically. This shift, carried out in the same direction of the liquid phase, simulates the movement of the solid phase in the opposite direction. Obviously, it is impractical to move the liquid inlet and withdrawal positions continuously. Nevertheless, approximately the same effect can be obtained by dividing the adsorbent bed into a number of fixed-bed columns and providing multiple access lines for the liquid streams between each column. Thereby, the four liquid access lines between each column can be used to perform a discrete movement of the inlet and outlet streams in the same direction of the liquid phase. In the Sorbex SMB technology developed by UOP, a complex rotary valve is used to periodically change the position of the eluent, extract, feed and raffinate lines along the adsorbent bed [10]. At any particular moment, only four lines between the rotary valve and the adsorbent bed are active. However, there are alternative techniques to perform the port switching, like the one developed by NovaSep (Vandoeuvre Les Nancy, France), a leading supplier of SMB industrial units that uses a set of individual on–off valves connecting the inlet and outlet streams to each node between columns.

7.3 Modeling of SMB processes

Following the two units described in the last section, the problem of modeling an SMB separation process can also be analyzed by two different strategies: one, by simulating the system directly, taking into account its intermittent behavior; the other by representing its operation in terms of a true countercurrent system. The first model represents the real SMB and considers the periodic switch of the injection and collection points. The second is developed by assuming the equivalence with the TMB, where solid and fluid phases flow in opposite directions. Both mathematical models are based on the following assumptions: an axially dispersed plug flow model is used to describe the fluid phase flow; a plug flow model is used to represent the countercurrent solid flow in the TMB approach; the adsorbent particles are considered as homogeneous material and mass transfer between fluid and solid is described by the linear driving force model. The adsorption equilibrium isotherms can be described by any kind of model, such as, linear, Langmuir, linear plus Langmuir, or bi-Langmuir models.

The transient SMB and TMB model equations are summarized in Table 7.1, which includes a mass balance over a volume element of the bed, a kinetic law for adsorption rate in the particle, initial and boundary conditions, global balances at each node, and the adsorption equilibrium isotherms. It should be pointed out that, owing to the switch of inlet and outlet lines, each fixed-bed column of an SMB unit travels through different sections, playing different functions during a whole cycle. In this way, the boundary conditions for each column change after the end of each switch time interval. This time dependence of the boundary conditions leads to a cyclic steady state for the SMB operation, instead of a real steady state achieved for the TMB model. In the TMB model, the solid phase is assumed to move in plug flow in the opposite direction to the fluid phase, while the inlet and outlet lines remain fixed. Model equations for the TMB model are equivalent to those presented for the SMB model, but include the term that represents the countercurrent movement of the solid phase. The resulting model parameters are the following: the ratio between solid and fluid volumes, $(1-\varepsilon)/\varepsilon$; the ratio between fluid and solid interstitial velocities, $\gamma_j = v_j/u_s$; the Peclet number, $Pe_j = v_j L_j/D_{Lj}$; the number of mass transfer units, $n_i = kL_j/u_s$; and the adsorption equilibrium parameters.

Table 7.1 Transient SMB and TMB model equations

Simulated moving bed model equations

Mass balance over a volume element of the bed k:

$$\frac{\partial C_{ik}}{\partial \theta} = \gamma_k^* \left\{ \frac{1}{Pe_k} \frac{\partial^2 C_{ik}}{\partial x^2} - \frac{\partial C_{ik}}{\partial x} \right\} - \frac{(1-\varepsilon)}{\varepsilon} n_k (q_{ik}^* - q_{ik}) \tag{7.1a}$$

Kinetic law for adsorption rate in the particle:

$$\frac{\partial q_{ik}}{\partial \theta} = n_k (q_{ik}^* - q_{ik}) \tag{7.1b}$$

Initial conditions:

$$\theta = 0: \qquad C_{ik} = q_{ik} = 0 \tag{7.2}$$

Boundary conditions for column k:

$$x = 0: \qquad C_{ik} - \frac{1}{Pe_k} \frac{\mathrm{d}C_{ik}}{\mathrm{d}x} = C_{ik,0} \tag{7.3}$$

Continued

Table 7.1 *Continued*

where $C_{ik,0}$ is the inlet concentration of species i in column k.
$x = 1$:
For a column inside a section and for extract and raffinate nodes: $C_{ik} = C_{ik+1,0}$ (7.4a)
For the eluent node: $C_{ik} = \frac{\nu_1^*}{\nu_4^*} C_{ik+1,0}$ (7.4b)
For the feed node: $C_{ik} = \frac{\nu_3^*}{\nu_2^*} C_{ik+1,0} - \frac{\nu_F}{\nu_2^*} C_i^F$ (7.4c)

Global balances:
Eluent node: $\nu_1^* = \nu_4^* + \nu_E$ (7.5a)
Extract node: $\nu_2^* = \nu_1^* - \nu_X$ (7.5b)
Feed node: $\nu_3^* = \nu_2^* + \nu_F$ (7.5c)
Raffinate node: $\nu_4^* = \nu_3^* - \nu_R$ (7.5d)

Multicomponent adsorption equilibrium isotherm:
$q_{Ak}^* = f_A(C_{Ak}, C_{Bk})$ (7.6a)
$q_{Bk}^* = f_B(C_{Ak}, C_{Bk})$ (7.6b)

True moving bed model equations

Mass balance over a volume element of the bed j:
$$\frac{\partial C_{ij}}{\partial \theta} = \gamma_j \left\{ \frac{1}{Pe_j} \frac{\partial^2 C_{ij}}{\partial x^2} - \frac{\partial C_{ij}}{\partial x} \right\} - \frac{(1-\varepsilon)}{\varepsilon} n_j (q_{ij}^* - q_{ij}) \quad (7.7a)$$

Kinetic law for adsorption rate in the particle:
$$\frac{\partial q_{ij}}{\partial \theta} = \frac{\partial q_{ij}}{\partial x} + n_j (q_{ij}^* - q_{ij}) \quad (7.7b)$$

Initial conditions:
$\theta = 0 : \quad C_{ij} = q_{ij} = 0$ (7.8)

Boundary conditions for column k:
$$x = 0 : \quad C_{ij} - \frac{1}{Pe_j} \frac{dC_{ij}}{dx} = C_{ij,0} \quad (7.9)$$
where $C_{ij,0}$ is the inlet concentration of species i in section j.
$x = 1$:
For the eluent node: $C_{i4} = \frac{\nu_1}{\nu_4} C_{i1,0}$ (7.10a)
For the extract node: $C_{i1} = C_{i2,0}$ (7.10b)
For the feed node: $C_{i2} = \frac{\nu_3}{\nu_2} C_{i3,0} - \frac{\nu_F}{\nu_2} C_i^F$ (7.10c)
For the raffinate node: $C_{i3} = C_{i4,0}$ (7.10d)
and $q_{i4} = q_{i1,0}, q_{i1} = q_{i2,0}, q_{i2} = q_{i3,0}, q_{i3} = q_{i4,0}$ (7.10e)

Global balances:
Eluent node: $\nu_1 = \nu_4 + \nu_E$ (7.11a)
Extract node: $\nu_2 = \nu_1 - \nu_X$ (7.11b)
Feed node: $\nu_3 = \nu_2 + \nu_F$ (7.11c)
Raffinate node: $\nu_4 = \nu_3 - \nu_R$ (7.11d)

Multicomponent adsorption equilibrium isotherm:
$q_{Aj}^* = f_A(C_{Aj}, C_{Bj})$ (7.12a)
$q_{Bj}^* = f_B(C_{Aj}, C_{Bj})$ (7.12b)

The equivalence between the TMB and the SMB models is made by keeping constant the liquid velocity relative to the solid velocity; that is, the liquid velocity in the SMB system is equal to the sum of liquid and solid velocities in the TMB model, $v_j^* = v_j + u_s$. Also, the solid velocity in the TMB model must be evaluated from the value of the switch time interval t^* of the SMB model, as $u_s = L_c/t^*$, where L_c is the length of one SMB column.

This implies that the internal liquid flow-rates in both systems are not the same, but related by $Q_j^* = Q_j + \varepsilon V_c/t^*$, where Q_j^* and Q_j are the internal liquid flow-rates in the SMB and TMB models, respectively, and V_c is the volume of one SMB column.

The differences between SMB and TMB model predictions, in terms of steady-state internal concentration profiles and also in terms of process performance like purity and recovery, depend on the number of subdivisions used in the SMB unit. In fact, the similarity would be perfect if the adsorbent bed of the SMB unit were divided into an infinite number of fixed-bed columns and using an infinitesimal switch time interval. Fortunately, a small subdivision of the bed is sufficient to ensure that an SMB unit performs close to the ideal TMB countercurrent operation. We concluded that the prediction of the SMB operation can be carried out through the equivalent TMB approach when the SMB unit is constituted by, at least, two columns per section (a total of eight columns) [11]. However, we should not forget that recent applications in the pharmaceutical industry use SMB systems containing a low total number of columns, for instance, a total of five or six columns. In these cases, as it was shown recently [12], the real and more precise SMB model should be used. Also, the SMB model will always be useful in characterizing the dynamic cyclic behavior of the concentration profiles. On the other hand, if the subdivision of the bed is sufficient, we shall use the simple TMB model, with obvious advantages in computing timesavings. Moreover, if we are interested to characterize only the steady-state operation, we can develop a steady-state TMB model that is simpler to implement. In fact, the original problem represented by a set of partial differential equations will be simplified to a set of ordinary differential equations. Other simplifications that can be introduced consist in neglecting axial dispersion and mass transfer resistances, leading to an equilibrium model [13,14] that, under certain kinds of adsorption equilibrium isotherms, can lead to explicit criteria for the choice of the SMB operating conditions for complete separation of a binary mixture.

7.4 Design of SMB processes

The design problem of an SMB unit consists in setting the flow-rates in each section and the value for the switch time interval to obtain the desired separation. Following the equivalence to the TMB operation, some constraints have to be met if one wants to recover the less adsorbed component A in the raffinate and the more retained component B in the extract. As it was pointed out earlier (see Fig. 7.2), these constraints can be expressed in terms of the net fluxes of each component in each section:

$$\begin{aligned} &\frac{Q_1 c_{B1}}{Q_S q_{B1}} > 1 \qquad \frac{Q_2 c_{A2}}{Q_S q_{A2}} > 1 \quad \text{and} \quad \frac{Q_2 c_{B2}}{Q_S q_{B2}} < 1 \\ &\frac{Q_3 c_{A3}}{Q_S q_{A3}} > 1 \quad \text{and} \quad \frac{Q_3 c_{B3}}{Q_S q_{B3}} < 1 \qquad \frac{Q_4 c_{A4}}{Q_S q_{A4}} < 1 \end{aligned} \tag{7.13}$$

where Q_1, Q_2, Q_3, Q_4 are the volumetric liquid flow-rates in the various sections of the TMB, Q_S is the solid flow-rate, c_{Aj}, c_{Bj} are the concentrations of species A and B in the liquid phase and q_{Aj}, q_{Bj} are the adsorbed concentrations of components A and B, in section j. The same constraints can be expressed alternatively in terms of fluid and solid interstitial velocities. Using the dimensionless model parameters defined earlier – $\gamma_j = v_j/u_s$, as the

ratio between fluid and solid interstitial velocities in zone j; and $\varepsilon/(1-\varepsilon)$, as the ratio between fluid and solid volumes, the constraints defined by Equation (7.13) become

$$\begin{aligned} \gamma_1 &> \frac{1-\varepsilon}{\varepsilon}\frac{q_{B1}}{c_{B1}} \qquad \frac{1-\varepsilon}{\varepsilon}\frac{q_{A2}}{c_{A2}} < \gamma_2 < \frac{1-\varepsilon}{\varepsilon}\frac{q_{B2}}{c_{B2}} \\ \frac{1-\varepsilon}{\varepsilon}\frac{q_{A3}}{c_{A3}} &< \gamma_3 < \frac{1-\varepsilon}{\varepsilon}\frac{q_{B3}}{c_{B3}} \qquad \gamma_4 < \frac{1-\varepsilon}{\varepsilon}\frac{q_{A4}}{c_{A4}} \end{aligned} \tag{7.14}$$

For the case of a binary system with linear adsorption isotherms, $q_{ij}/c_{ij} = K_i$ is constant, and very simple formulas can be derived to evaluate the TMB flow rates [15]. For nonlinear systems, however, the evaluation of the flow-rates is not straightforward. It is well known that the adsorption behavior must be commonly described with more complex models, such as the nonlinear competitive adsorption isotherm. For this kind of system, the adsorbed concentration of a component in equilibrium with its concentration in the liquid phase depends not only on its own but also on the concentration of all other species. This means that the ratio between the adsorbed-phase and fluid-phase concentrations that influences the net fluxes of both components in the TMB operation [see Equations (7.13) or (7.14)] is no longer constant but concentration-dependent.

Morbidelli and coworkers developed a complete design of the binary countercurrent separation processes by SMB chromatography in the frame of equilibrium theory, assuming that mass transfer resistances and axial dispersion are negligible, and that the adsorption equilibria can be described through a variable selectivity modified Langmuir isotherm [14]. The conditions to achieve complete separation were evaluated considering the equivalent TMB operation and defining a region for complete separation in terms of the flow-rate ratios in the four sections of this equivalent TMB unit. This separation region is the area in a $\gamma_3 \times \gamma_2$ plot where both extract and raffinate are pure (γ_2 and γ_3 are the ratios between fluid and solid interstitial velocities in sections 2 and 3, respectively). This plot, first proposed by Morbidelli and coworkers [13,14], is an important tool in the choice of the best operating conditions, providing that the constraints in sections 1 and 4 are fulfilled; that is, the flow-rate ratios in sections 1 and 4, γ_1 and γ_4, are chosen away from their critical values. Figure 7.4 shows a typical $\gamma_3 \times \gamma_2$ plot that can be found for a nonlinear TMB separation. Depending on the γ_2 and γ_3 values, we can find four regions: one where none of the extract and raffinate streams are pure, two regions where only one of the two streams is pure, and one region where both of the extract and raffinate streams are pure. Of course, we must operate the system with operating conditions inside this last region, called the separation region, since the natural objective is to obtain simultaneously pure extract and raffinate streams. Moreover, we should operate the system with operating conditions near the vertex point of this separation region. The vertex is the point at the boundary of the separation region most distant from the diagonal $\gamma_3 = \gamma_2$ (see Fig. 7.4), and represents the best operating conditions in terms of system productivity and solvent consumption. In fact, system productivity can be defined as the total amount of feed introduced into the system per volume of adsorbent bed and per unit of time, and is proportional to the difference $\gamma_3 - \gamma_2$.

The separation regions, originally built by using the equilibrium theory model, can also be evaluated using more precise models that take into account the resistances to mass transfer [16–20]. The presence of mass transfer resistance can affect significantly the performance of the SMB operation, reducing the size of the separation region and modifying the optimum SMB operating conditions [16,17,21]. Moreover, when mass transfer resistance

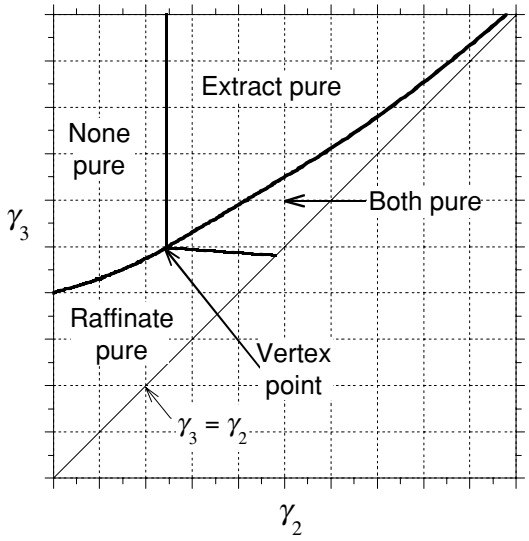


Figure 7.4 Typical $\gamma_3 \times \gamma_2$ plot for nonlinear TMB operation.

is neglected, equilibrium theory states that the critical flow-rate ratios required to achieve separation depend exclusively on the equilibrium data. However, in the presence of mass transfer resistance, those critical values are more restrictive and should be evaluated through simulation [22–24].

7.5 Simulation of SMB processes

The TMB and SMB models presented can be used to study the influence of the various model parameters on the system performance. System performance can be evaluated by using the following three main parameters: purity, productivity and solvent consumption.

(a) Purity:

$$\text{Extract purity} = \frac{C_B^X}{C_A^X + C_B^X} \tag{7.15}$$

$$\text{Raffinate purity} = \frac{C_A^R}{C_A^R + C_B^R} \tag{7.16}$$

(b) Productivity:

$$\text{pr} = \frac{Q_F(C_A^F + C_B^F)}{V_T} = \frac{\varepsilon}{N_c t^*}(\gamma_3 - \gamma_2)\left(C_A^F + C_B^F\right) \tag{7.17}$$

$$\pi = \text{pr}\frac{N_c t^*}{\varepsilon} = (\gamma_3 - \gamma_2)\left(C_A^F + C_B^F\right) \tag{7.18}$$

(c) Solvent consumption:

$$\text{sc} = \frac{Q_E + Q_F}{Q_F\left(C_A^F + C_B^F\right)} = \frac{1}{\left(C_A^F + C_B^F\right)}\left(1 + \frac{\gamma_1 - \gamma_4}{\gamma_3 - \gamma_2}\right) \tag{7.19}$$

This section will present the results obtained using the equivalent countercurrent TMB model to study the influence of two main model parameters: the adsorption isotherm parameters and the mass transfer coefficient (mass transfer resistance). A more complete study of the influence of other model parameters can be found elsewhere [16,25].

7.5.1 *Influence of the equilibrium adsorption isotherms*

A binary linear + Langmuir isotherm model is used as illustrative example:

$$q_i^* = mC_i + \frac{Qb_iC_i}{1 + b_AC_A + b_BC_B} \tag{7.20}$$

where $i = A, B$ represents the component (A is the less retained, B the more retained component). This model is often used to describe the adsorption isotherms for chiral mixtures, since it is a competitive model and can predict the concentration dependency of the selectivity factor:

$$\alpha = \frac{(q_B^*/C_B)}{(q_A^*/C_A)} = \frac{m(1 + b_AC_A + b_BC_B) + Qb_B}{m(1 + b_AC_A + b_BC_B) + Qb_A} \tag{7.21}$$

As shown by Equation (7.21), the selectivity factor depends on both species concentrations and is not constant like, for instance, for the competitive Langmuir isotherm model. At low concentrations, the adsorption isotherms are near linear and the previous equations become

$$q_{i,\text{linear}}^* = (m + Qb_i)C_i \tag{7.22}$$

$$K_i = \left.\frac{q_i^*}{C_i}\right|_{\text{linear}} = m + Qb_i \tag{7.23}$$

$$\alpha_{\text{linear}} = \frac{K_B}{K_A} = \frac{m + Qb_B}{m + Qb_A} \tag{7.24}$$

The examples shown in this section use isotherm parameters described in Table 7.2. Cases 1, 2 and 3 have the same m, Q and b_A parameters, but differ for b_B values. In consequence, K_A is the same for all cases, but not K_B, and so, α_{linear}. Figure 7.5 shows the system selectivity as a function of both species concentrations and illustrates three completely different situations.

Cases 1, 4 and 5 have the same m, but different Q, b_A and b_B parameters. However, the products Qb_A and Qb_B are the same for all situations and, consequently, they have the same K_A, K_B and α_{linear}. Figure 7.6 shows the system selectivity as a function of both component concentrations for cases 1, 4 and 5. All cases show the same selectivity in the linear range of the adsorption isotherms (low concentrations), but present different values in the nonlinear region owing to different b_i values [see Equation 7.21]. This fact will influence the form of the separation regions, mainly for higher feed concentrations. The $\gamma_3 \times \gamma_2$ separation

Table 7.2 Linear + Langmuir isotherm parameters used in simulations

	Isotherm parameters				Linear range		
Case	m	Q (g/L)	b_A (L/g)	b_B (L/g)	K_A	K_B	α_{linear}
1	1.00	10.0	0.10	0.15	2.00	2.50	1.25
2	1.00	10.0	0.10	0.20	2.00	3.00	1.50
3	1.00	10.0	0.10	0.30	2.00	4.00	2.00
1	1.00	10.0	0.10	0.15	2.00	2.50	1.25
4	1.00	5.0	0.20	0.30	2.00	2.50	1.25
5	1.00	2.5	0.40	0.60	2.00	2.50	1.25

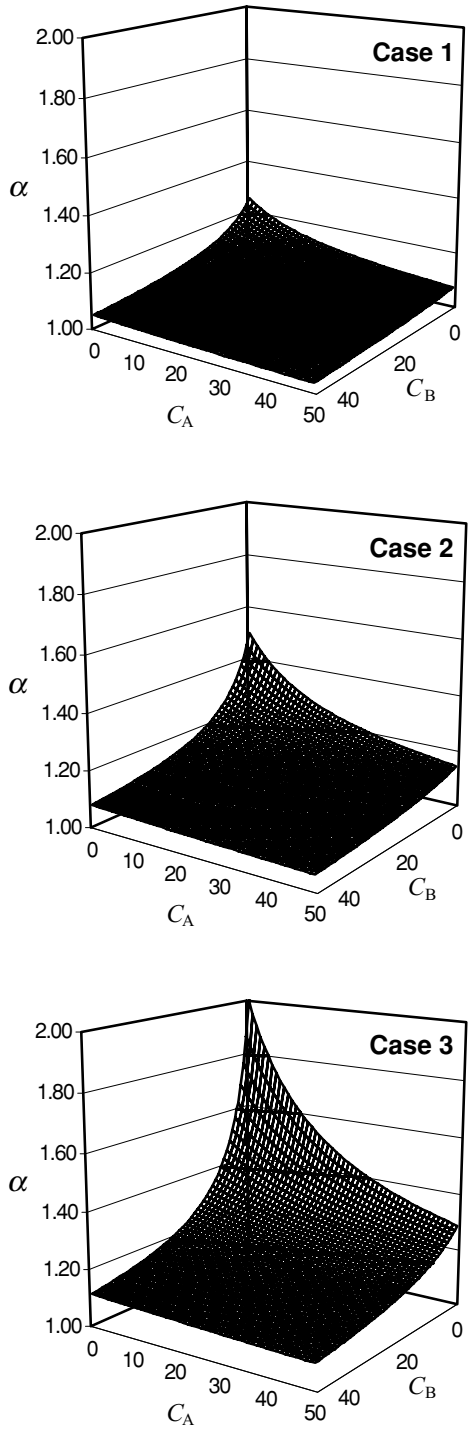


Figure 7.5 System selectivity α as a function of both species concentrations: cases 1, 2 and 3. For adsorption isotherm parameters, see Table 7.2.

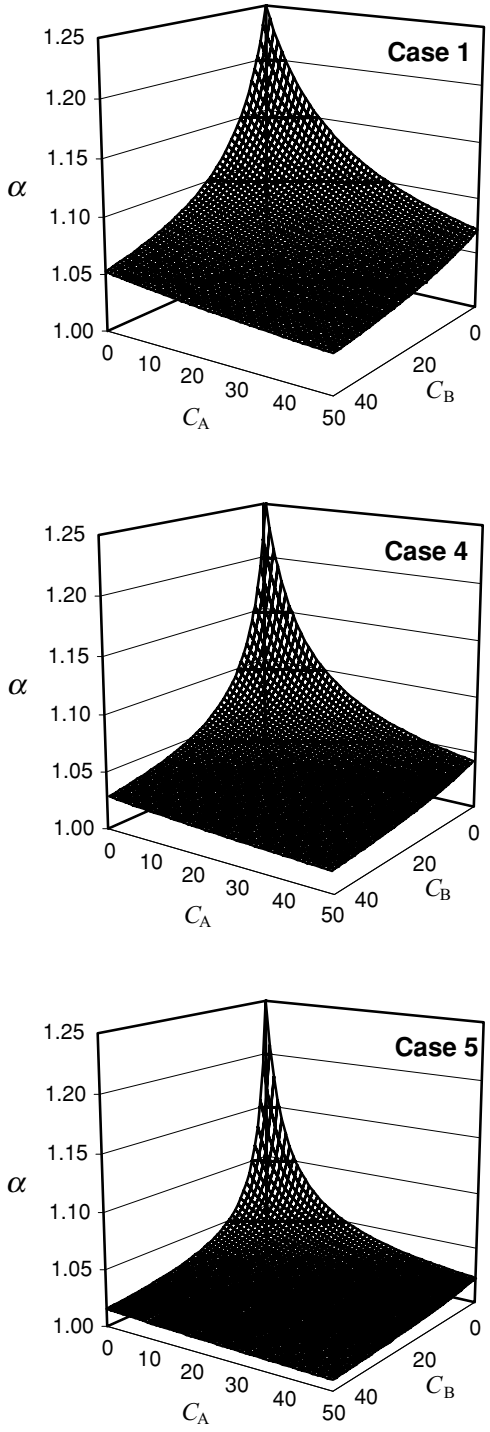


Figure 7.6 System selectivity α as a function of both species concentrations: cases 1, 4 and 5. For adsorption isotherm parameters, see Table 7.2.

regions were obtained using the equivalent countercurrent equilibrium theory model [14] (negligible axial dispersion and mass transfer resistances) with bed porosity $\varepsilon = 0.4$.

The influence of adsorption isotherm parameters on the $\gamma_3 \times \gamma_2$ separation region is shown in Fig. 7.7 for cases 1, 2 and 3, and for feed concentrations of $C_A^f = C_B^f = 0.1$, 2 and 10 g/L.

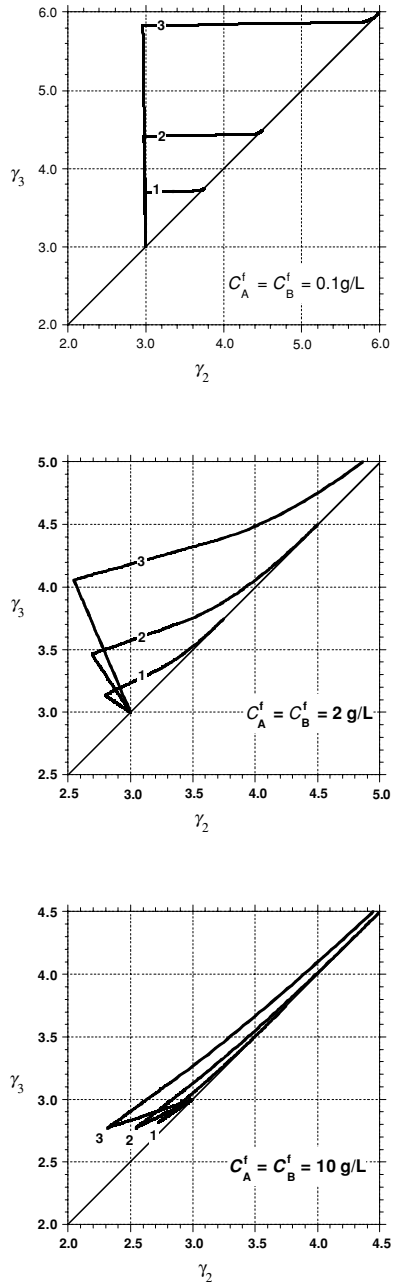


Figure 7.7 Influence of adsorption isotherm parameters on the $\gamma_3 \times \gamma_2$ separation region: cases 1, 2 and 3 for feed concentrations of $C_A^f = C_B^f = 0.1$, 2 and 10 g/L. Separation regions obtained using the equilibrium theory model. For adsorption isotherm parameters, see Table 7.2.

The typical rectangular triangles are obtained for the linear range of adsorption isotherms ($C_A^f = C_B^f = 0.1$ g/L); for higher concentrations, the area of the separation regions diminish and become more narrow and closer to the diagonal $\gamma_3 = \gamma_2$. Completely different situations are obtained for the three cases 1, 2 and 3, since adsorption isotherms are, also, significantly different. This difference also influences the system productivity and solvent consumption (see Fig. 7.8). Interesting results are obtained when we take into account the adsorption

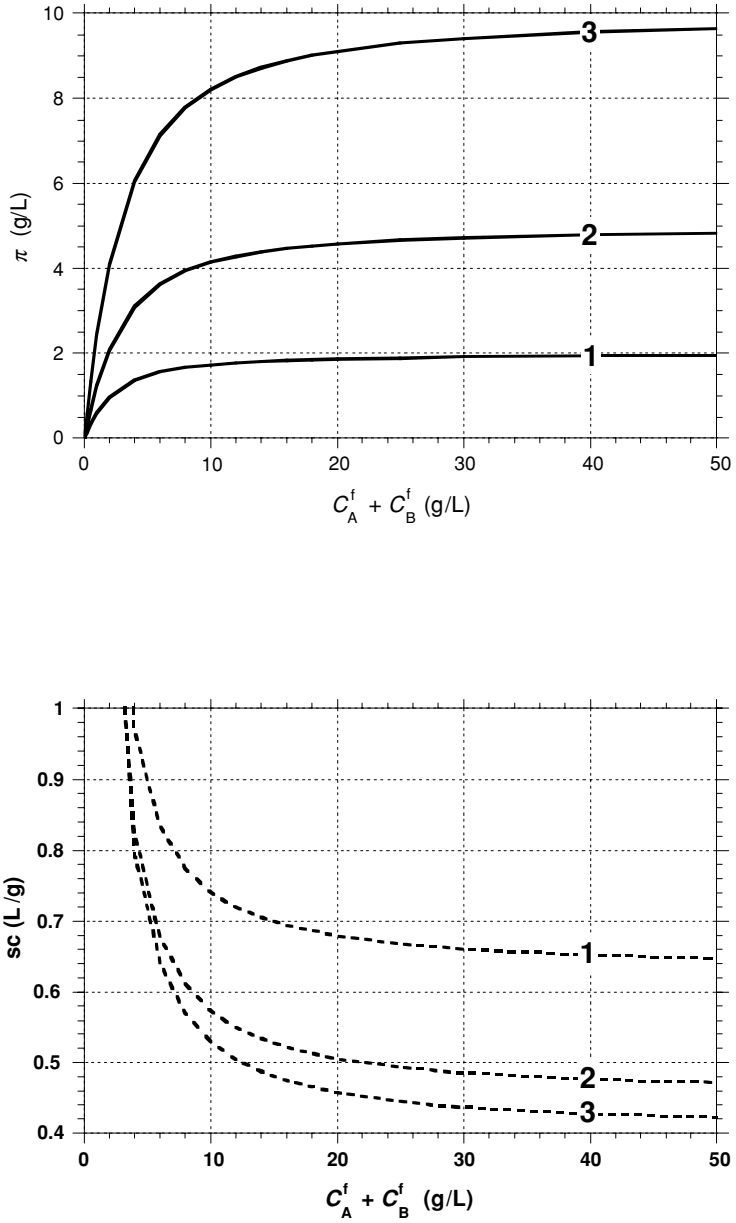


Figure 7.8 Influence of adsorption isotherm parameters on the system productivity and solvent consumption: cases 1, 2 and 3. System productivity and solvent consumption are evaluated at the vertex points of the separation regions obtained using the equilibrium theory model.

isotherm parameters for cases 1, 4 and 5. For low concentrations (see Fig. 7.9, $C_A^f = C_B^f = 0.1$ g/L), the $\gamma_3 \times \gamma_2$ separation regions obtained for the three cases are not significantly different, when compared with the same situation for cases 1, 2 and 3 (compare Figs. 7.7 and 7.9, for $C_A^f = C_B^f = 0.1$ g/L). This occurs because cases 1, 4 and 5 have the same

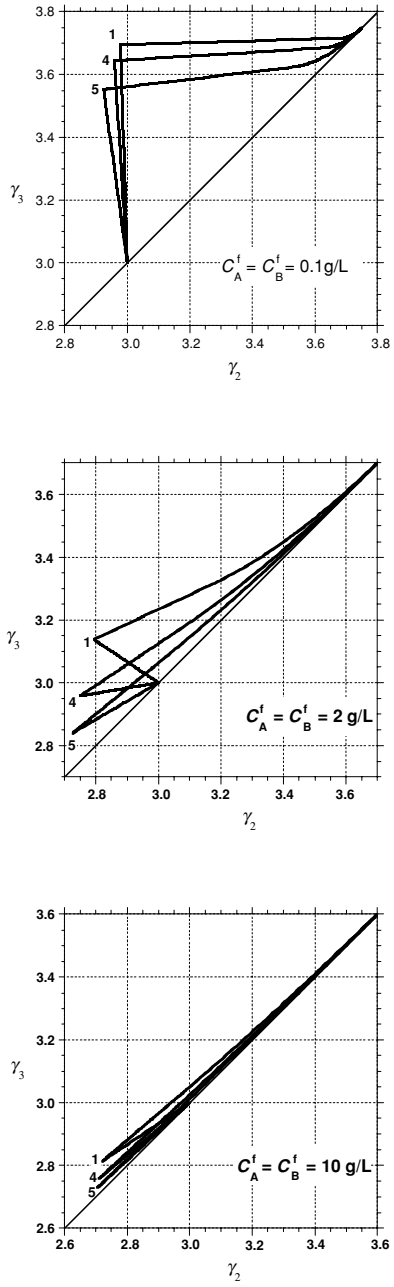


Figure 7.9 Influence of adsorption isotherm parameters on the $\gamma_3 \times \gamma_2$ separation region: cases 1, 4 and 5 for feed concentrations of $C_A^f = C_B^f = 0.1$, 2 and 10 g/L. Separation regions obtained using the equilibrium theory model. For adsorption isotherm parameters, see Table 7.2.

isotherm parameters in the linear range ($K_A = 2.00$, $K_B = 2.50$, $\alpha_{linear} = 1.25$). However, completely different separation regions are obtained for higher concentrations (see Figure 7.9, $C_A^f = C_B^f = 2$ and 10 g/L). Figure 7.10 shows that cases 1, 4 and 5 also present different behaviors in terms of system productivity and solvent consumption.

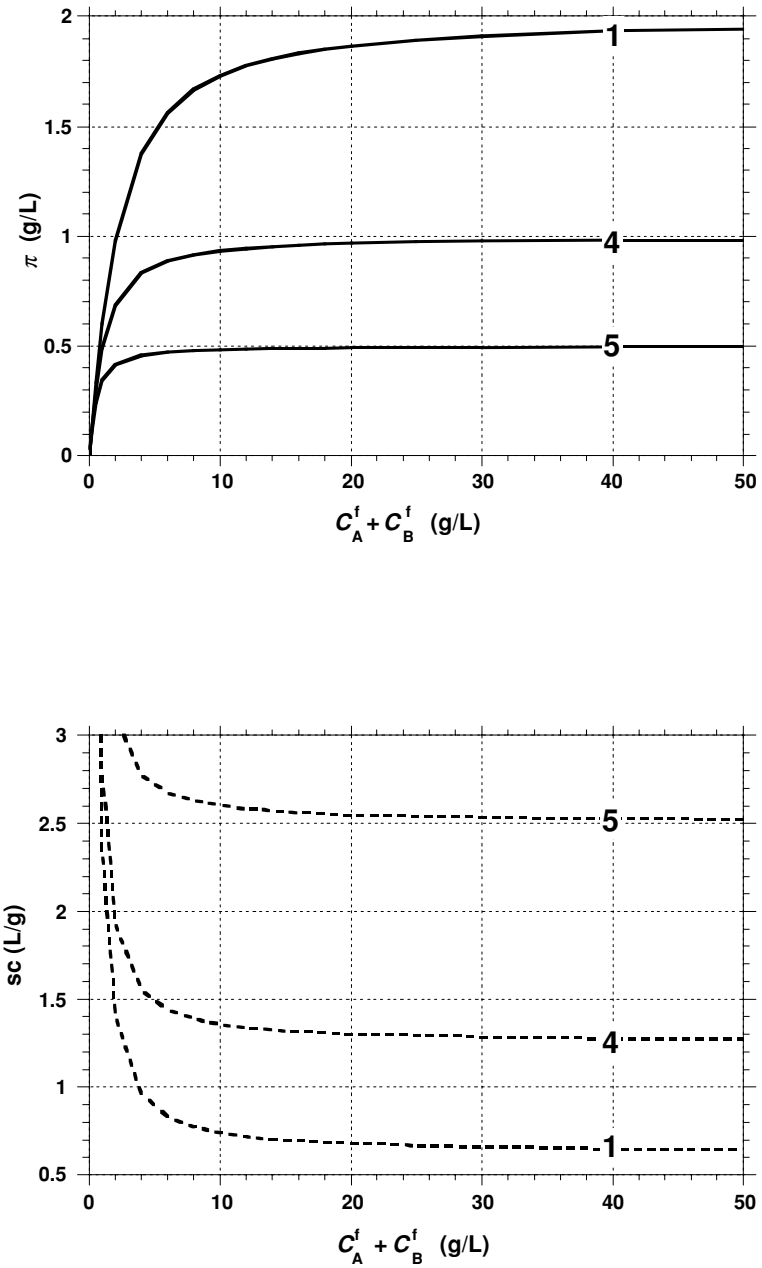


Figure 7.10 Influence of adsorption isotherm parameters on the system productivity and solvent consumption: cases 1, 4 and 5. System productivity and solvent consumption are evaluated at the vertex points of the separation regions obtained using the equilibrium theory model.

The previous results illustrate the crucial importance of the equilibrium adsorption isotherms on system performance. Also, it is shown that the evaluation of the SMB operation and system performance needs the complete knowledge of the equilibrium adsorption isotherms, not only in the linear range, but certainly in the nonlinear and competitive region.

7.5.2 *Influence of mass transfer resistance*

Case 1 (see Table 7.2) was used to study the influence of mass transfer resistance on the $\gamma_3 \times \gamma_2$ separation regions and on system productivity and solvent consumption. The separation regions were obtained by simulation using the TMB model presented in Section 7.2, which considers axial dispersion and mass transfer resistance. In all simulations, bed porosity was considered as $\varepsilon = 0.4$ and Peclet number as $Pe_j = 2000$ (negligible axial dispersion). The ratios between fluid and solid interstitial velocities in sections 1 and 4 were fixed away from their critical values ($\gamma_1 = 5.625$, $\gamma_4 = 2.000$).

Figure 7.11 shows the influence of mass transfer resistances (mass transfer coefficient, $k = 0.125, 0.15, 0.2$ and $0.4\ s^{-1}$) on the form of the $\gamma_3 \times \gamma_2$ separation regions for three feed concentrations: $C_A^f = C_B^f = 0.1$, 1 and 5 g/L. Inside each separation region both extract and raffinate are at least 99.0% pure. Also shown in each graph is the result obtained considering negligible mass transfer resistance ($k \to \infty$; equilibrium theory model). The conclusion is obvious: mass transfer resistance reduces the size of the separation region, diminishing the region of operating conditions that allow certain purity criteria. This is true for both low and high concentrations, i.e. for both linear and nonlinear ranges of the equilibrium adsorption isotherms. Consequently, system productivity and solvent consumption are also influenced by mass transfer resistance (see Fig 7.12). The previous results show that when mass transfer resistance is important, we shall replace the use of the simple equilibrium theory model by a more realistic model that takes into account these phenomena.

7.6 SMB related techniques

SMB technology is one of the most powerful techniques for preparative chromatography. Recently, various authors and companies have proposed SMB-related techniques. Among them is the use of new operating strategies to be used in SMB units with a low number of chromatographic columns, and the modification of SMB operation to perform ternary separations.

7.6.1 *Varicol processes*

The Varicol process was recently proposed by NovaSep [26,27] and is based on a nonsynchronous shift of the inlet and outlet valves in a multicolumn system, in contrast to the SMB operation where this shift is synchronous. This new process makes possible operation with a number of columns per section that is not constant in time, and can show advantages over the classical SMB operation, particularly when using a low number of columns (recent applications in the pharmaceutical industry use SMB systems containing, usually, four to eight chromatographic columns).

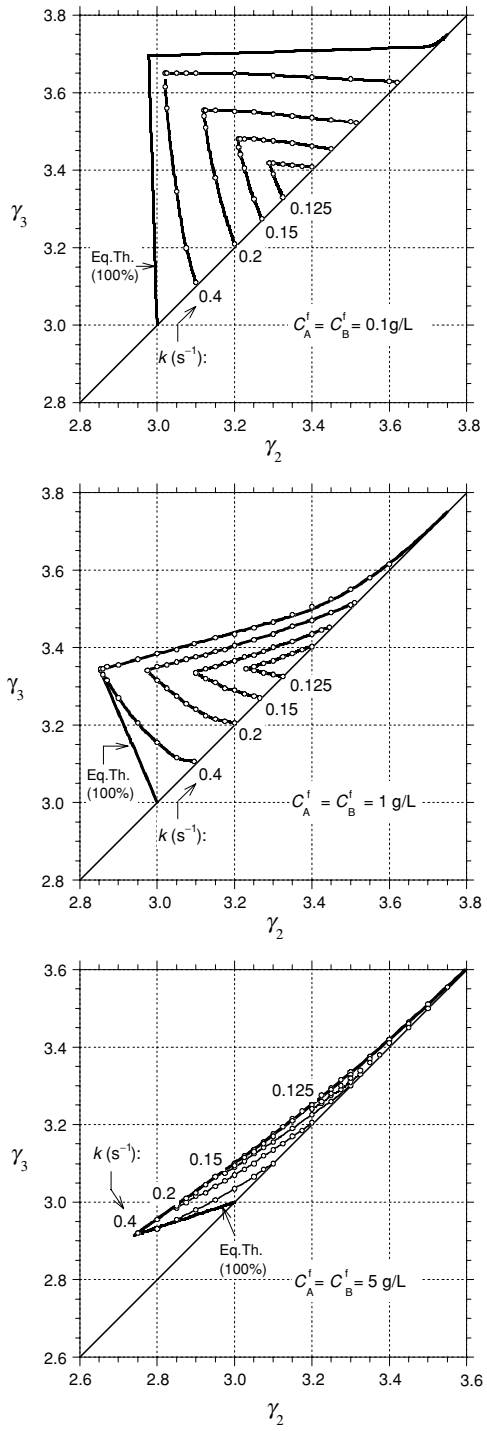


Figure 7.11 Influence of mass transfer resistance on the $\gamma_3 \times \gamma_2$ separation region for case 1 and feed concentrations of $C_A^f = C_B^f = 0.1$, 1 and 5 g/L. Separation regions obtained by simulation using the steady-state TMB model. Inside each separation region both extract and raffinate are, at least, 99.0% pure. Also shown is the separation region obtained using the equilibrium theory model ($k \to \infty$; 100% pure extract and raffinate).

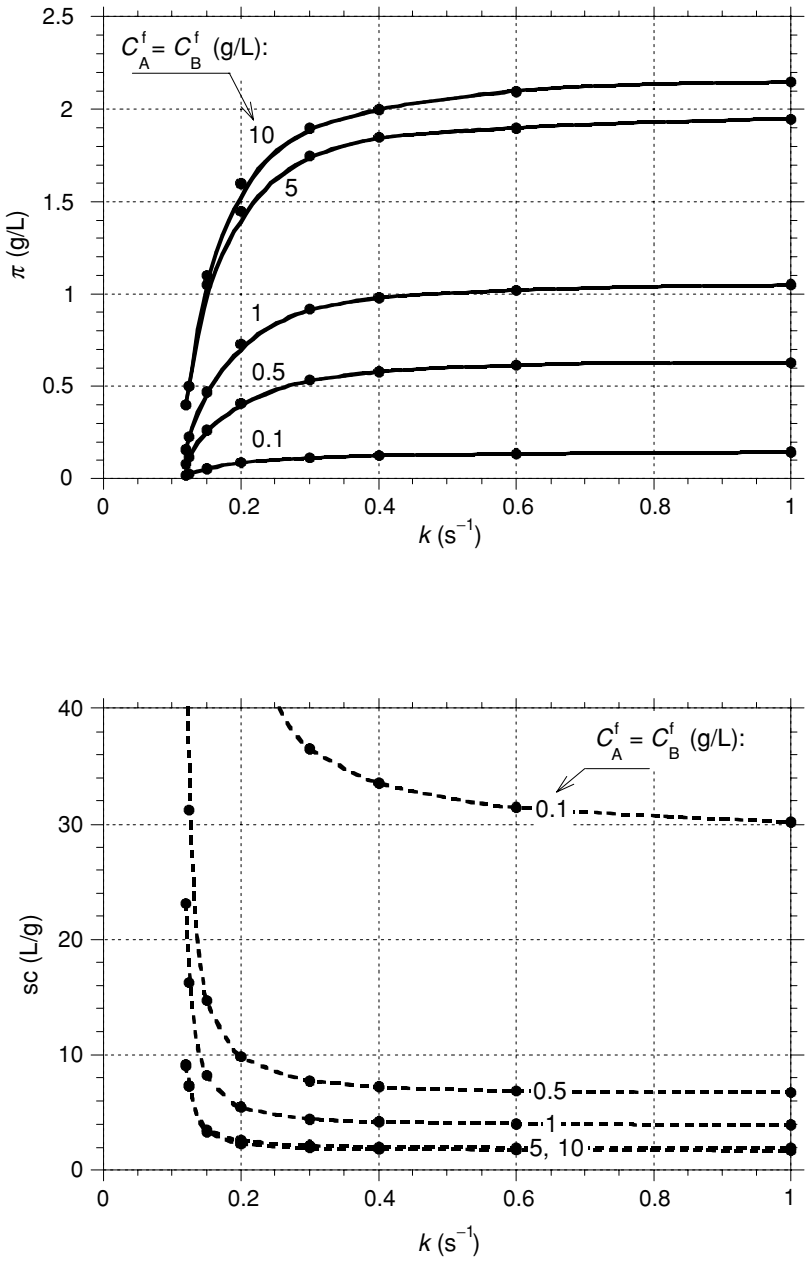


Figure 7.12 Influence of mass transfer resistance on the system productivity and solvent consumption for case 1 and feed concentrations of $C_A^f = C_B^f = 0.1$, 0.5, 1, 5 and 10 g/L. System productivity and solvent consumption are evaluated at the vertex points of the separation regions obtained by simulation using the steady-state TMB model.

Figure 7.13 shows an example of Varicol operation and compares it to the classic SMB system. Consider an SMB system with a 1211 configuration (see Fig. 7.13, left). At the beginning of each switch time interval ($\theta = 0$), all the inlet and outlet streams will jump one column forward, in the direction of the liquid phase. This jump is synchronous, and so, for this example, sections 1, 3 and 4 will always have one column, while section 2 will always have two. Consider now an SMB 1121 configuration: in this case, the feed inlet is at the

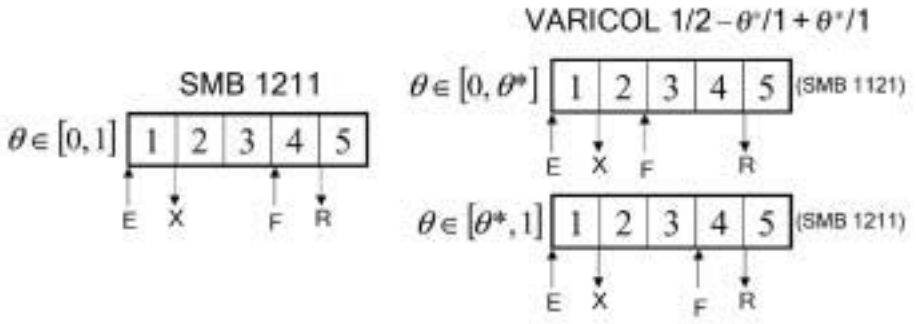


Figure 7.13 Example of a Varicol operation: five-column SMB 1211 configuration (left) and five-column Varicol $1/2 - \theta^*/1 + \theta^*/1$ configuration, with $0 \leq \theta^* \leq 1$ (right).

beginning of the third column (see Fig. 7.13, upper right). At a certain moment during the switch time interval ($0 \leq \theta = \theta^* \leq 1$), the feed inlet will jump to the beginning of the fourth column, while all the other lines (eluent, extract and raffinate) remain fixed (see Fig. 7.13, lower right). From now on, the configuration is not 1121 but 1211, and will stay like this until the end of the switch time interval is reached. We may say that during the whole switch time interval we have two different SMB configurations: during the interval $0 \leq \theta < \theta^*$, the SMB 1121 configuration; during the interval $\theta^* \leq \theta \leq 1$, the SMB 1211 configuration. This example represents a Varicol operation, since the system is operating with a number of columns per section that is not constant in time. For this example, sections 1 and 4 always have one column; however, sections 2 and 3 have, respectively, $2 - \theta^*$ and $1 + \theta^*$ columns; that is, we are in the presence of a Varicol $1/2 - \theta^*/1 + \theta^*/1$ configuration.

As it was shown recently [12], the simulation of SMB units with a low number of chromatographic columns must use the real and more precise SMB model. Figure 7.14 illustrates this necessity: for a 1211 configuration (one column in sections 1, 3 and 4; two columns in section 2), a feed concentration of $C_A^f = C_B^f = 1$ g/L and a mass transfer coefficient of $k = 0.2\,s^{-1}$, there is no 99.0% separation region predicted by the real and more precise SMB model. However, completely different results are obtained if we wrongly use the TMB or the equilibrium theory approaches. Figure 7.14 clearly shows that the evaluation of the SMB units using a low number of chromatographic columns shall avoid the use of the ideal countercurrent TMB or equilibrium theory models, and a more realistic SMB model, which takes into account the periodic shift of the injection and collection points, is needed.

Figure 7.15 presents the 99.0% $\gamma_3 \times \gamma_2$ separation regions obtained for the same operating conditions of Fig. 7.14 but using the Varicol $1/2 - \theta^*/1 + \theta^*/1$ configuration. Model equations to simulate the Varicol process are the same as used in the SMB model, only they must take into account the nonsynchronous shift of the inlet and outlet lines. Figure 7.15 clearly shows the advantages of the Varicol operation when using a low number of columns, since completely different separation regions are obtained when changing the θ^* value (remember that no 99.0% separation region is obtained for any five-column classic SMB configuration). Figure 7.16 stresses the influence of Varicol configuration in terms of system productivity and solvent consumption. For this case, the best situation occurs for $\theta^* = 0.5$, i.e. for a Varicol $1/1.5/1.5/1$ configuration. However, even better performance can be obtained by considering the optimization procedure as not confined to the two central sections, but as extended to the two adjacent sections, 1 and 4.

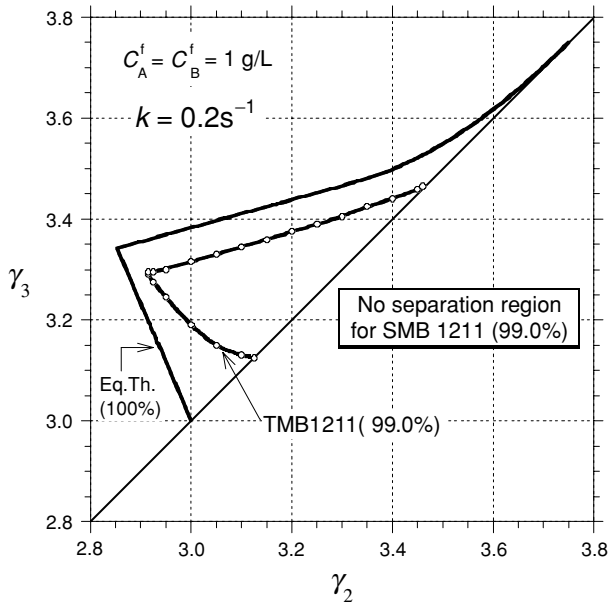


Figure 7.14 $\gamma_3 \times \gamma_2$ separation regions for case 1, feed concentrations of $C_A^f = C_B^f = 1$ g/L, and a 1211 configuration (a total of five columns, two columns in section 2). Shown are the separation regions obtained using the equilibrium model ($k \to \infty$; 100% pure extract and raffinate) and the TMB model for a mass transfer coefficient of $k = 0.2$ s^{-1} (99.0% pure extract and raffinate). There is no 99.0% separation region using the SMB model with a 1211 configuration and for $k = 0.2$ s^{-1}.

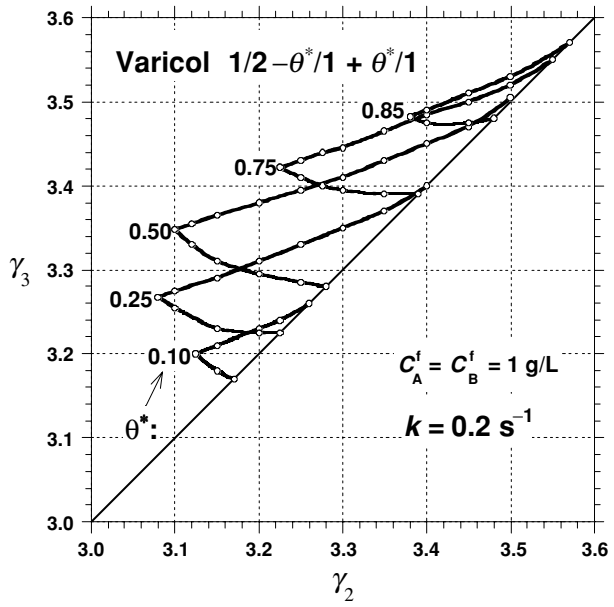


Figure 7.15 $\gamma_3 \times \gamma_2$ separation regions for case 1, feed concentrations of $C_A^f = C_B^f = 1$ g/L, and a mass transfer coefficient of $k = 0.2$ s^{-1} (99.0% pure extract and raffinate). Separation regions obtained using the Varicol operation with a total of five columns and a configuration of $1/2 - \theta^*/1 + \theta^*/1$ configuration, with $\theta^* = 0.10, 0.25, 0.50, 0.75$ and 0.85.

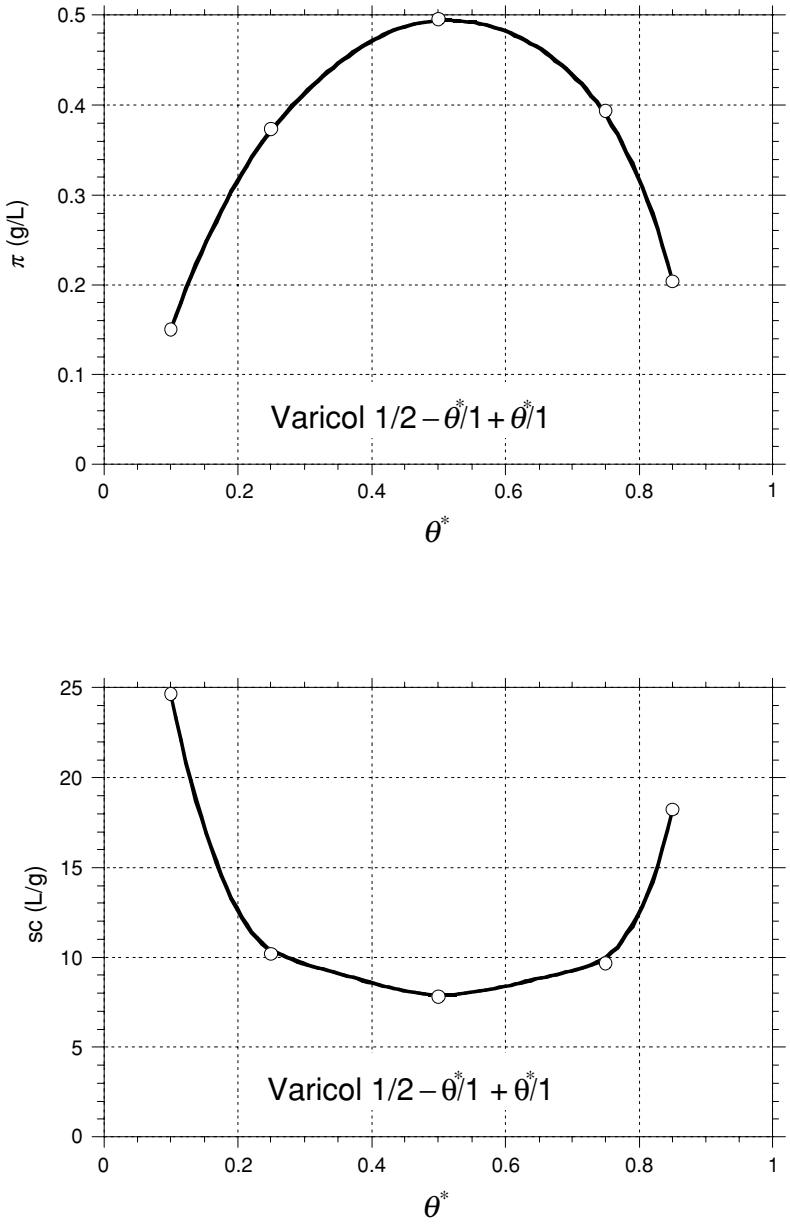


Figure 7.16 Influence of θ^* on the system productivity and solvent consumption for case 1, feed concentrations of $C_A^f = C_B^f = 1$ g/L, and a mass transfer coefficient of $k = 0.2$ s^{-1} (99.0% pure extract and raffinate). System productivity and solvent consumption are evaluated at the vertex points of the separation regions obtained by simulation using the Varicol $1/2 - \theta^*/1 + \theta^*/1$ operation.

In conclusion, the use of SMB units with a low number of chromatographic columns presents the advantage of a simpler and more economic solution, but may lead to a significant loss of process purity or system productivity. The Varicol process, a modification of the classic SMB operation by introducing a nonsynchronous shift of the inlet and outlet lines, can overcome these disadvantages and present performances closer to the ones obtained with SMB units with a higher subdivision of the adsorbent bed.

7.6.2 *Pseudo-SMB processes*

Recently, SMB operation has been modified to allow the separation of ternary mixtures. The process described in this section was proposed by the Japan Organo Company (http://www.organo.co.jp/technology/hisepa/en_hisepa/) using patented technology [28, 29]. This technique of pseudo-SMB chromatography is being applied in the separation of complex multicomponent mixtures, for instance, the separation of beet molasses mixtures into raffinose, sucrose, glucose and betaine [29] and in the production of raffinose from beet molasses [30].

Like SMB, it is also a cyclic process, in which the following two steps constitute one cycle:

(1) In the first step, the feed and eluent streams enter the system and the component B, with intermediate affinity, is recovered. This step can be modeled as a series of preparative chromatographic columns.
(2) In the second step, there is only one inlet flow of eluent and no feed. The more retained component C is recovered in the extract and the less adsorbed species A is recovered in the raffinate. This step can be modeled as a pseudo-TMB with no feed. At the end of this step, the intermediate species B is located downstream of the feed point.

Figure 7.17 shows the traditional TMB system and the schematic diagram of both step 1 and step 2 of the JO process. As described in Section 7.1, in the traditional TMB operation, there are four sections separated by the inlet and outlet streams: section 1, between the eluent and extract ports; section 2, between extract and feed ports; section 3, between feed and raffinate ports; and section 4, between raffinate and eluent ports; the fluid leaving section

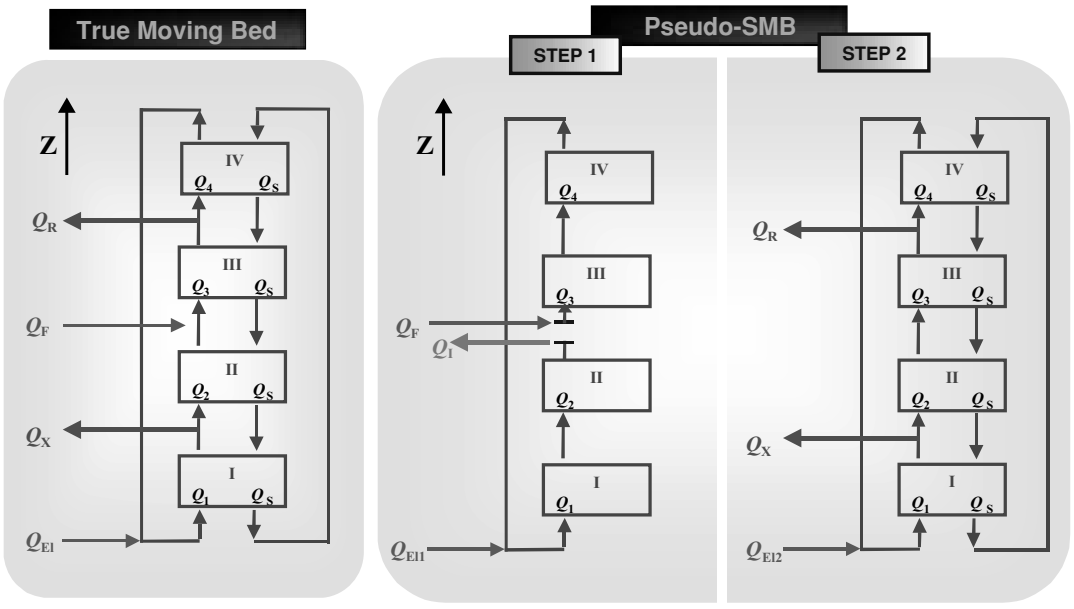


Figure 7.17 Schematic diagrams for TMB and Pseudo-SMB operation.

4 is recycled to section 1. In the pseudo-SMB model of the JO process, we still consider four sections for convenience: in step 1, the feed flows through sections 3 and 4, and the sum of feed plus eluent flow-rates flow through sections 1 and 2; in step 2, equivalent to a TMB operation, the flow-rates in sections 2 and 3 are the same, since there is no feed. The detailed mathematical model for the JO process can be found elsewhere [31] and uses the same assumptions for the TMB and SMB models presented earlier in this chapter (includes axial dispersion and the mass transfer resistance as described by a linear driving force model).

The operating conditions for the pseudo-SMB JO system, i.e. the choice of the internal flow-rates in each section and the duration of steps 1 and 2 in order to obtain the desired separation, can be evaluated taking into account the velocity of propagation of concentration of a species i and the constraints for steps 1 and 2.

Conditions for step 1: the duration of the step 1, t_{s1}, is assumed to be the time necessary to feed the intermediate component, B, only in the first column of section 3 on the upstream of the feed. The time t_{s1} can be calculated from the pulse response of a fixed bed.

Conditions for step 2: the determination of the internal flow rates for step 2 are based on the following assumptions: component A (less retained species) moves with the liquid in order to stay centered in the raffinate; component B (intermediate species) moves with the solid in order to stay totally downstream of the feed point in section 2; and component C (more retained species) moves with the solid in order to stay centered in the extract. A more detailed description of the determination of these conditions can be seen in literature [32].

One example of multicomponent separation using the pseudo-SMB JO process was tested using the ternary system shown in Table 7.3. This system represents the separation of three pure components present in sugar molasses, namely sucrose, fructose and betaine. The characteristics of the physical system (from Japan Organo Catalog, 1998) are shown in Table 7.4. The assumed feed flow-rate and the duration of step 2 are $Q_F = 350$ mL/min and $t_{s2} = 66$ min [29]. The duration of step 1 is $t_{s1} = 17.8$ min.

Table 7.3 Values of mean retention time, adsorption parameters (linear isotherms) and mass transfer coefficients for the ternary mixture

Component i	$\bar{t}_i$ (min)	K_i	k (s^{-1})
Sucrose (A)	10.76	0.19	0.20
Fructose (B)	13.91	0.44	0.13
Betaine (C)	16.51	0.65	0.11

Table 7.4 Characteristics of the SMB columns for pseudo-SMB operation

Column number (total)	12
Column length (cm)	120
Column diameter (cm)	10.84
Column volume (L)	11.1
Number of columns per section	3
Bed porosity, ε	0.4

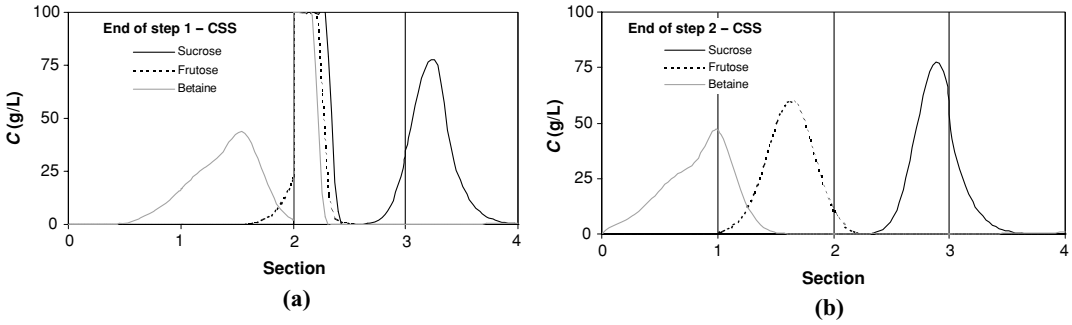


Figure 7.18 Pseudo-SMB operation. Concentration profiles of sucrose, fructose and betaine, at the end of (a) step 1 and (b) step 2. CSS = cyclic steady state.

Figure 7.18 shows the concentration profiles in the liquid phase at the end of (a) step 1 and (b) step 2, obtained after cyclic steady state was achieved. The feed containing the three solutes at species concentration $C_0 = 100$ g/L is injected in step 1 of the cycle during $0 < t \leq t_{s1}$ with flow rate Q_F, at the inlet of section 3. At the same time, there is an intermediate stream, Q_I, rich in fructose, coming out from the end of section 2. During the second step of the cycle for $t_{s1} < t \leq t_{s2}$, the feed flow and the flow of intermediate species are stopped. There are two outlet streams: one at the end of section 1, the extract, Q_X; and the other at the end of section 3, the raffinate, Q_R. At the same time, it is assumed that the solid starts moving in the opposite direction of the fluid. The less retained component, sucrose, moves in the direction of the fluid, but both fructose and betaine move in the opposite direction, i.e. in the direction of the solid phase.

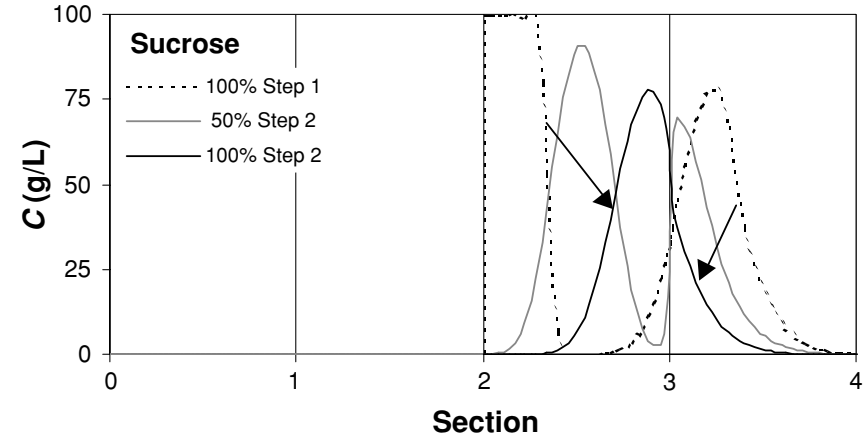
Figure 7.19 shows the movement of each component in the liquid phase during its recovery. Namely, it shows

(a) the evolution of the internal liquid sucrose profile during step 2 (from the end of step 1 to the end of step 2): during this time, t_{s2}, sucrose is recovered at the bottom of section 3, in the raffinate stream;
(b) the evolution of the internal liquid fructose profile during step 1 (from the end of step 2 to the end of step 1): during this time, t_{s1}, fructose is recovered at the bottom of section 2, in the intermediate stream;
(c) the evolution of the internal liquid betaine profile during step 2 (from the end of step 1 to the end of step 2): during this time, t_{s2}, betaine is recovered at the bottom of section 1, in the extract stream.

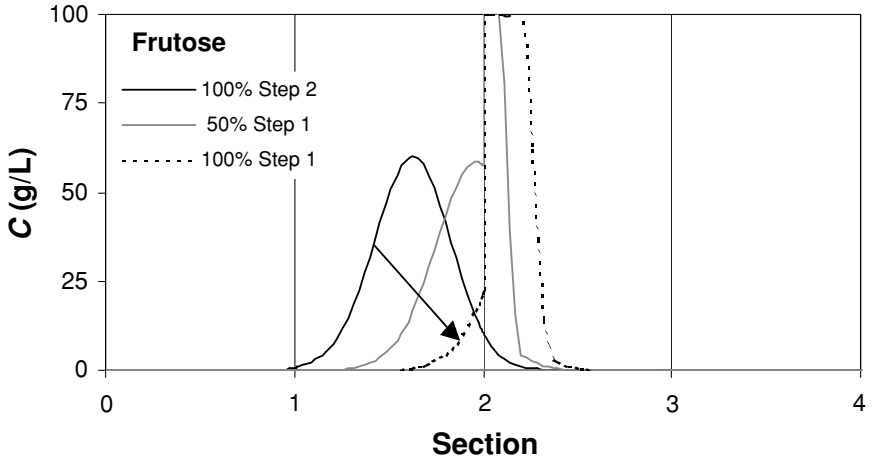
This example shows how SMB operation can be modified to improve its classical goal of binary to ternary separations.

Notation

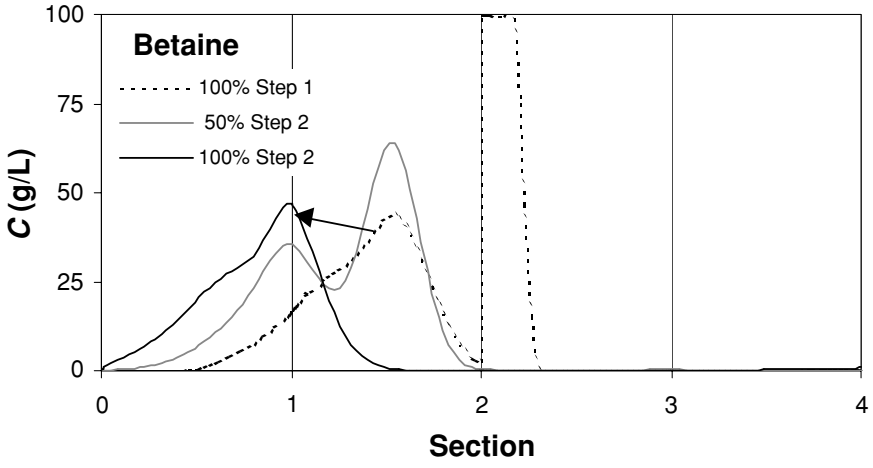
b	adsorption isotherm parameter
C	fluid phase concentration
D_L	axial dispersion coefficient
K	adsorption isotherm parameter (linear range)



(a)



(b)



(c)

Figure 7.19 Pseudo-SMB operation. Evolution of the concentration profiles during the recovery of (a) sucrose in step 2, (b) fructose in step 1 and (c) betaine in step 2.

k	mass transfer coefficient
L_c	length of an SMB column
L_j	length of a TMB section
m	adsorption isotherm parameter
n	number of mass transfer units
N_c	total number of columns in the SMB unit
Pe	Peclet number
pr	system productivity, defined as in Equation (7.17)
Q	adsorption isotherm parameter
Q	volumetric liquid flow-rate in the TMB
Q^*	volumetric liquid flow-rate in the SMB
Q_s	solid flow-rate
q	average adsorbed phase concentration
q^*	adsorbed phase concentration in equilibrium with C
sc	solvent consumption, defined as in Equation (7.19)
t^*	switch time interval
$\bar{t}$	mean retention time
t_{s1}	duration of the step 1 in the pseudo-SMB JO process
t_{s2}	duration of the step 2 in the pseudo-SMB JO process
u_s	interstitial solid velocity in the TMB operation
V_c	volume of an SMB column
v	interstitial fluid velocity in the TMB operation
v^*	interstitial fluid velocity in the SMB operation
x	dimensionless axial coordinate

Greek symbols

α	selectivity
γ	ratio between fluid and solid interstitial velocities in the TMB operation
γ^*	ratio between fluid and solid interstitial velocities in SMB operation
ε	bed porosity
θ	dimensionless time
π	productivity parameter, defined as in Equation (7.18)

Subscripts

E	eluent
F	feed
I	intermediate (in pseudo-SMB JO process)
R	raffinate
X	extract
i	component index (i = A, B for binary, or i = A, B, C for ternary systems)
j	section index ($j = 1, 2, 3, 4$)
k	column index ($k = 1, \ldots, N_c$)

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Preparative Enantioselective Chromatography
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8 Preparative-scale supercritical fluid chromatography

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8.1 Introduction

The first phenomenon of the 'critical state' was observed in 1822, when Cagnaird de la Tour noted a lack of discontinuity upon the disappearance of a meniscus when going from the gaseous to the liquid phase [1]. In 1962 Klesper expanded the utility of the critical state and developed the first preparative supercritical fluid chromatography (SFC) utilizing an eluent in the critical state for the separation of a porphyrin mixture [2]. SFC was later interfaced with a mass spectrometer in 1978 by Randall and Wahrhaftig [3]. Then, in 1982, Hewlett-Packard introduced the first, commercially available, analytical SFC instrument at the Pittsburgh Conference.

In the early years, preparative SFC was very challenging. Most laboratories that implemented the technology built their own instrumentation and optimized these instruments for their very unique purposes. One of the most difficult aspects of preparative SFC was the quantitative recovery of samples. The difficulty was due to formation of aerosols as the eluent was immediately returned to the gaseous state at ambient pressure during fraction collection. Before marketing a commercial instrument, manufacturers spent considerable effort in designing a suitable fraction recovery device to enhance recovery and reduce formation of aerosols. For several years, individual laboratories constructed preparative SFC units, but it was not until 1990 that the first preparative SFC system became commercially available, from Prochrom, Champigneulles, France [4]. Since then, SFC technology has been substantially improved upon. Laboratory- and pilot-scale systems are now commercially available for a variety of separations, i.e. chiral and achiral. SFC instrumentation is now more robust and the issues with fraction collection have been resolved, thereby yielding a viable technique for preparative chromatography. This chapter will discuss the use of SFC in the purification of chiral and achiral molecules.

8.2 History of SFC at GlaxoSmithKline

In late 1987 Chris Bevan from Glaxo, United Kingdom, started to use analytical SFC. The analyses were particularly successful for Glaxo's novel cyclic peptides, which had not been chromatographed by SFC before and had given problems by HPLC. Bevan used his analytical SFC for several years and in 1988 scaled up the system to use a 1 in. diameter preparative column. In 1989, he built various items of specialized equipment to allow large volume sample injection in CO_2, preparative flow rates and sample collection. The preparative instrumentation was built in the Greenford, UK, workshops [5]. Capillary SFC was practised at SmithKlineBeecham (King of Prussia, PA) in the early 1990s and packed-column SFC in 1994. Daniel Morgan of Glaxo, Research Triangle Park, NC, started using analytical SFC in 1994. His first project was a steroid that required two reverse-phase HPLC methods to

separate three components. Each HPLC method required 30 min to complete using SFC, Morgan found a method with only 15 min runtime. In 1995, he interfaced the SFC to a mass spectrometer to identify the impurities [6,7]. Morgan has since used SFC–MS for bioanalysis [8,9]. In 1994, Manon Villeneuve, Glaxo (Research Triangle Park, NC), also started to use analytical SFC for chiral separations [10] and preparative SFC in 1997. The successful use of the technique continued at several GlaxoSmithKline (GSK) sites with Verona in 2000 and Harlow (UK) in 2001, both using SFC on analytical and preparative scales.

In 1996, Glaxo (Research Triangle Park, NC) purchased a preparative SFC unit (Prochrom SuperC-10). The unit was installed in 1997 and was very quickly used to separate Glaxo compounds. The evolution of SFC in the Research Triangle Park laboratories is shown in Figs. 8.1 and 8.2. Within the first year of installing a preparative SFC system, the number of in-house compounds separated on SFC almost doubled (Fig. 8.1) and then continually increased, whereas the amount of material separated remained virtually the same with the exception of the year 2003 (Fig. 8.2). The increase in 2003 is mainly due to the acquisition of a larger scale preparative SFC system, which can accommodate larger quantity requests.

8.3 Principles of SFC

Above a particular temperature and pressure, known as the 'critical point', a substance can no longer remain a gas or liquid, but becomes a supercritical fluid. The physical properties of a supercritical fluid are intermediate between those of a gas and a liquid (see Fig. 8.3). Table 8.1 shows the parameters necessary to create supercritical fluids from common solvents. It can be seen from this table that carbon dioxide requires a low temperature and pressure to become a supercritical fluid. In addition, carbon dioxide is safe, non-explosive and non-toxic compared with other fluids and for these reasons is the most commonly used eluent in SFC. The unique feature of SFC is that the solvent power strongly depends on the fluid density, which can be adjusted by controlling the pressure and temperature.

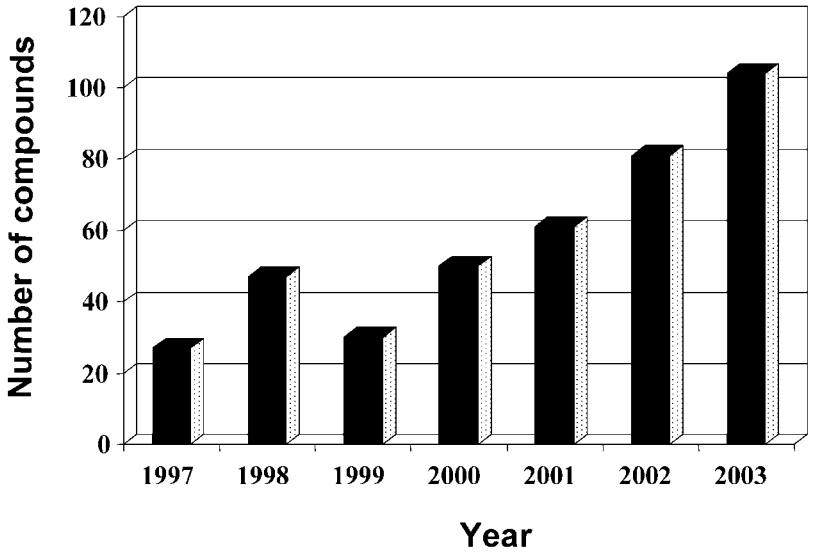

Figure 8.1 Number of compounds separated on preparative SFC since 1997.

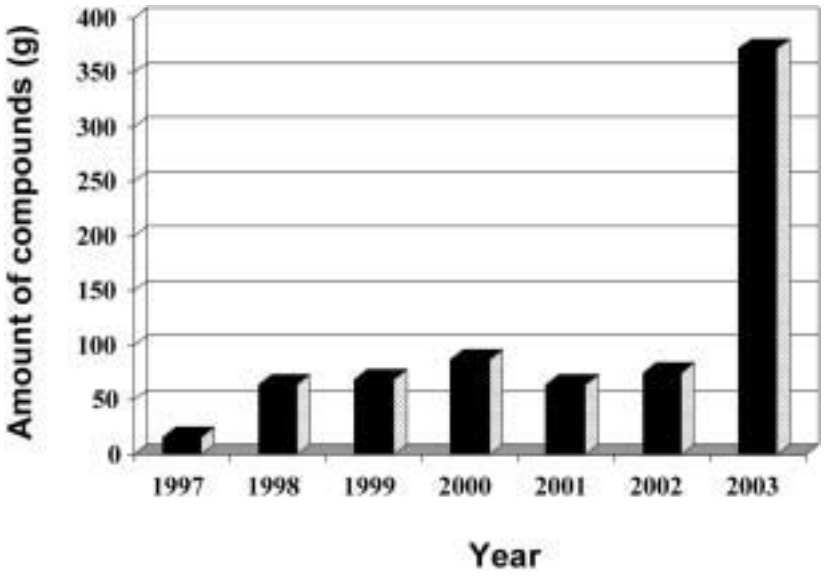

Figure 8.2 Amount of compounds (g) separated on preparative SFC since 1997.

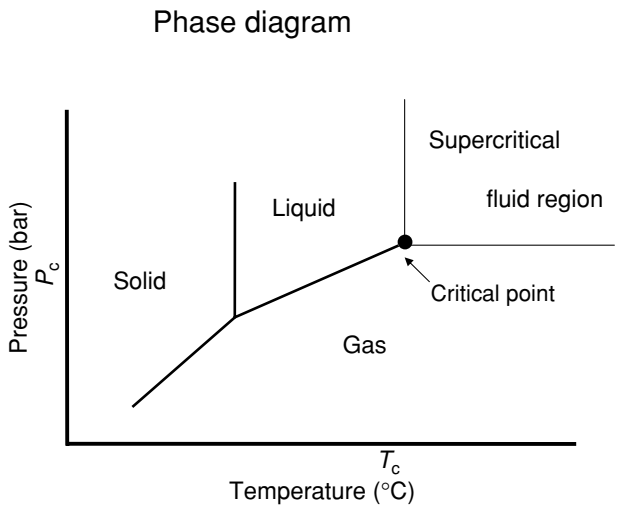

Figure 8.3 Phase diagram.

For SFC, the eluent is compressed and heated to produce a supercritical fluid. The eluent is passed through a packed column where the separation of a mixture occurs. Decompression of the eluent to the gaseous state occurs after the column and pure samples are collected in different collection vessels. Fraction collectors differ slightly in design between commercial manufacturers, but the principle remains constant. Cyclone separators separate the gaseous portion of the eluent from the liquid products (the fractionated sample and any liquid co-solvent such as methanol). The gaseous eluent (in which the products and any modifier used have very low solubility) can be recycled by cooling to the liquid state before repressurization. Figure 8.4 shows a simplified picture of how this process works.

Table 8.1 Critical parameters of various supercritical fluids

Compound	T_c (°C)	P_c (bar)	ρ_c (kg/m^3)
Carbon dioxide	31.3	72.9	468
Nitrogen oxide	36.5	71.4	457
Xenon	16.6	58.8	1155
SF6	45.5	37	734
Ethane	32.4	48	203
Propane	97	42	217
Butane	152	38	225
Pentane	197	33.3	237
Diethyl ether	193.6	41.7	265
Methanol	240.5	78.9	272
Ethanol	243.4	63	276
Ammonia	132.3	111.3	235
Water	374.4	226.8	323

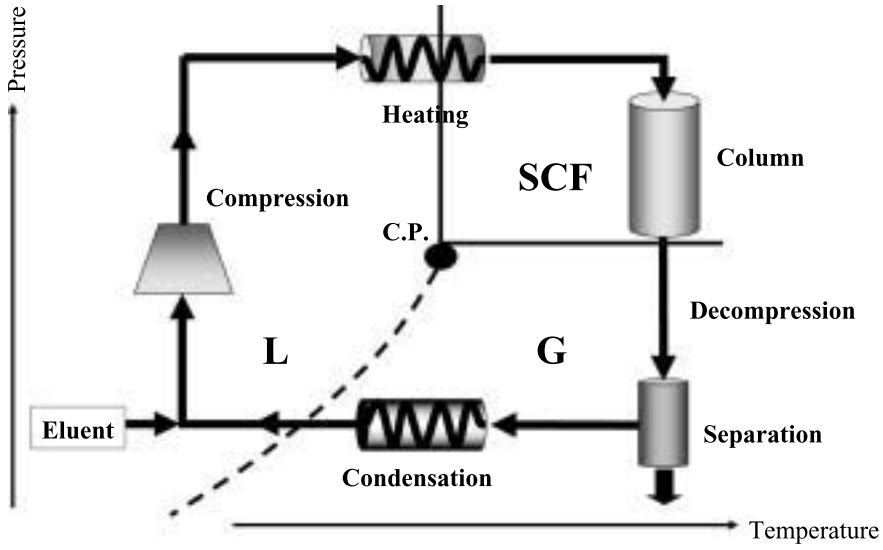

Figure 8.4 Principle of supercritical fluid chromatography.

It should be noted that depending on the chromatographic conditions used, we do not always work in the supercritical region, the gaseous or the liquid regions, but in a region that is a combination of liquid and supercritical.

8.4 Advantages of SFC

Since our group (Villeneuve *et al.*) began using analytical SFC in 1994, the results have continuously shown that the technique is very powerful for both chiral and achiral separation.

Preparative SFC has several advantages over preparative HPLC [11]. The most impressive advantage of preparative SFC is speed, due to low viscosity of the mobile phase and high diffusivity of the solutes [12–14]. On average, SFC requires one-third the runtime of a comparable HPLC separation. In addition, one can typically obtain better resolution,

better peak shape and shorter evaporation time than for a comparable HPLC separation [4,5,11,15–20].

In LC the increased flow rate as a consequence of geometrically scaling up to a preparative column causes the pressure drop across the column to increase. In efforts to reduce this consequence, most preparative HPLC separations are performed in columns packed with particle sizes of 10 or 20 μm. SFC has the advantage of producing a low pressure drop across the column, again due to the low viscosity of the mobile phase [4]. This is a major advantage, because a smaller particle size may be used in preparative SFC, creating more inherent efficiency for preparative SFC. Reduction of the pressure drop across the column becomes critical when employing some brands of chiral columns. Typical chiral columns used are polysaccharide-based packing materials coated on silica. It is recommended to operate these columns with a low pressure drop in order to not damage or strip the chiral selector from the stationary phase. In order to maintain low pressures in chiral HPLC, one is, therefore, limited to low flow rates. As the dimensions of the column are increased, moving from an analytical to preparative scale, the effect of this low flow-rate requirement is exacerbated. By using SFC, the flow rate can be scaled proportionately as the column dimensions increase, so the retention times for analytical scale and preparative scale are approximately the same.

Preparative SFC can reduce the large solvent consumption seen in preparative HPLC [12]. Preparative HPLC uses 5–20 times more solvent than preparative SFC, thus increasing the possibility for the introduction of solvent-associated impurities in HPLC. As a result, the concentrated samples are typically more pure than those obtained from preparative HPLC. With the small amount of solvent used by preparative SFC, the time to concentrate the fractions is also shorter. A considerable amount of time and energy can be spent after separation by preparative HPLC to concentrate the large amount of solvent used. For example, most chiral separations are performed by normal phase chromatography using, on average, 85% hexane as the mobile phase. Using SFC, hexane is replaced by the considerably less toxic supercritical CO_2, which has a similar polarity and is the majority of the mobile phase. Many achiral preparative separations are performed by reverse-phase chromatography and require large amounts of water to be evaporated from the collected fractions. Most SFC separations are performed with methanol as the co-solvent, which is quicker to evaporate. On the basis of the above factors, plus solvent and disposal costs, one can calculate that, on average, the operation cost for preparative SFC will be half of that of preparative HPLC [13,14].

8.5 Drawbacks of preparative SFC

Solubility problems are encountered in many purification techniques, including both preparative HPLC and SFC. However, solubility presents a greater challenge in SFC and is thus the major drawback of preparative SFC. Solubility within HPLC eluents can be easily tested in the laboratory, but solubility experiments in the supercritical state demand specialized equipment, such as a phase monitor, able to withstand the high pressure required to maintain the supercritical fluid.

It is widely accepted that if a compound dissolves in methanol, it will be suitable for SFC using a CO_2/methanol mixture eluent [21]. Furthermore, samples that do not dissolve in methanol are often considered unsuitable for SFC. It is our experience that solubility limitations can be overcome by replacing the methanol with a mixture of methanol and

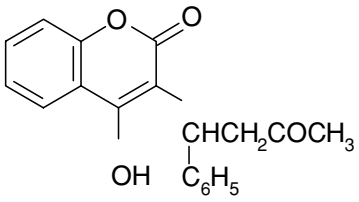

Figure 8.5 Structure of Warfarin.

chloroform as the co-solvent and diluent. Chloroform has been used because most compounds submitted to our laboratories are soluble in chloroform. A note of caution: manufacturers of polysaccharide-based chiral columns recommend that a maximum of 20% by volume of chloroform can be used in SFC with the Chiralpak AD, OD, AS and OJ columns. (It is strongly advised by manufacturers to avoid the use of chloroform with these chiral stationary phases (CSPs) for HPLC applications.) Warfarin®, the structure of which is shown in Fig. 8.5, has poor solubility in methanol, and was used to demonstrate how solubility issues can be solved in SFC.

Initial analysis of the enantiomers of Warfarin indicated that sufficient resolution was obtained; thus, the only method development performed involved an analysis of different CSPs. The separation conditions were 15% methanol, 210 bar, 40°C, 2 mL/min for all four chiral columns. Figure 8.6 shows that the Chiralcel OD-H column gave the best resolution

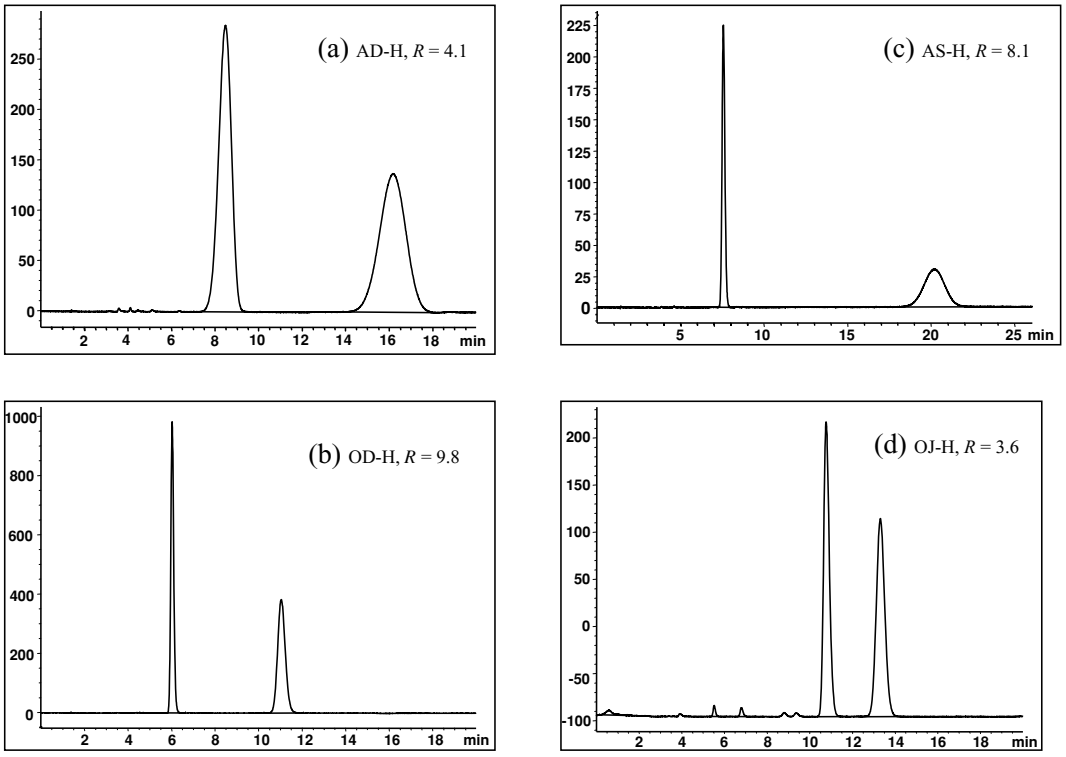

Figure 8.6 Warfarin injected on analytical SFC. Chromatographic conditions: 15% methanol, 210 bar, 40°C, 2 mL/min (a) Chiralpak AD-H column, (b) Chiralcel OD-H column, (c) Chiralpak AS-H column, (d) Chiralcel OJ-H column.

and peak shape. The analytical SFC method was then transferred to preparative SFC. Because of solubility issues in methanol, Warfarin was first dissolved in chloroform, and then methanol was added to give a final concentration equivalent to 1 g in 5.5 mL of chloroform + 2.0 mL of methanol. For this example, preparative SFC was performed on 20 mm and 30 mm i.d. columns, with 140 mg and 350 mg injections respectively. Figure 8.7 shows the preparative chromatograms. The loading capacity is directly related to the cross-sectional area of a column. The correlation between the two scales is calculated using the area of each column.

$$\text{Area} = \pi r^2, \qquad r = \frac{1}{2}\,\text{i.d.}$$

$$\text{Area (2 cm i.d.)} = 3.14\ \text{cm}^2, \qquad \text{Area (3 cm i.d.)} = 7.065\ \text{cm}^2$$

The ratio of loading capacity between a 20 mm and a 30 mm column is 2.25; therefore the data shows good correlation between a 20 mm and a 30 mm i.d. column. Purity of 99.0% was obtained for the first eluted enantiomer and 98.8% for the second enantiomer (see Fig. 8.8). The total recovery by weight was 96.3%.

In addition to solubility, another drawback of preparative SFC is the added complexity of the hardware. The preparative SFC systems are generally more complicated and the learning curve for troubleshooting of the equipment is longer than that of preparative HPLC. With many interdependent processes controlling the chromatographic parameters, the source of the problem is not always clearly defined. One must approach the troubleshooting of a preparative SFC in a highly methodical manner. Pressure and temperature are very important factors on SFC and require more precise pressure and temperature controls. In addition, due to the high operating pressure of SFC, safety relief valves are necessary. The use of many

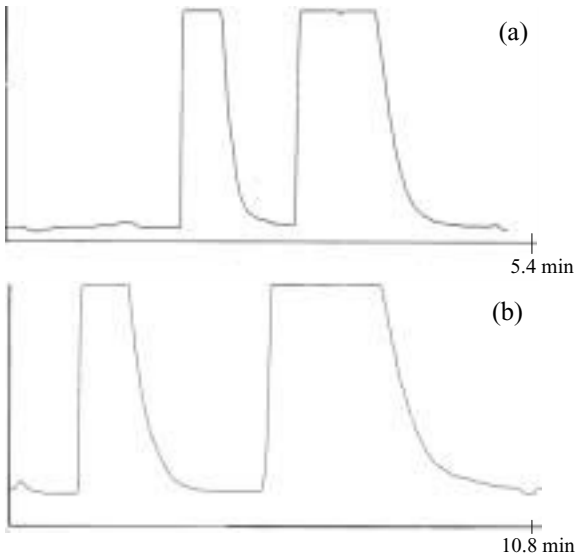

Figure 8.7 Warfarin injected on preparative SFC. Chromatographic conditions: (a) Chiralcel OD-H (20 mm × 250 mm), 200 bar, 40°C, 45 g/min of CO_2, 10.5 mL/min of 10% chloroform in methanol, 140 mg injected; (b) Chiralcel OD-H (30 mm × 250 mm), 200 bar, 40°C, 75 g/min of CO_2, 13.0 mL/min of 10% chloroform in methanol, 350 mg injected.

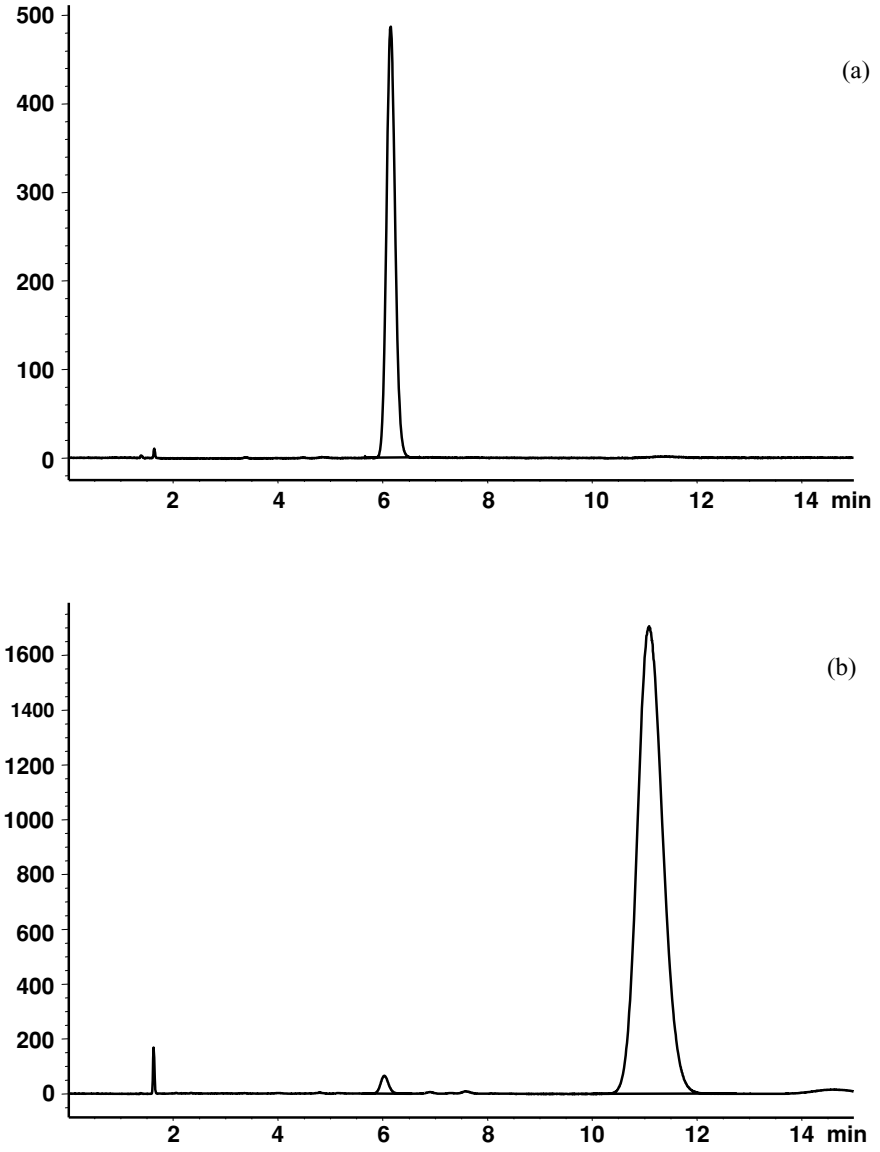

Figure 8.8 Purity of Warfarin's enantiomers after preparative SFC. Chromatographic conditions: Chiralcel OD-H, 15% methanol, 210 bar, 40°C, 2 mL/min (a) 99%, (b) 98.8%.

more control systems within the overall equipment means that the process control software can be complicated and costly. The capital cost of preparative SFC is therefore higher compared to preparative HPLC, although, as noted earlier, savings in solvent consumption and disposal and the reduced energy cost of evaporation using preparative SFC will compensate for the difference of the investment between preparative SFC and preparative HPLC.

8.6 Use of SFC

SFC can be used for both chiral and achiral separations. Stationary phases that can be utilized in normal phase mode can also be utilized in SFC, with the caveat that the columns

need to be able to withstand high pressure and be tested on SFC. It is important to remember that when ordering columns, one needs to specify that the column will be used for SFC.

In our laboratory, method development is performed in analytical SFC mode using 4.6 mm columns with 5 μm particle size. Typically, analytical methods are scaled to our preparative SFC system using a 10 mm, 20 mm or 30 mm i.d. column, 5 μm particle size, depending on the quantity of material to be separated. Our initial method development is to inject the samples on a Chiralpak AD column with methanol as the co-solvent, using either a 5–25% or 5–50% co-solvent gradient. This determines the relative solvent strength required to elute the compounds, which may vary in polarities. We employ a pressure of 210 bar, a temperature of 40°C and a flow rate of 2 mL/min. Preliminary results from the gradient method determine the starting point for isocratic method development. Automated method development can then be initiated using column and solvent selection valves [10]. On average a suitable method can be found within 24 h. Separation requests for samples smaller than 200 mg are typically performed on a 10 mm i.d. column, 200 mg to 2 g on a 20 mm i.d. column and larger than 500 mg on a 30 mm i.d. column. There are of course exceptions depending on solubility and system availability.

8.7 Chiral separation using SFC

The commercially available compound Flurbiprofen®, shown in Fig. 8.9, was used to demonstrate our protocol of chiral method development by SFC. The objective was to find a suitable analytical SFC method for both enantiomers and then to transfer the method to preparative SFC. Resolution is given by the equation

$$R_s = \frac{2\,(t_2 - t_1)}{w_2 + w_1}$$

Runtime and percentage of co-solvent were the criteria used to select the method of choice. If two different methods give approximately the same resolution, but one uses 10% alcohol while the other uses 30%, we will choose the method using 10% alcohol since less solvent would be used and evaporation time and energy costs will be less. Such *modus operandi* would be applicable unless there are solubility issues and a higher percentage of co-solvent is required.

Flurbiprofen was injected on four CSPs: Chiralpak AD, Chiralcel OD, Chiralpak AS and Chiralcel OJ. The unique feature of SFC is that the solvent power strongly depends on the density, which can be adjusted by controlling the pressure and temperature. The effects of varying the pressure, temperature and the percent alcohol have been shown elsewhere [16]. The information from the initial method development determined that the AD-H was the best column for Flurbiprofen. Comparison of the resolutions obtained for methanol, ethanol and isopropyl alcohol (Fig. 8.10) shows that methanol and

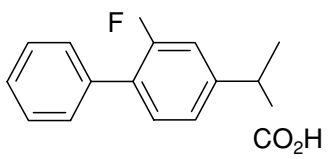

Figure 8.9 Structure of Flurbiprofen.

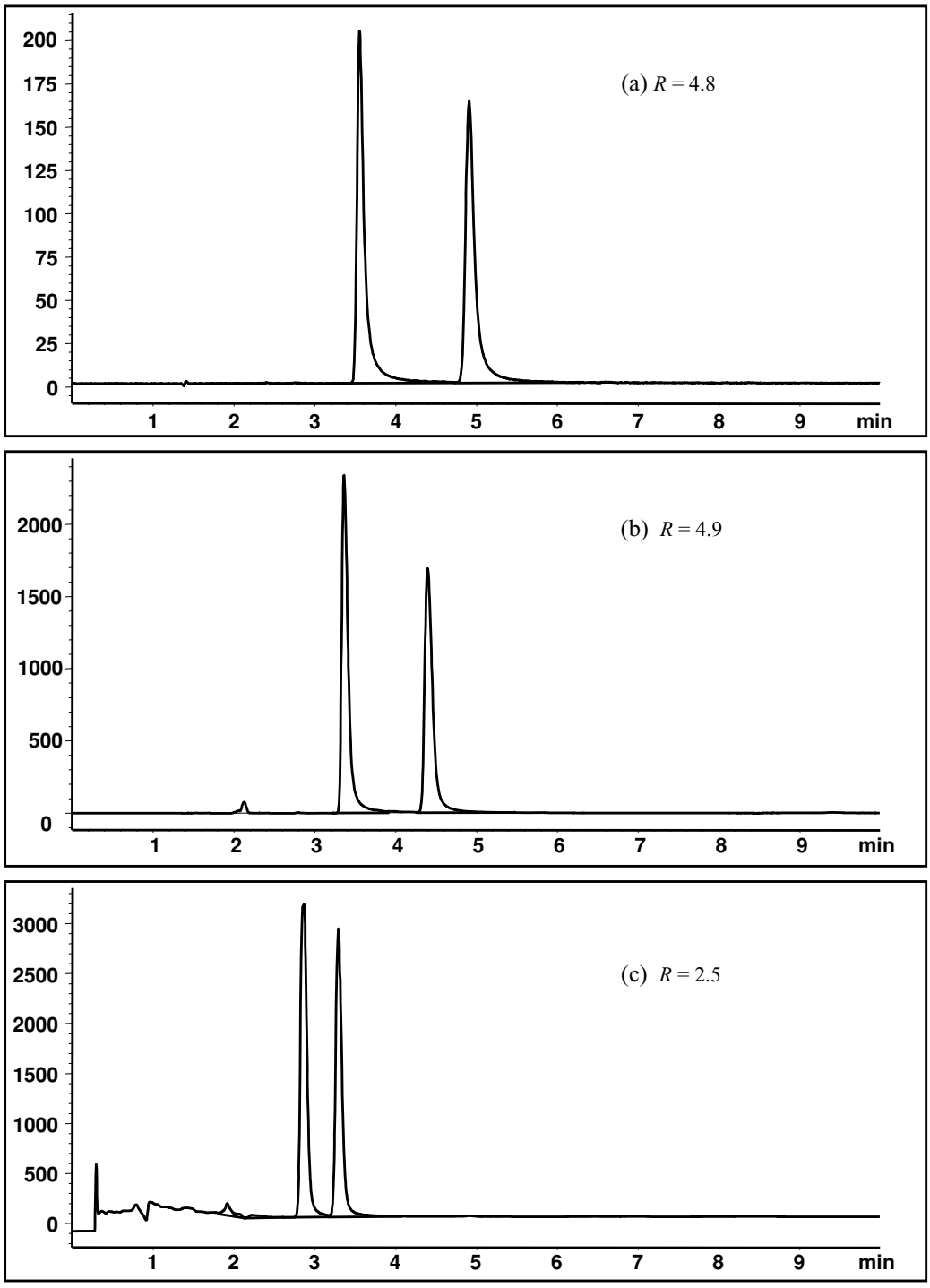

Figure 8.10 Flurbiprofen injected on analytical SFC using Chiralpak AD-H column. Chromatographic conditions: 210 bar, 40°C, 2 mL/min (a) 15% methanol, (b) 15% ethanol, (c) 15% IPA.

Table 8.2 Summary of the resolution of Flurbiprofen's enantiomers on Chiralpak AD-H column using different operating conditions

15% Methanol			15% Ethanol		
Pressure (bar)	Temperature (°C)	R_s	Pressure (bar)	Temperature (°C)	R_s
210	30	9.6	210	30	4.8
140	30	10.9	140	30	5.6
105	30	11.5	105	30	6.2
210	40	4.8	210	40	4.9
140	40	6.1	140	40	5.6
105	40	6.0	105	40	6.2
210	50	4.2	210	50	4.7
140	50	7.3	140	50	5.1
105	50	8.4	105	50	5.1
210	60	2.9	210	60	4.2
140	60	4.8	140	60	4.2
105	60	6.0	105	60	5.2

ethanol as the co-solvents give the best resolution. Using a Chiralpak AD-H column with 15% cosolvent, 210 bar and 40°C, we varied the pressure and temperature. Table 8.2 summarizes the resolution obtained for all methods that were tested and shows that methanol at 105 bar and 30°C gave the best resolution. For comparison, using this method, Flurbiprofen was also injected on OD-H, AS-H and OJ-H columns. Figure 8.11 shows the chromatograms and it can be seen that the AD-H column remained the column of choice for this sample. The analytical SFC method was then transferred to our preparative SFC.

For preparative SFC, Flurbiprofen was dissolved in methanol at a concentration of 167 mg/mL. The column used was a 20 mm × 250 mm Chiralpak AD-H with the following conditions: 75 g/min of CO_2, 13 mL/min of methanol, 105 bar, 27°C. Each injection was of 350 mg; a representative chromatogram is shown in Fig. 8.12. The isolated fractions had purities of 98.9 and 99.2%, respectively (Fig. 8.13). The total recovery by weight was 96.5%.

8.8 Achiral separation using SFC

Historically, small-scale (<200–300 mg) achiral purification has been performed by reverse-phase HPLC. SFC has been proven to be very efficient for the separation of chiral molecules [10,11,15–19]. Most SFC work is performed using methanol, which is more volatile than water and reduces the evaporation time post-purification. Because of the success of SFC in the separation of chiral molecules within GSK, we decided to use SFC for the analysis and purification of achiral molecules as well. The achiral SFC chromatograms of a GSK proprietary compound are shown in Fig. 8.14. The purity by UV of the material prior to purification was estimated as 55%. After preparative SFC purification, the compound of interest was returned to the organic chemist at a purity of 98.3% by UV.

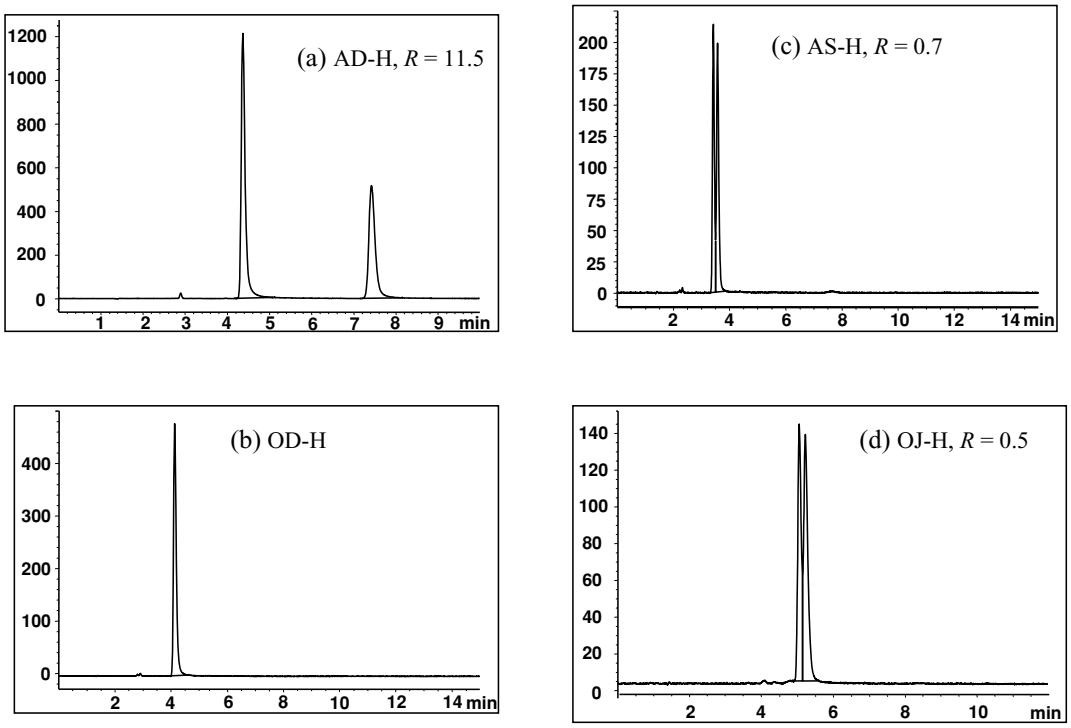

Figure 8.11 Flurbiprofen injected on analytical SFC. Chromatographic conditions: 15% methanol, 105 bar, 30°C, 2 mL/min (a) Chiralpak AD-H column, (b) Chiralcel OD-H column, (c) Chiralpak AS-H column, (d) Chiralcel OJ-H column.

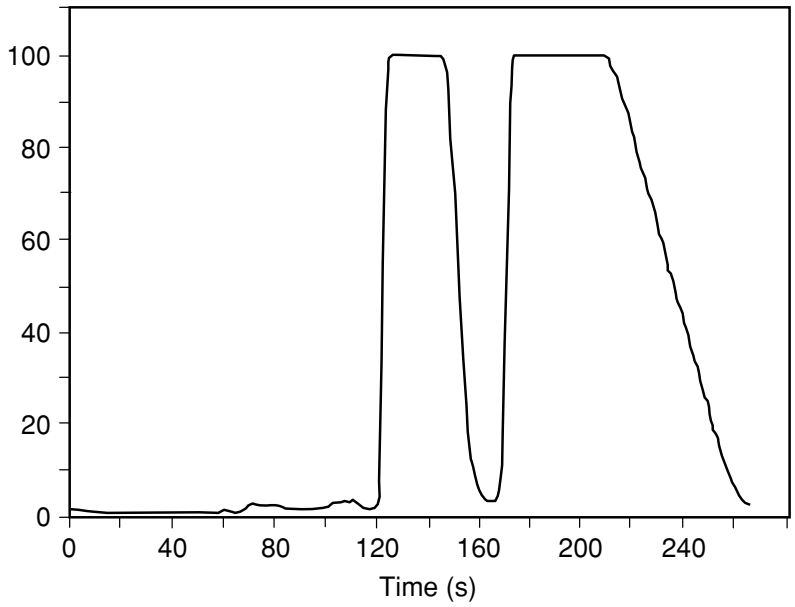

Figure 8.12 Flurbiprofen injected on preparative SFC. Chromatographic conditions: Chiralpak AD-H (20 mm × 250 mm), 105 bar, 30°C, 75 g/min of CO_2, 13 mL/min of methanol, 350 mg injected.

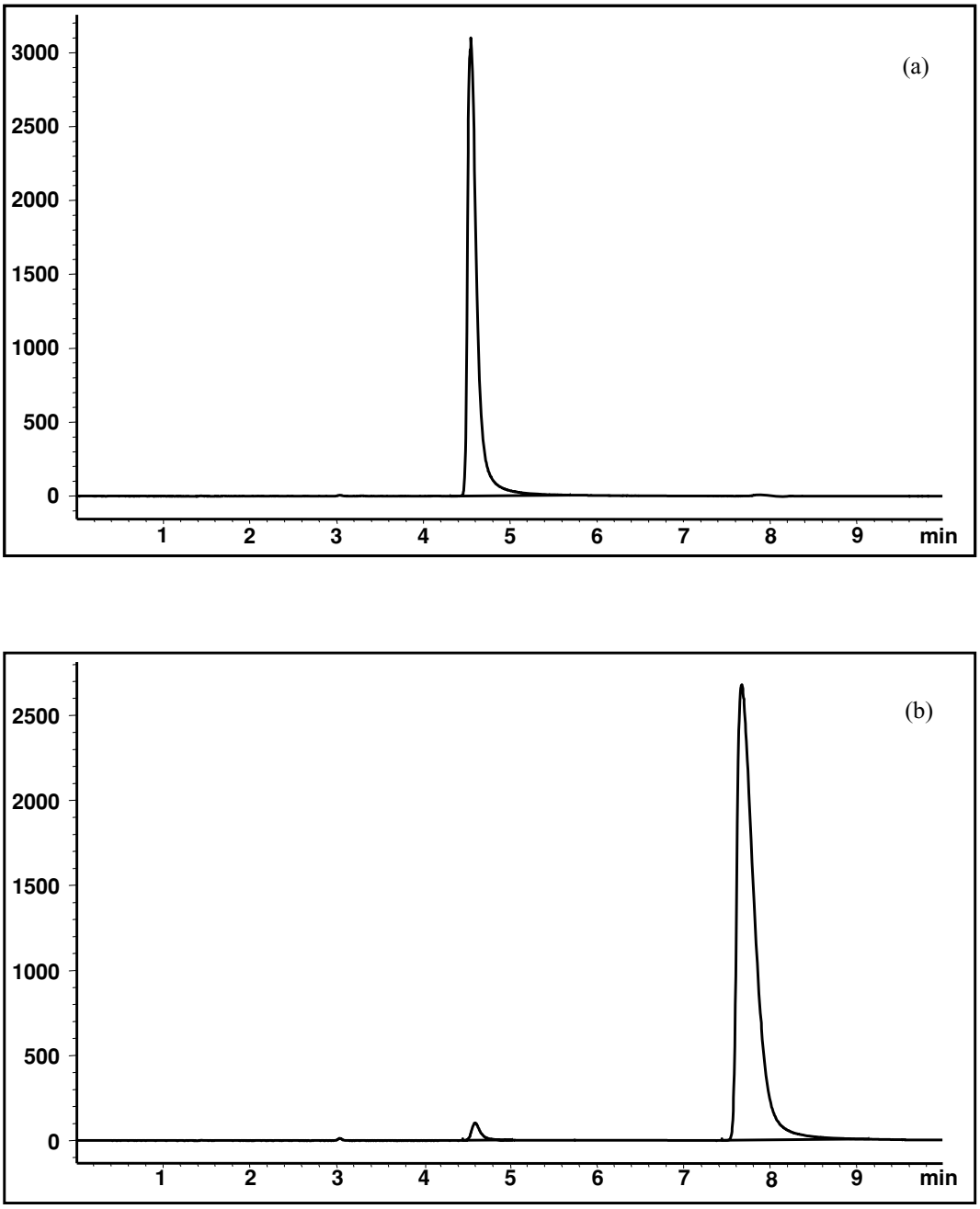

Figure 8.13 Purity of Flurbiprofen's enantiomers after preparative SFC. Chromatographic conditions: Chiralpak AD-H, 15% methanol, 105 bar, 30°C, 2 mL/min (a) 99%, (b) 97.9 %.

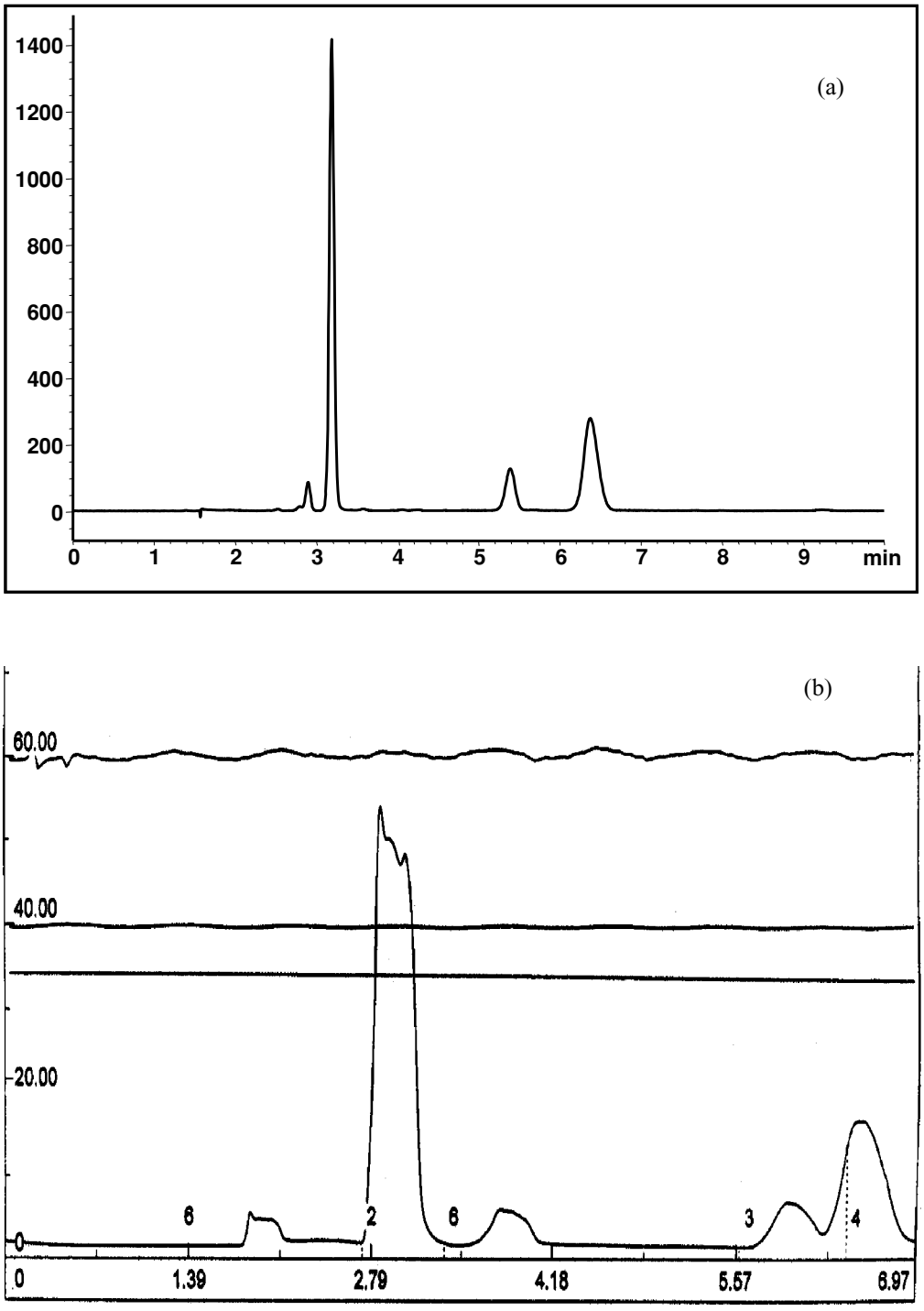

Figure 8.14 Achiral SFC chromatograms of a GSK proprietary compound. Chromatographic conditions: diol column (a) analytical SFC of original sample, 10% methanol, 140 bar, 27°C, 2 mL/min; (b) preparative SFC on diol column (20 mm × 250 mm), 36 g/min of CO_2, 4 mL/min of methanol; (c) purity after preparative SFC, 10% methanol, 140 bar, 27°C, 2 mL/min.

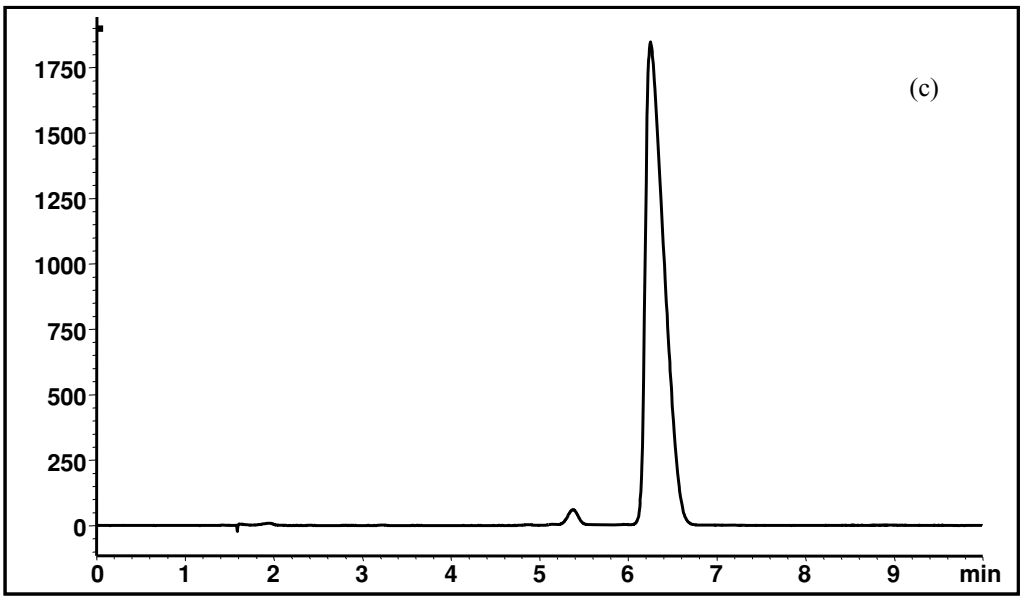

Figure 8.14 *Continued*

A test mixture containing nine commercially available compounds having a wide range of polarity was prepared. The goal was to compare the elution of each compound with that obtained by reverse-phase HPLC. The chromatograms shown in Fig. 8.15 demonstrates that all nine compounds eluted within 8 min on both LC and SFC. It should be noted that the broadening of the peaks for compounds 6, 7 and 8 in the SFC chromatogram could be due to the lack of additive in the cosolvent or due to the decreased solubility of these compounds in methanol/CO_2 compared to water/acetonitrile.

8.9 Consideration of preparative SFC

Several preparative systems are commercially available and should be selected depending on the specific needs of a laboratory or process/pilot-scale plant. Preparative SFC manufacturers include Jasco, Gilson, Berger, Thar and Novasep. Table 8.3 shows the specifications of each system.

While the Novasep SuperSep 10, the Berger MiniGram SFC, or the Gilson or Jasco preparative SFC can be used for g/day separations, it is more efficient to use either the Novasep SuperSep 20/30, the Berger MultiGram or the Thar SuperPure 50 for separation of more than 10 g/day. For separations of metric tons/year, the Novasep SuperSep 150 can be considered.

8.10 Future direction and development

Currently in our laboratories, the analytical chemists operate the SFC systems, whereas reverse-phase achiral analytical and preparative HPLC can be run in walk-up (open access) mode by the synthetic organic chemists. Development of similar walk-up SFC capability

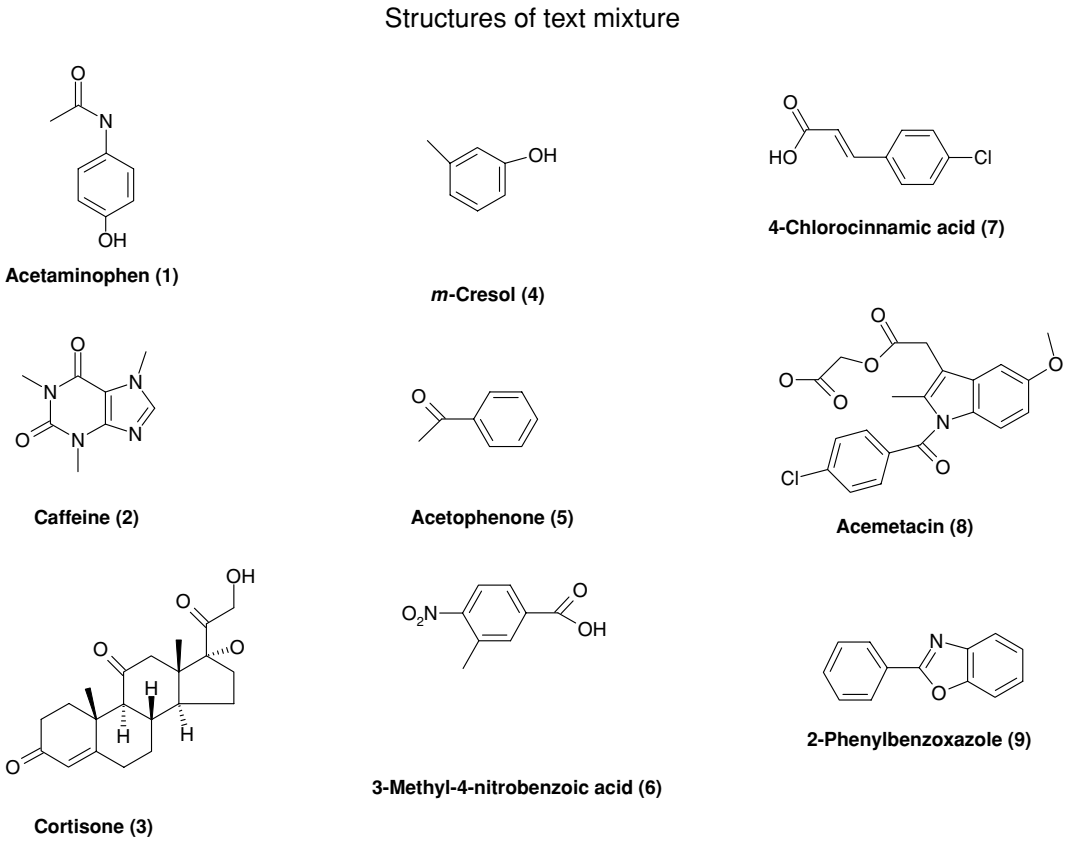

Figure 8.15 Structures of test mixture.

is underway and it is our hope that it will be deployed in our laboratories in the near future. In addition, we currently have the capability to use mass spectrometry, followed by parallel HPLC purification on plates (typically 96-well) of achiral molecules. We are currently researching methods to multiplex an SFC system to allow us to carry out similar parallel SFC analysis and purification.

8.11 Conclusion

SFC is a complementary technique to HPLC. It has been proven that SFC is very powerful for the separation of chiral and achiral molecules on both an analytical and preparative scale within many industries, including the pharmaceutical industry. Most of the time, an analytical method suitable for preparative SFC is found in less than 24 h and may be readily scaled up for preparative purification efforts. Solubility issues can be avoided by using chloroform as the diluent and added to the co-solvent as well. Both analytical and preparative SFC systems are now very robust and reproducible. SFC systems are used on a daily basis and separate 95% of all requests from the single sample purification group at GSK in Research Triangle Park, NC.

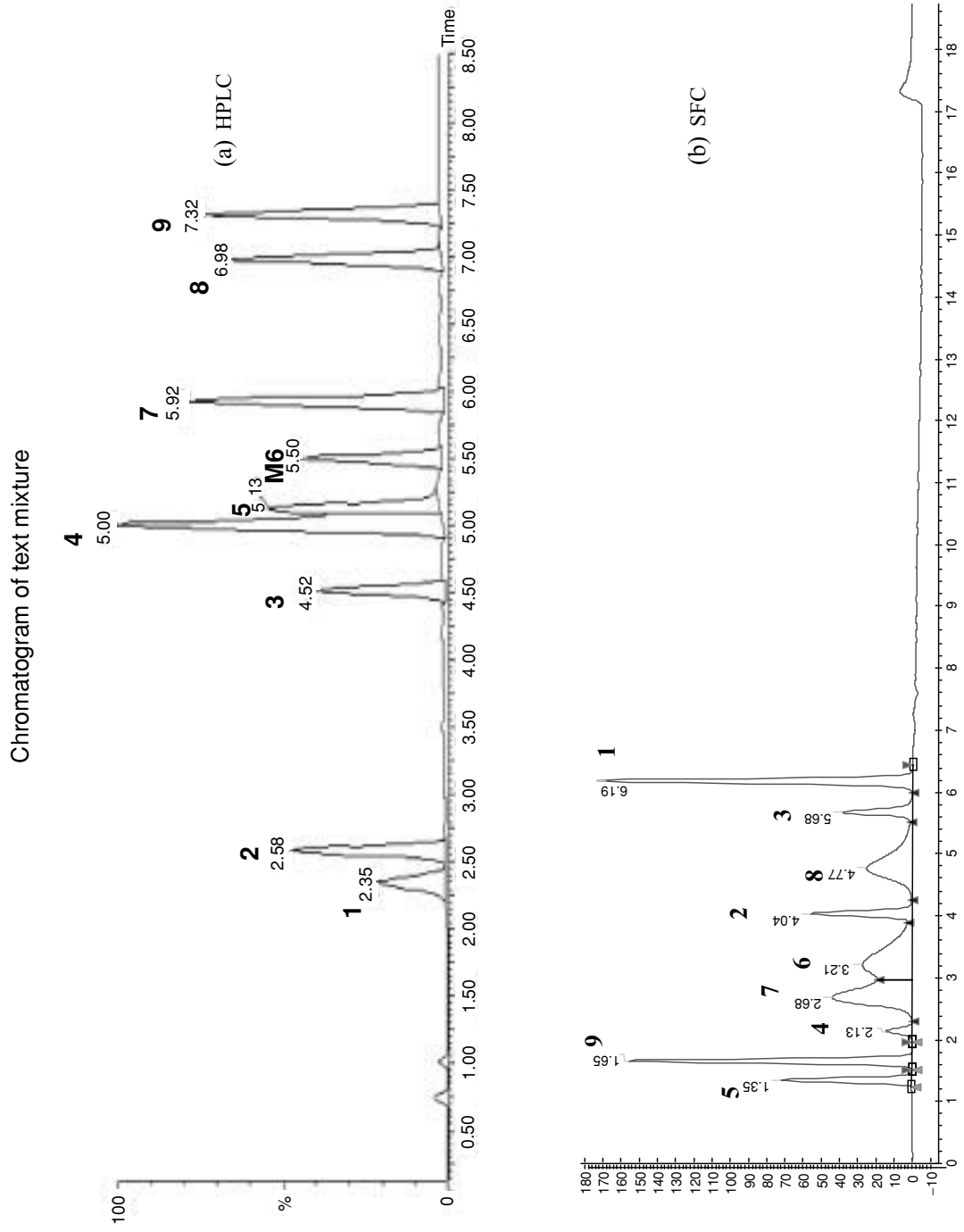

Figure 8.15 *Continued* Chromatogram of test mixture on (a) HPLC, Waters SymmetryShield RP18 (3.9 mm × 150 mm), 10–90% acetonitrile/water (0.1% formic acid), 1.5 mL/min; (b) SFC, silica column (4.6 mm × 150 mm), 5–50% methanol, 210 bar, 40°C, 2 mL/min.

Table 8.3 Specifications of commercially available preparative SFC systems

Manufacturer	Max. column i.d. (mm)	Max. flow rate	Max. pressure (bar)
Gilson	20	25 mL/min total	ca 250
Jasco	20	20 mL/min total	ca 300
Berger MiniGram preparative SFC	10	9.9 mL/min total	250
Berger MultiGram	20	50 mL/min total	400
Thar SuperPure 50	20	5–50 g/min CO_2	380
Thar SuperPure 200	75	200 g/min CO_2	380
Thar SuperPure 350	100	350 g/min CO_2	320
Novasep SuperSep 10	15	45 g/min CO_2	300
Novasep SuperSep 20/30	30	180 g/min CO_2	300
Novasep SuperSep 30/50	30 or 50	300 g/min CO_2	300
Novasep SuperSep 50	50	1 kg/min CO_2	300
Novasep SuperSep 80	100	2 kg/min CO_2	300
Novasep SuperSep 150	150	6 kg/min CO_2	300

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Preparative Enantioselective Chromatography
Edited by Geoffrey B. Cox

9 Equipment for preparative and large size enantioselective chromatography

HENRI COLIN, OLIVIER LUDEMANN-HOMBOURGER and FÉLICIE DENET

9.1 Introduction

Equipment for preparative and large size enantioselective chromatography does not differ much from equipment for non-optically active molecules. However, enantioselective separations are mostly binary ones and there are some specific aspects of the equipment for such separations. Whatever the type of application considered, one should never forget that the separation takes place in the column. It is the heart of the unit. This is true whether analytical or preparative chromatography is considered, whether the goal is to purify enantiomers or any other type of compounds, whether the operation is continuous or discontinuous and whether the mobile phase is a liquid or a supercritical fluid (or even a gas). And this is particularly true for modern preparative chromatography where rather short columns (i.e. 100–300 mm in length) packed with rather small particles (i.e. 5–20 μm) are used. These columns offer high efficiencies and are operated at high velocities (5–20 cm/min) in order to maximise productivity and/or minimise purification costs. In the following discussion, it is considered that a preparative column is a column with an internal diameter exceeding 50 mm. This is obviously an arbitrary limit but it is more or less the maximum diameter of commercially available pre-packed columns.

When discussing columns, several items should be addressed: the column technology, the column design, the packing material and the packing procedure. Enantioselective stationary phases are not discussed here since they are the topic of a dedicated chapter.

Particular emphasis is put on the column because this is the most critical part of the equipment, but obviously, the column is not the only part of the equipment to be considered. A good column associated with a poorly designed unit is often worth nothing. Enantioseparations, except from the viewpoint of the stationary phase, do not require the use of specially designed pieces of equipment, except for a few aspects such as the type of detection used. What applies to any separation, and particularly to any binary separation, applies to enantioseparations as well.

The design of the equipment has to be optimised for the chromatographic technique to be used. For instance, if single-column recycling is considered (see below), special attention should be given to dead volumes in the recycling section, and particularly the eluent pump. In 'simple' elution chromatography, the volume of the pump head(s) is usually not a concern.

The purpose of this chapter is to discuss equipment for preparative chromatography in general, with special emphasis for enantioseparations. A first part of the chapter will address the column itself (bed formation, technologies available, column design, etc.), whether it is used in a batch or a continuous process. In a second part of the chapter, specific items related to equipment for preparative batch chromatography are discussed. The third section

addresses preparative chromatography with supercritical eluents, and finally the last section is devoted to continuous chromatography (simulated moving bed and VARICOL). (Note: the following discussion does not include radial flow column designs.)

9.2 The heart of the chromatographic process: the column

A good chromatographic column is a column that produces the expected separation. This involves thermodynamic considerations (related to the chemical nature of the mobile and stationary phases), as well as kinetic considerations. Kinetics describe how the bands of solutes broaden as they migrate down the column. Parameters involved in kinetics are the column design (including but not restricted to its length), the particle size (or rather size distribution), the mobile phase velocity, the product diffusion coefficients and the bed structure. This last point is particularly critical and not so easy to assess. It is related to physical properties of the packing material (size distribution, shape, 'chemistry'), the column technology and its design, and the packing procedure itself. Clearly enough, the mechanical stability of the bed over time is of utmost importance. A column which would offer adequate quality for a limited time only would be almost useless.

The technology and the design of preparative columns must be such that they bring answers to several questions, among which [1]:

- *Column packing* (and unpacking). This is particularly critical for large size columns (i.e. columns with an internal diameter exceeding 300–400 mm). The packing operation must be safe, reproducible, easy and reasonably fast, and compatible with regulatory norms. Obviously, it must also respect the integrity of the packing material.
- *Bed settling/reorganisation*. With time, and because of the friction created by the flow of eluent in the column, the particles of packing material reorganise themselves to produce a denser and better organised bed (see discussion below). This is accompanied by a reduction of the bed volume (bed shortening). The column technology must be such that this bed shortening does not result in an 'empty' section (devoid of packing material) where diffusion and back-mixing phenomena could take place with adverse effects on the column performance.
- *Scalability*. The column performance should be directly scalable. In other words, and under otherwise identical conditions, the column performance should not depend on the column diameter.
- *Fluid distribution*. A 'plug flow' distribution should be obtained in order to avoid additional band distortion and broadening.
- *Practical issues*. Among these one should mention the possibility to pack the column with any type of packing material (including fragile and soft media), to control temperature and to adjust the bed length (for full process optimisation). In particular, it should be possible to pack short beds (i.e. 100 mm) in wide columns (i.e. 1000 mm i.d.) for continuous chromatography applications.

9.2.1 *Packing technique, bed formation and bed consolidation*

Before discussing column technology and design, it is worth considering how beds of particles are formed. This is particularly relevant for columns of large size. A lot of work has been done in this field (see [2–5], for instance). Comprehensive reviews on column technologies and packing techniques as well as bed formation and consolidation have been published [1,6].

Chromatographic columns are packed using a slurry technique (except for dry packing procedures). During the packing process, unstable particle bridges or arches can be formed across the column diameter. Some of these bridges break during the packing process and some remain once the packing is completed. During column operation, several phenomena will tend to break the remaining bridges. These can be the friction of the eluent against the particles, mechanical vibrations, temperature changes, etc. In analytical columns, the remaining bridges are stabilised by the support from the column wall ('wall support'). The loss of wall support happens when the ratio of column diameter to column length exceeds a certain value (not clearly known, but typically above 0.2). Above this limit, the particles at the centre of the packed bed are no longer stabilised by the column wall because they are too far away. The remaining bridges can collapse within a few minutes of operation of the column after it has been packed, or after a longer period of time. But usually they do ultimately collapse. Unless an appropriate column technology is used, bed consolidation results in the formation of channels and voids with detrimental effects on the column efficiency. The loss of performance is obviously of great concern when columns are used for industrial purifications. The way to eliminate the detrimental effects of bed consolidation (but not bed consolidation itself) is to adapt the column volume continuously to the changing bed volume. This can be achieved by using columns with an appropriate technology, basically columns equipped with dynamic axial or radial compressions (DAC and DRC) [7,8].

With the DAC approach, continuous axial bed compression is achieved by the pushing action of a piston, whereas with the DRC the squeezing of the column wall produces radial compression.

The extent and the speed of bed consolidation depend on the magnitude of the compression applied, bed size (length and diameter), physical properties of the particles (shape, average size, size distribution, mechanical resistance, etc.) and local conditions (nature of the solvent, possibly temperature, etc.). The presence of a layer of organic material on the particles (i.e. bonded phases) may act as a lubricant and have an effect on the speed of the consolidation process. It is worth mentioning at this point that silica particles do have some elasticity (Young's modulus of glass is about one third that of steel) and can be deformed by compression. This particle deformation has an effect on both external and internal bed porosities. It seems reasonable to predict that materials with a large pore diameter and/or pore volume are more deformable (and thus compressible). Such materials are typical of chiral stationary phases (CSPs). Stationary phases based on polymers are obviously more compressible than those based on silica.

Because of the existence of frictional forces between particles, bed consolidation is not an instantaneous process. Upon increasing compression, the particles do not rearrange 'smoothly' and uniformly. Consolidation proceeds by the formation of local conditions producing chaotic and irreversible equilibrium ruptures. Indeed, upon compression,

particles can roll more or less smoothly on top of each other or around each other, but sudden avalanches do happen as well which result from the rupture of the unstable bridges. These bridges can be broken rather far away from the point where compression is applied. Accordingly, bed consolidation resulting from a given compression proceeds by steps and tends towards a limit in a quasi-exponential way, the order of magnitude of the pseudo time constant being a few hours and increasing with the bed volume. It is reasonable to predict that the pseudo time constant depends on the particle size distribution and the particle shape. Beds made from monodispersed spherical particles are likely to consolidate faster than those made from irregular particles with a large size distribution.

The time required to reach bed stabilisation upon compression is not only the result of the frictional phenomenon previously described. Another effect, of a hydrodynamic nature, is the time necessary to remove the liquid located between the particles. The magnitude of the effect increases when the bed volume increases and/or the particle size decreases.

If the bed compression is released, the bed length does increase (relaxation) but it remains less than what it was prior to applying compression. The bed expansion taking place when compression stops is created by a sudden increase of the space between particles (corresponding to the column external porosity) [2]. The particles that were deformed by compression recover their original shape (provided they have not been damaged) but the movement of particles relative to each other resulting in a decrease of the interparticulate space (bed consolidation) is irreversible [3].

According to the theory of soil mechanics, most of the bed consolidation process takes place within a few seconds after compression has been applied. This has indeed been a common observation with DAC columns: it takes just a few minutes for the piston to stop its initial obvious movement once the bed is formed [9]. The bed length then continues to shorten with time, but very slowly. There is a consensus, however, that bed consolidation is never fully achieved [4]. The fact that the bed length is stable after a certain time is not necessarily the indication that full consolidation is achieved. There is indeed friction of the particles against the column wall (the wall support previously mentioned), which counteracts bed compression, to some extent. Being able to eliminate bed friction against the wall would probably result in more compact beds, but this friction provides faster stabilisation of the bed, even though consolidation is not fully achieved, and this is of practical interest. The contribution of wall friction decreases with increasing column diameter. As already mentioned, the wall support is intense enough to provide very efficient stabilisation of the bed for analytical size columns. This does not apply to preparative columns.

9.2.2 *The wall region*

There are several phenomena occurring near the wall and at the wall. As previously discussed, there is friction of the particles against the wall. During the packing process, this friction produces a radial heterogeneity of the bed density, the bed very near the wall being not as dense as the core of the column. The consequence is a peculiar flow velocity profile in the column (when it is used), the solvent near the wall (and accordingly the injected products) migrating faster than in the rest of the column. This has a negative effect on the column efficiency. The existence of this wall region has been demonstrated using magnetic resonance techniques [10]. The thickness of the wall region is very small, however, typically

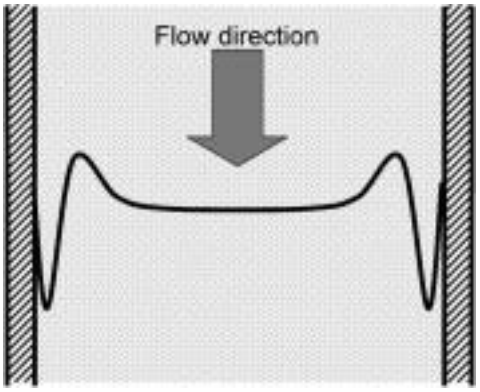

Figure 9.1 Typical shape of the eluent velocity profile across the section of a large column. The magnitude of the changes is exaggerated for better understanding [11].

a few particle diameters. This thin wall region can be clearly seen when unpacking a column in which a coloured substance has been injected and partly eluted. It is reasonable to assume that under normal conditions, this wall effect is negligible.

The existence of another wall region ('second wall region'), extending further inside the bulk of the column, has also been suggested. In this region, the bed would be denser than in the rest of the column (with a concomitant reduced velocity), because of the heterogeneous distribution of the stress during the packing operation [11]. Contradictory results have been reported concerning the thickness of this region, but it would be far greater than the previously discussed one and could exceed 1 cm. The velocity distribution over the column cross-section would then be as shown in Fig. 9.1, resulting in peaks with a small front (the product migrating very near the wall) and a tail (the product migrating in the second wall region).

It is not clear for the authors whether this second wall region really exists or not. It is likely that in several cases the particular shape of the velocity profile could be explained by the column design. More experiments are presently being performed to get better understanding (H. Colin, O. Ludemann-Hombourger and F. Himbert, data to be published) of this region. What is clear, however, is that it is desirable to obtain a velocity profile across the column which is as flat as possible.

9.2.3 *Heat dissipation*

There is another phenomenon contributing to produce a peculiar velocity distribution near the column wall, and unless some precautions are taken, this phenomenon always results in asymmetrical peaks, even with perfectly packed columns. This phenomenon is heat dissipation. During percolation of the mobile phase though the packed bed, heat is generated because of the friction of the solvent on the particles. The larger the pressure drop in the column, the more heat is generated. This phenomenon results in a longitudinal temperature gradient (the eluent at the column outlet being warmer than at the inlet) and a radial gradient [12]. The temperature between column inlet and outlet depends on many parameters (particle diameter, flow velocity, etc.) and is typically between 1 and 5°C. It can be more, however, particularly when short beds packed with very small particles and operated at high velocity

are considered. Provided the temperature is homogeneous over the column cross-section, the longitudinal temperature gradient does not affect the column performance. In terms of kinetics, it translates into a local plate height decreasing (slightly) from the column inlet to the outlet [13].

Heat dissipation also induces a radial temperature gradient which can potentially be much more harmful than the longitudinal gradient [12]. It is reasonable to assume that the column wall is isothermal (for preparative columns, the wall is a thick piece of steel of rather short length). The wall temperature is often controlled by the use of a jacket, or by placing the column in an oven. Because of the existence of the longitudinal temperature gradient, it is likely that the centre of the column is at a different temperature than the wall for most of its length. Accordingly, a radial temperature gradient is created, and its magnitude changes from the column inlet to the outlet. The radial gradient induces an eluent velocity gradient (because of the effect of temperature on viscosity), which produces distortion of the bands of solutes. In addition to this kinetic phenomenon, a thermodynamic effect should also be considered. Since heats of adsorption are negative, in most cases retention decreases with increasing temperature (i.e. the equilibrium constant is reduced). In other words, solutes migrating in sections of the column where the mobile phase is warmer are less retained. As can be seen, the two effects (kinetic and thermodynamic) act in the same direction and do not compensate for each other: solutes migrating in warmer sections of the column move faster because the solvent velocity is higher and the adsorption constant is less. Depending on the temperature difference between the column wall and the solvent at the column inlet, tailing, fronting or symmetrical peaks can be obtained with perfectly packed columns of ideal design (see Fig. 9.2, for instance). As a matter of fact, if the solvent at the column inlet and the column wall are at the same temperature (which might be considered as an ideal situation), peaks will always tail. The wall temperature can be adjusted to compensate for heat dissipation. In order to get symmetrical peaks, it is necessary that the wall temperature is between the solvent inlet and outlet temperatures. The ideal wall temperature depends on the solute considered (it is related to the solute heat of adsorption) and is typically 1–3° C above the incoming mobile phase temperature.

Controlling wall temperature thus appears to be a good tool to help reduce adverse effects occurring near the column wall and related to the packing structure, as well as those due to the delay for the molecule to reach the wall at the column inlet (and to reach the outlet pipe at the column outlet; see discussion below). Increasing the wall temperature is a simple way to speed up solvent and products migrating near the wall.

It should also be mentioned that the thermal expansion coefficients for the packed bed and the column wall are not identical. This should not create significant problems, however, unless the column is operated at 'very high' temperature (i.e. more than 70–80°C) and is subjected to repeated heating–cooling cycles, in which case cracks of thermal origin could appear in the bed with detrimental effects on the column efficiency.

9.2.4 *Column technology*

In terms of column technology, there is a consensus today that the best (and probably only) approach, feasible at any size, is DAC. The piston can be driven by a hydraulic jack, or pushed by a liquid, or by a spring, for instance [1]. Radial compression is an alternative but it does not seem to be available at (very) large size. It also requires the use of pre-packed

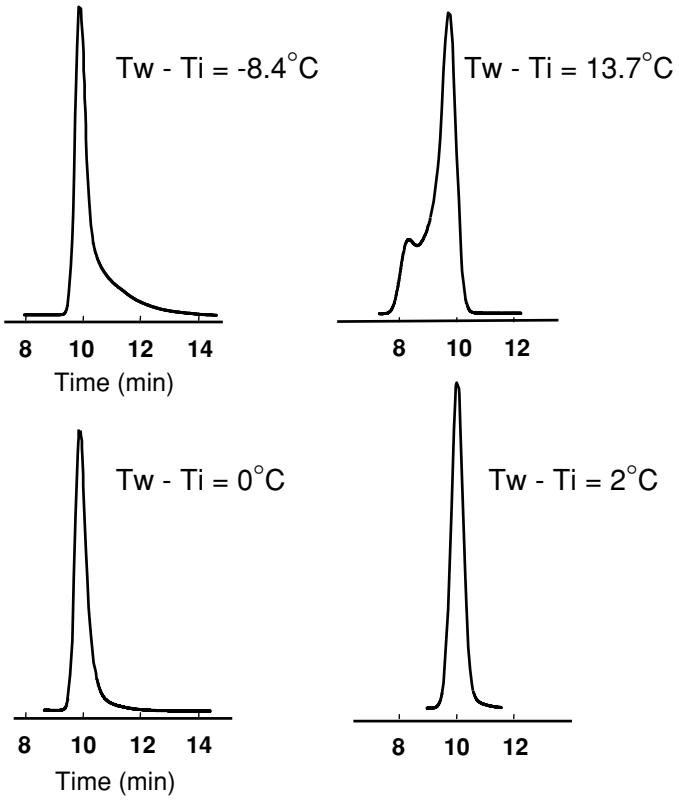

Figure 9.2 Effect of the temperature difference between the eluent entering the column and the column wall (experimental chromatograms). Column length: 25 cm, column diameter: 11 cm. Sample: acetophenone. Eluent: methanol (205 mL/min). Tw: wall temperature, Ti: solvent temperature at column inlet.

cartridges, thus reducing the operator's freedom compared to DAC. Under appropriate conditions, the DAC technology ensures bed stability and reproducible performance. DAC can be scaled-up to very large size with remarkable reproducibility in performance. This has been verified with columns up to 1600 mm i.d. [14]. A scale-up example is shown in Fig. 9.3.

Various designs for DAC columns are available from different companies [1]. Some designs are interesting at small scale for simplicity and price considerations but cannot really be produced for large diameter columns. More information is given in Ref. [1].

When fragile and/or soft packing materials are used, special care must be taken to ensure that the compression energy is compatible with the mechanical properties of the particles. Also, the shrinking–swelling phenomena observed with some organic materials under certain mobile conditions should not result in overcompressed beds. To address this issue, columns with an 'intelligent' piston are available. With such columns, a given bed compression can be programmed and the hydraulic system is able to adjust the piston position so that a constant compression is maintained.

In terms of column technology, another special situation is encountered in supercritical fluid chromatography (SFC; see Section 9.4). Here, rather elevated pressures must be used in order to attain the supercritical state. The fluid pressure in the column varies a lot, depending on whether the column is in use or not. The effective pressure exerted by the piston on the bed is the difference between the compression pressure and the fluid pressure under the piston.

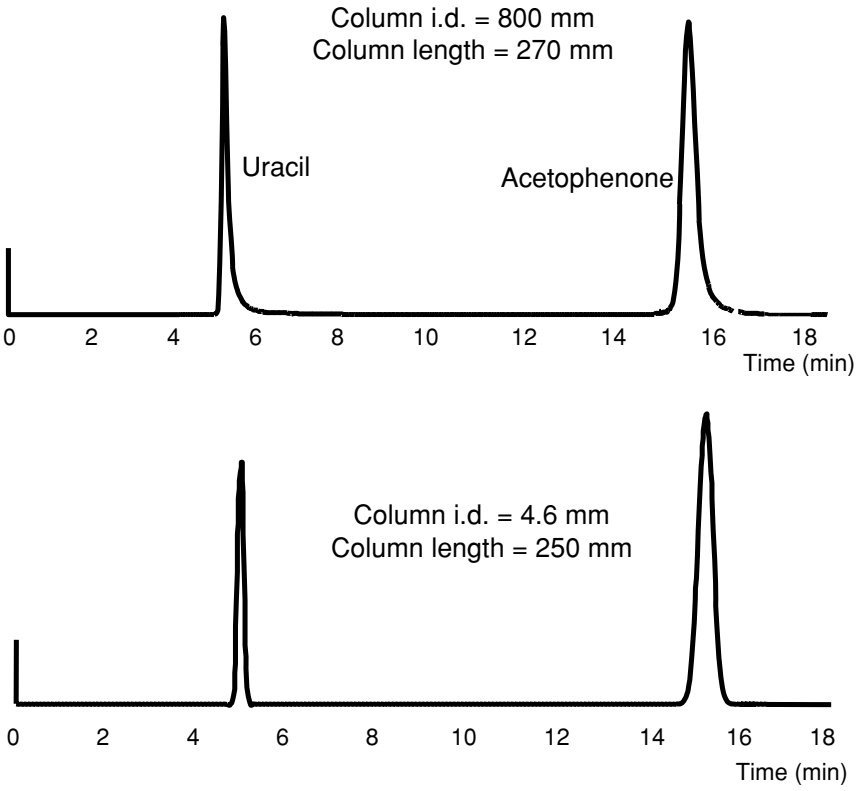

Figure 9.3 Scale-up between a 4.6 mm × 250 mm analytical column and a 800 mm × 270 mm DAC column. Packing material: C18 10 μm spherical. Eluent velocity: 5 cm/min. Eluent: 80/20 (v/v) acetonitrile/water.

The compression pressure should then be adjusted, depending on the internal pressure of the column, since too high a piston pressure may crush the stationary phase, whereas a too low pressure may prevent the piston from effectively compressing the bed. It seems that only DAC columns are suitable for large-scale SFC. These columns are equipped with 'smart' compression units that maintain adequate bed compression without allowing the packing material to be overpressurised when the mobile phase pressure is released.

9.2.5 *Column design*

As far as the column design is concerned, key points are safety, the achievement of the proper distribution of the liquid in the column, the possibility of direct scale-up, convenience of use and the possibility to pack beds of adjustable length. This last point is particularly critical for production columns (large size columns) and even more important for continuous chromatography applications, which often require the use of (very) short beds (i.e. 100 mm or less; see Section 9.5). Indeed, for given operating pressure and production capacity, the optimum bed length is almost proportional to the square of the particle size whereas the column diameter is only slightly influenced by this parameter [15]. Decreasing the particle size results in reduced column length and bed volume. Smaller particles then mean reduced investments and cheaper separations. This is particularly true for chiral separations, where the cost of the stationary phase is high and not significantly influenced by the particle size. As a matter of fact, and contrary to what is often thought, large diameter columns (the

production capacity of a column is proportional to its cross-section) of short length, packed with small particles should be used for production-scale chromatography. It is important to mention that for several reasons (such as equipment pressure rating and heat dissipation, for instance), it is not recommended to use particles less than 5 μm in size.

For batch processes and pressure rating of 70 bar (1000 psi), the optimal particle size is typically around 10–15 μm. For continuous chromatography, reduced column efficiencies are required compared to batch chromatography, and very short columns (less than 100 mm in length) are often used. The optimal particle size in this case is about 20–40 μm.

The design of the column ends must be such that a plug flow situation is attained over a few millimetres of migration in the bed. A typical design includes a frit and some sort of chamber above the frit to help distribute the liquid. Most of the time, a mesh-type frit is used, the 'active' mesh being 2–5 μm porosity. In order to help distribute the liquid evenly over the column cross-section, the permeability of the frit must exceed that of the bed. If this is usually not a problem for columns packed with small particles, it may be more difficult to achieve when short beds packed with medium size media (i.e. 20 μm particles) are used. This is typically the case for columns used for enantioseparations in continuous chromatography (see below). In this case, introducing a pressurisation chamber of adequate design above the frit is almost a necessity. Another major advantage of the mesh-type frits is the fact that the radial frit permeability is greater than the axial one. As a result, liquid in the frit tends to go to the edge of the frit rather than going through it, thus promoting the sample distribution. It is important to mention that the quality of the frits (inlet and outlet) is of paramount importance as far as the performance of a column is concerned. It is the experience of the authors that most of the problems related to loss of efficiency, shoulders and double peaks, etc., are related to dirty and partially plugged frits.

Adequate design of the column ends can be made by proper modelling of the fluid dynamics. An example of flow modelling is given in Fig. 9.4 where the calculated fluid

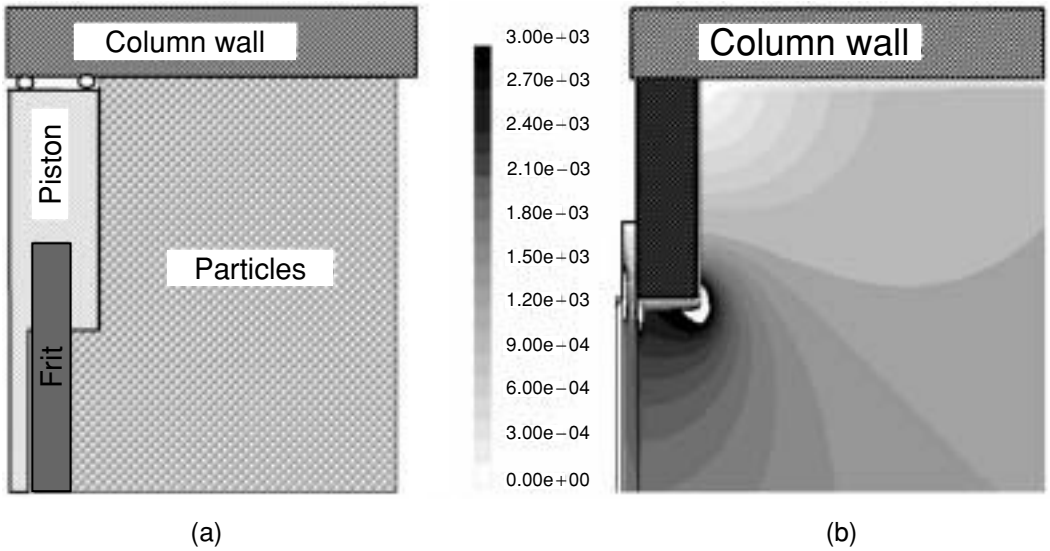

Figure 9.4 (a) Simplified configuration of the inlet of a 1600 mm DAC column. (b) Calculated velocity contour.

velocity distribution at the inlet of a 1600 mm i.d. column is shown. With proper column design, it is easily possible to achieve scale-up factors exceeding 30 000. Modelling is also an efficient tool to test alternative solutions to help in achieving a plug flow distribution. For instance, the use of a jet breaker may be shown sometimes to create more harm than good.

The design of the column ends must also be such that all solute molecules injected reach the top of the bed simultaneously. This is theoretically impossible since, even if the design of the column inlet is such that a perfect piston flow situation is achieved right at the column inlet, it necessarily takes less time for a molecule entering the column via the inlet pipe to go straight into the bed, rather than migrate to the side and reach the wall. The time difference may have an effect on the column performance under certain conditions [16]. Obviously, the situation is the same at the column outlet: it takes more time for the molecules migrating near the wall to reach the outlet pipe than for the molecules migrating at the centre of the column. Radial mass transfer in the mobile phase does help reduce the magnitude of this 'geometrical' effect, but in large diameter columns with short bed length, the effect may be critical. Using a warm column wall can reduce the effect very significantly (see above).

9.3 Equipment considerations for batch chromatography

The following discussion is limited to two aspects of equipment design (not including the column) for batch chromatography: recycling and detection.

9.3.1 *Recycling*

Recycling, under various forms, is a very interesting way to increase throughput for enantioseparations and more generally speaking, binary separations (see [17–20] for instance).

Recycling consists in injecting a large quantity of feed (so large that there is not complete separation of the two products) and returning the column effluent to the column as many times as necessary to get satisfactory purity and recovery yield. Under ideal conditions (see below), and assuming that the first peak of cycle n does not interfere with the last peak of cycle $n - 1$, recycling n times is equivalent to using a column $n + 1$ times longer, but with the pressure drop of one column only and the amount of stationary phase of one column only. This is clearly a big advantage when expensive stationary phases are used. This is 'simple recycling'. It is also possible to collect some amounts of each product at each cycle and recycle only the fraction of the chromatogram where the products co-elute. This is 'shave recycle'. Other strategies have been described and particularly the combination of shave recycling and re-injection of crude each time the impure fraction is returned to the column. After a certain number of cycles, and provided the conditions are appropriately selected, a steady state is achieved. This is 'steady-state recycling', patented under the name Cyclojet [18,21,22]. The principle of recycling techniques is described in detail in Chapter 6.

Recycling techniques can be performed with one or two columns. When only one column is used, the recycled fraction has to go through a certain section of the piping of the unit ('recycling section'), and particularly through the eluent pump before being injected. It is important to design the unit in such a way that additional band broadening occurring in the recycling section does not impair the quality of the separation. In other words, the variance

associated with the recycling section should only be a small fraction of the band variance created by the column. Under certain conditions, and particularly when columns of small volume offering large numbers of plates are used, this puts strong constraints on the design of the unit. The following discussion is limited to linear chromatography.

The variance created by the column, σ_C^2, is given by

$$\sigma_C^2 = V_R^2/N \tag{9.1}$$

where V_R^2 is the retention volume of the solute and N the column efficiency. V_R^2 is related to the capacity ratio k', the total porosity ε_T and the column geometrical volume V_G according to

$$V_R = V_G\varepsilon_T(1 + k') \tag{9.2}$$

The variance of the recycling section, σ_{RS}^2, is related to the design of the piping and particularly to the existence of poorly swept areas. In this respect, the contribution of the eluent pump may be particularly critical. In order to minimise the extra-column effects and to prevent the loss of performance due to the recycle loop, it is essential that σ_{RS}^2 is small compared to the column variance. It is best, but not simple, to design the system such that it is under turbulent flow conditions everywhere in the recycling section. Under these circumstances, the volume of the recycling section is not critical and it only creates a time delay that can be easily taken into consideration. In reality, the situation is more complicated and σ_{RS}^2 depends on the flow rate.

Equations (9.1) and (9.2) suggest that working with strongly retained products (i.e. $k' > 2$) makes the situation (much) easier concerning equipment design since the constraints on σ_{RS}^2 are much wider. The same is true with columns of large volume. The equations also indicate that using columns offering large numbers of plates creates more constraints if performance is not to be lost due to the recycling loop. Modern preparative batch chromatography is based on the use of efficient columns (small particle size) of optimised volume. In addition, it is recommended to use small k' values (<1) in preparative chromatography for production rate considerations. These remarks illustrate the fact that designing a unit with optimum performance is far more than assembling pipes, valves and pumps.

9.3.2 *Detection*

Detector specifications for preparative applications are obviously different than for analytical ones. For instance, because of the large solute concentration values in the column effluent, it is often desirable to reduce detection sensitivity. It is also desirable to extend the detector dynamic range. Usually short path length detector cells are employed to accomplish these objectives. Despite this, it is often the case that the detector signal is overloaded, or, at least, non-linear. This restricts the usefulness of detection in preparative chromatography. It is also known that because of the peculiar peak shapes often encountered in non-linear chromatography, the detector signal can suggest the presence of more than two compounds even when binary feeds are processed (as in the case of enantioseparations). The detector signal must then be used with great care, particularly when it is used to trigger fraction collection.

As far as enantioseparations are concerned, detection can be specific (based on optical properties: polarimetry and circular dichroism) or non-specific (UV absorption, refractive index, light scattering, for instance). The most common detection technique in preparative

chromatography is certainly UV absorption. It is a very well-known technique and it will not be discussed here. UV detectors for preparative applications are characterised by flow cells with short path length (typically 1 mm compared to 10 mm for analytical purposes), and large dynamic range, ideally to 5 or 10 OD (optical density).

Besides the UV detector, there are two specific detectors for enantioseparations: the polarimeter and the circular dichroism detector [23–25]. The optical activity detectors deliver signals that are related to the concentration and configuration of the analytes. It is possible, at least in theory, to use the combination of UV and optical activity detection to assess the concentration of each isomer in areas of the chromatograms where they co-elute. This makes it possible to calculate the enantiomeric excess (ee) and this information can be used to control fraction collection. Indeed, in the linear range of the detectors, the UV signal is proportional to $(C_a + C_b)$, whereas the polarimeter signal is proportional to $(C_a - C_b)$, C_i being the concentration of species i. A previous calibration of the detector signals permits direct measurement of the concentration of each enantiomer, at least in theory.

It is the experience of the authors, however, that practical attempts to use this combined detection strategy in preparative chromatography are not so successful. There are several reasons for this. For one, the detectors are often operating in a non-linear domain because of the high concentration of solutes eluting the column; this is particularly the case in continuous chromatography (see Section 9.5). It must also be mentioned that the method is very sensitive to the accuracy of the detector calibration and is strongly disturbed by potential impurities or by a drift of the baseline, situations that are not rare in preparative chromatography. Unless one is operating under 'touching band' conditions, the normal mode of preparative chromatographic operation is to define cut points through the collection of fractions, in which case it is easy to relate the collection points to the signal from a single, usually UV, detector. For touching band separations, the separation can be monitored by any detector since one is not looking for quantitative data but simply an indication if the peak has eluted or not.

9.4 Supercritical fluid chromatography

9.4.1 *Principle*

The interest in using supercritical fluids, and more particularly supercritical carbon dioxide, in preparative chromatography has been discussed several times (see Chapter 8, for instance) and will not be addressed in this chapter. For preparative chromatography, carbon dioxide is the ideal supercritical fluid for many reasons. The critical point is easily accessible ($P_C =$ 73.8 bar and $T_C = 31°C$), the toxicity is low as well as the price. In addition, CO_2 recycling is rather simple. Although other potential supercritical fluids exist, they are not generally used. In the following, the discussion only concerns the use of carbon dioxide (pure or modified) as supercritical fluid.

The limitations to the use of supercritical CO_2 in preparative chromatography are mainly related to the molecular weight and polarity of the products to be purified. Typically, the molecular weight should not exceed 1500 and the molecules should not be 'too' polar. Although there are not yet numerous examples of applications of SFC published (confidentiality is partly the explanation), the situation is presently changing (see Chapter 8, for instance). Medium and large units are available and are in use today.

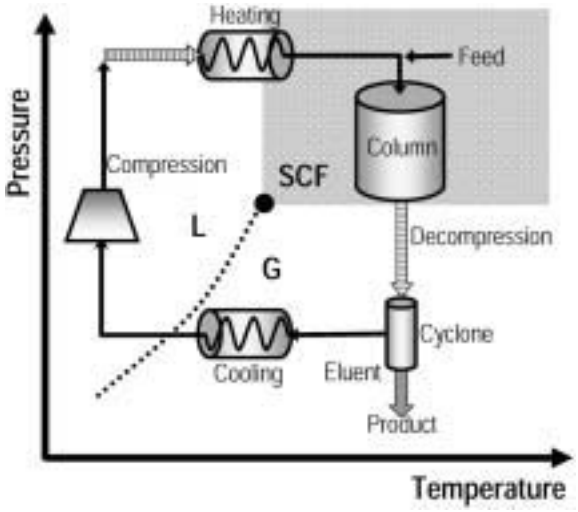

Figure 9.5 Principle of supercritical fluid chromatography.

9.4.2 *Technical aspects*

SFC can be simply described by a loop around the critical point in the phase diagram (see Fig. 9.5) (see [26], for instance). For the sake of simplicity, the use of co-solvent is not considered below. Aspects related to the column itself have been discussed earlier in this chapter and will not be further addressed.

The eluent source is either liquid or gaseous CO_2. After entering the unit, the fluid is cooled and compressed to reach the separation pressure P ($P > P_C$). Once at the right pressure, the fluid is heated to reach the desired temperature. Pressure and temperature should be precisely controlled as they influence the solvent density, which in turn controls its eluotropic strength. The feed is then injected and the chromatographic separation performed. At the column outlet the stream is decompressed and the fog obtained is directed to the collection manifold. Carbon dioxide being gaseous, it becomes a very poor solvent and the products 'precipitate' (see later); CO_2 can then be recovered and recycled.

9.4.3 *Eluent*

Carbon dioxide is delivered in high-pressure cylinders for analytical and laboratory-scale units or in high-capacity tanks for larger pieces of equipment. Liquefied CO_2 inside a container is at the pressure corresponding to a gas–liquid equilibrium (at 20°C this pressure is about 60 bar). CO_2 can be withdrawn from the reservoir (cylinder or tank) either as a liquid or a gas. Liquid withdrawal must be done without any significant pressure drop due to the possibility of liquid phase evaporation. Gas withdrawal is preferred when low-quality CO_2 is used since the purity can be easily improved using an adsorbent bed. However, a heating system must be used to prevent the reservoir from freezing.

For general use, the CO_2 purity should be not less than 99.9%. For pharmaceutical applications, it must be about 99.995%. The humidity in particular must be precisely known and controlled since the presence of water could dramatically affect the separation performance, depending on the sensitivity of the stationary phase to water.

Liquid modifiers used as polar additives in supercritical fluid are typically HPLC or analytical grade solvents. The most common ones are methanol, ethanol and acetonitrile, but other solvents can be used as well, with potentially interesting selectivity effects.

9.4.4 *Pumping*

SFC units require high-pressure pumps (typically 300 bar delivery). Pumps are preferred to compressors to avoid any oil contamination. Piston or membrane pumps are used (except for some analytical instruments operated with syringe pumps). Membrane pumps have the advantage of zero leakage and reduced contamination. To avoid cavitation, pressure shocks or even diaphragm damage, a subcooler is generally placed between the CO_2 source (even if it is a cylinder with a deep tube delivering liquid) and the pump's inlet valve(s). The liquid CO_2 pressure at the pump inlet is typically around 60 bar.

As supercritical CO_2 is a very non-polar solvent, a polar organic solvent (also referred to as modifier or co-solvent) is usually added to increase the mobile phase polarity in order to broaden the application range. Modifier addition can be done at high pressure (at the outlet of the CO_2 pump) or at low pressure (at the inlet of the CO_2 pump; see Fig. 9.6). Low-pressure addition is particularly interesting for technical reasons, since the operating pressure of the modifier pump is no longer 300 bar but 60 bar. Moreover, the CO_2 pump acts as a dynamic mixer, guaranteeing a homogeneous mixture between the CO_2 and the modifier. In the case of high-pressure addition, the heat exchanger located at the CO_2 pump outlet acts as a static mixer.

For optimum control of the CO_2 and modifier flow rate (and then chromatographic retention), it is recommended to use high-pressure flowmeters on the CO_2 and the modifier lines.

Pressure is a critical parameter because it controls the solvent strength. Back-pressure regulator or analogue valves are commonly used to control pressure. They are generally located after the column. Pressure regulation can be done by the control software based on

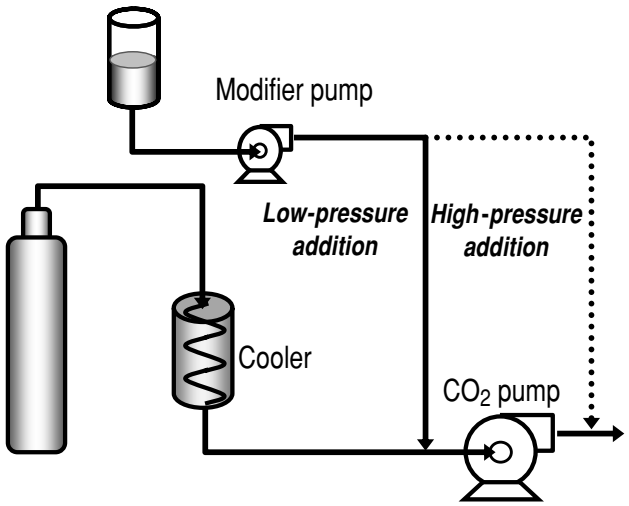

Figure 9.6 High- and low-pressure modifier addition.

pressure information provided by a properly located pressure sensor. Great attention should be paid to the dead volume created by these elements to avoid any remixing of the product streams before collection.

For some applications (mainly chiral), it can be interesting to work at a temperature lower than that required for true supercritical operation ('subcritical' conditions). This requires the cooling of the eluent at the outlet of the pump. The heat exchanger located after the pump can be used for such a purpose.

9.4.5 *Injection*

Once the mobile phase has reached the proper pressure and temperature conditions, the feed can be injected. Different techniques can be used:

- Six-way injection valve: This is very similar to the technique used for analytical injection. This is well suited for development purposes and small-scale preparative work. The injected volume can be changed by changing the loop volume.
- For larger injections (and larger pieces of equipment), the six-way valve can be replaced by a combination of several two-way valves (see Fig. 9.7). With proper sequencing of opening and closing, these valves make it possible to fill the loop, perform the injection and depressurise the loop (in order to refill it). This system is recommended for medium size units, particularly when they are dedicated to a given separation with a given injected quantity.
- Finally, direct injection into the column can be considered. The feed pump directly injects the feed into the eluent stream at the column inlet. This system is very flexible (the injected quantity can easily be controlled) but it requires the use of a high-pressure feed pump. It is not recommended for injection of small volumes.

9.4.6 *Detection*

Detection is generally made under supercritical conditions before decompression. Detection considerations are similar to those pertaining to liquid chromatography except that the detector must withstand pressure. For preparative purposes, UV detection is most commonly used.

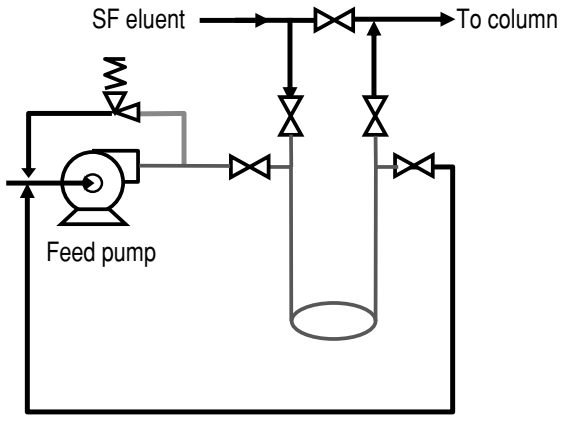

Figure 9.7 Principle of injection.

9.4.7 *Product recovery*

The strong dependence of solubility properties on pressure for supercritical CO_2 is used for solute recovery. The transition from 'good' (supercritical) to 'poor' (compressed gas) solvent results in separation of the solute and organic modifier from the CO_2 stream. Under ideal conditions, an adiabatic decompression of supercritical CO_2 leads to the formation of a biphasic system, either liquid–gas (above the triple point pressure of about 5 bar) or solid–gas (below this pressure). However, the efficient implementation of solute recovery is not so easy to perform, and without adequate hardware, a large part of the solute will be lost in a spray of fog from the back-pressure regulation valve [27].

For preparative purposes the most efficient way to separate solutes and any modifier present from the gaseous CO_2 stream is to use cyclonic separators [28]. Such a cyclone 'breaks' the two-phase mist created during the depressurisation of the column effluent. The separation principle in a cyclonic separator is based on the density difference between the liquid (or solid) phase and the gas phase. Typically, values of solute density – or of the solute dissolved in modifier – are in the range 800–1000 kg/m^3, without any significant influence of pressure. The density of gaseous CO_2, on the contrary, changes dramatically with pressure (from around 20 kg/m^3 at 10 bar to 110 kg/m^3 at 50 bar). Liquid–gas separation is then favoured by operating at low pressure.

In practice, the gas–(modifier)–solute mixture enters the cyclone via a tangential inlet, creating a vortex which forces the particles/droplets to migrate to the separator wall (Fig. 9.8). The cleaned gas forms a core in the centre of the cyclone, and leaves through a vortex finder at the top of the device.

Cyclones are sized depending on the eluent flow rate and modifier concentration. A bottom phase withdrawal can be done by an automatic valve system (under software supervision)

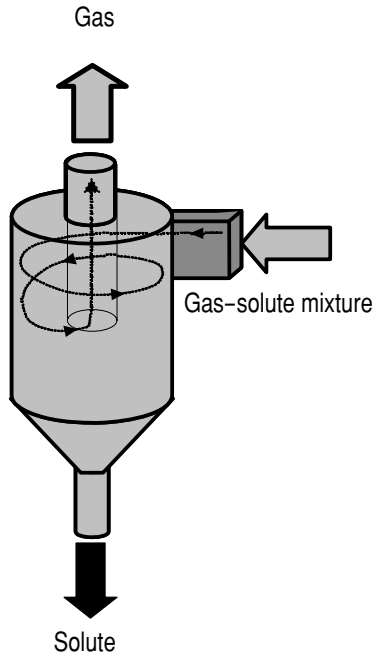

Figure 9.8 Principle of cyclonic separator.

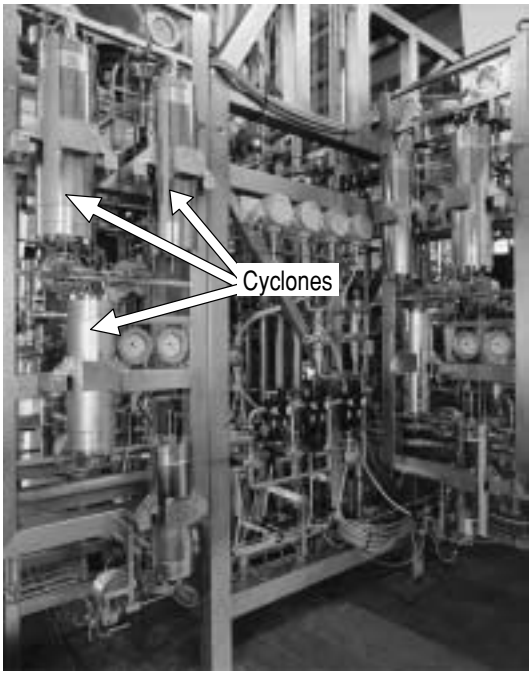

Figure 9.9 Picture of cyclonic separators for an industrial SFC unit equipped with a column of 150 mm i.d. Courtesy of NOVASEP.

into intermediate collection chambers. This has the advantage of performing a 'mild' depressurisation of gas-saturated liquid and avoids splashing.

For small size units, when eluent recycling is not necessary, pressure in the cyclones is set at 10 bar. For higher capacity units (see Fig. 9.9), the flow rate involved being much larger, CO_2 recycling becomes a necessity. In this case, the CO_2 pressure at the cyclone outlet should be larger or equal to the pressure at the pump inlet, i.e. it should be between 40 and 60 bar.

With cyclones of appropriate design, the solute recovery ratio can reach 99%. However, even the best design and optimised operating conditions do not make it possible to achieve total solute recovery (100%). Accordingly, the gas recovered in the separators always contains some traces of solutes, as well as potentially significant quantities of modifier (see below). As a consequence, recovered CO_2 has to be cleaned before being recycled.

9.4.8 *Eluent recycling*

The relative simplicity of online eluent recycling is clearly a significant advantage of SFC over liquid chromatography where eluent recycling requires evaporation units. Depending on the eluent composition (pure CO_2 or modified CO_2), recycling is done differently.

9.4.8.1 *Purification of CO_2 without modifier* Since the solutes involved in liquid chromatography are not volatile, it is likely that CO_2 at the cyclone outlet contains mechanically entrained droplets (aerosols) of non-trapped products. These contaminants can be removed using a high-efficiency coalescer located downstream of the cyclonic separators. The most efficient technology for this purpose makes use of very fine packs of fibrous materials.

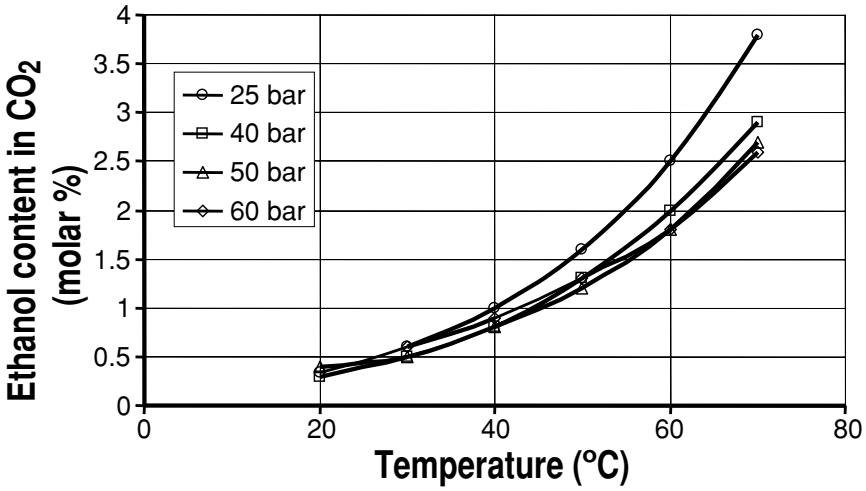

Figure 9.10 Solubility of ethanol in CO_2 as a function of temperature and pressure.

Droplets down to 0.1 μm can be trapped this way. The final cleaning is often done using cartridges filled with activated carbon and/or molecular sieves.

9.4.8.2 *Purification of CO_2 with (liquid) modifier* In this case, the gaseous CO_2 at the outlet of the cyclones contains modifier in liquid and vapour form. The liquid form is associated with microdroplets of modifier (which also contain some amounts of solutes) and corresponds to 'mechanical losses'. An aerosol trap of proper design can be used to eliminate these droplets. There are also 'thermodynamic losses' corresponding to the vapours of modifier mixed with CO_2. The theoretical value of the modifier vapour pressure can be calculated from Gibbs' law. Data is given for ethanol, a commonly used modifier, in Fig. 9.10.

As can be seen, the ethanol content in CO_2 depends considerably on temperature and less on pressure. Theoretically, below 30°C, thermodynamic losses do not exceed 1% from 25 to 60 bar. Practically, owing to the very short residence time of both phases in the cyclonic separators, equilibrium conditions are never reached. As a consequence, the ethanol content in the gas phase may be much higher than the theoretical value. Since retention times are often very dependent on the modifier concentration, this parameter must be carefully adjusted. A gas–liquid contactor with controlled modifier level coupled with an efficient demister (to avoid mechanical liquid entrainment) can be used to adjust precisely the amount of modifier.

As shown in Fig. 9.10, a convenient temperature operating range is between 30 and 40°C, under which conditions the amount of ethanol in the CO_2 is between 0.5 and 1%. To achieve larger amounts, it is necessary to work at higher temperature, but even at 60°C it is difficult to reach 3%. For separations requiring larger modifier concentrations, the balance must be added with the modifier pump.

9.4.9 *High-pressure technology, safety aspects*

SFC involves high pressure. Accordingly, units must be designed taking into consideration mechanical and safety requirements. Specific safety devices should be installed at appropriate locations, depending on the size of the unit [29]. These devices can be mechanical

items such as safety valves, rupture disks, etc., or electrical ones like pressure switches. Most often, safety is also provided within the automation and control of the chromatograph (pressure and temperature alarms).

9.5 Multicolumn continuous chromatographic processes

Batch chromatographic processes are certainly the easiest and fastest way to apply preparative chromatography to practical problems. However, other chromatographic processes can be imagined to improve productivity and reduce eluent consumption compared to the classical elution on a column, involving the use of several chromatographic columns connected and organised in a loop arrangement [30–34].

An innovative idea was developed in the late 1950s [35]. The basic concept is to improve the driving force of the separation [36] using a chromatographic process with countercurrent between the solid and the eluent phase (true moving bed, TMB). Practical implementation of this principle led to the development of the well-known simulated moving bed (SMB) process, which was then widely applied in the petrochemical and sugar industries [37]. In the last decade, SMB units have been developed to answer the specific separation needs for fine chemistry. A strong interest in chiral applications of pharmaceutical compounds emerged rapidly because of the common development of the technology and of new efficient CSPs. SMB processes are now widely used for chiral resolutions in pharmaceutical industries [38,39].

More recently, a new multicolumn continuous chromatographic process called VARICOL has been invented [40–42]. This process has a higher flexibility compared to SMB and leads to better performance. A significant advantage is to reduce the required number of columns in the process, which simplifies the system and reduces the purification costs (simpler equipment, less CSP) [43–45]. The process is already applied for the resolution of chiral molecules at industrial scale. It is estimated that for 2003, the annual industrial production capacity of purified enantiomers using both SMB and VARICOL technologies is 1200 t.

The description of the equipment corresponding to these two processes is presented in this section. Process alternatives like Power Feed [46] or ModiCon [47] have also been developed, but no industrial applications have been reported so far. The main points developed in this chapter are also valid for these processes, where a time modulation either of the feed flow rate (Power Feed) or of the feed concentration (ModiCon) during the switch period is applied.

9.5.1 *Simulated moving bed*

9.5.1.1 *Process implementation* The number of zones of an SMB process depends on the separation problem. The most classical configuration is the four-zone system, which is equivalent to the true moving bed process. Other configurations are described in the literature [48,49]:

- The fourth zone (used for the desorption of the less retained compound) is removed, leading to a three-zone open-loop system: the total stream from zone III is then collected at the raffinate outlet. This configuration leads to a higher raffinate dilution

compared to the classical four-zone process. It is mainly used when eluent consumption is not a concern and/or when the raffinate is the by-product.

- A fifth zone is added, mainly to recover a third fraction containing a strongly retained compound. An additional desorption inlet stream is required to desorb the strongly retained product.
- Other 'exotic' configurations with 8, 9 [50] or even 60 zones [51] are published for the resolution of the feed in more than two fractions, but the practical interest is limited due to the reduction of the number of degrees of freedom of the system.

For chiral applications, the four-zone approach is mainly applied and the technical description presented below will focus on this classical configuration.

An SMB process is based on the connection of columns (6 to 10 for chiral applications) in series, with two inlets (feed and eluent) and two outlets (extract and raffinate) lines, which can be connected between each column. A recycling flow is required for ensuring the elution of the product in the columns.

Different technologies are used for the connection of the inlet/outlet lines:

- Each stream is connected to the inlet of each column (manifold), where two-way valves are located (one valve for each line). At a given time, only one valve among feed, eluent, raffinate and extract is open [52].
- Multiport valves are used to select the right location of each stream [53,54].
- A rotating column carousel can also be used, but mainly for bio- or sugar separations under low-pressure conditions [55].

The second method is widely applied for petrochemical applications [56], but the first option using two-way valves is the selected technology for industrial chiral applications: the robustness is much better compared to complex multi-port valves.

Different process concepts can be applied for the SMB process, depending on the method selected for recycling the eluent [38].

- *Internal recycling* is the common approach applied for chiral applications (Fig. 9.11a). A recycling pump is integrated within the eluent loop (the 'internal loop'), where the columns are connected in series. The recycling pump is always located between the two same columns and 'turns' with the columns. The positions of the inlet/outlet lines are moved continuously in the direction of the recycling flow. With this concept, the recycling pump is located successively in zones I, II, III and IV, where the flow rates are different. A variation of the recycling pump set point during the cycle is therefore required. This is perfectly mastered on pilot and industrial machines.

Common SMB configurations used two pumps for inlet streams (feed, eluent) and two pumps for outlet streams (extract and raffinate). The following mass balance is a prerequisite in this closed-loop system:

$$Q_{\text{feed}} + Q_{\text{eluent}} = Q_{\text{raffinate}} + Q_{\text{extract}} \tag{9.3}$$

where Q_i is the flow rate of stream i. Stability of the pressure in the internal loop is directly based on the flow mass balance and a standard way to regulate the process is based on the control of the pressure at the inlet of the recycling pump (corresponding to the lowest pressure in the loop) with a modulation of one or more inlet/oulet stream to stabilise

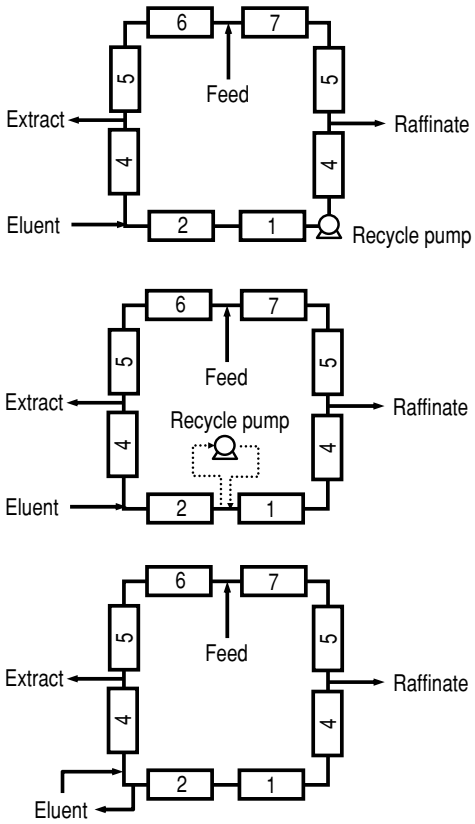

Figure 9.11 Principle of eluent recycling in SMB. (a) Internal recycling; (b) external recycling; (c) use of a single pump for eluent addition and recycling.

the process. It is possible to replace the extract and raffinate pumps by analogue valves associated with flowmeters.

The main limit of internal recycling is the dissymmetry induced by integrating the recycling pump within the internal loop. In the 'ideal' SMB process, each zone should have the same volume. This is not the case when a recycling pump is introduced in the internal loop: one zone has a dead volume (the volume of the pump) that disturbs operation and leads to a loss of performance (decrease of extract and/or raffinate purities). This dissymmetry can be easily overcome by a compensation of the resident time in the recycling pump using either a shorter column (to compensate for the pump volume) or a delay for switching some valve [57–59].

- Instead of using an internal recycling pump, an *external recycling* approach can be considered (Fig. 9.11b). The recycling pump position is fixed in the eluent loop and is always located at the position (eluent inlet between zones IV and I) where no solute should normally be present. No dead volume correction is therefore required with this configuration and the recycling pump flow rate is constant during the cycle. There are, however, two main drawbacks with this concept compared to internal recycling:
  - The recycling pump must move around the loop together with the inlet/outlet lines and more valves are required between each column (two additional valves) [60].

The technical implementation is more complex and the risks of technical failures are increased.

– When the recycling pump is moved by one position, the associated column is instantaneously depressurised from the highest pressure (at the outlet of the recycling pump) to the lowest pressure (at the inlet of the pump) of the loop. These sudden and frequent pressure fluctuations may be detrimental to the stability of the packed bed and potentially the lifetime of the stationary phase. This is particularly true when stationary phases based on wide pore silica are used, which is common for enantioseparations.

Another alternative is to modify the internal recycling operation: the recycling pump is removed and the stream from zone IV is injected together with the eluent in zone I by a single pump (Fig. 9.11c) [61]. The process scheme is simplified compared to the previous one and only one additional valve is required compared to an internal recycling scheme. However, sudden pressure changes are also present.

As far as these three main approaches are concerned, the expected process performance is equivalent when the system is correctly designed. However, internal recycling is preferred by the authors at industrial scale to ensure the optimal lifetime of the stationary phase, which is particularly critical for enantioseparations considering the contribution of the stationary phase investment to the global process economics [38,62].

9.5.1.2 *Process control* Selecting the right technology is the first step to success for an industrial process. Selection of the right operating conditions is the second key.

Efficient mathematical tools [63,64] together with efficient methodologies [65,66] have been developed for selecting the right operating parameters. Calculations are based on preliminary experimental measurements of the required data for describing the chromatographic process: thermodynamics (adsorption isotherms), kinetics (column efficiency) and hydrodynamics (column permeability). It is also necessary to select the optimal operating pressure and particle size for the selected stationary phase [15].

Once the calculated operating conditions are applied to the SMB unit and the first results are obtained, it may be necessary to fine-tune the operating conditions. The first check is to measure the experimental extract and raffinate purities. These measurements should only be performed once a steady state has been reached. Assuming that the columns were initially equilibrated with the selected eluent, it must be pointed out that the raffinate and extract purities are always higher during the transient regime than at steady state. Ten to twenty cycles are commonly required to reach the steady state. Two measurements of the experimental purities at cycle n and $n + 10$ are useful to confirm that steady state is reached.

It must be pointed out that the raffinate and extract purities (and concentrations) vary significantly during the switch period because of the continuous displacement of the internal concentration profiles in the recycling loop and that small differences always exist between columns (length, retention and/or efficiency). It is therefore essential to collect extract and raffinate over a complete cycle in order to measure the averaged purities.

Experimental measurement of the internal concentration profiles is also required to check the consistency of the predicted results and to adjust the operating parameters for improving the obtained purities. These profiles can be measured using a collection valve located at a given position in the recycling loop [67] (classical solution) or using an online measurement of the enantiomer concentrations [68].

9.5.1.3 *Process optimisation: the right choice* The most common objective function when designing an industrial process is the separation cost. The influence of the different parameters involved in the separation cost (investment, eluent, manpower, stationary phase, maintenance, etc.) depends strongly on the application. Let us consider two different applications: the resolution of sugar compounds (e.g. glucose and fructose) and the resolution of a racemic mixture.

For sugar applications, the columns of the SMB unit are typically filled with cheap ion-exchange resins with a large particle size (200–500 μm). Twelve to twenty-four columns are commonly used with a bed length of 1 m. Even if productivity (linked to the system size and the amount of resin) is the primary consideration as far as economics are concerned, the eluent consumption is a critical parameter to minimise for such applications owing to the high energy consumption required to recover the purified sugars from the aqueous fractions.

For chiral applications, the situation is quite different. The CSP is expensive, the cost being mainly related to the complexity of the chiral selector and the allied chemistry. The particle size only has a minor influence on the cost. Optimisation of such processes leads to reduction of the packing particle size to reduce the required amount of packing for the selected application [15]. The optimal number of columns in order to minimise the separation cost is also reduced and six to eight columns is often an optimal choice for industrial applications. The process economics are in this case strongly dependent on the specific productivity and the required amount of stationary phase has to be minimised.

Another way to improve the process economics is to change the purification process to get a higher flexibility and better performance than with SMB. This can be achieved using the new VARICOL process.

9.5.2 *VARICOL*

The VARICOL process is a multi-column continuous chromatographic process that has been recently developed [40,41]. This process offers a greater flexibility and better performance than SMB. Improvement of the purity, recovery, productivity and the reduction of eluent consumption and the number of columns (and then the amount of stationary phase required) are all possible. This often has a strong effect on the cost of enantioseparations.

9.5.2.1 *Basic principle* The VARICOL process is based on columns connected in a recycling loop with two inlet lines (feed and eluent) and two outlet lines (extract and raffinate) [42]. However, instead of simulating true moving bed by a synchronous shift of all inlet/outlet lines (as it is for SMB), the lines are shifted asynchronously in the VARICOL process. As an example, Fig. 9.12 presents the position of the inlet/outlet lines of a five-column VARICOL system during time (chronogram). It is noticeable that the number of columns contained in each zone is changing during the time, whereas this number is fixed in an SMB process. A separation zone can even temporally disappear in the VARICOL process (zones I and IV in Fig. 9.12). The VARICOL configuration can be defined by an average number of columns in each zone [41]. For the example presented in Fig. 9.12, the average column configuration is 0.35 column in zone I, 1.00 in zone II, 0.40 in zone III and 1.25 in zone IV.

Playing with the asynchronous shift of the lines allows the average number of columns in each zone to be modified. This specific property of VARICOL is used to allocate the total

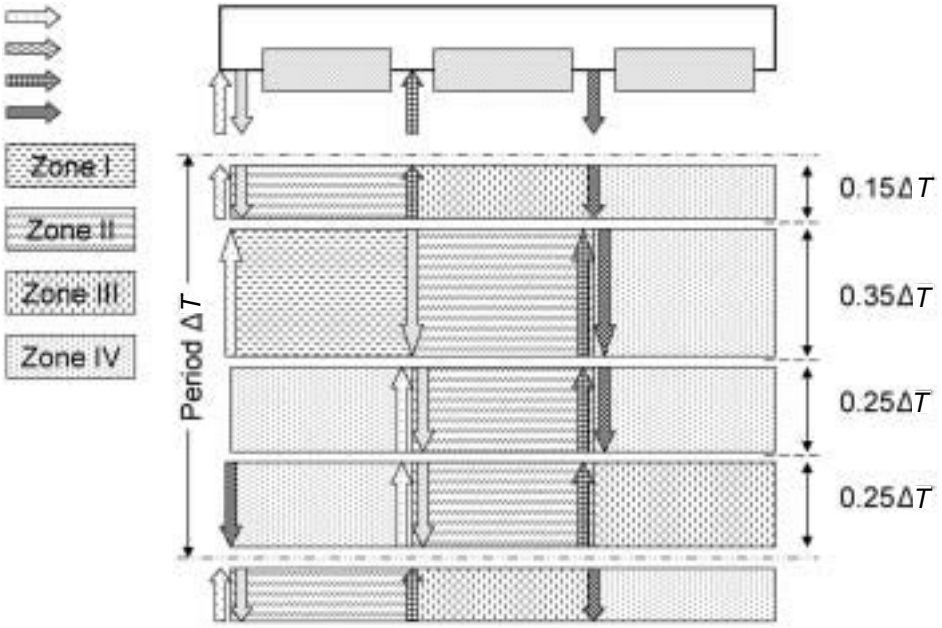

Figure 9.12 Chronogram of a five-column VARICOL system.

column length (and efficiency) in the different zones of the process according to the optimal requirement for the separation. For an SMB process, reducing the number of columns limits the number of available columns in each zone since an integer number of columns is required in each zone. This is not the case with a VARICOL process: the column allocation can be freely adjusted, even with a low number of columns [42,44].

An industrial-scale VARICOL unit used for the separation of chiral molecules is shown in Fig. 9.13 for a yearly production exceeding 100 t. The five columns have an internal diameter of 1 m. Design of the column is mastered to ensure a perfect flow distribution in the columns, even with very short bed length (about 10 cm). See Section 9.2 for column considerations.

9.5.2.2 *Technical specificities* The main points discussed for the design of SMB equipment are valid for a VARICOL process. However, there are some technical considerations specific to the VARICOL.

The inlet/outlet lines are shifted asynchronously. The proper design for the displacement of the lines must be selected accordingly. For example, a column carousel cannot be used for a VARICOL process, whereas it is perfectly suitable for an SMB process. The use of two-way valves on each line between each column is the most convenient design.

It must be pointed out that the temporary superposition of lines (superposition of raffinate and eluent in Fig. 9.12, for instance) leads to obvious consequences for the technical design of a VARICOL unit [42]. Between each column, the two outlet lines must be connected to the recycling line before the eluent and feed lines (following the direction of the recycling flux) as shown in Fig. 9.14. Using this design, the feed flux does not pollute the extract or raffinate streams when the number of columns is temporarily zero in zones II and III, respectively. The eluent flux will also not unnecessarily dilute the extract or raffinate flux when the number of columns is equal to zero in zone I or IV.

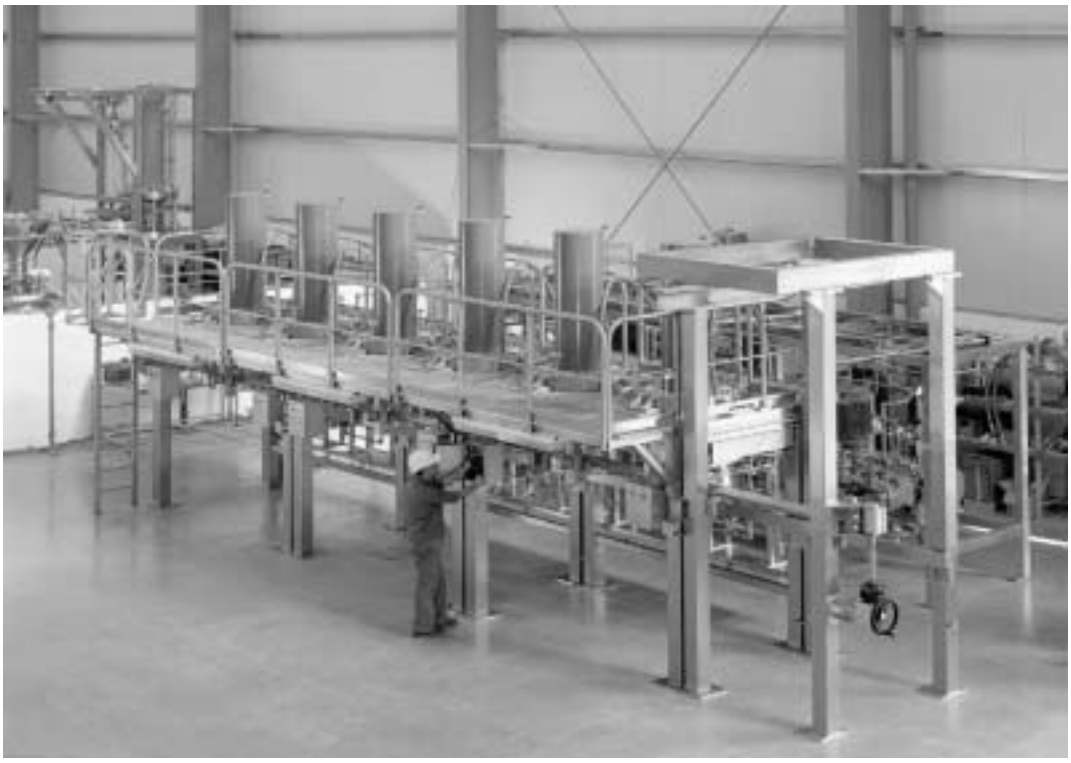

Figure 9.13 VARICOL unit equipped for five columns of 1000 mm i.d. Courtesy of NOVASEP.

9.5.2.3 *Solvent recycling* Chiral separations by chromatography involve a relatively large amount of solvent. The eluent consumption to separate a given amount of racemate, EC, can be characterised by

$$\mathrm{EC} = \frac{Q_{\mathrm{feed}} + Q_{\mathrm{eluent}}}{C_{\mathrm{feed}} Q_{\mathrm{feed}}} \tag{9.4}$$

EC is linked to the limited concentration of the feed stream (limited by both the solubility of the racemate and the stationary phase capacity) and to the dilution of the purified products inherent to chromatography.

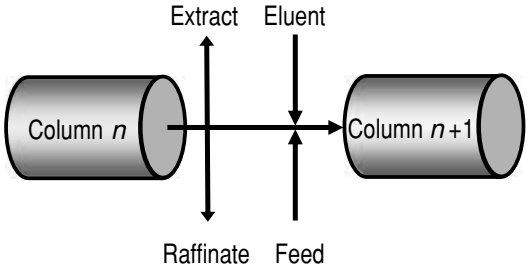

Figure 9.14 Manifold arrangement for a VARICOL unit.

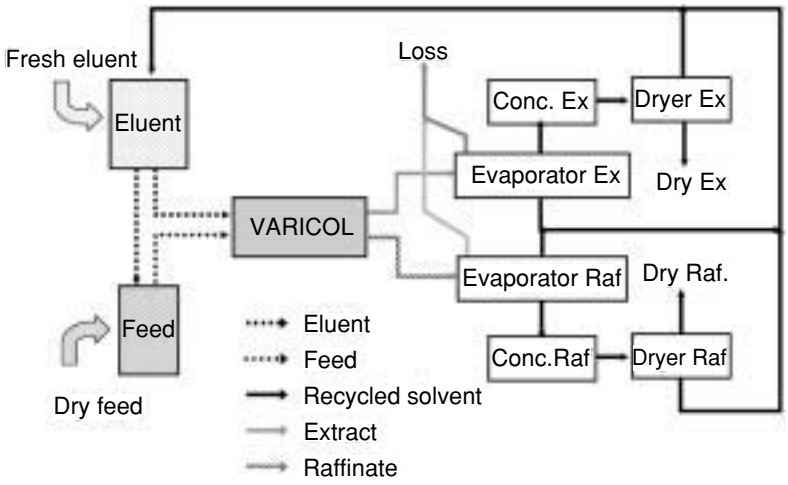

Figure 9.15 Principle of continuous purification with eluent recycling.

For chiral applications, the eluent consumption is typically between 100 and 1000 L/kg of feed. Considering the costs associated with buying solvents and destroying them after use, the process is only realistic at industrial scale if solvents are recycled. Figure 9.15 shows the layout of a classical industrial implementation of a VARICOL process including solvent recycling. The purified, but diluted, extract and raffinate streams are concentrated in evaporation units to a final concentration close to the limit of solubility of the purified enantiomer in the solvent. The evaporated solvent is condensed and can be recycled. The concentrated solutions of purified enantiomers are often dried to recover the purified products. The solvents evaporated during this process can also be condensed and recycled. In such a case, the global solvent requirement is linked to the solvent losses and to the residual solvent content in the dried purified products. Depending on the evaporation/drying technology, the final solvent consumption can often be reduced to less than 1 L/kg of purified enantiomer.

When the eluent is a pure solvent, the condensed stream can be directly recycled (with a periodic control of potential traces of enantiomers or other impurities). Binary solvent mixtures are often used for chiral separations and an adjustment of the condensate composition may be required before recycling, because of the difference in boiling temperatures of the two solvents.

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Preparative Enantioselective Chromatography
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10 Case study in production-scale multicolumn continuous chromatography

MICHEL HAMENDE

10.1 Introduction

From late 1993 onwards, demand forecasts for a new enantiomerically pure drug from the UCB Pharma research portfolio have driven the need to explore and compare several synthetic routes as alternatives to the original, which involves synthesis of the racemic compound followed by salt crystallisation. At the onset of the project, the available alternative technologies to synthesise this molecule that contains one chiral centre were syntheses of the racemic compound, followed by chromatographic resolution or synthesis using commercially available chiral precursors [1].

A simplified comparison of several synthetic routes was carried out in terms of manufacturing costs, investment, environmental impact and complexity of industrialisation for a given quantity. The results (see Table 10.1) are clearly in favour of chromatographic resolution, mainly in terms of manufacturing costs and environmental impact.

From these results, the decision was taken to investigate in detail the use of chromatographic resolution techniques. It should be noted that since then, other techniques have been developed and are available at industrial scale; these will be briefly discussed in Section 10.5.

10.2 Chromatographic process research

10.2.1 *Introduction*

Process research using chromatographic techniques has dealt with several domains of optimisation such as the selection of the racemic mixture, the choice of the chromatographic conditions and the selection of the chromatographic mode (batch or continuous). It should be noted that each of these key parameters is closely interrelated.

10.2.2 *Selection of racemate to separate*

10.2.2.1 *Generalities* The choice of the racemate to separate is crucial for the entire process economy. A first rule of thumb is to make the separation as early as possible in the drug synthesis as it will minimise the overall amount of associated reactant, solvent and wastes. This general rule must however be balanced with other criteria such as the possibility to recycle the unwanted enantiomer, the influence of the racemate impurities on the separation process, the separation performance of the chromatographic process and the absence of racemisation in the subsequent steps (see Section 10.2.3).

Chromatographic techniques always lead to the isolation of the unwanted enantiomer; it is consequently important to study the possibility of reuse or recovery of the unwanted

Table 10.1 Comparison of several synthetic routes in terms of manufacturing costs, investment, environmental impact and complexity

	Salt crystallisation	Chromatographic resolution	Synthesis from chiral precursors
Manufacturing costs	100%	24%	48%
Investment	100%	76%	62%
Environmental impact	100%	11%	33%
Complexity	Low	High	Low

enantiomer in the drug synthesis or in another application. This reuse must be carefully studied, as sometimes the chemistry to recover the unwanted enantiomer, for instance by racemisation, can be complex and negatively impact the overall process economy.

Purity of the racemate is also essential as organic or inorganic impurities can cause negative mechanical, chemical and physicochemical effects. Mechanical damage can be manifested as corrosion or pressure drop increase, whilst chemical effects can be, among others, unwanted reactions with the stationary phase or mobile phase or adverse adsorption/clogging of the mechanical equipment and/or the selection sites of the stationary phase.

10.2.2.2 *UCB example* In the case of the specific UCB example, the original synthesis consists of several chemical steps leading to a crude active pharmaceutical ingredient (API), which is purified by recrystallisation and is then milled. As the clinical trials were done with the API from the original synthesis, it was mandatory to keep the purification and milling unchanged in order to simplify the comparison between the two sources of API. This comparison had to be done in terms of impurity profile, physical properties, galenic machinability and properties of the dosage form as finished product.

For the new synthesis, which uses a chromatographic step, followed by the isolation of the desired enantiomer, the racemic compound can be separated either at step 1 or step 2 of the chemical synthesis. Figure 10.1 summarises the possible scenario of separation and synthesis.

With strategy 1, the step 1 of the new synthetic route generates a racemic chemical intermediate, which is purified and isolated by distillation. This intermediate, which is a liquid at ambient temperature, can contain up to 6% of process-related impurities; it is very soluble in most organic solvents and can be easily separated with commercially available stationary phases and non-exotic organic solvents as eluent (step 2a). However, the chemical step (step 3a) after the chromatographic separation uses conditions that may cause partial racemisation. In conclusion for strategy 1, the labile chiral centre and the process-related impurities are clearly disadvantages for an ideal synthetic route. In this particular case, therefore, the general rule of placing the optical resolution immediately after insertion of the chiral centre is difficult to follow.

With strategy 2, the second step of the synthesis produces a pure crystalline salt, which is already the racemic mixture of the drug substance. This salt, at a chemical purity of 99.5%+, is soluble in most organic solvents and shows excellent separation capabilities with commercially available stationary phases and industrial mobile phases.

In-depth analysis of the impurities has shown the presence of non-process-related impurities such as sodium carbonate or metallic ions at trace levels and also the presence of

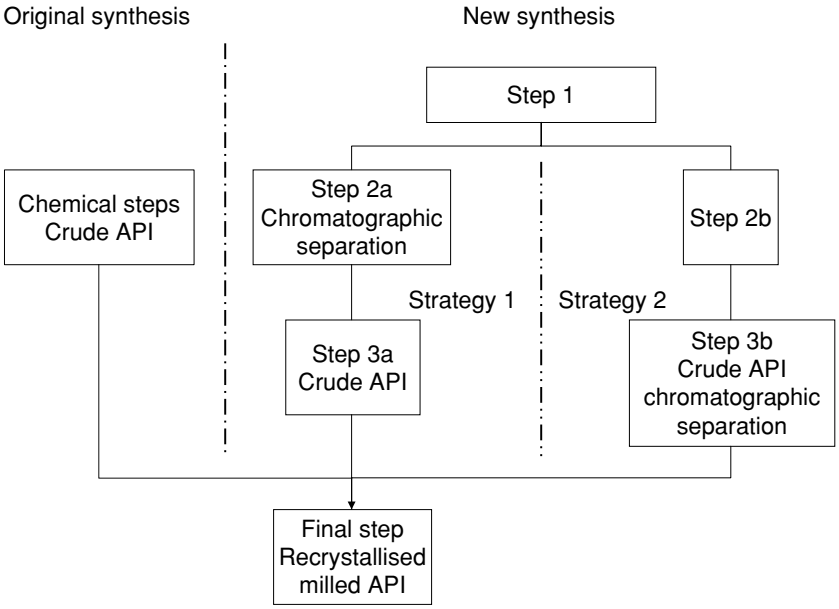

Figure 10.1 Possible scenario of separation and synthesis processes.

process-related impurities such as traces of alkali at a maximum concentration of 0.01%. Further laboratory trials have shown that these traces of alkali are evenly distributed in both enantiomers during the separation process. Moreover, it appeared that these small amounts of alkali were sufficient to cause a partial racemisation during the separation and isolation of the enantiomers by thermal evaporation of the solvents. As this racemic compound is already the last intermediate before the drug substance, it was therefore necessary to remove these traces by a further recrystallisation.

This recrystallisation has led to the same chemical purity as for the crude salt but with much lower levels of alkali (<0.001%). In this case, laboratory trials did not show any partial racemisation during the thermal isolation process. The recrystallised salt was therefore chosen as the candidate for the separation.

10.2.3 *Choice of the chromatographic conditions*

10.2.3.1 *Generalities* The choice of stationary phase and mobile phase is crucial, as this choice will directly determine the adsorption isotherms and therefore the performance of separation.

For a given stationary phase and mobile phase at a constant temperature, selectivity and resolution must be measured and can be accessed by single injections in an analytical column under analytical conditions. No theory will predict the best choice of stationary phase and mobile phase for a given racemic compound; only experiments will lead to the combination of choice. It should be noted that software tools like CHIRBASE [2] may help to minimise the number of experiments necessary.

In this optimisation, several key factors must be considered separately for the stationary phase and the mobile phase.

10.2.3.2 *Key factors for the stationary phase* The stationary phase should be chosen to give high selectivity and high resolution whilst resisting both mechanically and chemically the eluent, the racemate and the equipment.

As the stationary phase is often very expensive (typical values are € 5000 to € 20 000/kg), it is therefore important to target the chemical and the mechanical resistance of the stationary phase in order to correctly estimate its lifetime.

Chemically, the stationary phase must be inert to the mobile phase and to the racemic mixture. It is well known that some chiral stationary phases (CSP) are sensitive to solvents like ketones or mixtures of alkanes and alcohols. Some CSPs must also be activated by a weak base such as triethylamine or by the racemate itself if it contains a weakly basic functionality.

Mechanically, it is important to know which maximum pressure and linear velocity the CSP can stand. High pressure can break the CSP whilst a too high linear velocity may abrade it. In any case, spherical CSP should be preferred to increase the mechanical resistance and to improve the fluid dynamics.

The selection of the particle size of the CSP must take into account that the resolution and the pressure drop per metre are inversely proportional to the particle size.

10.2.3.3 *Key factors for the mobile phase* Besides meeting the requirement, in combination with the stationary phase, to give good resolution, the mobile phase should be optimally chosen with regard to the chromatographic conditions, the ease of recovery and the responsible care of the industrial environment (safety, hygiene).

For the chromatographic conditions, viscosity is one key element as the pressure drop is inversely proportional to the liquid viscosity. Doubling the viscosity reduces the maximum flow rate at constant pressure by 50%. Increasing the temperature may circumvent that issue by diminishing the viscosity but it can be detrimental to the separation efficacy.

As will be discussed later, recovery of the solvent is a key element for an economically viable and an environmentally sound process. Heat of evaporation must also be considered as it directly determines the heating and cooling needs of the unit. Moreover, the evaporation of a complex mixture typically leads to a change in the composition. Offline sampling must be carried out prior to the readjustment of solvent composition. Online techniques using the electrical properties of the eluent can be used in the case of binary mixtures such as alkanes/alcohols. More sophisticated systems using near-infrared technology can also be applied for more complex systems. Periodic calibration must be carried out on these online measurement techniques. In conclusion, where applicable, a single solvent or a binary mixture is the best choice.

Environmental, health and safety issues should never be overlooked; typically some solvents should be avoided because of their high toxicity and/or their capacity to build up static electricity charges.

10.2.3.4 *UCB example* In the specific UCB example, stationary phases that can be used for this separation are the phases Chiralcel (OD, OG, OJ) and Chiralpak (AD, AS) (available from Daicel Chemical Industries), Chirose (C1) (available from Chiralsep, France), Kromasil (PM 493, etc.) (available from EKA Nobel) and Phamachir (available from A.I.T., France).

For this application, it was demonstrated by internal studies and by using the Chirbase database [2] that the Chiralpak AD type phase from Daicel was the most efficient candidate at a temperature between 20°C and 30°C. More studies were performed to determine if a dedicated AD type phase in which the percentage of polymer is modified could be beneficial. Finally, the productivity increase did not overcome the additional cost of the tailor-made CSP.

Regarding the choice of the particle size of the CSP, commercially available Chiralpak AD 20 μm was tested with respect to 10 μm or 50 μm possible alternatives. Practically, both alternatives appeared to be non-economically viable as the 10 μm CSP would have cost up to 50% more than the commercially available 20 μm CSP. A 10 μm packed system would have required a minimum bed length for a plug flow distribution inside the columns and would need equipment able to operate at higher pressure and pressure drop; this solution was therefore not economical. In the same way, 50 μm particles would have required more CSP to achieve the same separation but would allow a higher flow rate for a constant pressure; furthermore, the 50 μm CSP was more expensive than the 20 μm commercial material and was therefore not considered to be economical.

Concerning the solvent, it initially appeared preferable to use a pure solvent in order to avoid any adjustment of the solvent composition. Considering the relatively low toxicity and the relatively high flash point of certain aliphatic alcohols, these were considered to be potential candidates. Azeotropically purified ethanol was selected principally as a result of its superior performance in terms of selectivity and resolution, as compared with methanol. However, additional trials demonstrated an increase in productivity by a factor of 2–3 by shifting from a single solvent to a binary eluent of the alkane–alcohol type, instead of azeotropic ethanol. A binary mixture was therefore chosen.

10.2.4 *Choice of the separation technique*

The main modes of chromatographic separation feasible for resolution of the racemic salt are batch preparative HPLC, possibly with peak recycling, and multicolumn continuous chromatography.

The first technique consists of periodic injections of feed solutions into a packed column in which a suitable solvent or combination of solvents is used to elute. Automation allows collection of the pure fractions and partly resolved fractions separately for further reprocessing.

Multicolumn continuous chromatography (MCC) is a general term for the continuous chromatographic separation technologies (both analytical and preparative) that are based on the continuous controlled injection of (usually binary) mixtures onto a series of linked columns filled with a stationary phase. The separated components of the mixture are then withdrawn continuously from the system. This approach would include, but is not limited to, simulated moving bed chromatography (SMB mode) [3], or modes where the inlet and outlet ports are shifted asynchronously (such as the Varicol mode) [4,5] or modes in which inlet and outlet flow rates and/or concentrations are changing in time during the switching period [6–9].

For the specific UCB example, it was necessary to optimise the productivity of the separation process by minimising the stationary phase quantity as well as the solvent quantity for recovery.

Table 10.2 Comparison of batch preparative HPLC and MCC technology in terms of productivity and solvent consumption

	Productivity (g racemate/kg SP/day)	Solvent consumption (L solvent/kg racemate)
Batch preparative HPLC	720	4200
Multicolumn continuous chromatography (MCC)	1200	580

By simulation using the software from Novasep (Helpchrom, formerly Licohelp), it was possible to determine the optimum for the batch or the continuous mode in terms of productivity (g racemate/kg/day) and solvent consumption (L solvent/kg racemate) at a purity of 99% and a recovery of 90%+ of the desired enantiomer for a given maximum pressure. From the results presented in Table 10.2, it was decided to investigate further the MCC technology.

10.3 Process development

Process development was conducted on both chemical steps and the chromatographic separation step to provide an overall optimised process.

For the chemical steps, the synthesis of the racemate was therefore optimised with respect to its purity and with respect to the overall yield before proceeding to the chromatographic step. The optimisation of the purification step was primarily conducted to ensure at least the same quality with respect to the original filed process but also to determine the level of unwanted enantiomer, which can be allowed after the chromatographic step. This last point was a key element for the entire process economy.

From these, optimisation of the chromatographic step could be carried out.

10.3.1 *Optimisation of the chemical steps*

10.3.1.1 *Optimisation of the racemic synthesis* The synthesis of the racemic compound can be carried out either directly or by racemisation of the unwanted enantiomer; material from both origins must show an identical impurity profile for the next step. In both cases, the need for a very pure racemic mixture requires a purification step by crystallisation. This has the drawback that it directly affects the overall yield. In order to circumvent this and to obtain an acceptable yield, the recrystallisation step of the racemate was chosen in such a way as to be able to purify both sources of crude racemic compound using a common solvent for both the crude and the purification steps, allowing the reuse of the mother liquors of recrystallisation as solvent in each of the crude steps.

10.3.1.2 *Optimisation of the recrystallisation step* The final crystallisation step for this new manufacturing route was designed in such a way that the drug substance from this route shows essentially identical chemical and physicochemical properties as the drug substance from the originally filed source. Therefore, the same solvent was chosen for both crystallisation processes. Nevertheless, the goals of this purification step are different for the

Table 10.3 Benefits of optimised recycling of solvents and racemisation of the unwanted enantiomer on the yield

	Non-optimised	Optimised
Equivalent of reactant per kilogram of drug substance	3.83	2.45
Overall yield (kg DS/kg reactant)	26.1%	41%

two routes. In the original route of synthesis, the objective is to eliminate process-related traces of impurities, whilst in the chromatographic process, the objective is to eliminate completely the unwanted enantiomer.

It was immediately apparent that this elimination of the unwanted enantiomer in the last purification step had to be done together with the optimisation of the chromatographic process. Numerous publications have dealt with the optimisation of crystallisation and chromatography [10]. For this specific example, owing to a favourable eutectic point, it was possible to select the final end temperature for the crystallisation process, which increases the ee from 97% to above 99.2% keeping the same chemical and physicochemical properties, with an overall crystallisation yield above 85%. With a chromatographic separation performance set at 90% recovery of the wanted enantiomer, it was therefore possible to calculate the benefit of racemisation and other recycling in terms of equivalent of reactant needed per kilogram of drug substance or the overall yield (see Table 10.3).

10.3.1.3 *Optimisation of the MCC chromatographic step* The MCC chromatographic step had to be optimised in terms of its operating parameters such as the stationary phase, the mobile phase and the technology itself, but it was also essential that it conformed with the local manufacturing environment and its related regulatory constraints (cGMP).

10.3.1.4 *Optimisation of the stationary phase* From the commercially available Chiralpak AD 20 μm, several variations were studied with Daicel, such as the modifications of the silica properties and changes of the AD polymer on the silica matrix. From these studies, it appeared that the commercially available phase was the most suitable and cost-effective.

A key question regarding CSPs was the expected lifetime; therefore, long-term or accelerated experiments simulating the stress during the separation process were carried out in collaboration with the CSP manufacturer. The tests were oriented towards the particle stability during the slurry preparation prior to the packing and during the packing itself.

Preparation of the slurry prior to the packing An early theory was that the slurry preparation for the packing process may cause breakage of particles owing to the high shear of the agitation. In order to verify this hypothesis, Chiralpak AD was slurried and stirred using a two-blade stirrer. Fine particles between 2 and 8 μm were counted in number over time with various stirring speeds and the results are presented in Table 10.4. From this table, it can be seen that there is no increase in fine particles caused by the slurry preparation.

Investigation of the packing process Chiralpak AD was packed in a preparative dynamic axial compression column (25 × 5 cm) 10 times at 40 bar packing pressure and the relative amount of fine particles between 2 and 8 μm was counted and the results are given in

Table 10.4 Influence of the stirring effect on the fine particles content

Stirrer speed (rpm)	Test duration (h)	Fine particles content (relative ratio)
0	0	100
750	0.17	82
1000	0.33	125
400	64	97
1500	4	93

Table 10.5. From this table, it can be seen that there is no real change for the Chiralpak AD after 10 cycles of packing and repacking. A second set of experiments in which the stationary phase was packed at varying piston pressures was carried out (see Fig. 10.2). Below 70 bar in the packed bed, there is no increase in fines; the use of 40 bar was therefore chosen as a safe bed packing pressure.

Packing and unpacking endurance test Endurance tests were carried out on the selected CSP during which the racemic mixture was injected periodically on an analytical column over 40 days. The selectivity, the resolution and the number of theoretical plates were recorded (see Fig. 10.3). No significant change in the separation characteristics of the stationary phase was observed during this test.

Specifications of the CSP From all the tests performed, specifications regarding the chiral stationary phase were finalised in order to guarantee the reliability of the separation. With this objective, work was carried out in conjunction with both the CSP and the MCC equipment manufacturer in order to establish the intrinsic and the performance specifications.

The intrinsic specifications are focused on the CSP characteristic regardless of the molecule to be further separated and these are the appearance by visual examination, the identification by FTIR, the particle size distribution, the loss on drying, the pore size and the polymer content by elemental analysis.

The performance specifications are directly linked to the racemic mixture; it was therefore established that, for an analytical column, with the eluent of the industrial separation, under a calibrated flow,

- the number of theoretical plates N [calculated on the basis of retention time (t_r) and peak width at mid-height (w), according to the formula $N = 5.54(t_r/w)^2$] should be higher than a minimal value for an analytical injection of the wanted enantiomer

Table 10.5 Influence of the number of packings/depackings on the file particles content

	Particles between 2 and 8 μm (relative ratio)
Virgin Chiralpak AD	100
10× packed Chiralpak AD	95

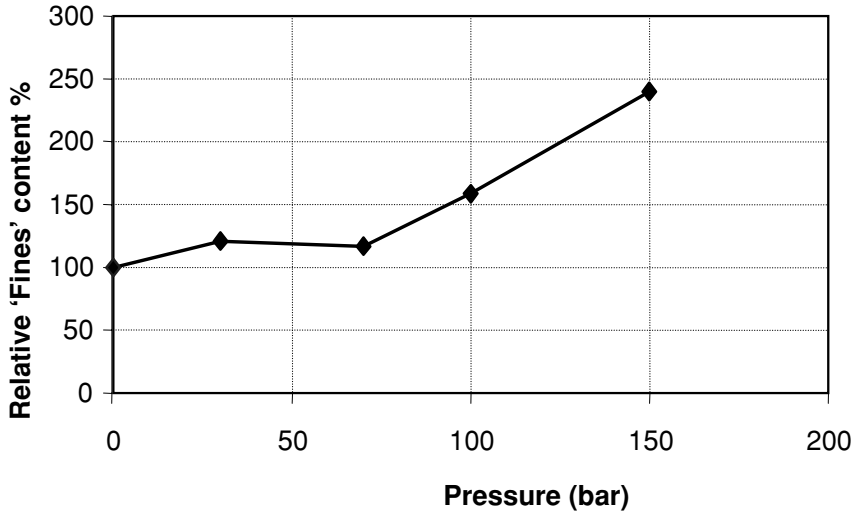

Figure 10.2 Relative percentage of 'fines' (2–8 μm particles) vs packing pressure (Note: 100% = content in virgin CSP).

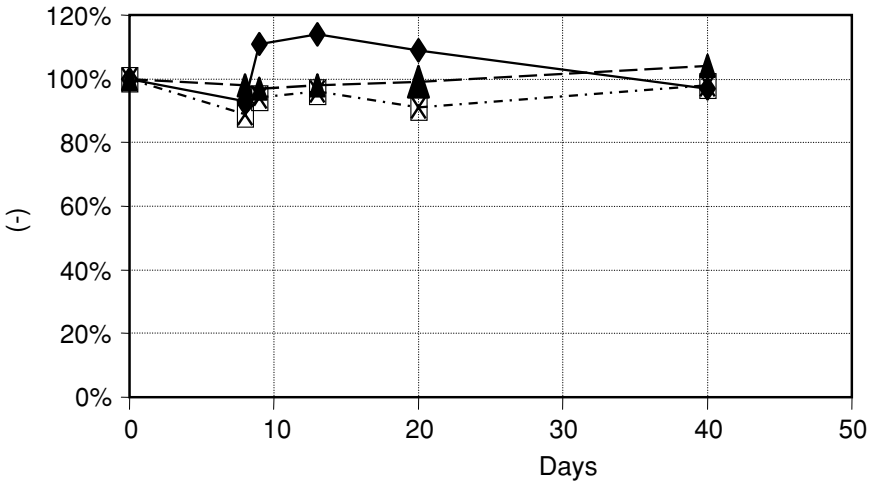

Figure 10.3 Relative change in column performance parameters over 40 days of endurance testing. Key: solid line = number of theoretical plates; dashed line = selectivity; dashed-dotted line = resolution.

- the retention factor of the least retained species (raffinate) k'_1 [$k'_1 = (t_{r1} - t_0)/t_0$, where t_{r0} is the solvent retention time] and the selectivity factor α [$\alpha = k'_2/k'_1$] must lie between a determined interval for an analytical injection of the racemic mixture
- the loadability factors f1 and f2 should be higher than a minimum value. [f1 and f2 are respectively defined as being the ratio between retention time for an injection of 25 μL and 100 μL of the wanted enantiomer at high concentration and retention time for the analytical injection (20 μL at 1g/L) of the same compound.]

10.3.1.5 *Optimisation of the mobile phase* Knowing from the preliminary search that a mixture of alkane/ethanol was the most suitable solvent, further selection had to be carried

out on the precise level of alkane, on the selection of the alkane itself and on the definition of their grade of purity.

Selection of the alkane level The composition must be selected in such a way that the separation performance (alpha and resolution) are optimised keeping the solubility sufficiently high in order to ensure that there is no precipitation during the solvent removal because of the azeotropic behaviour of the mixture.

From the solubility table (see Fig. 10.4), alkane levels above 40% v/v were discarded as the objective was to have a solubility higher than 50 g/L at ambient temperature. It must also be added that initially, alkane compositions in alcohols in the range 50–85% were not recommended for use with the CSP. On the other hand, from Table 10.6, it is clearly seen that the selectivity and resolution are favoured by increasing the amount of alkane in the ethanol/alkane mixture.

From this, the eluent was selected as a mixture of alkane/ethanol, where the alkane level is at 40–50%.

Selection of the alkane type In parallel, environmental, health and safety data were collected regarding several alkanes including the flash point and threshold limit value (TLV). From Table 10.7, it can be seen that *n*-hexane was eliminated owing to its low TLV, whilst

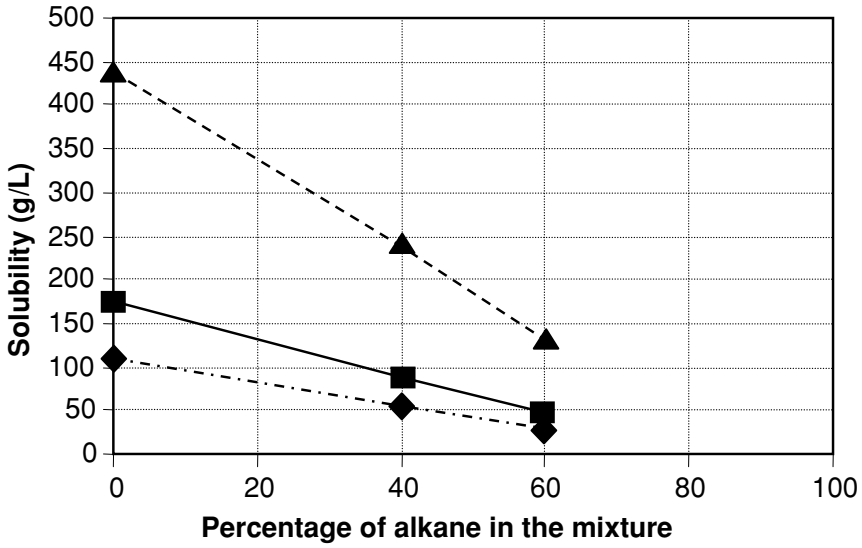

Figure 10.4 Racemate solubility in alkane/ethanol. Key: dashed line = 50°C; solid line = 30°C; dashed-dotted line = 20°C.

Table 10.6 Influence of the amount of alkane in a binary eluent on the selectivity and resolution

	Selectivity	Resolution
100% ethanol	1.88	2.89
60% ethanol/40% alkane	1.65–2.47	3.71–6.18
50% ethanol/50% alkane	1.75–2.58	4.14–7.50

Table 10.7 Flashpoints and TLV values for several alkanes

Alkane	Flash point (°C)	Threshold limit value (TLV)
n-Pentane	−40	600
n-Hexane	−22	25
Cyclohexane	−18	250
Methylcyclohexane	−4	400
n-Heptane	−4	300
n-Octane	13	300
Isooctane	−12	n.a.
n-Dodecane	58	n.a.

pentane, cyclohexane and isooctane candidates were discarded because of their low flash point values. To discriminate between the remaining candidates, the selection was based on the criterion of the lowest viscosity in order to maximise the throughput at constant pressure. The mixture of heptane and ethanol was found to have the lowest viscosity (see Table 10.8).

Selection of the solvent grade Preliminary studies were performed in HPLC-grade solvent but this quality was not available in large quantities and at a suitable price. Therefore, various solvent qualities and packaging types were evaluated in order to define the suitable quality for the industrial separations. Relative changes in the retention times and UV absorbency test were the parameters to be recorded.

The results of these tests are summarised in Tables 10.9 and 10.10. From these tables, it appeared that, for *n*-heptane, 99% quality (whether further distilled or not) gave the closest absorption and retention times with respect to the *n*-heptane of HPLC grade. This quality was therefore selected.

Ethanol denatured with methanol and food quality absolute ethanol provided similar chromatographic performance and UV absorbency. Because of the similarity in their cost, and the fact that a record of the quantity of ethanol used must be kept for customs and excise duty, food quality absolute ethanol was chosen for this separation.

In order to avoid problems linked to plasticisers in lined drums, galvanised iron was selected as the manufacturing material for the drums containing *n*-heptane. Drums with a food quality FDA-approved inner lining were selected for the absolute ethanol packaging.

10.3.1.6 *Optimisation of the MCC technology* Following the theoretical and laboratory-scale feasibility studies performed in 1995 with the MCC equipment manufacturers and with the existing R&D unit at UCB [11], the separation of the racemate was studied from

Table 10.8 Viscosity and relative flow rates for several binary eluents

Ethanol/alkane	Viscosity at 30°C (cP)	Relative max. flow rate at constant pressure (%)
Ethanol/methylcyclohexane (50:50)	0.78	81
Ethanol/heptane (50:50)	0.63	100
Ethanol/octane (50:50)	0.72	88
Ethanol/dodecane (50:50)	1.04	61

Table 10.9 Influence of the ethanol and *n*-heptane grades on the retention times

		Relative changes in retention time (%)	
Ethanol quality	*n*-Heptane quality	Enantiomer A	Enantiomer B
HPLC	HPLC	0 (reference)	0 (reference)
HPLC	99%	−0.09%	−0.18%
HPLC	Distilled 99%	0.02%	0.02%
HPLC	95%	0.36%	0.71%
HPLC	Distilled 95%	0.16%	0.08%
Denatured with MeOH	HPLC	0.47%	−0.13%

Table 10.10 UV absorbency test for several solvent grades

Solvent	Quality	Absorbency (mAU)
n-Heptane	HPLC	0
	99%	115
	Distilled 99%	11
	95%	915
	Distilled 95%	550
Ethanol	HPLC	0
	Denatured with MeOH	−11

1996 to 2001 using various sizes of equipment in order to industrialise and scale up the entire process and to deliver product for the next chemical and pharmaceutical formulation steps. It should be noted that two modes of MCC operation have been investigated: the simulated moving bed (SMB) mode and the Varicol mode [3–5].

For the specific example of the UCB compound, all these separations were conducted with the performance targets of minimum 90% recovery of the wanted enantiomer at a minimum ee of 97% (i.e. a maximum of 1.5% of the unwanted enantiomer) under a maximum operating pressure of 40 bar.

Several units from different equipment manufacturers and at several sites were used for this study:

- an eight-column system of 50 mm i.d., a six-column system of 200 mm i.d. from Novasep as R&D and scale-up units
- a five-column system of 350 mm i.d., five- and six-column unit of 450 mm i.d., all from Novasep as launching units
- a six-column system of 800 mm i.d. and 3 five-column units of 1000 mm i.d., all from Novasep as production units.

Several key parameters were recorded during the R&D, scale-up, launching and production units in order to assess the reproducibility of the process:

- The MCC internal concentration profile and consequently its performance only depends on four dimensionless internal flow parameters defined as R_k, where k refers to zone I, zone II, zone III and zone IV. For a given system size, the flow rate in each zone

and the cycle time has to be selected to reach the target purity for each outlet stream, depending on the feed solution to be separated. The required elution volume V_k can then be defined in each zone for a given column to absorb or desorb the enantiomer concerned. This elution volume is equal to the flow rate in the zone multiplied by the switching time (ΔT) of the inlet/outlet lines. In the zone k, the elution volume on each column is $V_k = Q_k \Delta T$. The required elution volume depends on the volume of the columns and is therefore dependent on the system size. It is therefore required to define a dimensionless parameter R_k for each zone k, independent of the column volume. $R_k = Q_k \Delta t / V_{\text{col}}$, where Δt is switching time (h) and V_{col} is column volume (L). [Usually the adimensional flow rates differ for an SMB mode and is in that case directly derived from the virtual true moving bed (TMB). $Q_{i_{\text{SMB}}} = Q_{i_{\text{TMB}}} + \varepsilon/(1-\varepsilon)$ and $Q_{i_{\text{TMB}}} = Q_{i_{\text{TMB}}}/M$; $Q_{i_{\text{TMB}}} = (R_i - \varepsilon)/(1-\varepsilon)$.]

$$Q_{\text{I}}(\text{MCC}) = Q_{\text{Liquid}}$$
$$Q_{\text{II}}(\text{MCC}) = Q_{\text{I}}(\text{MCC}) - Q_{\text{Extract}}$$
$$Q_{\text{III}}(\text{MCC}) = Q_{\text{II}}(\text{MCC}) + Q_{\text{Feed}}$$
$$Q_{\text{IV}}(\text{MCC}) = Q_{\text{III}}(\text{MCC}) - Q_{\text{Raffinate}}$$

- The throughput (kg racemate separated per day)
- The productivity (kg racemate/kg CSP/day)
- The solvent volume to recover (L/kg racemate)

The results are presented in Table 10.11. The adimensional flow rates and the solvent consumption can be plotted with respect to the relative size of the unit in a semi-logarithmic scale (see Figs. 10.5 and 10.6). It can be seen that if scale-up factors remain constant even the size of unit increases by a factor of 400! From this comparison table, it was also important to compare the SMB modes with the Varicol mode. From Fig. 10.7, it was established that a six-column Varicol configuration does not increase the productivity but diminishes the solvent consumption by 9%, whilst the five-column Varicol configuration reduces the solvent consumption by 19% and increases the productivity by 64% with respect to the six-column SMB configuration.

In conclusion, from the initial laboratory set-up, it was therefore possible to scale up the process by a factor of 400 using the same key process parameters whilst increasing the productivity and reducing the solvent consumption.

Some other advanced operation modes of MCC units are being studied by research groups and will be briefly discussed in Section 10.5.

Table 10.11 Adimensional flow rates and solvent consumption for several MCC units used

Mode/unit	Production year	Relative size	R_{I}	R_{II}	R_{III}	R_{IV}	L solvent/kg prod.
SMB 6-50	1998	1	1.93	1.20	1.36	0.98	388.89
SMB 6-200	1996	16	1.98	1.22	1.34	0.95	540.27
SMB 6-450	1997–2000	81	2.00	1.35	1.48	1.04	492.71
Varicol 6-450	2000–2001	81	1.94	1.34	1.47	1.06	441.30
Varicol 5-350	2001	49	1.86	1.23	1.33	0.97	539.71
Varicol 5-450	2001–2002	81	1.95	1.29	1.44	0.99	394.56
Varicol 5-1000	2002	400	1.92	1.31	1.47	1.02	412.32
Varicol 5-1000	2003	400	2.02	1.32	1.49	1.07	360.91

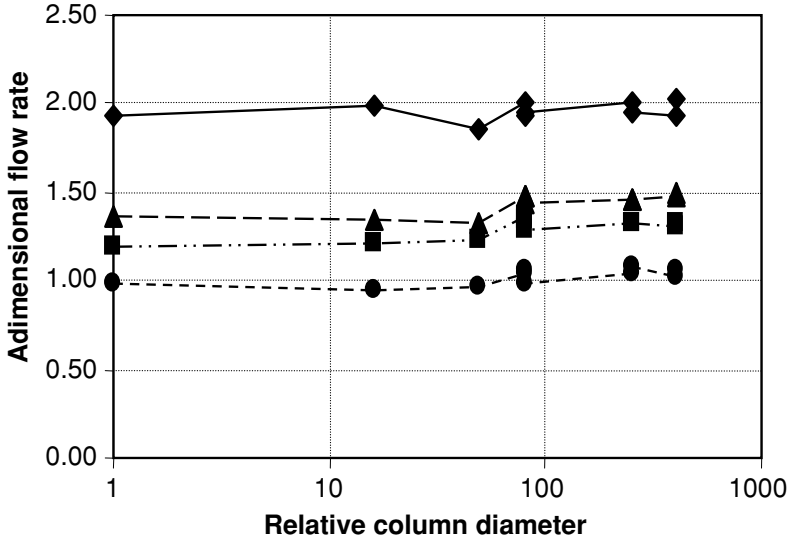

Figure 10.5 Adimensional flow rate vs relative size of the unit. Key: solid line = zone 1; dashed-double dotted line = zone 2; long dashed line = zone 3; short dashed line = zone 4.

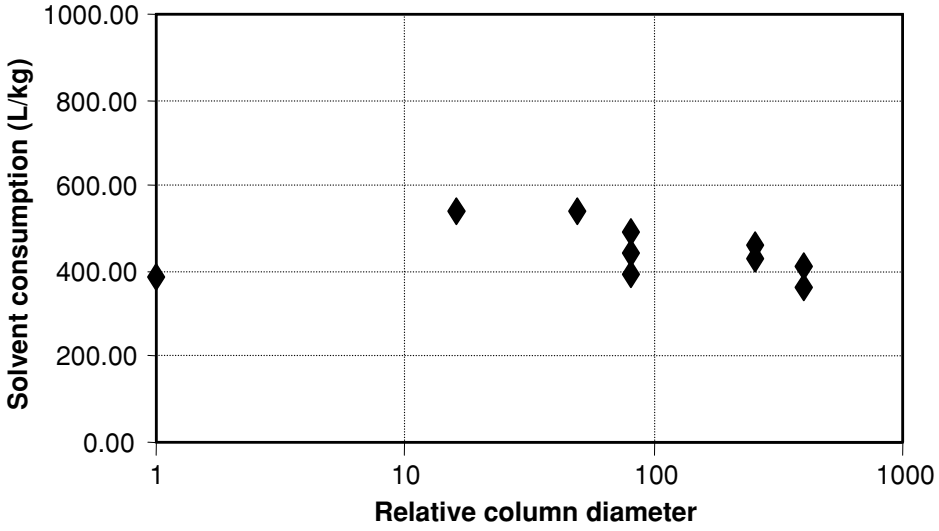

Figure 10.6 Solvent consumption per kilogram of product vs relative column diameter of the chromatographic system.

10.4 Production facts

10.4.1 *Introduction*

Specifically, for UCB, as this was the first time that a continuous process was implemented for a chemical process, training of the production personnel, set-up of the required controls and batch traceability were the key factors to focus on from the development stages. Even

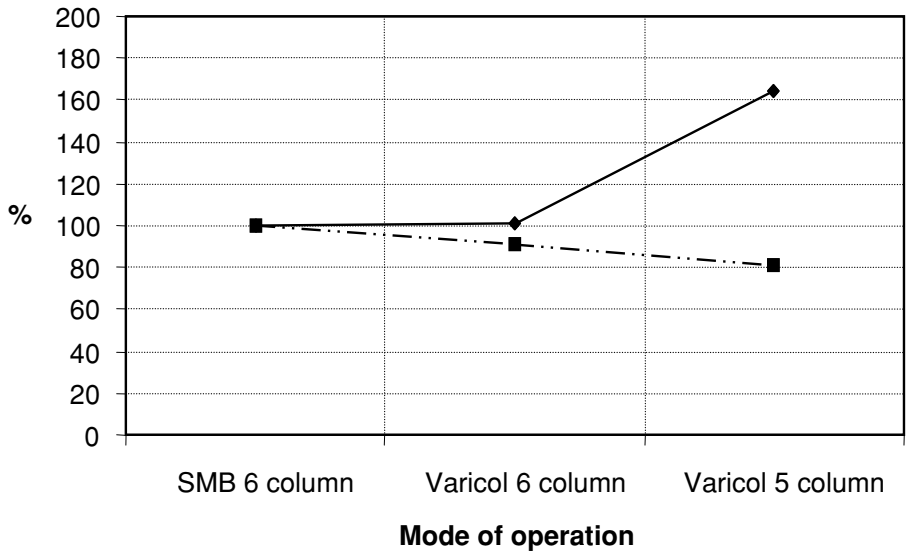

Figure 10.7 Comparison of productivity and solvent consumption in SMB and Varicol modes. Key: solid line = relative productivity; dashed line = relative eluent consumption.

though the technology was easily scaled up, it had to be not only a qualified and validated process but also a system easy to operate and to maintain.

10.4.2 *Implementation in a cGMP production environment*

From the early development to the industrial production stages, key personnel followed basic and advanced courses given by Novasep and attended the most well-known symposia in the field (SPICA in Europe, Prep in the US).

Concerning the measurement, the control and the regulation of the key parameters, besides the usual instrumentation of the mechanical parts of the MCC unit, key in-process controls were installed to establish the consistency, reliability and maintainability of the process.

Therefore, from the development stages, it was established that, for the specific UCB example, the feed, the eluent composition and the distillate from the isolation process had to be verified prior to each batch by an offline analysis. The monitoring of the ethanol composition in the eluent had to be permanently controlled and regulated by an online measurement. In addition to this, periodic sampling of both the extract and raffinate was implemented to determine their respective chiral purities.

During the development, automatic collection of fractions inside the columns was also installed to provide the internal product concentration profile inside the columns. This additional tool has enabled better understanding of the separation process at the development stage and appeared to be an effective monitoring and educational tool for the production personnel. An example of the internal profile is given in Fig. 10.8; this internal profile enables visualisation of the separation inside the columns.

Another key element was the definition of the notion of batch in a continuous process and consequently to demonstrate the full traceability of the process. The following process flow diagram was therefore created (see Fig. 10.9); the racemic mixture (2) is dissolved

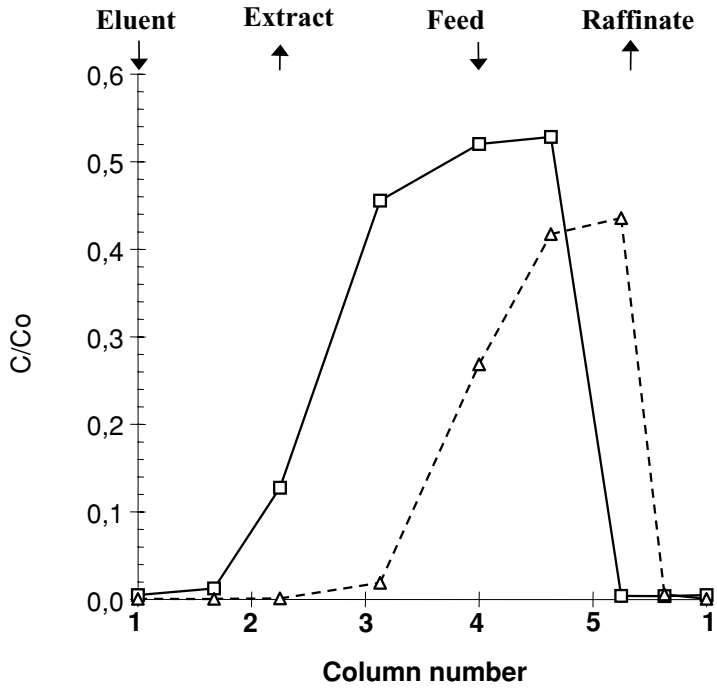

Figure 10.8 Internal concentration profile. Key: solid line = Enantiomer A; dashed line = Enantiomer B.

in its eluent (1 or 1′) in two separate feed preparation vessels (3 or 3′). One vessel (3) is in distribution mode at constant and regulated flow (5) to the MCC unit whilst the other (3′) is in preparation mode and vice versa. Meanwhile, the eluent (6) is continuously fed also to the MCC unit via the eluent tank (4). From the continuous inlets (feed and eluent), two outlet flows (extract (7) and raffinate (8)) are distributed to their respective falling film evaporator unit to give concentrated fractions of extract (11 or 11′) and raffinate (9 or 9′) accordingly collected in two separate vessels. A batch was therefore defined as the distribution and emptying of an integer number of charges in a vessel. The enantiomers from each collected concentrated extract (19) and raffinate (15) are isolated batchwise in a Nauta drying equipment to give the crude API (22) and the enantiomer to be racemised (18). The distillates (10 and 12) from the falling film evaporators are collected in the eluent tank (13) where the composition is permanently monitored to keep it constant. Because of the azeotropic behaviour of the binary mixture of the eluent, slow enrichment of the *n*-heptane occurs and is neutralised by the make-up tank (14), which is enriched in ethanol. The content of the make-up tank can be either of the distillates from the Nauta dryer (20 and 16 to give 24 as the combined flow), after compliance to its specifications, or fresh *n*-heptane and ethanol (23). High-boiling fractions from the isolation process are discarded (21 and 17).

10.4.3 *Qualification*

As each of the units had to be operated according to cGMP standards, full qualification was required. For this purpose, the process has been divided into two sections: the equipment and

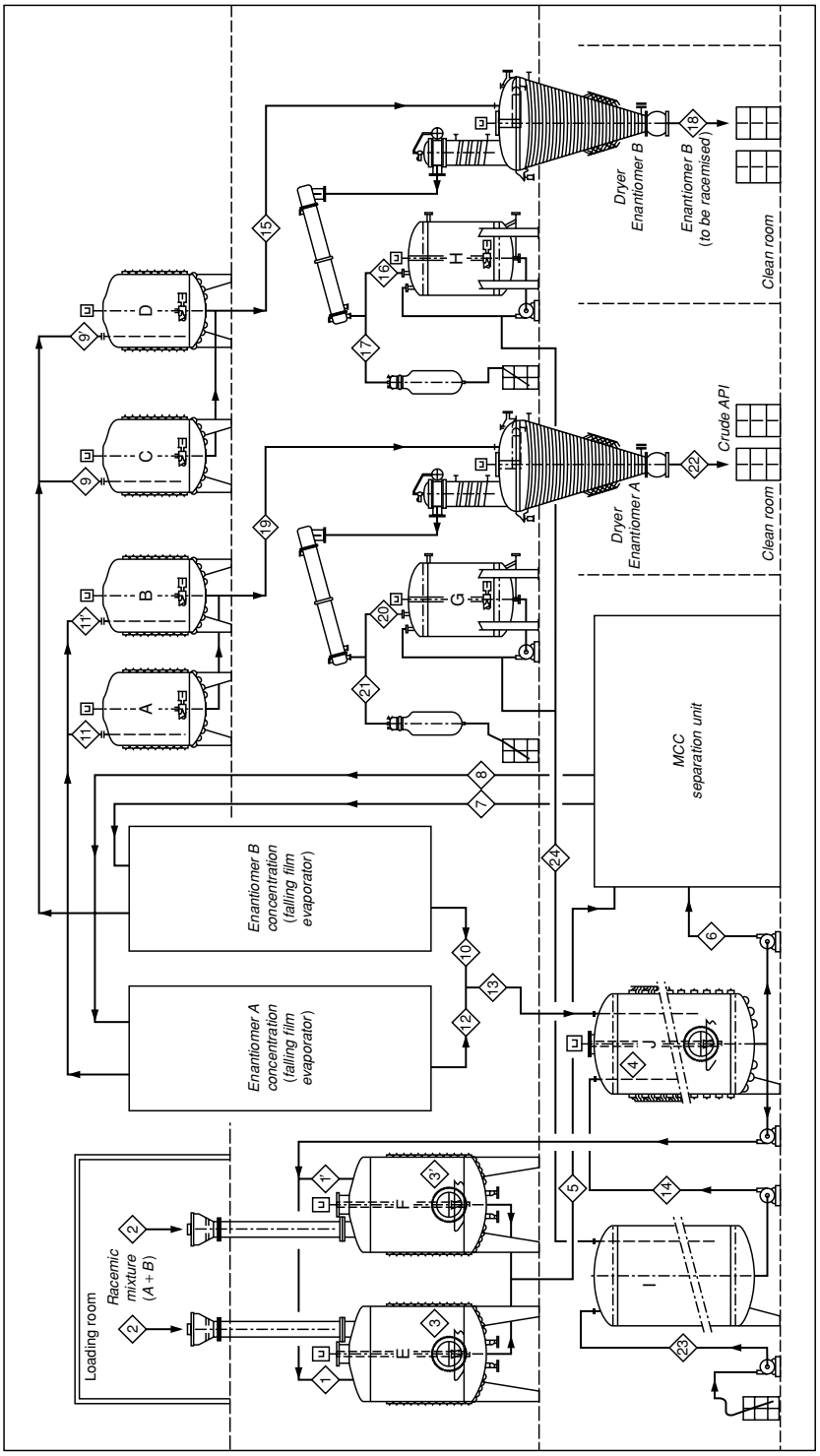

Figure 10.9 Process flow diagram for the chromatographic processing. A: Enantiomer A concentrated solution tank 1; B: Enantiomer A concentrated solution tank 2; C: Enantiomer B concentrated solution tank 1; D: Enantiomer B concentrated solution tank 2; E & F, feed preparation vessels; G & H, receivers; I, solvent make-up tank; J, eluent tank.

the control systems. For each of these, manufacture and qualification of some equipment were subcontracted to third parties, namely the MCC and related evaporators to Novasep and the control systems to Novasep and DCS integrators. The third parties were first audited to verify their systems compliance to the ICH Q7A, GAMP 4 and CFR 21 §11 rules and guidelines.

In order to build a homogeneous set of qualification documents, each individual qualification document was controlled and authorised by UCB experts for the factory acceptance tests (FAT) and site acceptance tests (SAT). These documents were linked to the UCB installation and operational qualification documents. Performance and process performance qualification documentation was entirely the responsibility of UCB. The tree of documents is depicted in Fig. 10.10.

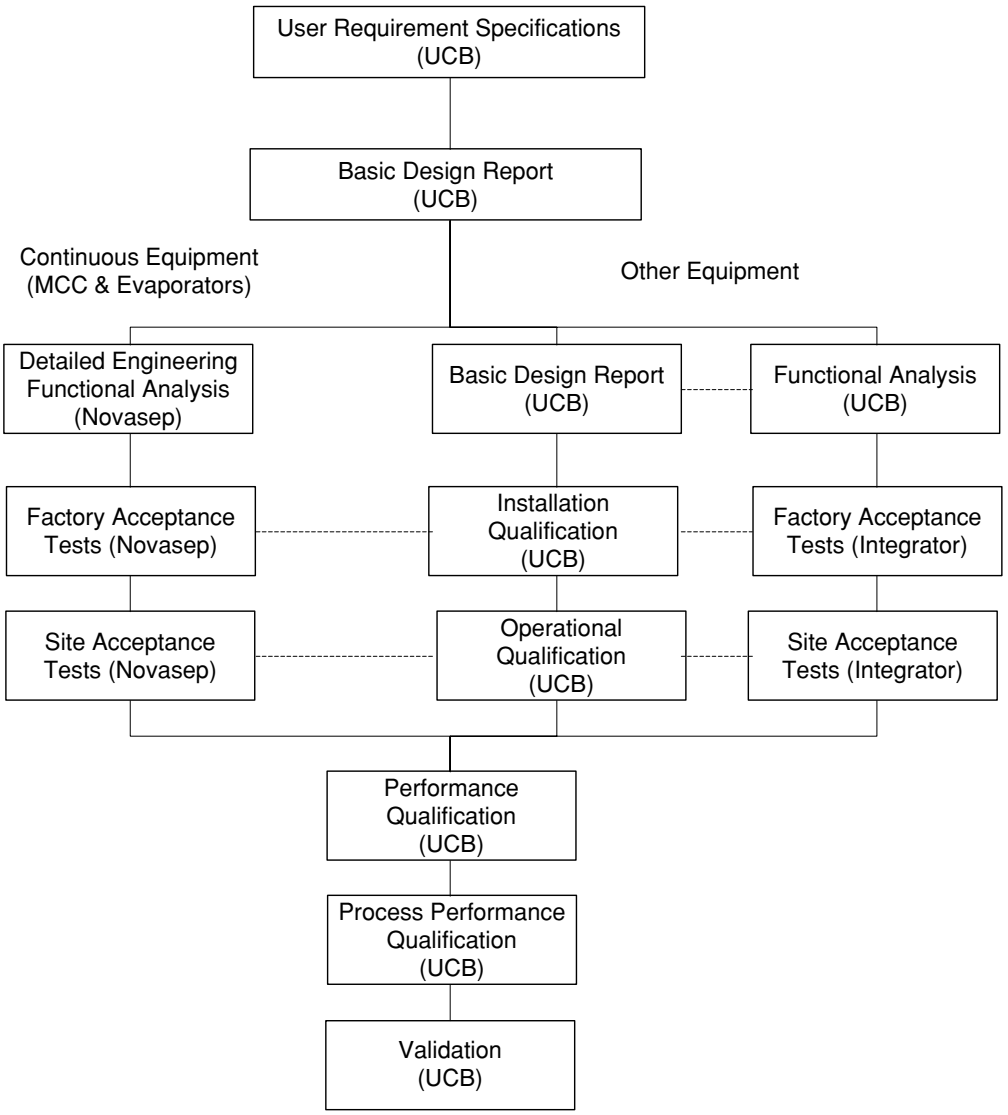

Figure 10.10 Qualification documents tree.

10.4.4 *Validation*

The entire process was documented by a complete process development report that among other items defines the critical process parameters and their allowed ranges for the MCC step. The impact of each critical process parameter for this step was verified by experiments and any combination effect of critical parameters was simulated using the Helpchrom software from Novasep (formerly Licohelp). Several key combination effects were also verified by practical experiments during the development work.

The validation was carried out with the current values of the critical parameters by the manufacturing of three consecutive batches. The compliance of the batches was verified according to the product specifications, to the critical process parameters and to the absence of any production deviations with respect to the Master Batch Record.

The production data are collected from the campaign reports and form part of an annual product review that maintains the validated status in the absence of major deviations.

10.4.5 *Production and maintenance data*

For the campaign reports, key parameters to be recorded are the optical purity and recovery yield of the desired enantiomer, the daily productivity and the solvent recovery yield. Specifically, for the UCB product, the mean purity of the desired enantiomer is at 98% ee with a recovery of 90% and a productivity of 1350 g of isolated enantiomer per kilogram of CSP per day (see Figs. 10.11–10.14).

The efficacy of the overall solvent recovery process was also a key element for economy. From Table 10.12, recovery efficiency reaches 99.7% for ethanol and 99.8% for *n*-heptane.

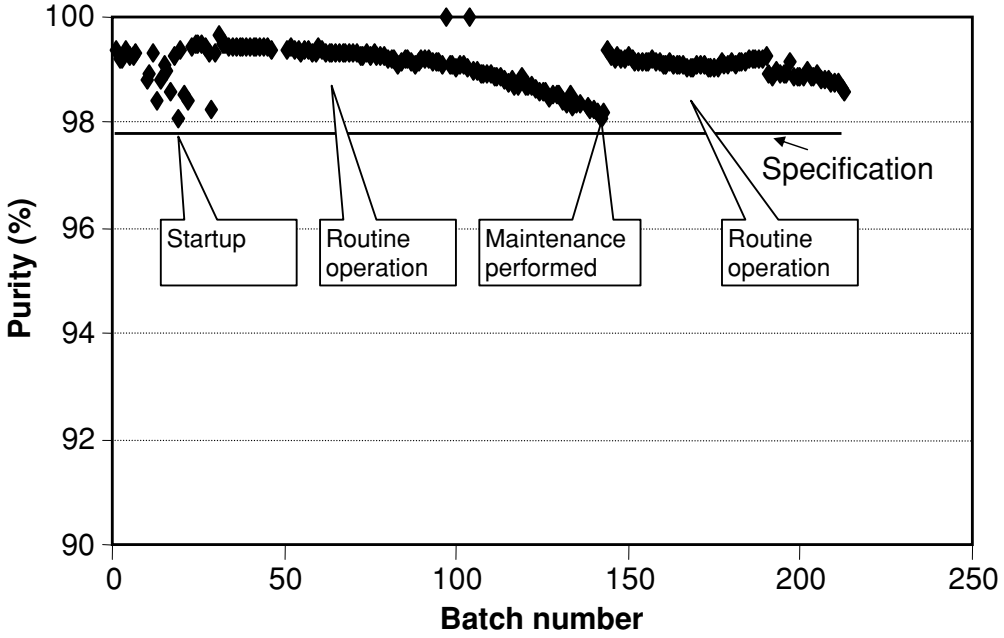

Figure 10.11 Chromatographic purity of the crude API in solution in a MCC 5-1000 unit (Varicol mode).

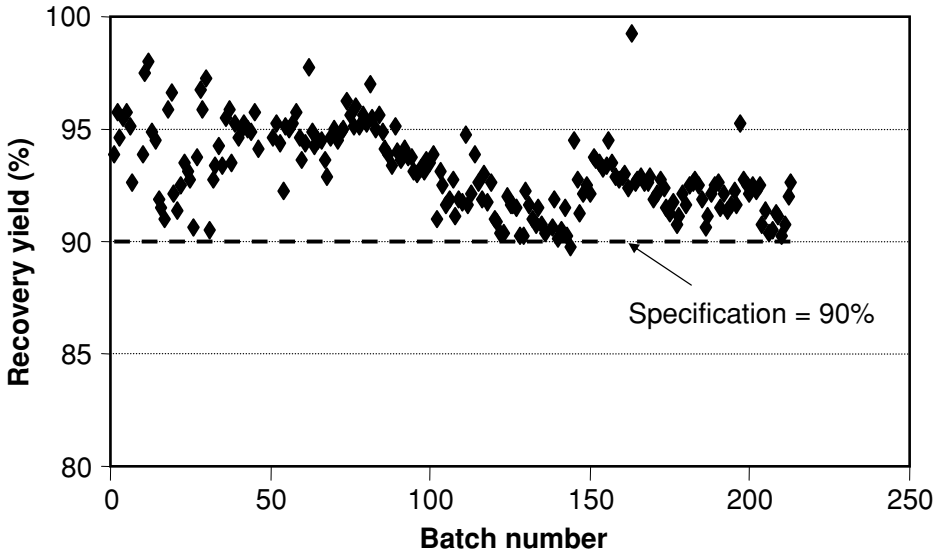

Figure 10.12 Recovery yield of the wanted enantiomer in a MCC 5-1000 unit (Varicol mode).

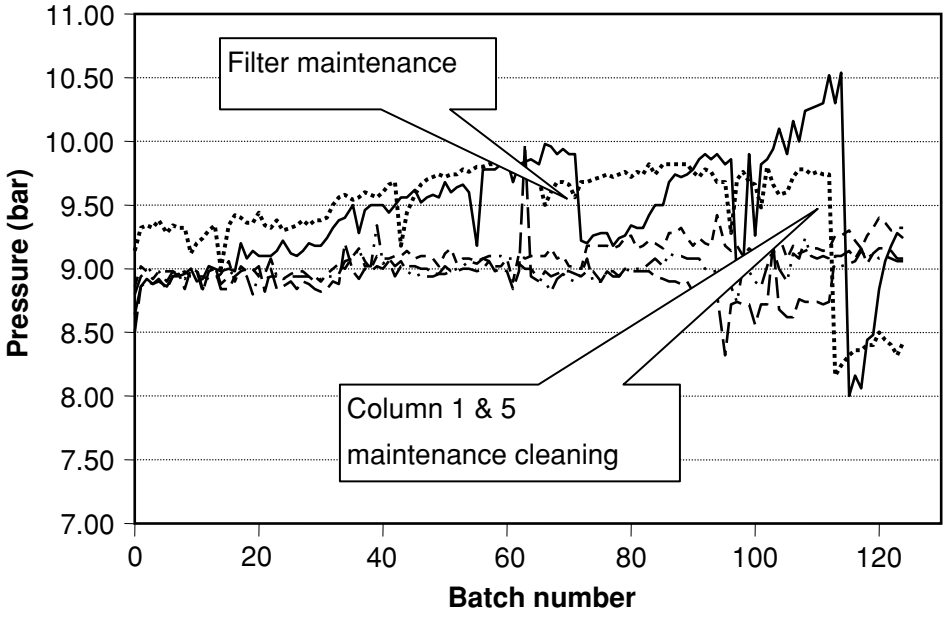

Figure 10.13 Pressure evolution over multiple batches per column in an MCC 5-1000 unit (Varicol mode). Key: solid line = column 1; short dashed line = column 2; long dashed line = column 3; dashed-double dotted line = column 4; dotted line = column 5.

In addition to the previous performance parameters, trend analysis of the pressure drop per column or of the separation performance directly provides key indications towards maintenance periods (see Figs. 10.13 and 10.14). For instance, from these figures, it was decided to perform a cleaning of the intercolumn filters and to unpack, clean and repack two

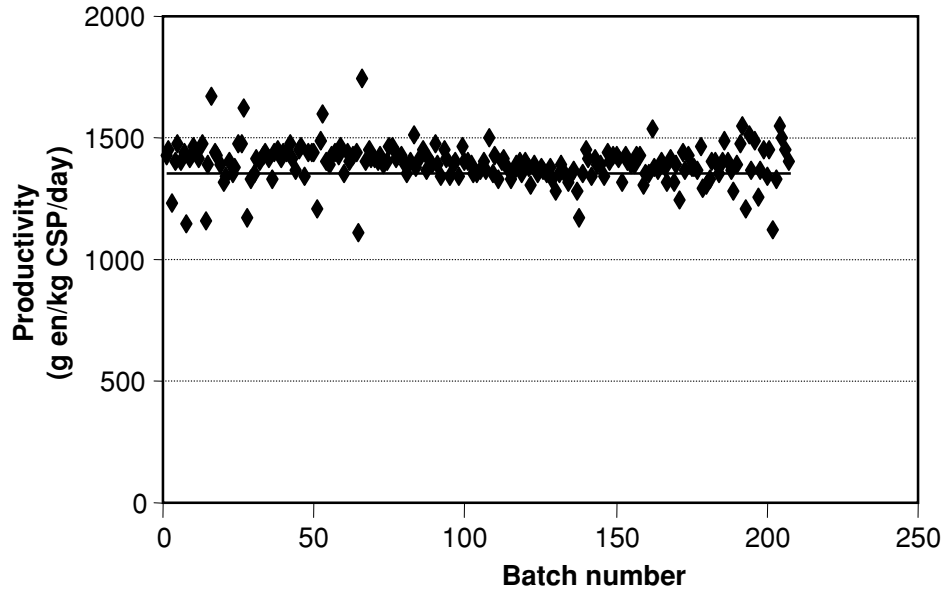

Figure 10.14 Evolution of productivity of the system over multiple batches in an MCC 5-1000 unit (Varicol mode).

Table 10.12 Solvent recovery efficacy

	Ethanol	*n*-Heptane
Solvent to be recovered (L/kg product)	243	162
Solvent losses (L/kg product)	0.68	0.36
Recovery efficiency	99.7%	99.8%

columns of the system. The frequency for such maintenance is about 1 year of continuous operation. (It should be noted that the CSP remained effective for 4 years of continuous operation in the MCC unit of 450 mm i.d. columns used for launching the process.)

Some photographs of the unit are presented in Figs. 10.15 and 10.16. Figure 10.15 shows a top view of the 5 × 1000 mm diameter MCC system installed at UCB in Braine, Belgium. Figure 10.16 shows the falling film evaporators associated with this unit, used for recovery of the solvent from the product streams.

10.5 Further areas of development

MCC technology has now become not only part of the educational background of any chemical engineer or process chemist as a new unit operation but it is extensively used by the chemical and pharmaceutical industries to produce enantiomerically pure drug substance from the separation of mixtures that have been prepared by conventional chemistry.

Development areas in chromatography are directed to fields that will have a positive impact on the future industrial environment. These fields are oriented to the fundamentals of

Figure 10.15 Photograph of an MCC 5-1000 unit at UCB Pharma (Braine, Belgium).

chromatography with the modelling of interactions between the stationary phase, the mobile phase and the molecules to be separated [12–17] or to very applied fields of MCC development with advanced controllers or other modes of operation (e.g. PowerFeed, Modicon) [6–9,18].

It should be noted that in order to assure a permanently optimised process and the largest protection during the life of the drug, it is not only important to focus on advancement in an existing technology but also to have a broader view with respect to the application of alternative technologies such as enantioselective enzymatic chemistry or enantioselective catalytic processes in the UCB example. MCC technology will always face competition with these alternative processes, as overall economics remains the key driving factor.

Figure 10.16 Falling film evaporators used for solvent recovery in an MCC 5-1000 unit at UCB Pharma (Braine, Belgium).

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11 Contract manufacturing and outsourcing considerations

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11.1 Introduction

Chromatographic processes have been known for more than a century, but it is only in the last 20 years that they have been considered as serious production tools for large-scale purification. Traditionally, chemists will use unit operations such as solvent exchange, crystallization, distillation and other techniques before they use chromatography for large-scale purifications. Chemists are familiar with thin-layer chromatography (TLC) and glass-column chromatography, but they usually have very little knowledge of the potential of high pressure preparative liquid chromatography (HPPLC). Nowadays, state-of-the-art chromatographic equipment is available that provides very high throughput while maintaining high purity and recovery of the product, performance that few other techniques can match (Fig. 11.1). Since the potential of chromatography to purify product at large scale is underestimated, very few companies have invested in large-scale chromatographic equipment.

Things changed with the development of chiral chromatography. Recent developments in new chiral stationary phases (CSPs) and in separation technology make the resolution of enantiomers a routine unit operation. Since the early 1990s the Food and Drug Administration (FDA) has been actively promoting the purification of single enantiomers whenever possible. This approach has also been taken by other regulatory agencies such as the European Medicine Evaluation Agency (EMEA) in Europe and the Ministry of Health and Welfare (MHW) in Japan. Pharmaceutical companies have reacted accordingly and a large percentage of new drugs (more than 80%) in development are single enantiomer drugs. Consequently, several fine chemical companies have identified chiral separation by chromatography as a potential business opportunity and have invested millions of dollars to make this unique separation capability available to their customers.

Chiral chromatography can be done using a single-column batch chromatography or using multicolumn continuous chromatography (MCC: simulated moving bed (SMB) or Varicol). However, for large-scale separation of enantiomers, the use of the latter technique is preferred. Like any other unit operation, the continuous mode is inherently more productive than the corresponding batch mode. Also continuous chromatography is an efficient binary separation technique that perfectly fits the requirements for chiral separation of enantiomers. The details of the technique are not presented here since a chapter of this book is dedicated to the principle of continuous chromatography (see Chapter 7).

The increasing need for purified single enantiomers and the limited equipment availability in pharmaceutical companies has generated the need for outsourcing, both for method development and production. After a brief overview of the regulatory aspects of single enantiomer and a discussion on the chiral market, the different aspects of contract manufacturing and outsourcing projects, relative to large-scale purification of enantiomers, will be discussed. We will also discuss the selection of a manufacturing partner as well as the steps required to make a project successful. Finally, we will present an overview on process economics.

Figure 11.1 Multicolumn continuous chromatography equipment (MCC 8 × 200) used for purification of material for clinical trial. Photography courtesy of Aerojet Fine Chemicals, Sacramento, CA.

11.2 The regulatory agencies and the chiral market

In a statement published in 1992 [1], the FDA has defined its position relative to stereoisomers and more specifically to enantiomers of active pharmaceutical ingredients (API). In this document, the FDA acknowledges the existence of technologies allowing the preparation of single enantiomers, e.g. asymmetric synthesis or large-scale chiral separation procedures (chromatography and chiral resolution). As a consequence the FDA has recommended the pharmaceutical industry to develop quantitative assays for individual enantiomers in the early development of a drug. This approach was also taken by both the European Union (EMEA) and Japan (MHW). Later on, the guidelines for the International Conference on Harmonization (ICH) unified the positions between all the agencies in guideline Q6A section 3.3.1 D 'test for chiral new drug substances' [2].

It is acknowledged that pharmaceutical companies have to demonstrate the benefit from using the single enantiomer instead of the racemate. In some cases, the two enantiomers of the same molecule have the same activity; for example, both enantiomers of ibuprofen have anti-inflammatory properties. In other cases, there is a significant difference in activity and pharmacokinetic properties of the two enantiomers; *d*-sotalol is a type 3 anti-arrhythmic while l-sotalol is a β-blocker. Sometimes the racemization of the single

enantiomer happens rapidly in the body and hence the need for pure enantiomeric drug is not justified.

Since in some cases one of the enantiomers can be toxic to humans (e.g. the *d*-isomer of levamisole induces vomiting, or the tragic birth defect associated with the (S) enantiomer of thalidomide), the regulatory agencies recommend that manufacturers should characterize the pharmacological effect of each of the enantiomers during the early stage of the project when therapeutic dose is established (e.g. efficacy, potency and specificity) [3]. This has a direct impact on the development of any enantiomeric drug. It creates the need for both enantiomers so that pharmaceutical companies can evaluate separately *in vitro*, or in animals and humans the behaviour of each enantiomer. The production of the two enantiomers requires the production of the racemic material and a non-destructive separation technique. Chiral chromatography is a perfect tool to achieve high purity and recovery of both enantiomers. It is also a rapid technique to achieve the separation of the racemic mixture and quite large quantities of both enantiomers can be isolated in a matter of few days.

In order to bring better drugs to the consumer, all three regulatory agencies (FDA, EMEA and MHW) are trying to entice the pharmaceutical companies to evaluate the benefit of the single enantiomer versus the racemic drug to the end user. They agreed that the data from the study of the racemate can be taken into account during the study of the single enantiomer and they recommended an abbreviated pharmacology/toxicology evaluation if the information for the racemate is already known. As a consequence, the amount of work required for clinical study is drastically reduced and hence the cost to launch the single enantiomer drug, compared to a completely new chemical entity, is reduced. The FDA in the United States is also granting a 5-year extension to market exclusivity to companies willing to develop the single enantiomer of an already existing racemic drug. This extension is normally granted to a new chemical entity, while the active enantiomer is often considered as a reformulation of an existing approved drug. Several pharmaceutical companies have in their pipeline a number of these chiral switch candidates and are actively looking into this opportunity.

The market for single enantiomer drugs is increasing steadily. This market is showing a constant double-digit growth every year and the forecasts are very optimistic for the years to come. According to market specialists, the revenues for chiral-related technologies (asymmetric synthesis, chiral chromatography, chiral resolution, bio-catalysis) will approach $15 billion in 2009 [4]. Out of $410 billion in sales of pharmaceutical products in 2001, $147 billion were attributed to single enantiomer drugs. The launch of racemate drugs drastically reduced in 2001 in favour of single enantiomer drugs [5]. As a result the number of technologies and techniques to achieve enantiomeric purity is increasing. The 2001 Nobel Prize for chemistry was granted to W.S. Knowles and R. Noyori for their work on chiral hydrogenation, as well as to K.B. Sharpless for his work on chiral oxidation. Despite all the efforts to discover new catalysts or biocatalysts, preparative chiral chromatography is still the fastest route to obtain a single enantiomer from an already marketed racemic drug. A chiral chromatography step is implemented after the production of the racemate. Since this part of manufacturing is well known and optimized in terms of cost, the addition of an extra step is simpler than the complete redesign of the overall synthetic route. The location of the chiral purification step in the overall reaction sequence is critical since 50% of the racemic product is discarded. Racemization of the unwanted enantiomer and recycling back in the purification step is one way to improve the economics. Thus, conducting a chiral separation

earlier in the reaction sequence is perhaps the most economical approach. For this reason companies are using preparative chromatography as a way to get the single enantiomer to the market quickly since time to market is crucial.

Large-scale continuous chromatography is now an approved unit operation in the manufacturing of pharmaceutical drugs. In 2001, the purification of the S enantiomer of Citalopram (Lexapro for anti-depression) using large-scale MCC was approved by the FDA for the company Lundbeck in Denmark. Several manufacturing processes using a chiral continuous chromatographic step are patented for the production of single enantiomer drugs (an MCC process for Sertraline, the single enantiomer of Zoloft for example, was patented by Pfizer, US Patent 6,444,854). Other drugs using an MCC unit operation are currently being reviewed by the FDA and more are in the pipelines of pharmaceutical companies.

11.3 Contract manufacturing

The need for single enantiomers has driven the industry to look for new technologies for preparing chirally pure materials. However, the technology is not always available in-house and investment in new technologies often requires a risk that companies are not always ready to take. As a consequence, the need to find a partner who already offers the desired technology exists. Several fine chemical companies have identified this need and have developed special technologies that they can offer to the industry.

The decision to outsource can be taken at different stages of the project. This decision often depends on the outsourcing policies of companies; some companies rely more on external manufacturers than others. However, the decision to outsource is based on certain needs and considerations.

11.3.1 *Time constraints*

The use of a contract manufacturing organization (CMO) can be a rapid solution to a time constraint problem. When capacity is not available in-house, it can be advantageous to use the resources and expertise of a CMO. Time to market is critical for the financial performance of a new drug. The ‘first of its class’ can potentially take more than 70% of the market. Even a better drug, but launched only few weeks later, can take years to take over the market.

For early phase development projects, there are a number of companies offering small-scale separation services that allow rapid turnaround for the project and in a matter of weeks the single enantiomers can be separated and sent back to the customer. However, for larger quantities requiring a larger size unit, the number of units available in the world is limited. As a consequence, careful planning is required to avoid delays.

11.3.2 *Risk of capital investment*

If equipment exists in-house, it is very likely that the company will consider using its own resources to perform the job. However, if the equipment is already in use for another project or simply does not exist, two choices are offered; the first is to install the

required equipment at the company facility and the second choice is to outsource the project. The first option allows the company to retain all the knowledge and know-how and at the same time increase its production capacity. However, it has some drawbacks. Large-scale chromatographic equipment is not readily available. The design of such equipment can take several months. Also, the equipment itself is expensive and a substantial amount of support equipment and infrastructure is required for its installation. Consequently, investment of millions of dollars is required to integrate the MCC unit into the manufacturing site.

There is a certain risk associated with the capital investment. If the project does not make it through clinical trials or a new, cheaper, synthetic route is developed, then this investment will not provide the desired return on investment (ROI). The use of a CMO allows the outsourcing company to reduce the risk associated with a new technology and hence allows the project to move forward faster. However, finding a partner that is able to take a project at any stage and carry it through, from early development phase up to the commercial phase is difficult. Few companies can offer the whole range of equipment to accommodate all requirements from R&D development to large-scale production. Several fine chemical companies have made the commitment to take the financial risk of installing equipment even if projects are still in the early development stage. These companies invest on the potential of the chiral market and the competitiveness of chromatography versus other resolution techniques. The typical size of unit currently available at CMOs is presented in Table 11.1. This table also gives an indication of the number of MCC units available for contract manufacturing.

11.3.3 *Expertise*

The use of an outside manufacturer is the quickest way to access expertise and know-how on a given technology. Chiral chromatography using MCC technology is a relatively new technique and only a few experts are available in the world. Chromatography is also at the interface of chemistry and engineering and hence requires a good mix of personnel trained for both specialties. For a pharmaceutical company, hiring personnel with the required skill may be less effective than finding a CMO to do the job.

Table 11.1 Typical multicolumn continuous chromatography unit sizes available for outsourcing (the number of units available is an indication only)

Column diameter (mm)	Column number	Number of units worldwide
10	8 or 12 or16	5–10
26	10 or 12	5–10
50	6 to 10	5–10
100	8	1–2
200	8	3–5
300	8	1–2
450	5, 6 or 8	1–2
600	5 or 6	1
800	5 or 6	1–2

Note: Systems with five columns are using Varicol technology (Novasep Patent).

The CMO team must demonstrate its capabilities to handle the project and bring value to its customer. A key reason to outsource a project is to find the right mix of expertise when it is not present inside the company. The CMO must have a team of trained personnel with experience and expertise in chiral separation technology. In addition, the CMO must also demonstrate expertise in other areas such as EH&S, chemistry, regulatory and quality, for instance. The customer must also have experienced personnel for evaluating the capability of the CMO.

The advantage of using CMOs is that most have handled multiple projects and hence have accumulated experience that will be useful for the new project.

11.3.4 *Intellectual property*

Prior to a project and even before any discussion is initiated, a confidentiality disclosure agreement (CDA) is usually signed between the two parties involved: the customer and the manufacturing site (CMO). This document usually covers all the information to be exchanged during the project.

In certain specific cases a third party has to be involved in the project. For example, this is the case when a CSP patented by Daicel is used for an SMB separation (Daicel US Patent 4,912,205, for example), or when a specific technique such as Varicol is used for a purification of an enantiomer (NovaSep US Patent 6,136,198). This requires disclosure of the project details to the owners of the patents and it may involve licensing. It is often the customer himself who deals with the patent owner. The manufacturing site is typically not involved for this part of the discussion since the license is usually relative to the molecule produced. Generally, when more than one party is involved in the project, all information will be circulated through the customer, who ultimately will decide which piece of information should be passed on to the involved parties.

11.3.5 *Location*

The need for a production site in a different location may be driven by the market. For a drug marketed in the United States, approval by the FDA is required and the production site can potentially be inspected. Having a manufacturing site that has a good history with FDA inspections as well as other regulatory agencies is beneficial to the new project.

The manufacturing site can also be located in an area of the world where manpower is cheaper. Some pharmaceutical companies will either build their own facility there or will rely on existing local manufacturing companies.

For early phase development, the location of the CMO is important for practical reasons. It is much easier to handle communication when the CMO is located in the same time zone as the customer. However, when a drug is at commercial scale and manufactured in several countries, communication is no longer such a critical issue since the process should be well developed and a good relationship based on trust and confidence should have been established between the customer and the production site.

11.3.6 *Primary or secondary supplier*

When a new drug is the pure enantiomeric form of an already existing blockbuster, the pharmaceutical company will look for a secure source of supply. This is achieved by selecting

several manufacturing sites. Depending on companies' outsourcing policies, the primary site can be the company itself or a CMO. The secondary and tertiary sites for production will most likely be a CMO. Pharmaceutical companies want to select a supplier with enough capacity in case one of the other suppliers is facing difficulties. They can then redistribute the share of the production among the different manufacturing sources available. It is also a good way to maintain competition and hence drive the price down.

A manufacturing site offering other types of technology as well as chemical production capacity can also provide the possibility to perform several steps of the synthesis, bringing more value to the project. The use of a CMO's capabilities reduces the cost of manufacturing.

11.4 Selecting the outsourcing partner

Once the decision to outsource a project is taken, the next step is to select the manufacturing site. This is a very important step. The CMO partner should be carefully selected since the future of a potential blockbuster drug is turned over to the CMO. Also, once a good relationship and trust have been established, the CMO can be used for other projects. A significant cost is associated with the changing of CMO, hence a long-term collaboration should be considered [6]. The selection of the CMO is based on several criteria and requirements.

11.4.1 *Expectations*

Objectives and milestones to be achieved have to be clearly defined and communicated accurately at the beginning of the project to avoid problems. These objectives should be established mutually and must be reasonable. The outsourcing company should expect the CMO to perform at the same level (or better) as compared to the project being performed in-house. However, one cannot expect the CMO to perform successfully if the level of expectation has been set too high. The manufacturing site very often has a structure that provides flexibility and speed but like any organization it has its own limitations. Understanding these limitations is essential for the success of the project. The CMO usually has numerous years of experience with different types of project and as a result may have valuable input to the process. Thus, the recommendations from the CMO should be taken into consideration and the objectives should be established mutually. A successful collaboration is based on open communication between the two parties involved and on establishing goals that are achievable.

11.4.2 *Audit*

The selection of the manufacturing site must be based on its ability to provide a high-quality product for both the chemical product and the paper product (documentation). This requires a thorough evaluation of the personnel involved with the project as well as the facility. Careful evaluation of the level of competence, experience and expertise of the team is a key to success. The audit is usually conducted in two phases: the first audit is a general audit and all potential CMOs are audited. The objective is to identify the right partner. This audit is usually not very thorough and is just used to evaluate among other things the potential of the CMO in terms of equipment, competence and quality. Once the choice is

made, a more thorough audit is conducted at the selected CMO site. The objective of the second audit is to assess more carefully the capability of the CMO in terms of regulatory compliance, EH&S and operations. The quality audit will focus on the systems in place. The auditor will normally focus on the standard operating procedure (SOP) system, the out of specification (OOS) and Variance reporting systems, the Preventive Maintenance program, the training plan, etc. Inspection of the facility is also mandatory to ensure that the manufacturing site follows its own procedures.

11.4.3 *Decision grid*

The selection of the right partner is finally not easy since several contract manufacturing companies offering very similar services are present on the market. Dr Levine from Bioprocess Technology Consultants presented in 2000 at the IBC USA conference in San Francisco his view on how the selection of an outsourcing company for the bioindustry should be done [7]. His presentation was adapted and presented in the *BioPharm* magazine a few months later (June 2001). He presented a table that allows a comparison of the contractors. The table lists 15 criteria and each criterion is weighted by a coefficient. Each contractor is given a score from 0 to 10 for each criterion. The weighted score is calculated giving a final score for each contractor. The potential contractors can then be compared on the same basis. The same table, with minor modification, can be used for the outsourcing of a chiral chromatography step as well as any other technology.

This selection table puts the emphasis clearly on three main criteria based on expertise, communication and production performance. The weight assigned to these areas is the highest. Other areas such as QA/QC and regulatory as well as cGMP manufacturing and price have a lower influence on the final score. In fact, it is expected that any company willing to become a successful contract manufacturer should be performing well in these areas.

11.5 Communication

Once the CMO has been selected, both organizations, i.e. the outsourcing company and the CMO, must exchange information to initiate the project. Both organizations must understand each other and how they function in order to make the project successful. For any project, there is a learning curve. It is the project manager's responsibility, from both organizations, to make sure that this curve is as steep as possible. The customer must provide to the CMO all the pertinent information regarding the preparation of the racemic feed, the specifications, the delivery schedule and the quantities required. The content of the technology package is discussed later in this chapter. The team lead on the CMO side must also communicate back to the customer the status of the project, i.e. achieved performance, unexpected delays, etc. Sometimes because of cultural differences, misunderstandings and mistakes can occur. But these can be minimized if a good relationship and a good line of communication are established between the two parties. A successful technology transfer requires a lot of attention from both sides to ensure a proper exchange of data. Good communication is a key to the success of the project.

11.5.1 *Contact matrix*

Personnel from several functions inside both the CMO and the customer organization (e.g. project manager, shipment, analytical chemist, R&D, quality, etc.) are involved with a project. It is useful to provide the customer with a list of the people involved in the project. In return, the customer fills a similar list and sends it to the CMO. This list, called the contact matrix, identifies clearly the personnel and their functions, as well as their responsibilities. This list also provides contact information such as phone numbers, fax numbers and e-mail addresses. Shipping addresses should also be specified on this document since during the course of the project, different samples will be exchanged between the two organizations (QC, R&D, Production). It allows easier communication since the right person can be contacted directly especially when the team is spread over several sites. However, the project lead or project manager from each party must be on the distribution list for each exchange of information in order to keep track of the project progress.

11.5.2 *Frequent updates*

Outsourcing a project requires trust. The project manager, who takes the decision to entrust the manufacturing site with the project has to have a high degree of confidence in the capability of the CMO to handle the project and to deliver what is expected. Once the project is transferred to the manufacturing site, there is little that the client's team can do. All is now in the 'capable' hands of the manufacturing site. Maintaining open communication during the course of the project is a requirement. A weekly conference call is a good way to keep the client aware of the progress on the project. Thus, potential problems can be anticipated and solutions discussed beforehand. It is also strongly recommended to call as soon as a problem arises and provide all the details about it so that a plan of action can be laid out. This regular update can be scheduled in advance on a weekly basis or requested when a certain milestone has been achieved (completion of the first batch, end of campaign, etc.). The update can also be initiated by the customer in order to get information or to provide new information regarding any change in the project scope.

11.6 Project requirements

The requirements associated with outsourcing vary depending upon the development stage of the project.

11.6.1 *R&D – method development*

At the early stage of the development, customers will normally request the CMO to provide them with small quantities of both enantiomers. This is important because ready availability of both enantiomers allows the outsourcing company to gather rapidly important information on both enantiomers. The separation method is not necessarily optimized as long as a few milligram/grams of each enantiomer are produced. This material will be used as a reference standard for toxicity studies or for the development of the downstream chemistry. This material will not necessarily be produced under cGMP since it may not be used in clinical

trials. Preparative chromatography, either in batch or continuous mode, is a non-destructive separation technique that provides both enantiomers rapidly in high purity and in a high yield.

The objective of the R&D phase is also to develop a preparative chiral chromatographic method with the intent to use it for large-scale production of the single enantiomer. In that case, it is necessary to identify rapidly the stationary phase that will give the best separation in terms of productivity (selectivity and saturation capacity). However, CSPs are expensive and having a library of preparative columns with a complete selection of all the commercially available stationary phases is not an economically viable option. Several companies are now offering 'screening' services. This service offers, in a week or so, to test many stationary phases. It is very useful to identify a number of separation conditions (stationary phase and eluent composition) that will be able to perform the chiral separation of the enantiomers. Once the packing material and the conditions are identified, it is necessary to perform complementary tests to assess the feasibility of the project at larger scale.

The determination of the parameters for a batch separation is relatively easy and a few days of work will lead to a relatively accurate production rate estimation. Scale-up from there is straightforward. The scale-up factor is simply the ratio of the squared column diameters. For example, if a load of 0.01 g can be injected onto a 4.6 mm i.d. by 250 mm long column with good separation performance, then the same performance should be achieved when injecting 18.9 g ($200^2/4.6^2 \times 0.01$) of the same sample on a 200 mm i.d. by 250 mm long column (providing that the packing material, the mobile phase and the linear velocity are kept the same).

The development of a method for continuous separations using MCC such as SMB or Varicol requires more work in the laboratory. Specific experiments have to be conducted to gather data to model the process. Parameters of a mathematical model are calculated to fit the equilibrium isotherm measured experimentally. The MCC parameters are then estimated leading to a productivity number. These calculations require trained personnel and will most likely be outsourced by companies that do not already use this technology.

The next step is to confirm the parameters obtained by simulation. We have experienced in many cases that the simulations can provide productivity data that is substantially different from the actual MCC performance. The chromatographic mechanisms involved in a chiral separation are rather complex. Existing mathematical models to represent the adsorption isotherm are not always accurate for this type of separation. The discrepancy between estimation and real data can have a severe impact on the potential large-scale production thus affecting the process economics. A decision to cancel the project may result from a poor estimation of the production rate. Production rate and productivity are discussed further in this chapter.

It is strongly recommended to confirm the mathematical modelling by running separation experiments on a small-scale unit. Small MCC units equipped with 1 cm diameter columns can provide valuable information on the production rate while using only 50–200 g of material and few litres of solvent. However, only a few of these units are available. Larger units using 2.5 or 5 cm i.d. columns require substantially larger amounts of material (a few kilograms) to achieve steady state. Unfortunately, such quantities of feed material are usually not available during the early stages of the development project.

Once the parameters have been confirmed on a small-scale unit, the scale-up is also straightforward. The same rule applies for the scale-up factor as for batch chromatography,

i.e. the ratio of the square diameter of the columns. However, if the number of columns is reduced when going to larger scale, then the scale-up will require some adjustment of the parameters.

Potential issues such as stability of the product in the mobile phase, stability of the stationary phase in contact with the product, stability during evaporation, influence and fate of impurities, etc. must be addressed during this phase of the development. The earlier the problems are detected the better the chances are of finding a good solution. Impurity profiles can be detrimental to the long-term stability of the separation. Identification of these stability problems during the early development phase is a necessity for the viability and the economics of the commercial-scale production.

For early phase development, time is a critical factor to take into consideration and cost is a secondary issue. Several suppliers of this type of small-scale manufacturing service exist that can provide a quick turnaround on projects at a reasonable cost.

11.6.2 *Clinical trial quantities*

During phases I, II and III the requirements change in terms of quantity. In most cases the purity of the material will be established during phase I of the project and increasing quantities will be required rapidly to supply the needs for the phase II and III clinical trials. Material prepared during these phases must be produced under current good manufacturing practices (cGMP). This requires the CMO to have a strong regulatory organization (QA, QC, QE). At the end of phase III, a robust, reliable and economical process must be defined. This process is most likely to be described in the CMC section of the New Drug Application (NDA) filed with the FDA or in the filing with other regulatory agencies. Later changes in the process will be quite difficult to implement. During this phase of the project the second enantiomer may or may not be required. Typically in this phase of the development there is a conflict of objectives. The main objective is to manufacture the single enantiomer at the required quality in the required amount for the clinical trials or for the downstream chemistry. But at the same time it is important to generate data on the robustness of the separation. Even if the scale-up in chromatography is straightforward, there are always small differences between the laboratory-scale run and the larger scale. These differences are due to the piping, the dead volumes, the pump capacity, etc. The optimization of the process must be looked at carefully since filing of the process may result at the end of the campaign.

In the early phase of the project (after laboratory-scale development), the quantity of racemic material to process is usually in the order of 200–500 kg depending on the project. At this scale, cost is more important since more stationary phase is required and cost of operating larger equipment is greater. However, the price is still not a critical criterion. Only few CMOs are equipped with MCC units capable of performing this scale of separation at a reasonable cost. As a consequence, early scheduling of the project is important in order to secure the access to the limited capacity available.

11.6.3 *Trial runs vs production runs*

There is often confusion between a trial run and a production run. The first one is very often skipped for the second. A process such as continuous chromatography is quite complex

and definition of critical parameters is essential for the robustness of the process. The operating domain is defined by pushing the parameters until the product purity decreases below specifications. As a result, out-of-specification material is prepared during this step. However, very often the need for material is so strong that the separation conditions are not pushed to the limit, and sub-optimized conditions are used for the production of the first lot of material. As a result, the separation is not conducted at the optimum performance. The product will be obtained at a very high purity and high recovery but at lower production rate. It will then be difficult for later production to provide material with a different purity since the clinical trials were conducted with high-purity material manufactured during phase I. The risk is that these conditions may end up in the NDA filing or used as the reference case for economical comparisons. Thus, conducting trial runs before production runs is very important for the thorough understanding of the process boundaries. It also helps to establish the production schedule as well as the price more accurately since productivity (production rate) will be measured at the required scale and will form the basis of future costing exercises.

11.6.4 *Commercial-scale quantities*

The choice to outsource production depends on several factors as mentioned earlier. If the chiral separation occurs at an early step of the reaction sequence and the product is considered a starting material, then most companies will outsource production to reduce cost. When the separation is closer to the final step, then the decision to outsource will be conducted on a case-by-case basis. Some companies, especially the large pharmaceutical companies, prefer to keep the final step of a drug's manufacture in-house. However, the risk associated with the investment in a new technology coupled with the expertise required to conduct the project rapidly and reliably is often an important factor that pushes the decision in favour of outsourcing. In any case, if the decision is made to outsource production, careful selection of the manufacturing site is required. Recommendations about the selection of a partner for the manufacturing of the single enantiomer have already been presented in this chapter.

Once the project reaches commercial scale the separation conditions are identified and well defined. Critical parameters must be defined and investigated for the filing of the process. The effect of these parameters on the product quality must be tested to assess the robustness of the separation. Usually, the temperature of the separation, the solvent composition, the flow rates and the feed concentration are considered critical parameters. A range of values must be defined for these parameters in which the process still produces material at the desired purity. However, these ranges must not be set too tight to allow process improvement at a later date.

Even if the MCC separation is a continuous process, a batch size has to be defined. Guidance from ICH Q7A [8] defines the batch size for a continuous process as a fraction of the production that is expected to be homogeneous within specified limits. It is defined either by a fixed quantity or by fixed production time. It is also a business decision since the financial value attached to a quantity of material in a batch can represent a financial risk for the company. In general, the batch size will be set by either the upstream or the downstream chemistry and will be defined, but not always based, on a fixed quantity produced. The size

of the batch also has an impact on supporting groups such as QA and QC; a small batch size will require more samples and batch records issued and reviewed.

When the production starts, the process will be validated. A minimum of three consecutive batches at full size will be required after the trial batch to verify that the process can reliably provide the desired enantiomer at the required purity. Usually the material produced during these validation batches is acceptable for commercial use.

11.6.5 *Schedule*

As indicated earlier, there are very few companies that offer chromatographic separation services at commercial scale. This generates an equipment availability issue. The total capacity available worldwide for chromatographic separation (e.g. chiral separation) is limited and hence careful planning is necessary. The planning for phase I and phase II material is critical. Delivery of the product on time is critical for the clinical study and hence any delays in production are not acceptable. In our experience, two major causes for the production delay are

- *Poor planning*. Unexpected delays can happen at any step of the production process, e.g. late delivery of a key material, product held in customs, equipment not ready in time, etc. If there was not a contingency built into the schedule, then any delay will cascade throughout the whole project and late delivery will result, putting the project in jeopardy.
- *Poor development of the chromatographic separation*. Despite the fact that chromatography scale-up is straightforward, proper evaluation of the separation performance is vital to the project. This is especially true for MCC (SMB or Varicol) since modelling and simulations are used to estimate the production rate. A demonstration of the process and the confirmation of the parameters calculated by simulation with a small-scale run are essential to ensure that the production will meet the customer expectation. If the production rate is lower than expected then the outsourcing company and the CMO will have to make a mutual decision. Either the minimum quantity to deliver has to be reconsidered or the delivery time has to be adjusted as a function of the actual production rate.

11.6.6 *Quantity*

The CMO's objective for a production campaign is to process all the racemic feed provided and to recover as much as possible of the desired single enantiomer at the required purity. However, for the first campaign, it is important to define minimum requirements (e.g. quantity) for the project. The separation may not perform as expected, either because the feed contains impurities that adsorb strongly on the CSP causing non-stable performance or because the production rate was overestimated and the production time exceeded the allocated time for the project. In most cases the manufacturing site will stretch the schedule to try to meet the target quantity requirements. However, this causes economic and scheduling problems for the CMO.

When the project reaches commercial stage, the production rate is well defined and the appropriate size equipment will be used to meet the annual requirement. The selection of the appropriate equipment size will be discussed in the section on process economics.

11.6.7 *Product quality*

For a chemical production, the product quality is measured against specifications established in the early phases of the project. However, other parameters have to be considered. Typicality of the material produced is important. Looking for new impurities generated even below the specification limit is critical. Although chromatography is a non-reactive unit operation, the product is nevertheless recovered after evaporation of the eluent and hence is submitted to temperature for a period of time. As a consequence, degradation of the product may be experienced.

Trending of the performance as well as mass balance on the process are also important to monitor. Out-of-trend material even within specification must be explained and hence should trigger investigation from both the quality and operations groups before subsequent batches fail specifications. MCC is a continuous process and as a consequence must be checked periodically for purity and recovery. Collection of samples of the extract and raffinate at regular intervals of time allows trending of the performance of the system as well as calculation of a mass balance. It helps to anticipate degradation of the CSP over time owing to impurity accumulation or mechanical degradation of the packing material. It also allows the verification of the steady state and hence the good operation of the system.

A large quantity of mobile phase is generally recycled internally in the system. Solvent evaporated through the falling film or other evaporation apparatus is reintroduced into the system via the eluent tank. Regular control of the recycled solvent quality is necessary to avoid:

- Possible carryover of material that may affect the separation performance,
- Volatile impurities that may accumulate over time and degrade the CSP and/or the chromatographic performance.

Industrial systems can recycle more than 99% of the solvent used in the MCC process; it is, however, common good manufacturing practice to discard a few percent of the recycled solvent to allow introduction of fresh eluent into the system.

The CSP is an important element of this unit operation. Cleaning of the CSP as well as storage at the end of the campaign have to be addressed properly in order to guarantee traceability of the chromatographic material.

The great advantage of preparative chromatography is the possibility to achieve the desired purity and recovery yield. For high purity (greater than 99.9%) and high recovery, the production rate will be low. The latter can be increased only if either the purity or the recovery requirements can be lowered. This is true for both batch and continuous preparative chromatography. However, for the latter the problem is somewhat more complicated since the MCC is a closed-loop system where several parameters have a great influence on the performance of the system.

The robustness of the process must be assessed properly during the R&D phase at small scale in order to set appropriate specifications for the purified enantiomer.

When it comes to commercial production, the process must be carefully defined and tested for robustness. A reprocess procedure must be established in order to ensure that product of desired quality is produced. It can either be a remixing of the extract and raffinate fractions to prepare a new racemic feed and then reprocessing it through the MCC unit, or the processing of the enriched fraction providing adjustment of the MCC parameters, or it can be a recrystallization. This will provide the product in the desired enantiomeric purity. Unfortunately, this is achieved by accepting a yield loss.

11.7 Transfer of information

At the beginning of any project, the CMO does not know the details of the project. The transfer of data from one side to the other side is critical and must be conducted in an efficient way to avoid miscommunication, delays and unnecessary work. This transfer of information is usually called a technology package. The manufacturing site knows its equipment and hence will have specific questions that the client may not have anticipated from previous experience. Communication must be established both ways to ensure a good transfer of the data and hence a successful project. The technology transfer is easier when the customer has some expertise in chiral chromatography and an understanding of the chromatographic process. This will help in understanding the requirements for the project as well as the limitation of the MCC technologies. A large part of the work during the technology transfer is to ensure that both the customer and the outsourcing manufacturer have the same understanding of the process and its limitations.

Below is a list of typical information that needs to be communicated to the manufacturing site prior to production. If the information is not available, then these data will have to be generated either by the client or by the manufacturing site.

11.7.1 *Feed characterization*

11.7.1.1 *The molecule* The customer should provide the manufacturing site with the molecular structure and the CAS number of the desired product. The reasons are multiple. First of all the molecule may react with the solvent. This may have been overlooked during the R&D phase since the quantities handled were much smaller. This, in turn, may cause a stability issue and in some cases a safety problem. The molecule may be heat-sensitive and part of the molecule may cleave off during the evaporation/recovery step. Technical solutions will have to be developed to circumvent these problems.

11.7.1.2 *Toxicity* If the feedstock is a racemic drug that is already on the market, then the customer must provide CMO with the toxicity data of the molecule such as a material safety data sheet (MSDS) and LD_{50} data, as well as the specific information relative to the handling and packaging of the product.

It is a common practice, when data is not available on the single enantiomer, to assume that the purified enantiomer is at least as toxic as the racemic feed material. When the information is not available, for a new chemical entity for example, data will have to be

generated or maximum precautions will have to be implemented when handling the product. This has an effect on the cost of the project and can potentially lead to delays.

11.7.1.3 *Impurities* An in-depth characterization of the feed quality is essential to the performance of the chromatographic step. Tight specifications have to be set for the feed. When the enantiomer is purified, ~50% of product is removed. Thus the concentration of an impurity eluting with the product can potentially be doubled. This may cause the product to fail the final specifications even if no new impurity was added to the product.

If the feed to be purified at large scale contains more impurities than the feed used for the method development work, then it is most likely that problems will arise during the purification step. Strongly retained compounds tend to affect the loadability of the stationary phase by using up active sites, and retention time reproducibility becomes a problem. MCC is a continuous process and hence requires stable conditions to operate efficiently. It is not an option to rinse the MCC with a strong solvent to remove strongly retained impurities and then restart the production. It is better to use batch chromatography under these conditions. If the CSP is affected by impurities then good performance cannot be achieved.

11.7.1.4 *Physical properties* Information available regarding the physical properties is important. When the racemic feed is received at the manufacturing site, it has to go through QC testing before being released for use in production. Usually the material is tested for appearance (e.g. white, off-white powder or viscous yellow oil for example), identity (by IR or NMR or HPLC retention time) and specific impurities (residual solvent, water content, residual metals, impurities, etc.). This type of information must be available as well as a sample of the material for reference standard.

11.7.1.5 *Solubility* The production rate of an MCC separation is closely related to the solubility of the racemic feed in the mobile phase. The racemic feed cannot be dissolved in a different solvent to improve the solubility as is sometimes done in batch chromatography. As a consequence, the feed solubility must be measured accurately as a function of the solvent composition and the temperature.

The difficulty of dissolving the racemic feed into the mobile phase to prepare the feed solution for the MCC must be evaluated since this can have an impact on the operations and the project costs through manpower and utilities requirements.

11.7.1.6 *Stability* The racemic feed will be dissolved in the mobile phase in advance before being used for most of the large-scale processes. The stability of the racemate in solution must be carefully evaluated. If there is evidence that the material undergoes decomposition or that new impurities are created by reaction with the solvent, then precautions have to be taken to minimize these degradations.

11.7.1.7 *Shipping* On a practical note, it is necessary to know what kind of shipping container is used for the racemic feed and if special storage (cold, ambient) is required. The transfer of the racemic from the container to the feed preparative tank may require special precautions. For example, if the material is dusty, static discharge (ESD) can occur. ESD as well as dust explosivity are major safety concerns when handling powders since explosion may result.

11.7.1.8 *Miscellaneous data* A CMO will often request the customer to provide data that is not directly relevant to the project. This includes, but is not limited to, the regulatory status of the compound (e.g. starting material or registered intermediate), the development phase, the therapeutic area, quantities at commercial scale, location of the chromatographic step in the reaction sequence, etc. This data allows the CMO to develop a better understanding of the project and plan for the future needs.

11.7.2 *Separation conditions*

The customer must clearly communicate the separation conditions, if available, to the CMO. In addition, other conditions that have been tried during the method development should also be communicated. Even conditions that did not work are a source of information and may help the manufacturing site to optimize the process.

11.7.2.1 *Selectivity/viscosity* Sometimes the separation conditions that lead to the best selectivity may not be the best choice if the solvent is very viscous. A lower selectivity and a lower viscosity may result in a higher production rate. Every MCC unit is rated for a given operating pressure, usually 40 bar. This can be translated into a maximum operating flow rate in the MCC unit. This flow rate depends directly on the viscosity of the mobile phase (assuming the CSP particle size is unchanged). Also the production rate is a direct function of the average flow rate, the selectivity and the saturation capacity of the CSP. The efficiency of the separation is affected by the flow rate but not significantly. For example, let us consider the case where solvent A has half the viscosity of solvent B but the selectivity using B is greater than using A. Very often the selection of the separation conditions will be based on the selectivity only. However, it may still be interesting to use A as a mobile phase since the average flow rate will be approximately twice as much as can be achieved with solvent B. The gain in selectivity and/or saturation capacity using solvent B must be significant compared to mobile phase A for it to be selected for the separation.

11.7.2.2 *Solubility* The solubility is also a driver for the selection of the mobile phase. Frequently, chiral separations use an alcohol (ethanol for example) and hexane or heptane as a non-polar solvent to provide some retention. The viscosity of such mixtures decreases when the content of alcohol decreases. Also in most cases the retention time increases and selectivity increases when the content of alcohol decreases. However, most of the compounds are not soluble in hexane or heptane and the feed concentration may then become a limiting factor for the separation. As a rule of thumb, if the solubility is maintained above 50 g racemate/L, then it is not a limiting factor for the production rate.

A compromise between the selectivity, the saturation capacity, the solubility and the viscosity has to be made to allow a satisfactory production rate.

11.7.2.3 *Modifiers* Modifiers are frequently used in chiral chromatographic separation to improve the peak shape and hence increase the efficiency of the column for acidic or basic molecules. As a result the production rate is improved since the efficiency has increased. The most common modifiers are acetic acid, trifluoroacetic acid (TFA), diethylamine (DEA) and triethylamine (TEA). These modifiers are usually recovered during concentration of the

product by the evaporation of the solvent. Thus the solvent composition has to be adjusted carefully during the separation. Usually a small variation in the modifier content has a strong effect on the separation. Hence robust conditions have to be developed to allow some flexibility on the modifier composition. In some cases, the modifier may be a non-volatile molecule. In such cases, it will be concentrated with the product and thus will not be recycled. Consequently, the required quantity of modifier has to be calculated for the entire campaign in order to ensure continuity of supply.

It is important to know if the column used for the method development was previously exposed to modifiers. 'Memory' effects of such columns have been reported in the literature. This memory effect can affect the retention times and hence the performance of the separation, which will then not be reproduced at large scale while using virgin CSP. Also, significant changes in retention time and selectivity have been experienced between samples from the same lot of CSP depending on their history. Thus, tracking the analytical column history is critical for reproducing separation performance on scale-up.

11.7.3 *The chiral stationary phase*

Usually the stationary phase is provided by the customer. The CSP is dedicated to the product and will be used once tested and released by the quality department. In certain cases, performance of the CSP has to be evaluated when lot-to-lot reproducibility is a potential problem for the separation. In that case, an analytical column is packed with the bulk packing material and the separation is tested with this column. For small-scale equipment (MCC 8×50), the quantity of packing required is around 1 kg, so one lot of material is sufficient which makes the QC testing quite simple. When the column diameter of the MCC unit increases, the quantity of CSP required also increases and several lots of CSP may have to be used. Each lot must then be evaluated for performance as well as a blend of the lots. When the MCC columns are packed, the lots are blended in equal amounts for each column in order to achieve reproducibility of performance from column to column. Table 11.2 gives an indication of the amount of CSP required as a function of the MCC column size.

Once the campaign is completed, the stationary phase is cleaned and either stored for the next campaign of the same product or shipped back to the customer.

A new lot of CSP has to be used for each different product to avoid any cross contamination. Even if there is no evidence of such contamination after intensive flushing of the CSP, the conservative approach is to avoid the use of the same CSP for different products. This tends to make projects more expensive since the CSP cannot be recycled for another product.

Table 11.2 Quantity of CSP required for different sizes columns for MCC unit

MCC unit	8 × 50	8 × 200	8 × 450	6 × 450	6 × 800	6 × 1000
CSP (kg)	0.8–1	13–15	65–81	47–58	147–182	230–288

Note: The sixth column is usually shorter on MCC using six columns to compensate for the recycle pump on Novasep systems.

11.7.4 *Analytical methods*

The quality control department will have to implement several HPLC methods for the chromatographic purification of a chiral compound. Usually these methods are provided by the customer and implemented by the quality department. Some of the methods may have to be modified to meet local requirements.

11.7.4.1 *Chiral method* This method should allow accurate measurement of the chiral purity of the material, feed and final product. This method must be tested for reproducibility, robustness and accuracy since this is the method used for the release of the final material. A reference standard of the single enantiomer must be available or generated. This method will have to be validated when the production of the single enantiomer reaches commercial scale.

11.7.4.2 *Achiral method* The feed and the final product must be tested for both specified and unspecified impurities. This method, usually a reverse-phase method, does not resolve the two enantiomers but is used to measure the different impurities in the sample. Again, this method must be tested for reproducibility, robustness and accuracy. This method will also have to be validated when the production of the single enantiomer reaches commercial scale.

11.7.4.3 *In-process method* Usually the in-process method uses the same mobile phase and stationary phase as used for the MCC separation. However, a different set of conditions can be used in order to achieve shorter analysis time. The objective of this method is to provide the operator/engineer with a snapshot of what is happening in the equipment. The cycle time for the analysis must be as short as possible while maintaining good resolution of the two enantiomers. This method is usually implemented on an HPLC unit located in the production area. It is used when developing the method and tuning the separation parameters. Once the MCC unit has reached steady state, then in-process samples are taken at regular time intervals and submitted to the QC laboratory to be analyzed using the chiral method.

11.7.4.4 *Cleaning method* This method is either derived from the achiral method or from the chiral method if the latter has enough sensitivity. A cleaning method is needed to verify that the equipment has been cleaned properly at the end of the campaign before going into the next product/project. The maximum allowable carryover (MACO) is calculated based on the toxicity of the molecule processed during this campaign and the dosage of the molecule of the following campaign. As a result the cleaning method must be able to detect very low levels of material in the solvent rinse.

Since chromatographic unit operations are conducted diluted and also no chemical reactions are conducted, swabbing of the chromatographic equipment is usually not required.

11.7.4.5 *Other methods* Other analytical methods such as gas chromatography are implemented to measure the amount of residual solvent present in the dry product. Water content is measured by the Karl Fischer analysis in the final product.

11.7.5 *Impurity specifications*

Specifications for both the feed and the final product must be clearly stated and approved by both the client and the manufacturing site. As mentioned earlier, the chiral separation may incidentally double the concentration of some impurities co-eluting with the product. Typical standard specifications for the final product are (but are not limited to) 98% achiral purity and 99.0% enantiomeric purity (relative to the other enantiomer). The usual specifications for unknown impurities is no greater than 0.1% but in some cases can be as low as 0.05%.

If the step following the MCC separation involves crystallization, it is important to know the potential for enantiomeric enhancement at this step as well as impurity removal in order that less stringent specifications may be set on the MCC product purity. A combined optimization of the MCC and the crystallization could potentially bring higher yields and production rates than the optimization of each individual step.

11.7.6 *Final product*

11.7.6.1 *Physical properties* The purified material has to be characterized like the racemic feed, e.g. solubility, stability, storage conditions but also the physical form of the product (powder, oil) colour and crystalline form. It is also essential to provide either a standard of the desired enantiomer or a method clearly to identify this enantiomer from the other in order to avoid any mistake.

11.7.6.2 *Shipping* The purified material will be shipped in containers. These containers must be defined by the customers to meet their requirements. The material will be either used in a downstream chemistry step or will be sent for formulation. In both cases the size of the container and the quantity of material per container has to be defined to avoid problems. Note that between Europe and the United States standards are different. Shipping drums and pallets have to be selected carefully to avoid potential problems during the transit of the material from one continent to the other. A common problem encountered is the use of shorter pallets in Europe. Once on the American side, the fork of the forklift will stick out of the shorter pallet and can potentially puncture drums on other pallets. This is a cause for financial loss but also a source of health and safety issues since a pharmaceutical compound can be released in an unprotected environment resulting in exposure of operating personnel.

11.7.7 *Other considerations*

It is important to know the manufacturing process used for the production of the racemic feed. For example, residual solvents used in the previous step such as acetone, ethyl acetate or water can be detrimental to the CSP and the separation. Stationary phases such as the Daicel coated polymers are sensitive to ‘good’ solvents such as acetone. This solvent will strip the polymer off the silica bead, destroying irremediably the chiral property of the stationary phase. Also, traces of acid from the previous step can corrode the equipment. Traces of dissolved salts from prior washing steps or the presence of finely divided metals, perhaps from catalysts or other sources, can also create long-term problems in the chromatographic

processing by precipitating out into the frit materials or at the head of the columns, causing pressure increases or flow distortions in the columns. In most cases pre-treatment of the racemic feed with charcoal or silica will alleviate the problem and provide a stable separation.

The knowledge of the downstream chemistry following the chiral separation is important as well. As we mentioned earlier, if there is a chiral enhancement in a crystallization step post-MCC, then the chiral specification can be relaxed and a higher throughput may be achieved. Also tight specifications on some impurities may be relaxed if they are removed in the following step, as long as they do not interfere with the chiral separation itself.

The content of residual solvent in the product delivered is important to know since residual solvent can affect the downstream chemistry (e.g. yield and impurity profile).

In a hypothetical case, when the purified material does not meet specifications, reprocessing options must be evaluated. If the material fails chiral purity, then it can be reprocessed on the MCC after being recombined with the other enantiomer produced at the same time. It is necessary to make sure that the reprocessed material matches the feed specifications in terms of enantiomeric ratio so the MCC parameters do not have to be adjusted. If the reprocessed feed is enriched in one of the enantiomers then the MCC parameters must be modified to match the individual load of the enantiomers in the system. This may lead to the production of material out of specification during the tuning of the parameters. If the product fails because of impurities, then another unit operation such as recrystallization may have to be considered. In that case a yield loss is to be expected. These methods should be discussed between the customer and the manufacturing site prior to the project.

11.7.8 *End of the project*

At the end of the project, the information flows the other way, from the manufacturing site to the client. Usually a campaign report is issued that summarizes the work conducted during the campaign. A wrap-up meeting through video conference or a visit at the production site is a good way to close the campaign and address the outstanding issues.

11.8 Economics

The cost of outsourcing chromatography is strongly dependent on the size of the project, its status (e.g. early development, phase I, II or III, commercial scale) and the cost structure of the CMO. In this section, we will discuss the important parameters to take into consideration when estimating the cost of a large-scale chiral separation in order to compare fairly with other routes used to achieve enantiomeric purity.

11.8.1 *Productivity*

All batch unit operations are evaluated based on a cycle time and the amount of material produced during that amount of time. The continuous nature of the MCC process makes it difficult to compare with other unit operations since it is difficult to define a cycle time.

However, to compare and scale up chromatographic processes, it is possible to calculate the productivity of the separation based on the amount of material processed per day per kilogram of stationary phase (kg feed/day/kg CSP). It is then possible to compare different sets of conditions and hence select the one that will give the highest productivity number and as a consequence the lower cost.

However, this productivity number can be misleading when it is used to compare different systems or technologies. For example, if the demonstrated productivity achieved on an 8×50 MCC unit is 1 kg feed/day/kg CSP, then the scale-up to an 8×200 unit will lead to a daily throughput of approximately 14.4 kg of feed per day (see Table 11.2 for the quantity of CSP per MCC unit). However, the scale-up to a 6×200 unit has to be calculated more carefully. The reduction of the number of columns from 8 to 6 usually does not affect the separation zone in the SMB (zone II and III) but only the other two zones (I and IV), which influences mainly the solvent consumption of the process. The daily production of the equipment can potentially increase since the operating pressure in the equipment is reduced, leaving room for an overall flow rate increase. This change in column number is rather frequently used to reduce the amount of CSP required for the separation at large scale and hence reduce the initial investment cost.

The productivity number should be used mostly for batch chromatography (single column of fixed length) as an indicator of the performance of the stationary phase since the price of the CSP is high. This number should be used carefully for continuous chromatography since it implies that the units being compared have the same characteristics, e.g. column number, column length and operating pressure.

11.8.2 *Production rate*

A better way to compare performance of different units or chromatographic conditions for MCC is to use the daily throughput or production rate (kg/day). In fact, this number is one of the few that have an influence on the project cost since it gives an indication of the time required to process the whole racemic feed or the size of equipment required to achieve the annual product for commercial scale.

When different column diameters are considered, it is easy to calculate the equivalent production rate at a given diameter using the scale-up factor presented earlier (ratio of the square diameter of the columns). Then fair comparison can be conducted. However, it is always assumed that column lengths are identical, which may not be the case. Under those circumstances a thorough analysis of the two processes is required to make a fair assessment of the process economics.

11.8.3 *Cost breakdown*

For any type of operation, the cost can be broken down into two categories: the fixed costs and the variable costs.

11.8.3.1 *Fixed costs* The CMO must cover these costs even if the equipment is idle (Fig. 11.2). The MCC unit, the ancillary equipment and the buildings are amortized over a certain period of time. This period depends on the location of the facility but is usually 10 years for the equipment and 30 years for the buildings. Since most of the equipment

Figure 11.2 Multicolumn continuous chromatography unit (MCC 6×800) used for the separation of an API at commercial scale. Photograph courtesy of Aerojet Fine Chemicals, Sacramento, CA.

installed in the world is new, the amortization cost is to be taken into consideration. This is usually a factor not in favour of MCC separations when this technology is compared to asymmetric synthesis since most of the equipment used for the latter is usually completely depreciated.

The overhead is also considered in the fixed cost since insurance and rent of equipment or buildings have to be paid. The project must also take its share of the overhead that covers the supporting groups such as the quality department, shipping, storage, maintenance, etc. Finally sales, general and administration expenses (SG&A) that cover the expenses related to payroll (salary, commissions, travel, sales and advertising) have to be considered in the fixed costs since they are calculated for the whole company.

11.8.3.2 *Variable costs* These costs are linked to the project and will be different from product to product.

The cost attached to raw materials, such as solvents and modifiers, must also include totes, drums, bags, cleaning solvent, glassware, personnel protective equipment (PPE), etc. When compared to chemical synthesis operations, large-scale chromatographic separations by MCC require less raw material. The amount of solvent and modifier is small compared to the quantity processed since most of the solvent is recycled from the recovery of the product (falling film evaporators and dryers). In certain cases, a recovery of solvent greater than 99% has been demonstrated at Aerojet Fine Chemicals, but a few percent of fresh eluent is usually introduced into the system to avoid potential accumulation of impurities. Usually the racemic feed is provided at no cost to the CMO as well as the CSP; as a consequence they both do not impact upon the pricing of the project for the CMO but will be part of the expenses associated with the project for the outsourcing company.

The cost of waste can be important depending on the nature of the solvents used and the product toxicity. Usually, the second enantiomer is discarded, unless its recycling is possible after racemization. If the product is toxic, then both the solvents (processing and cleaning solvents) and the second enantiomer have to be collected separately from the normal waste stream of the plant and then incinerated. Under these conditions a certificate of disposal is usually requested by the customer.

The direct labour can be a large cost contributor for commercial-scale projects. The number of operators per shift is fixed and hence this part of the cost is not dependent on the size of the equipment or the production. Even if the equipment is idle it is important to maintain the qualified workforce and there is a cost attached to that.

The final variable cost is the cost of the utilities. However, this cost is very often considered constant and low for the MCC separation since operations are often conducted at ambient temperature or slightly above ambient. The need for utilities is essentially at the product recovery stage where steam and vacuum are needed. Most of the solvents used for separations are organic solvents such as ethanol, methanol or acetonitrile and hence the energy requirement for evaporation is rather low compared to water. This cost is, however, not negligible since it also depends on the location of the facility and the cost of energy.

11.8.4 *Clinical trial quantities*

The quantity of racemate to purify depends on the amount of material required for the downstream chemistry and eventually for the clinical trial. The quantity processed at pilot scale is indicated in Table 11.3 based on the clinical phase requirements. These numbers are an indication only and may be different for each case since the chromatographic separation may be conducted at different steps in the synthesis scheme.

Table 11.3 Quantity of single enantiomer usually required for clinical trials

Phase	Minimum quantity (kg)	Maximum quantity (kg)
I	50	200
II	300	500
III	500	2000

The equipment size required for the separation will be dictated by three parameters:

- The first is the time to market. The faster the product is purified, the quicker the product gets to clinical trials and eventually to the market.
- The second factor is the size of MCC units available to perform the separation (Table 11.1). Since the worldwide capacity is limited, only a few options are offered to the outsourcing companies to process their material.
- Finally, the cost of the stationary phase will influence the selection of the equipment. The high price of the CSP makes it an important cost contributor and hence the customer will try to use the smallest amount of CSP possible for the project.

Processing the material in a large diameter MCC unit will give a very short production time but at the same time a large cost due to the amortization of the equipment, and the cost of the CSP. The CSP has to be purchased for the project and can potentially be only a one-time purchase if the drug does not make it through clinical trials. This item then becomes a major contribution to the price of the project for the first campaign. For later campaigns, if the CSP can be reused, then the cost of the project will only be the cost associated with the CMO. Customers will also be charged for the solvent used during the campaign.

As discussed in previous sections, the CMO usually calculates the cost of using the equipment based on the manpower, the utilities, the facility (e.g. the amortization) and the overhead. In summary, for this type of campaign, the cost of the project is mainly based on the time required for processing the racemic feed.

11.8.5 *Commercial-scale quantities*

When the product enters commercial scale, the equipment size required is calculated based on the projected sales. The cost of the project will then be calculated based on the quantity of product manufactured. A price agreement has to be settled between the customer and the CMO. Usually this agreement contains phases and milestones to achieve for both the CMO and the customer. The quantity of material to process is determined with a minimum and maximum. The unit price can then be reduced as the product reaches peak sales and the required quantities of single enantiomer have increased. Usually a manufacturing agreement is also established between the customer and the CMO.

At this stage of the project, the equipment and the CSP are both amortized over a long period, e.g. 10 years for the equipment and 3–5 years for the CSP. As a consequence, the contribution of the CSP cost to the total price of the single enantiomer becomes small. The main contributors to the cost are now the manpower and the facility, which is more traditional for a unit operation in the fine chemical industry.

11.9 Conclusion

A new technology such as chiral chromatography has demonstrated performance, robustness and reliability on many occasions. The number of projects requiring a large-scale chiral chromatography step is growing and hence the need for equipment is increasing. However, since it is a relatively new technology, there is a limited capacity available. The demand for outsourcing chiral chromatography has grown noticeably over the last 10 years. Only a few fine chemical companies have invested capital in the installation of equipment to supply separation and manufacturing service to the pharmaceutical companies. A few drugs are already filed with the FDA using an MCC step and more are in the pipeline. As more and more projects are taken from method development all the way to large-scale production, the number of MCC units installed will grow. Improvements in the equipment, the stationary phases and also the monitoring of the equipment performance and the modelling of the process are expected in the next 5–10 years.

The establishment of an outsourcing relationship is a long process since trust has to be established. Open communication and proper evaluation of both parties' capabilities and limitations is essential to establish the required level of confidence. However, the final parameter on which to base the decision to move forward for the outsourcing company is the final cost of the product in terms of dollars per kilogram manufactured. Every CMO is expected to be cGMP-compliant, with capable people and good systems in place, but they must also provide a cheaper solution to be considered as a potential partner.

Finally, the outsourcing of a chiral separation follows the same rules as the outsourcing of any other unit operations. It is based on competence, trust, communication and cost.

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Preparative Enantioselective Chromatography
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Appendix: advanced concepts

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A.1 Introduction

The intent of the Appendix is to address a few concepts in greater detail than is necessary for the main text of this book to show how one may optimise simulated moving bed (SMB) separations without a computer simulation, to illustrate some of the techniques used in computer simulations, to outline isotherm measurement and to provide some points to begin further and deeper study of preparative chromatography.

The newcomer to preparative chromatography who wishes to learn more of the mathematical theory in great detail is recommended the book by Guiochon, Golshan-Shirazi and Katti [1].

A.2 Development of SMB separations

Conventionally, SMB separations are designed using some method of estimation of the adsorption isotherms and computer modelling to reach a set of starting operating conditions. This is then followed by small adjustments to the conditions to reach the desired product purity and recovery with the maximum feed flow. In certain circumstances, however, the adsorption isotherms for the solutes of interest are not well fitted by the mathematical models currently available. This means that the operating conditions that are developed from the modelling are at best approximate and may be quite wrong. In this case, there is an alternative, empirical approach that can be taken.

The first step is to determine the retention times of the components at low sample concentration in a column similar to or which can be related to the SMB system's columns. At first, one can assign an arbitrary flow rate (to be refined later) to zone 2 of the SMB unit. The retention times of the solutes are then calculated for columns in this zone from the analytical capacity factors and the assumed flow rate. In order for the lesser retained solute to move in the desired direction during SMB operation (i.e. from zone 2 to zone 3), the switch time for the columns must be longer than the retention time of that solute in a column of zone 2. Equally, in order for the second component to move from zone 2 to zone 1, the switch time must be shorter than the retention time of that component in the columns of zone 2. This gives a range of switch time; the larger the selectivity of the separation, the larger is this range of possible switch times. The choice of the initial value of the switch time is made according to some ideas of how the solutes move through the column under load. If the first eluting solute moves only to shorter retention time under load, then the switch time is safely chosen to correspond to the elution time of the tail of the solute peak when injected as a pulse. If the isotherm is 'S' shaped and the solute peak first moves to longer retention time, then the switch time should correspond to the end of the longest eluting peak envelope. Doing this ensures that eventually the largest feed flow

can be accommodated. Once the switch time is known, then it is possible to calculate the flow rates in the other sections that allow the components to move in the correct directions in the zones. This is most easily demonstrated with an example.

Suppose that we have a separation with the first eluting component having a capacity factor of 1 and a selectivity of 2. The SMB system for which the calculation is being performed uses columns 10 × 5 cm in dimensions, corresponding to a column void volume of 141 mL. If we select (arbitrarily) a flow rate of 200 mL/min in zone 2, then the retention times of the two components will be 1.41 and 2.12 min. This is the outside range for the switch time. Ultimately, because of the displacements that can occur at high concentration in the system, the switch time could become lower than this range, though it should be noted that such a selection can cause some small difficulties at start-up and shutdown of the system. To be safe, we actually choose the retention time of the tail of the first peak to be the starting switch time value. If we suppose that our columns are packed with 20 μm chiral stationary phase (CSP), then under the chosen conditions, this will occur at around 1.6 min. In zone 1, both solutes have to move toward zone 2, and so the retention time of the more retained peak must be less than the chosen switch time of 1.6 min. Thus, the minimum flow rate in zone 1 must be given by $200 \times 2.12/1.6 = 265$ mL/min. This means that the extract flow rate will be 65 mL/min, the difference between the zone 1 and zone 2 flow rates. The flow rate in zone 3 will depend eventually on the concentration of the solutes and the isotherm shape, but we can calculate the limits by supposing a linear isotherm, which will give the maximum possible feed flow. Here, the retention time of the second component must still be greater than the switch time. The maximum flow rate in zone 3 will give a retention very close to 1.6 min, so that the maximum limit for the zone 3 flow rate will be 265 mL/min. In practice, this flow will always be less than this maximum. The zone 4 flow rate is less easy to calculate, because in this zone the speed of migration of the first component is controlled both by the flow rate and also by the concentration of that component in the zone. The maximum flow rate in zone 4 (i.e. the minimum raffinate flow rate) will be given again by supposing a linear isotherm. Here the retention time of the first component must be greater than the switch time (to ensure that this component does not exit the column before it switches) and so the flow rate in this zone must be no greater than $200 \times 1.41/1.5 = 176.25$ mL/min, i.e. a raffinate flow rate of at least 24.75 mL/min plus the difference between zone 3 and zone 2 flows – i.e. the feed flow rate.

Once we have these values, then we can set up the SMB unit to operate in a 'safe' way by choosing the zone 2 flow rate (still in this case 200 mL/min), a zone 1 flow rate of at least 265 mL/min (we would probably choose a value 10% greater than this = 291.5 mL/min, so that the extract flow rate would be 91.5 mL/min), a small feed flow to reduce overload effects of perhaps 5 mL/min, which makes a zone 3 flow of 205 mL/min and a zone 4 flow less than 176.25 mL/min (again, a 10% safety margin would give us 160 mL/min in this zone and hence a raffinate flow of 45 mL/min). At this point we can calculate the average flow rate through the system to ensure that the operating pressure stays within the limits imposed by the system or by the CSP. Supposing a 2:2:2:2 configuration, the average flow would be $(2/8 \times 291.5 + 2/8 \times 200 + 2/8 \times 205 + 2/8 \times 160)$, which comes out to be 214.125 mL/min. Calculation of the expected pressure drop is simple using the Darcy equation; for methanol, this flow rate should result in a pressure of around 16 bar across the columns, although there also has to be some allowance made for the pressure losses in

Table A.1 Flow rates corresponding to limit flow for different solvents in a 5 cm commercial SMB unit

Solvent	Viscosity (N s/m^2)	Flow rate (mL/min)
Acetonitrile	0.00038	279
Methanol	0.00058	183
Ethanol	0.00120	88
Hexane/ethanol (9:1)	0.00043	246
Hexane/2-propanol (9:1)	0.00045	234

the internal tubing, valves, etc., of the SMB unit. Table A.1 shows the practical flow rates that have been employed to give a pressure drop of around 30–35 bar using eight columns of approximately 10 cm length in a commercial 5 cm SMB unit. If the average flow is too great or little for the optimal SMB operation, then the flow rates are changed in proportion to the ratio of the assumed and the desired average flows while the switch time is changed in inverse proportion to this ratio.

At this point the SMB unit is programmed and started. To ensure that the system is not immediately overloaded, a feed solution more dilute than will be eventually used is often employed for the first experiment. The most useful parameters to monitor in this stage are the extract and raffinate purity along with the internal concentration profiles in the column set. These latter profiles may be monitored in one of two ways. Traditionally, a conventional HPLC six-port injection valve is inserted between two columns, conventionally between the last and the first column in the set. At defined moments in the switch cycle, a sample is taken by setting the valve to the 'inject' position and allowing the loop (for a 5 cm i.d. SMB a maximum loop size of around 0.5–1 mL is adequate) to fill, after which the valve is moved to the 'load' position and the loop is flushed with a known volume (at least 2 $\times$ the loop volume) from a syringe, collecting the effluent for later HPLC analysis. This is best done at least twice during each switch period (one point per switch is insufficient for a good internal profile representation). If the switch time is too short to allow two such collections to be made during one cycle, then the collections are made over two consecutive cycles, the first time the collection is made after (for example) one third of the switch time has elapsed, the second cycle it is made after two thirds of the switch time has elapsed. In this way, the profiles of the concentrations of the two components can be plotted against the time (or position) through the cycle. A typical plot is shown in Fig. A.1.

A useful alternative, which eliminates the time required for the analysis of the fractions, is to insert not a valve into the column set but the cell of a polarimeter detector [2]. Although this does not give a response for the individual concentrations of the two components, it gives the difference between them. This is illustrated in Fig. A.2 for the same separation of the enantiomers of guiaphenesin in a small-scale (1 cm i.d.) SMB unit as seen in Fig. A.1.

With very little experience, it is easily possible to envision what is occurring in the SMB unit from either of the profiles that can be obtained by these techniques. As an example, Fig. A.3. shows three polarimeter profiles under different SMB conditions. These were obtained by changing the temperature of the system, but the same effects can be found from changes in either the switch time or the solvent composition. In trace (a), all is adjusted correctly and the product streams are pure. In (b), the switch time is too short (the retention

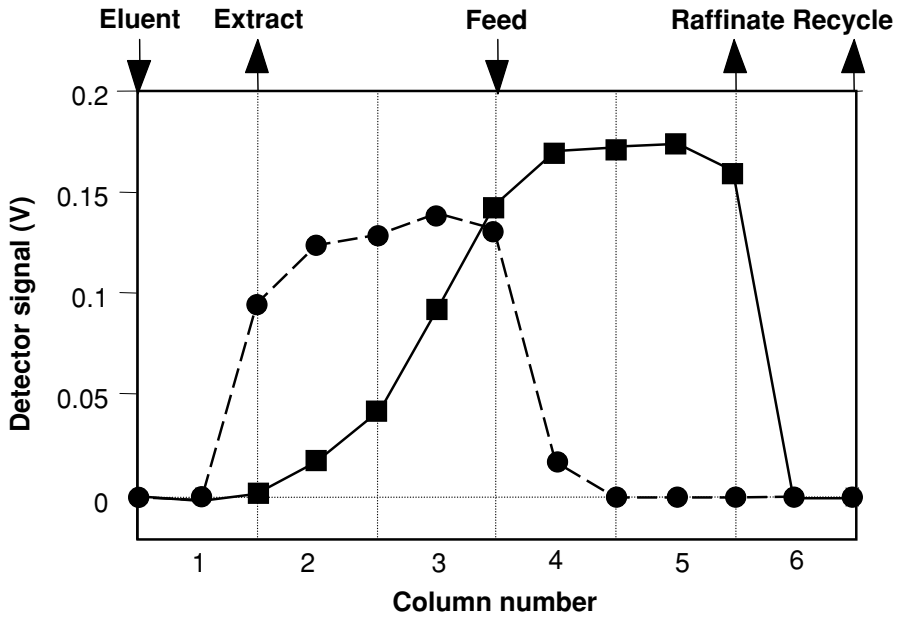

Figure A.1 Internal SMB profile, measured by fraction collection and analysis. Solid line: raffinate product (+) enantiomer. Dashed line: extract product (−) enantiomer. Sample: Guaiphenesin; concentration = 30 g/L. Columns: CHIRALCEL OD 6 × 10 × 1 cm. Mobile phase: 60% EtOH/hexane. Temperature: 28°C. Flow rates: zone 1 = 15 mL/min; feed = 2.32 mL/min; extract = 5.64 mL/min; raffinate = 3.18 mL/min; T (switch) = 47 s.

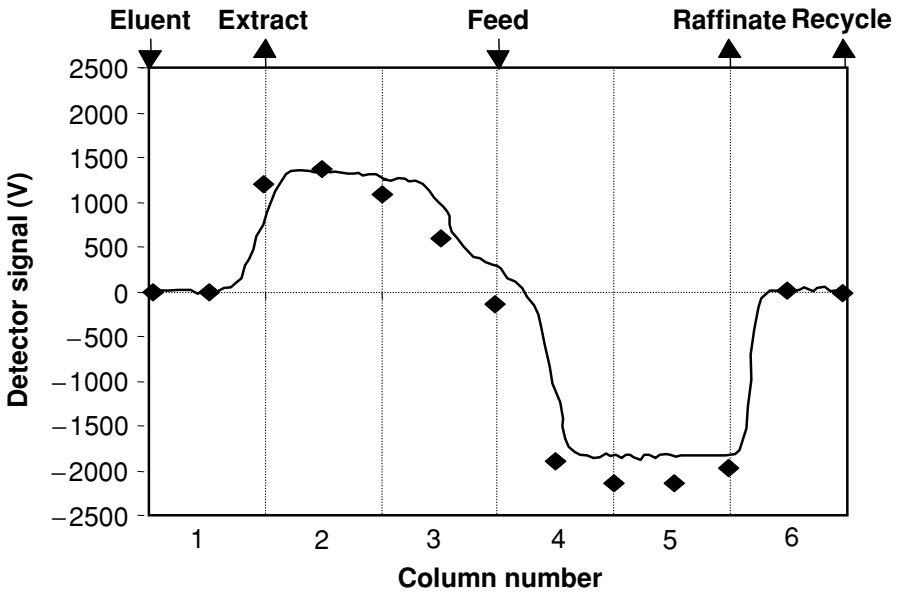

Figure A.2 Internal SMB profile measured by in-line polarimeter. Line: polarimeter output. Points: predicted profile from fraction collection experiment of Fig. A.1. Conditions as Fig. A.1.

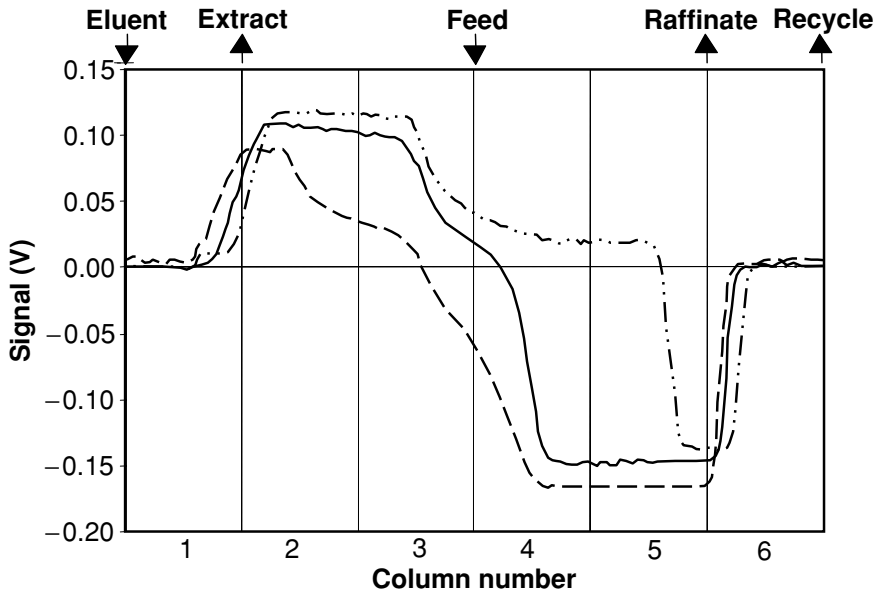

Figure A.3 Internal SMB profiles from polarimeter. (a) Solid line: 28°C; raffinate = 99.5% ee; extract: 99.3% ee. (b) Dashed line: 23°C; raffinate = 99.5% ee; extract: 94.5% ee. (c) Dashed-dotted line: 32°C; raffinate = 55.0% ee; extract: 99.2% ee. Other conditions as Fig. A.1.

is too great because of the lower temperature) and the raffinate product is found in the extract stream. In (c), the switch time is too long (the retention is too small because of high temperature) and the extract product now moves in the 'wrong' direction in the system and elutes in the raffinate stream. In this latter case, the flat zone between the feed position and the narrow peak at the raffinate port is diagnostic. The system is optimised usually by first reducing the switch time until the extract stream begins to be polluted by the faster moving product. At this point the internal profiles will resemble those in Fig. A.4a. The zone 1 flow (and thus the extract flow) may need to be increased to prevent the slower moving product from being carried through to zone 4. Once this point is established, the feed concentration is brought to its desired value (usually around 80–90% of the maximum possible) and the feed flow is incrementally increased until the front of the second component begins to move forward through zone 3 towards the raffinate port. If the profile is monitored continuously with a polarimeter, this front is easy to track in real time and the establishment of the maximum feed flow is easy to do. If the profiles are obtained by collecting samples through the cycle using the valve, then this process is longer because it is essential to reach equilibrium after each change, but equally simple as the changes in the profile are readily seen and tracked, even though there is a good chance of reaching the overloaded condition where the extract product elutes in part through the raffinate port during the process (see Fig. A.4b). Adjustment of the feed flow to allow the desired purity of the raffinate flow completes the basic parameter setting. During the feed adjustment, the raffinate flow may need to be adjusted to prevent the faster eluting component from escaping from zone 4 into zone 1. Once the switch time and feed flow are set, then the extract and raffinate flows can be reduced to the minimum necessary to maintain the separation without products leaking between zones 1 and 4. This is done, of course, such that the zone 2 and 3 flows remain

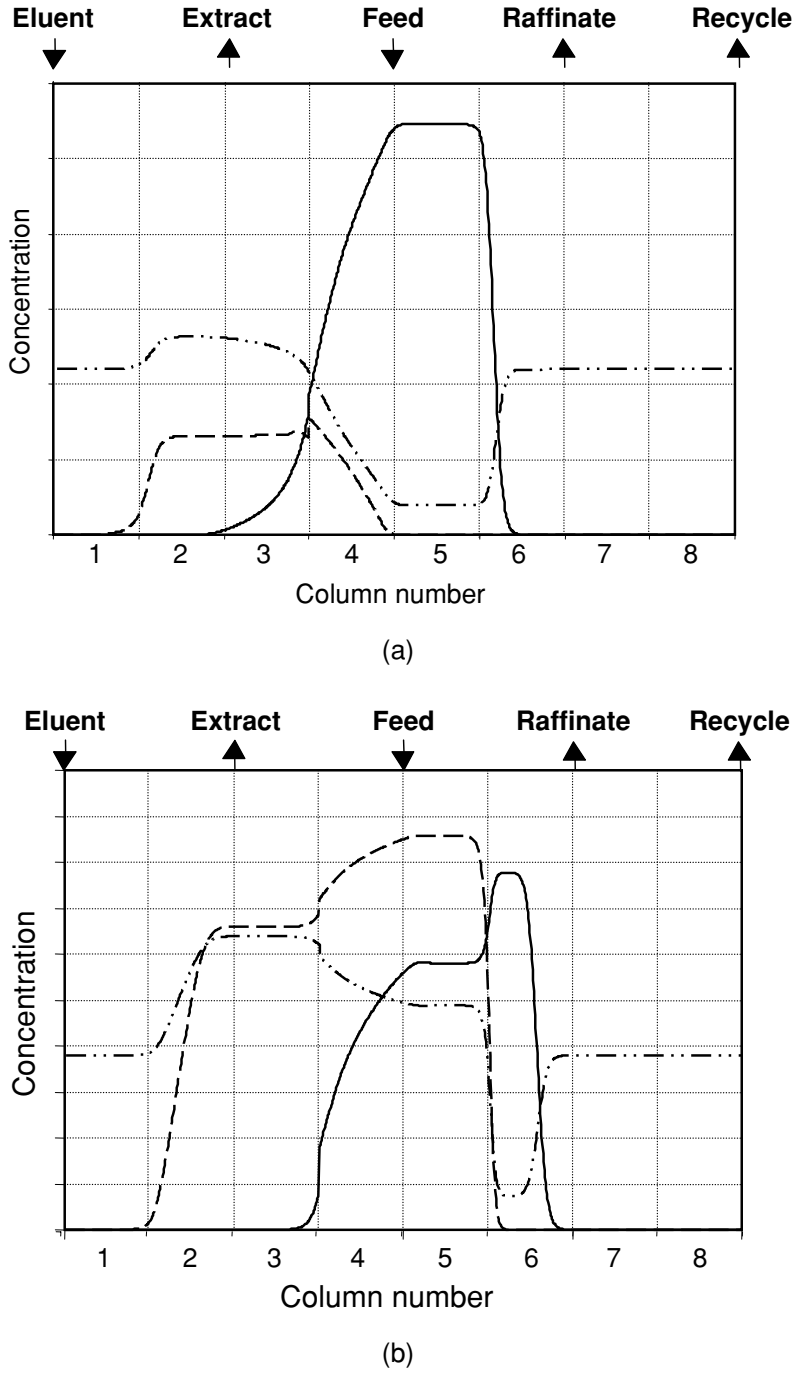

Figure A.4 Typical SMB internal profiles (a) at the early stage of method development and (b) at sample overload. Solid line: raffinate product; dashed line: extract product; dashed-dotted line: polarimeter output.

constant during this process. At this point, mostly no further optimisation is done, although some refinements are possible.

Further adjustments can be made since often displacement effects come into play, where the high extract component concentration causes the lower concentration tail of the faster moving component to elute more quickly in zones 1 and 2, thus bringing the extract purity to a value higher than that desired. It is possible – at the potential expense of the robustness of the separation – to reduce the switch time to bring the extract purity back to the desired value, compensating if necessary the zone 1 flow to prevent leakage between zones 1 and 4. This allows a further increase in feed flow to bring the raffinate purity back to that desired.

Another possible refinement is to reduce the number of columns in the system. This can be done most simply by decreasing the number of columns in zones 1 and 4, perhaps increasing the extract and raffinate flows to ensure that there is no cross-zone leakage.

A frequently employed configuration is to have one column only in zones 1 and 4, maintaining two columns in each of zones 2 and 3. This has the effect of reducing the number of columns and the quantity of packing material used at the expense of an increase in the solvent consumption. Further refinement can be made – mostly for large-scale operation where the financial impact can be significant – by use of the Varicol variation on SMB. Other variations are also possible where the feed flow is changed during the switch period ('power feed') or the feed concentration is changed. All of these refinements allow some improvement over the conventional SMB process, though none can exceed the performance of the theoretical true moving bed process to which they all approximate.

A.3 Computer simulations

Much of the more theoretical content of the chapters in this book have relied on computer simulations. Enough work has been done since the start of these computational techniques to be sure that they represent with reasonable accuracy the processes they describe. In addition, the weak points of computer calculations are now also well understood. At the present day, there are few chromatographers who venture into the complex world of SMB separations without a suite of computer programs at hand to assist with the determination of the operating conditions. The aim of this section is to introduce the basics of computer simulations; for further reading to gain a greater depth of understanding of the various computational methods that may be used, a recently published book by Guiochon and Lin [3] is recommended.

Computer simulation of chromatography has been carried out since the first PC programs were written in the early 1980s. The early versions were simple and, owing to the lack of performance of IBM XT computers, were able to simulate separations with only a limited number of theoretical plates; a typical simulation with 1000 plates could take an overnight computer run! Computer programs were based either on the Craig model of chromatography (based on the Craig countercurrent extractors used extensively at one time for partition-based separations) [4] or on the Rouchon model [5].

A.3.1 *Craig model*

The Craig model is simple to envisage and program, if slow to execute. It considers the column as a series of compartments (cells) each of which contains both mobile and stationary

phase and can be thought of as approximating to a theoretical plate. The program functions by a series of equilibrations and transfers. Samples are introduced into the first cell, the contents of which are equilibrated. The mobile phase, plus the solute that remains in solution in the first cell, is then transferred into the next cell and either fresh mobile phase or more sample (depending on the injection volume, which is measured in terms of the number of cell mobile phase volumes) is placed into the cell. The transfers continue, moving solvent plus any dissolved solute from one cell to the next to the end of the set of cells that make up the column. The mobile phase transferred from the final cell represents the column effluent. The series of transfers are followed by redistributing the total solute content in each cell between the mobile phase and stationary phase in each according to the adsorption isotherm. After this equilibration step, the mobile phase in each cell is once more transferred to the next. In this way a solute is transferred along the set of cells, is equilibrated between the two phases and is eventually 'eluted' as the mobile phase is transferred out of the end cell. The sample is 'injected' by putting the desired concentration of solutes into the first cell. The model suffers from some disadvantages. One of these is simply that any solute which is not transferred into the stationary phase experiences no band broadening whatsoever and emerges as a very narrow rectangular peak. Early eluting peaks have narrower band width than they should while later eluting peaks have lower efficiency. Another disadvantage is that each cell has to be 'equilibrated' each time the mobile phase moves from one cell to another. This is a slow process since the total quantity of solute (calculated from the stationary phase and mobile phase concentrations and volumes) is redistributed between the phases using an iterative, numerical calculation.

The column is represented in the program as an array (or several arrays) that contains the mobile and stationary phase concentrations of the components. These are first set to zero. The mobile and stationary phase volumes in each cell are obtained by dividing the mobile phase volume (from the elution volume of a non-retained peak) or the stationary phase volume (conventionally given by subtraction of the mobile phase volume from the empty column volume) by the number of stages in the column. The simulation is carried out by an iterative procedure representing the flow of solvent through the column. The sample is introduced as a concentration in the mobile phase of the first cell. This is calculated from the injection volume by first calculating the number of individual cell volumes that make up the injection volume. Because one cannot have a fractional cell volume, the concentration is corrected. The total weight of each solute in the cell is calculated and this is then distributed by an iterative procedure between the mobile and stationary phases in the cell by means of the isotherm. The mobile phase concentrations of the two (or more, depending on the whim of the programmer) solutes are guessed (in the fragment of code in Fig. A.5, this is one tenth of the input concentration, but it could equally well be any non-zero number) and this guess is often the previous value of concentration in that cell. The stationary phase concentration is then calculated from this guess, after which a new total mass of solute in the cell is calculated from these concentrations. The mobile phase concentration is modified by multiplying by the ratio of the real total weight of solute to the freshly calculated value and the stationary phase concentration is recalculated. The total weight converges after a few cycles to the actual weight of solute and the calculation is stopped after the difference between the values becomes small enough. When the difference chosen to stop the calculation is too large, the peaks are distorted, often having flat tops. The calculation is done for each cell in the array that contains a finite weight of solute. At the end of this cycle, all cells are now equilibrated.

Make an array containing a structure with 4 elements, all doubles*, for mobile and stationary phase concentrations of the two components (Am, As, Bm, Bs respectively). The size of the array is the number of Craig stages. Set all array elements to zero (empty column)

Input the chromatographic data: column size; mobile phase volume (from to) or porosity data; flow rate; capacity factors; saturation capacity (same for both components); sample concentration; injection volume

Calculate internal column volumes, mobile phase volume (Vm) and stationary phase volume (Vs) per stage

Calculate number of stages (M) corresponding to the injection volume

Calculate the load injected, and then a new concentration in terms of the volume of the number of stages just calculated [concentration = load/(M*Vm)]

Calculate isotherm parameters a(i) = k'(i)*Vm/Vs, b(i) = a(i)/ws

Start the calculation loop

Decide if the injection is taking place (the first M cycles); if so, put the sample concentrations in the first stage mobile phase; if not, set the concentration to zero to correspond to fresh solvent. (Note: Code examples are written in C++.)

```
if (LoopCounter<inj_points)   // "Injects" sample for the number of cell volumes used for injection
{
    Array1[0].Am = Conc1;
    Array1[0].Bm = Conc2;
}
else                          // When no more sample is left, introduce mobile phase into 1st cell
{
    Array1[0].Am = 0;
    Array1[0].Bm = 0;
}
```

Start a loop which takes each stage in turn, calculates the total solute in the stage

```
for (j=0;j<NoOfStages;j++)          // increments Z
{
    if ((Cell[j].Am)}}(Cell[j].As)}}(Cell[j].Bm)}}(Cell[j].Bs))
    {
        TotalA = Cell[j].Am * Vm + Cell[j].As * Vs;   //total weight A in cell
        TotalB = Cell[j].Bm * Vm + Cell[j].Bs * Vs;   //total weight B in cell
```

Guess some value to seed the iterative redistribution of the cell contents between the phases

```
        oldAm = Conc1/10;               // seed value for iteration
        oldBm = Conc2/10;               // seed value for iteration
```

start a loop for the iterative redistribution

```
        do
        {
```

Calculate stationary phase concentration from mobile phase concentrations

```
            newAs = a1 * oldAm / (1+ b1 * oldAm + b2 * oldBm);
            newBs = a2 * oldBm /(1+ b1 * oldAm + b2 * oldBm);
```

Calculate the new total weight of A and B in the cell from the new concentrations

```
            newTotalA = newAs * Vs + oldAm * Vm;
            newTotalB = newBs * Vs + oldBm * Vm;
```

Figure A.5 Outline of the Craig simulation programme.

Change the initial guess at mobile phase concentrations by the ratio of the actual total weight of material in the cell to the calculated value

```
if(newTotalA) oldAm = oldAm * TotalA/newTotalA;
else oldAm = 0;
if(newTotalB) oldBm = oldBm * TotalB/newTotalB;
else oldBm = 0;
```

Calculate the difference between actual and calculated weight

```
double diff1 = fabs(TotalA - newTotalA);
double diff2 = fabs(TotalB - newTotalB);
```

Find the larger difference and test if it is small enough not to matter

```
if (diff1 > diff2) diff = diff1;
else diff = diff2;
```

If it is small enough, stop

```
} while (diff > 0.0000000001);
```

Assign new concentrations to the cell

```
        Array1[jj].Am = oldAm;
        Array1[jj].Bm = oldBm;
        Array1[jj].As = newAs;
        Array1[jj].Bs = newBs;
    }
}                           /*end of j loop - continue*/
```

Put last cell mobile phase content into a product array

```
ProductArray[LoopCounter] = Array1[data.GetEfficiency()];
```

Move the mobile phase in each cell to the next one

```
for (int i = NoStages; i > 0;i--)
{
    Cell[i].Am = Cell[i-1].Am;
    Cell[i].Bm = Cell[i-1].Bm;
}
```

Add graphics if needed…
Increment the cycle counter ready for the next time the function runs

```
LoopCounter++;
```

Test to see if the total number of cycles is reached; if so, stop the timer and start any other functions that need to run to close the simulation, display the chromatogram stored in the product array, etc.

```
if (LoopCounter == data.GetEfficiency()* (2+k2))
{
    Timer1->Enabled = false;
    Etc
}
                            * double = floating point, double precision.
```

Figure A.5 *Continued*

The mobile phase concentration of the last cell in the array is taken to be the output from the column and is usually stored in another array until the simulation is complete before the final chromatogram is reconstituted. The mobile phase concentrations in the array are then moved one cell downstream and the system is then ready for another equilibration. In a Windows program, this is triggered by a timer that allows other functions to be carried out between cycles. If the program simply uses a loop for the control of the sequential equilibrations, Windows freezes until the run is complete. The number of cycles has to be large enough to allow the most retained component to elute from the column. Normally this can be achieved in a number of cycles given by multiplying the number of stages (cells) by $(2 + k')$.

The chromatogram is reconstituted from the output array by recalling that each movement of solvent (equilibration cycle) corresponds to an elapsed time. The time represented by the cycle is given by dividing the mobile phase volume of the cell by the flow rate through the column.

Figure A.5 shows the section of the program that makes the iterative calculation. This is essentially the function called by a timer that controls the program and prevents it from taking all the computer's resources and blocking the normal function of the operating system. The function is called every few milliseconds. This time delay can be varied according to the speed of the computer such that normal operation of the operating system is not impaired by the CPU-intensive loops. The timer is switched off by the routine once sufficient loops have run to perform the separation. In this fragment, the array that contains the concentration data is an array of structures that contain mobile and stationary phase concentrations of each component. In this way, each structure represents one cell in the Craig machine. This could equally well be done with four arrays, each containing one concentration value at each position. The remainder of the program consists of a way in which to enter the initial data, the timer function that calls the loops to run the calculation and finally a routine that outputs the results either into a file or into a graphical representation of the chromatogram.

A.3.2 *The Rouchon model*

The Rouchon model, on the other hand, is fast because it considers that the mobile phase concentration controls that in the stationary phase. This means that instead of using several cycles of the numerical convergence used for the Craig model for each equilibration in each cell, the program simply assigns the stationary phase concentration from the mobile phase solute concentrations and the adsorption isotherm. The algorithm solves the differential equation for the mass balance in the column by using the method of finite differences. Instead of infinitesimal differences between the calculated points, finite differences that are related to physical parameters of the system are used. The method uses a space–time grid, with the distance increment along the column corresponding to the theoretical plate height and the time interval calculated from the solute capacity factor and flow rate. In this way, it turns out that the numerical dispersion due to the errors introduced by the use of the finite differences is identical to the kinetic dispersion in the column for the solute of the capacity factor chosen. The consequence of this is that the column efficiency calculated is correct only for a peak with the capacity factor chosen for the calculation of the time interval. Peaks that elute earlier have lower apparent efficiency while peaks eluting later have a higher efficiency. In practice, this means that the simulations are reasonable where there

is small selectivity and the peaks are close. Where selectivity is large, the results are more suspect. For preparative separations, the best compromise is to use the analytical capacity factor for the first component since the zone of overlap is close in retention to this value. It should be noted, however, that in practice the algorithm does not calculate parts of a peak that are greater than twice the value of capacity factor chosen; thus, for large selectivity separations, a different value has to be taken which is either the average k' for the two peaks or the capacity factor for the second peak. In any event, use of the algorithm for large selectivity separations will give errors in the calculations due to the poor efficiency values given to the peaks. In such situations, more complex simulation algorithms are needed.

The equation used for the fastest, simplest calculations is derived as follows.

We start with the partial differential equation of chromatography

$$\frac{\partial C}{\partial t} + F\frac{\partial q}{\partial t} + u\frac{\partial C}{\partial z} = D_{\mathrm{L}}\frac{\partial^2 C}{\partial t^2} \tag{A.1}$$

where F is the phase ratio, C is the mobile phase concentration, q is the stationary phase concentration and D_{L} is the longitudinal mass transfer parameter.

To solve this numerically, we have to convert this into a finite difference equation rather than a differential one, by assuming discrete values for the time and distance steps.

It turns out for the numerical solution that the errors involved with the assumption of a finite difference model relative to the differential equation closely equal the band spreading represented by the right-hand side of Equation (A.2). If we choose the right values for the time difference and the space difference, then we can set the right-hand side to zero, which considerably simplifies the situation.

Equation (A.1) then becomes (with a little rearrangement)

$$\frac{\partial C}{\partial z} + \frac{1}{u}\left(\frac{F\,\partial q}{\partial t} + \frac{\partial C}{\partial t}\right) = 0 \tag{A.2}$$

We choose [2] the values for δt and δz to be $2H/u_z$ and H respectively, where H is the HETP (height equivalent to a theoretical plate) of the column. The band velocity for the solute concerned u_z is equal to $u/(1+k')$, where u is the mobile phase velocity in the column. As noted above, the actual value of capacity factor chosen for the calculation depends on the selectivity of the separation. The way in which the differences are calculated make a difference to the final algorithm for the calculation. Here we take the change in concentration in the z direction as being from z to $(z+\delta z)$ at time $(t+\delta t)$ and the change in time direction as being from t to $(t+\delta t)$ at the distance z. Other possibilities exist.

This brings Equation (A.2) into the following form:

$$\frac{C^{z+\delta z}_{t+\delta t} - C^{z}_{t+\delta t}}{H} + \frac{1}{u}\left(\frac{C^{z}_{t+\delta t} - C^{z}_{t} + (q^{z}_{t+\delta t} - q^{z}_{t})\frac{V_{\mathrm{s}}}{V_{\mathrm{m}}}}{\frac{2H(1+k')}{u}}\right)$$

which rearranges to

$$C^{z+\delta z}_{t+\delta t} = C^{z}_{t+\delta t} - \frac{\left(C^{z}_{t+\delta t} - C^{z}_{t} + \frac{(q^{z}_{t+\delta t} - q^{z}_{t})V_{\mathrm{s}}}{V_{\mathrm{m}}}\right)}{2\,(1+k)} \tag{A.3}$$

In practice, the method uses two arrays, one representing the situation at a time t and the other at a time $t + \delta t$. The array elements are different distances along the column; the first is the injection point, the last is the column outlet. Each point on the second array is calculated from two others, one in the first array and the other in the second array, both with a smaller z value, with Equation (A.3). Figure A.6 shows the code for the program fragment equivalent to that for the Craig method of Fig. A.5. Again, the concentration in the mobile phase on the final step at each time increment is used as the output concentration for the separation, being stored in an array with a time increment of δt, which is subsequently used to construct the chromatogram.

Make an array containing a structure with 2 elements, both doubles*, for mobile phase concentrations of the two components. The size of the array is the number of plates in the column, which can be calculated from the column parameters, flow rate, etc. Set all array elements to zero (empty column)

Input the chromatographic data as for the Craig programme.

Calculate the column volume, mobile phase volume (Vm) and stationary phase volume (Vs)

Calculate number of plates (M) corresponding to the injection volume

Calculate the load injected, and then a new concentration in terms of the volume of the number of plates just calculated.

Calculate isotherm parameters a(i) = k'(i)*Vm/Vs, b(i) = a(i)/ws

Calculate the value (speedk) of capacity factor for the calculation.

Calculate the δt (=2*to*(1+speedk)/N) and δz (=L/N) values for the calculation.

Start the calculation loop

Decide if the injection is taking place (the first M cycles); if so, put the sample concentrations in the first stage mobile phase

```
if (LoopCounter<inj_points)
{
    Array2[0].Am = Conc1;                    // is zero by default = fresh mobile phase.
    Array2[0].Bm = Conc2;                    // set to input concentration during loading
}
```

Start a loop which takes each stage in turn

```
 for (j=0;j<total_plates - 1;j++)        // increments distance along the column
{
```

If the mobile phase concentration is non-zero,

```
    if (Array1[j].Am }} Array2[j].Am)
    {
```

calculate the stationary phase concentration for distance j along the column from the isotherm for time = t:

```
        CsA1 = a1* Array1[j].Am /(1+b1* Array1[j].Am * b2* Array1[j].Bm);
```

Figure A.6 Outline of the Rouchon simulation program.

calculate the stationary phase concentration for distance j along the column from the isotherm for time = t + δt:

```
CsA2 = a1* Array2[j].Am /(1+b1* Array2[j].Am * b2* Array2[j].Bm);
```

Calculate the mobile phase concentration at distance j + 1 along the column at time = t + δt:

```
Array2[j+1].Am = Array2[j].Am -(Array2[j].Am-Array[j].Am+(CsA2- CsA1)*Vs/Vm)/ ((1+Speedk)*2);
```

If the value of mobile phase concentration is insignificant, set it to zero to reduce the numbers of pointless calculations

```
  if (Array2[j+1].Am < 0.00000001) Array2[j+1].Am = 0;
}
```

Do the same calculations for the second component:

```
if (Array1[j].Bm }} Array2[j].Bm)
{
  CsB1 = a2*Array1[j].Bm/(1+b1*Array1[j].Am* b2*Array1[j].Bm);
  CsB2 = a2*Array2[j].Bm/(1+b1*Array2[j].Am* b2*Array2[j].Bm);
  Array2[j+1].Bm = Array2[j].Bm -(Array2[j].Bm-Array1[j].Bm+(CsB2-CsB1)*Vs/Vm)/
                                                ((1+Speedk)*2);
  if (Array2[j+1].Bm < 0.00000001) Array2[j+1].Bm = 0;
}
```

Put the content of the last plate into the product array

```
ProductArray[LoopCounter] = Array2[total_plates];
```

Put values from Array2 into Array1:

```
for (int i=0;i<total_plates;i++) Array1[i] = Array2[i];
```

Reset Array2 to zero

```
MPCell Fell;
fill(Array2.begin(),Array2.end(),Fell);
```

The remainder follows the Craig programme in testing for the correct number of cycles (=number of plates), taking care of graphics and stopping the timer.

Figure A.6 *Continued*

A.4 SMB simulation software

SMB simulations are an important part of the prediction of operating conditions and economics of large-scale separations and as such there are several commercial and a number of proprietary programs. Some of the programs are complex and include many parameters while others are extremely simple. The main problem with all is the lack of good and easy adsorption isotherms with which they can work and at present it is probably true to say that

the main source of error is the fact that the isotherms available fit the experimental data only rather poorly in many cases. Under these circumstances, it seems to a first approximation that even the simple programs do a reasonably good job of prediction, at least to the point where experimentation can complete the optimisation and many of the simulations carried out with such software fit the experimental data at least as well as those carried out using the other programs.

One simple approach to SMB simulations uses the Rouchon algorithm (see above) for the calculation. Each zone in the SMB unit is considered separately, with the input to one zone being the output of the previous zone. The output of the zone is stored in a buffer, ready for use as input to the subsequent zone. There are two difficulties. One is that the flow rate in each zone is different. This means that for each zone the time increment is different from the preceding and succeeding zone. Therefore the output of any one zone first has to be extrapolated to fit the time increment for the next. This is fast and easy to achieve. The other difficulty arises when one wishes dynamically to change the flow rate in any one section. Because the time increment in the calculation is related to the flow rate and also has a fixed absolute value because the switch time is an important operating parameter, the increments in flow rate in a section are fixed by the increments in time and are thus fixed by the calculation. This means that fine-tuning and accurate representation of small flow rate changes are not possible, especially where the column efficiency is low. Of course, one can always compensate for the limited flow rate values by changing feed concentration for the calculation to allow the correct mass flow of solute, but this can be misleading.

The basic system consists of four zones, to each of which are allocated a number of columns. The flow rate in each zone is different from the others and so the number of plates in that zone has to be calculated to ensure that the simulation is realistic. The different flow rates and efficiency influence the time step used for the calculation for each zone. This means that since the switch time is fixed, there are a different number of calculation steps that are made for each of the zones. The calculation starts with the first zone, all concentrations in the columns at the start of the simulation being assumed to be zero. As the calculation progresses, the output from the last column in each zone is collected in a buffer – an array which contains the mobile phase concentrations of the two (or more) components as a function of the step number, which can be related to the retention time in that zone through the time element. At the extract and raffinate positions the buffer is also used to give the concentration – time data for the output streams; the flow rate of each stream is known from the difference in flow rates between the previous and succeeding zones. The simulation is run separately for each zone through one switch period. At the end of that period, the buffer is redimensioned for input into the following zone. The number of points in the buffer is changed to match the number of time points used for the following zone and the mobile phase concentrations at each new point are interpolated from those in the original buffer. These concentrations are then used as the input concentrations for the new zone, being introduced to the first stage in the same time sequence as they emerged from the previous zone. At the feed position, the input from the buffer is added to the incoming feed concentrations, proportioned for the different relative flow rates of zone 2 and the feed. At the end of the calculation for the fourth zone, the first switch period is complete and one column from each zone – that is to say the array containing the concentrations that correspond to the first column in each zone – is moved to the last position in the preceding zone. The first ‘column’ in the first zone is moved to the end of the fourth zone. Because

there are different flow rates in each zone, there are different numbers of plates in each zone and the arrays that represent the moving columns also have to be resized, much as is done to the output buffers, as they are moved from zone to zone. This cycle continues until stopped by the user. One big advantage of this calculation procedure is that the feed concentrations, various flow rates and the switch time can be changed during the simulation, giving a 'real-time' modification of the conditions that allows rapid optimisation of the process. The major disadvantage, as noted earlier, is that the changes in flow rate have to fit the step sizes in the program since the flow rate is closely related to the time interval δt.

A.5 Measurement of adsorption isotherms

The determination of the adsorption isotherms can be done directly or by inferring the parameters from other experiments. A recent review [6] describes many of the processes for isotherm parameter estimation.

A.5.1 *Direct measurements*

Classically, the isotherm points can be measured by a direct process: a known weight of the CSP is suspended in a solution of known initial volume and solute concentration and the solute concentration is measured after equilibration. The change in concentration of the solute gives the quantity adsorbed on the CSP and the isotherm point is obtained from the calculated solute concentration in the CSP and the measured mobile phase concentration after equilibrium. A related procedure passes a known concentration of solute through a column until equilibrium is reached. At this point the column is flushed with pure mobile phase, collecting all material that elutes. The total weight of solute collected is that which was in solution in the void volume of the column (which must be known) plus that adsorbed on the CSP. The isotherm point is determined by subtracting the quantity of solute calculated to have been in solution from the total. This difference is that adsorbed on the CSP from which the stationary phase concentration corresponding to the mobile phase concentration is employed in the experiment. Both these processes have the disadvantage that only one point is measured in each experiment and relatively large quantities of solute are needed. This is not necessarily a problem once a large-scale separation is envisaged since it is probable that sufficient quantities of the enantiomers have been previously prepared by preparative HPLC. Where the sample is expensive or is not available in large quantities, other procedures must be used.

A.5.2 *Frontal elution*

The frontal technique consists of first equilibrating a column of known void volume and CSP content with pure mobile phase and then switching the solvent to a known concentration of the solute in the mobile phase. The time taken for the solute front to elute is measured. The isotherm point is taken from the difference between the front elution volume and the column void volume multiplied by the concentration of the solute. This is the quantity adsorbed on the CSP at the concentration applied. A similar approach can be taken for competitive isotherms, where known mixtures of the solutes are used. This produces a more complex

front with two elution waves from which the various concentrations can be measured, thus reaching a competitive isotherm point.

A major problem with frontal elution techniques is the interference of impurities which often interfere with the measurements and distort the fronts. The other problem is that at high concentrations, the multicomponent fronts are very close together, sometimes merging completely, making the points inaccurate or impossible to determine.

A.5.3 *Perturbation method*

The perturbation method equilibrates the column with a known concentration of solute in the mobile phase and then a small injection of the solute at a slightly different concentration is made. The retention time of the peak is measured. If the phase ratio in the column is known, the distribution coefficient is calculated from the capacity factor and the stationary phase concentration is obtained from this distribution coefficient and the mobile phase concentration of the solute.

This method has some problems, notably in that the high concentrations needed for the isotherm points make it very difficult to measure small perturbations, either because the detectors become saturated or because one uses a relatively insensitive detector to avoid overloading it, with the result that the small perturbation has to be rather large to be detected, which adds error to the measurement.

All of the above procedures can be performed with pure solutes or mixtures of solutes such that both individual and competitive isotherms can be determined. They also have the common property that no isotherm equation is assumed for the calculation of the individual points, although usually the points thus obtained are fitted to one of the popular isotherm models afterwards.

Other techniques use chromatographic data. These are typically easier to perform and use less solute, but they also can be misleading.

A.5.4 *Elution at characteristic points*

One technique is elution at characteristic points. This is based on the observation that a solute at a specific concentration moves at a constant velocity through the column. The detector is first calibrated so that the response for a given concentration is known. A mass-overloaded injection is made and the retention times on the peak tail related to chosen concentrations are measured. Again, the isotherm points can be calculated from the capacity factors, the column phase ratio and the known concentrations derived from the detector calibration. This does not give competitive isotherm points and requires a high column efficiency to be useful since otherwise the band spreading effects substantially influence the retention times measured in the experiment. A value of at least 5000 theoretical plates is required.

A.5.5 *Peak retention measurements*

A convenient procedure is that of making a series of injections at different loads and measuring the peak retention times. Although it is one of the least accurate methods, its simplicity makes it one of the more popular procedures. Normally such procedures rely on the assumption of an isotherm model for success. If the column has a high number

of theoretical plates and a single solute is used, then the simple relation [Equation (2.8), Chapter 2] due to Guiochon may be used. For columns of lower efficiency, a related equation was proposed by Snyder *et al.*, although this approach, too, is limited to single components or very well resolved peaks [7]. With both these approaches, it is usually best to make the calculation for several values of load, emphasising the higher loads in the calculation of the saturation capacity since the approximations involved usually mean that saturation capacities calculated from low loads are not accurate. In this case, the isotherm is constructed from the value of the capacity factor at analytical load and the phase ratio to calculate the a term and the saturation capacity derived from the equations.

When two components are being separated, it is necessary to take a different approach. This can be done assuming that the overlap for the second peak can be ignored and the standard equation due to Guiochon may be used. The area of the first peak (corresponding to the known load) is used as a limit for a series of numerical back-calculations which predict the shape of that peak. This is somewhat complex to program.

Recently, an approach has been proposed [8] that relies on relating the relative capacity factors (i.e. k' at overload divided by k' at analytical load) from a loading study to those calculated from a series of computer simulations. It was found that for a given isotherm equation, columns with more than 500 plates and for a range of k' values conventionally used for preparative chromatography, the curves relating the k' ratio to the ratio of load to column saturation capacity fall very closely to the same line. Further, for a range of selectivity that is also preparatively useful, the curves were also closely similar. The main differences in the curves were due to the isotherm model. Separations were calculated using both the conventional Langmuir isotherm model and also the ideal adsorption solution theory model that allows solutes to have different saturation capacities within the general constraint of Langmuir behaviour. These standard curves, differing by the ratio of one saturation capacity to the other, were used to estimate the average saturation capacity for the solutes by varying the saturation capacity such that the standard curve fitted the experimental data. The set of curves giving the best fit were used to estimate the isotherm parameters while the inevitable small errors due to the approach were compensated by running some simulations to predict the experimental retention data and 'fine-tuning' the saturation capacity accordingly. This approach allows the rapid estimation of the isotherm behaviour from a limited set of experimental data involving only the injection of the racemic mixture. It does suffer from the fact that a certain general isotherm shape is assumed and the data is fitted to it. Figure A.7 shows a typical solution to the fitting, which is done graphically, adjusting the saturation capacity on a sliding scale until the points and lines coincide. Different standard curves are tried until a good fit to the data is found.

A similar approach using an empirically modified curve derived for a single component in the case of a Langmuir isotherm was described some time ago by the group of Snyder [9].

A.6 Ideal Adsorbed Solution theory

The principal limitation of the competitive Langmuir adsorption isotherm is that it does not allow the use of different saturation capacities for the solutes. A limitation of the more complex isotherms is that they are mostly single component in nature and modifying them to incorporate competitive behaviour is difficult at least. The Ideal Adsorbed Solution

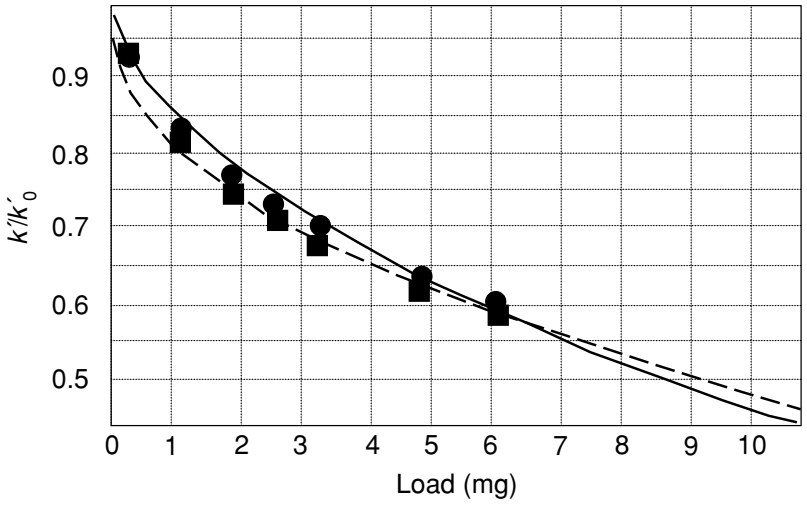

Figure A.5 Fit of standard curve to experimental peak positions. Experimental data: $k_1' = 1.04$, selectivity = 3.02. Standard curve for ratio of saturation capacities (ideal adsorbed solution theory): 105:95; average saturation capacity = 97 g/L.

theory is an attempt to circumvent these problems by allowing the combination of any single component isotherms into a competitive framework. As with all of the more complex operations, there are no rigorous mathematical solutions and the ideal adsorbed solution theory relies on numerical solutions to its equations except in one, simple case, that of the Langmuir isotherm. A publication by Seidel–Morgenstern [10] – although not recent – gives some introduction to the theory; interested readers can use the references in this paper to obtain more information.

We have two isotherms for the two pure components:

$$q_1^\circ = f_1(C_1^\circ)$$

$$q_2^\circ = f_2(C_2^\circ)$$

One chooses a fictitious pure component concentration (C_i°) for each solute, which results in the same 'spreading pressure' (the theory came from gas adsorption) for the two isotherms.

$$\int_0^{\tilde{C}_1} \frac{f_1(C_1^\circ)}{C_1^\circ} \mathrm{d}C_1^\circ = \int^{\tilde{C}_2} \frac{f_2(C_2^\circ)}{C_1^\circ} \mathrm{d}C_2^\circ \tag{A.4}$$

Because the sum of the mole fractions has to be 1,

$$\frac{C_1}{\tilde{C}_1} + \frac{C_2}{\tilde{C}_2} = 1 \tag{A.5}$$

We set

$$\frac{C_i}{\tilde{C}_i} = z_i \tag{A.6}$$

so

$$q_T = \frac{1}{\sum_{n=1}^{2} \frac{z_i}{f_i(\tilde{C}_i)}} \tag{A.7}$$

and

$$q_i = q_T z_i \tag{A.8}$$

We start with values of C_1 and C_2 (mobile phase concentrations) for which we need q_1 and q_2. We guess a value of $\tilde{C}_2$ (greater than C_2) and then can calculate $\tilde{C}_1$ from Equation (A.5). Both sides of Equation (A.4) are calculated numerically, using the approximation

$$\int_0^{\tilde{C}_i} \frac{f_i(C_i^\circ)}{C_i^\circ} \mathrm{d}C_i^\circ = \sum_0^{\tilde{C}_i} \frac{f_i(C_i^\circ)}{C_i^\circ} \Delta C_i^\circ \tag{A.9}$$

$\tilde{C}_2$ is then corrected (for example by multiplying the original value by the ratio of the integral for product 1 divided by that for product 2) and the cycle is repeated until the change in concentration is minimal (the ΔC_1° and the difference in $\tilde{C}_2$ values to be determined).

The value of q_T is then calculated from the two individual isotherms and the fictitious concentrations [(Equation (A.7)] and the individual q_i values are then obtained from Equation (A.8).

For a Langmuir isotherm, the approximation of the integral in Equation (A.4) becomes

$$\sum_0^{\tilde{C}_1} \left[\left(\frac{a_1 C_1^\circ / (1 + b_1 C_1^\circ)}{C_1^\circ} \right) \Delta C_1^\circ \right] \quad = \quad \sum_0^{\tilde{C}_2} \left[\left(\frac{a_2 C_2^\circ / (1 + b_2 C_2^\circ)}{C_2^\circ} \right) \Delta C_2^\circ \right] \tag{A.10}$$

The value of q_T is found from substituting the isotherm into Equation (A.7):

$$q_T = \frac{1}{\frac{z_1}{a_1 \tilde{C} / (1 + b_1 \tilde{C}_1)} + \frac{z_2}{a_2 \tilde{C}_2 / (1 + b_2 \tilde{C}_2)}} \tag{A.11}$$

For the competitive Langmuir isotherms, the results show that the ratio of the fictive concentrations is equal to the ratio of ***a*** terms:

$$\tilde{C}_1 = \tilde{C}_2 \frac{a_2}{a_1} \tag{A.12}$$

From Equations (A.5) and (A.12),

$$\tilde{C}_1 = C_2 \frac{a_2}{a_1} + C_1 \tag{A.13}$$

and

$$\tilde{C}_2 = C_2 \ + \ C_1 \frac{a_1}{a_2} \tag{A.14}$$

Incorporation of the ideal adsorbed solution theory into a computer simulation of chromatography slows the calculation considerably. Fortunately, there is an acceptable approximate solution for the Langmuir isotherm that is due to Levan and Vermeulen, which has been successfully applied to chromatographic simulations [11]. The procedure of Levan and Vermeulen results in an expansion series for the isotherm. The two-term expansion is a

good approximation to the full theory; there is reported to be little difference between the results of this and the three-term expansion. Use of this equation is just as fast as using the Langmuir isotherm since there are only four parameters that describe the isotherm and no iteration is required. There are a few extra calculations to be made but they add only a few extra lines to a simulation program and the code is quite straightforward. There is little purpose in repeating the clear exposition of this approach here and the interested reader is directed to the original publication [11].

A.7 The modified Langmuir isotherm

One isotherm that is found frequently in papers describing SMB separations is the modified Langmuir isotherm. This is essentially the familiar Langmuir isotherm, modified to allow for the different assumptions made by chromatographers with an engineering background and those from a chemical or analytical background. An 'analytical' chromatographer calculates the void volume of a column from the elution time of a non-retained peak. An 'engineering' chromatographer prefers to calculate the void volume of the column as the volume outside the particles in the column. Thus, for the first, the pore volume is part of the mobile phase since it is filled with mobile phase and all solutes (except for very large ones) diffuse in and out of this volume freely and, under most HPLC conditions, at rates that allow equilibrium between the mobile phase outside the pores and that inside them. For the engineer, the pore volume is part of the particle. This is because in many engineering contexts, the particles are large and the mass transfer in the pores from deep inside the particle to the outside can be slow and is certainly not fast enough for equilibrium conditions to be assumed. This difference goes deep into the equations that either 'type' of chromatographer uses. In an isotherm, for example, the solutes that are dissolved in the mobile phase in the pores are taken to be in the stationary phase according to engineering practice. This means that the quantity of solute adsorbed is in this case given by the amount in the stationary phase ($= qV_s$) plus that remaining in the mobile phase in the pore ($= CV_p$). Thus, instead of the concentration in the stationary phase being q, as an 'analytical' chromatographer would have it, the engineering version has the concentration as given by

$$\text{Concentration} = \frac{qV_s + CV_p}{V_s + V_p}$$

where V_p is the pore volume of the particle. This adds a new term to the Langmuir isotherm:

$$q' = \lambda C + \frac{AC}{1 + BC}$$

The λ parameter represents the pore volume of the particle. The A and B terms are also modified from those normally used by 'analytical' chromatographers since capacity factor no longer has meaning and the phase ratio is also different. The effect of this is zero, provided that the value of λ remains that due to the pore volume. Frequently, however, this is not the case, since the isotherm is being fitted to data that is not, in fact, Langmuir in nature. A larger value of λ means that a linear isotherm character is being added to the Langmuir part. While this may sometimes fit the observed data better, there is always the concern that it will give optimistic values at high concentrations because of the linear

character. One reason often given for the better fit sometimes found with this isotherm is that it approximates to a bi-Langmuir isotherm, although from the equation it is clear that the second saturation capacity has to be very large. Probably the fitting is fortuitously better at the low concentrations normally used for the isotherm determination. If one is using the 'engineering' definitions, then the modified Langmuir isotherm is important. If one is using the 'analytical' definitions, then it is not used.

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