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Double Emulsions for Controlled-release Applications—Progress and Trends

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I. INTRODUCTION

Double emulsions are complex liquid dispersion systems known also as "emulsions of emulsions," in which the droplets of one dispersed liquid are further dispersed in another liquid. The inner dispersed globule/droplet in the double emulsion are separated (compartmentalized) from the outer liquid phase by a layer of another phase (1—9).

Several types of double emulsions have been documented. Some consist of a single, internal compartment while others have many internal droplets and are known as "multiple-compartment emulsions." A schematic presentation of some double emulsions is shown in Fig. 1. The most common double emulsions are of W/O/W, but in some specific applications O/W/O emulsions can also be prepared. The term "multiple emulsion" was "coined" historically because microscopically it appeared that a number (multiple) of phases were dispersed one into the others. In most cases it was proven that in practice most systems are composed of double (or duplex) emulsions. A more suitable and more accurate term for such systems should be, therefore, "emulsified emulsions."

Potential applications for double emulsions are well documented and many of these applications have been patented (10-14). The important applications are in agriculture, pharmaceuticals, cosmetics, and foods. In most cases, double emulsions are aimed for slow and sustained release of active matter from an internal reservoir into the continuous phase (mostly water). In some applications the double emulsions can serve also as an internal reservoir to entrap matter from the outer diluted continuous phase into the inner confined space. These applications are aimed to remove toxic matter. In other applications, double emulsions are reservoirs for improved dissolution or solubilization of insoluble materials. The materials will dissolve in part in the inner phase, in part at the internal interface, and occasionally at the external interface. Applications related to protection of sensitive and active molecules from the external phase (antioxidation) have been recently mentioned (15, 16). Many more applications are expected to emerge in the near future. Special attention must be paid to the most promising new application of double emulsions as intermediate systems in preparation of solid or semisolid microcapsules (17—19).

II. THE EMULSIFIERS

Double emulsions consist of two different interfaces which require two sets of different types of emulsi-fiers. In O/W/O double emulsions the first set of emulsifiers, for the internal interface, must be hydro-philic, while the second set of emulsifiers, for the external interface, must be hydrophobic (Table 1). For W/O/W double emulsions the order of the emulsifiers is the opposite; the inner emulsifiers are hy-

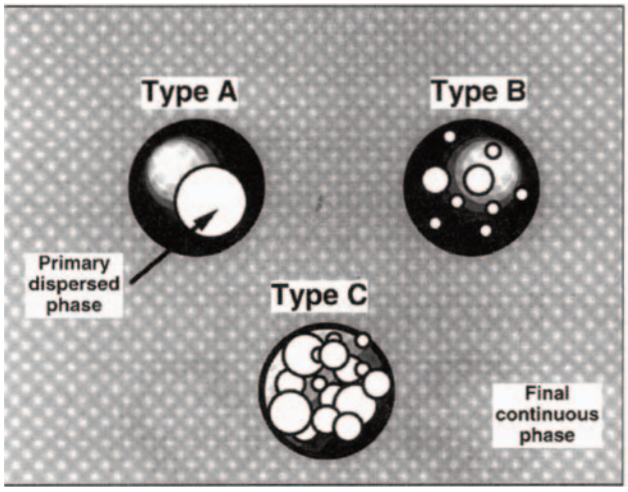


Figure 1 Schematic presentation of the three common types of multiple-emulsion droplets.

drophobic while the outer ones are hydrophilic. In many cases a blend of two or more emulsifiers in each set is recommended for better stabilization results. This review will discuss mostly W/O/W double emulsions since most of the important applications require such emulsions. Some O/W/O emulsions applications are also given.

In early reports on the formation of double emulsions only one set of emulsifiers and an inversion process were used. Such preparations were done in one step, but the stability was in most cases questionable. It was difficult to control the distribution of the emulsifiers within the two interfaces. There was fast migration of the emulsifiers between the phases that destabilized the emulsions. In most recent emulsion formulations the emulsions are prepared in two steps. At first, a high-shear homogenization is applied to the water that is added to the solution of the oil and the hydrophobic emulsifiers, to obtain a stable W/O emulsion. In the second step the W/O emulsion is gently added with stirring (not homogenization) to the water and hydrophilic emulsifiers solution (Fig. 2). The droplet size distribution of a typical classical double emulsion ranges from 10 to 50 μm.

Some more sophisticated preparation methods have been reported in the literature out of which two are interesting and worth being mentioned, the "lamellar phase dispersion

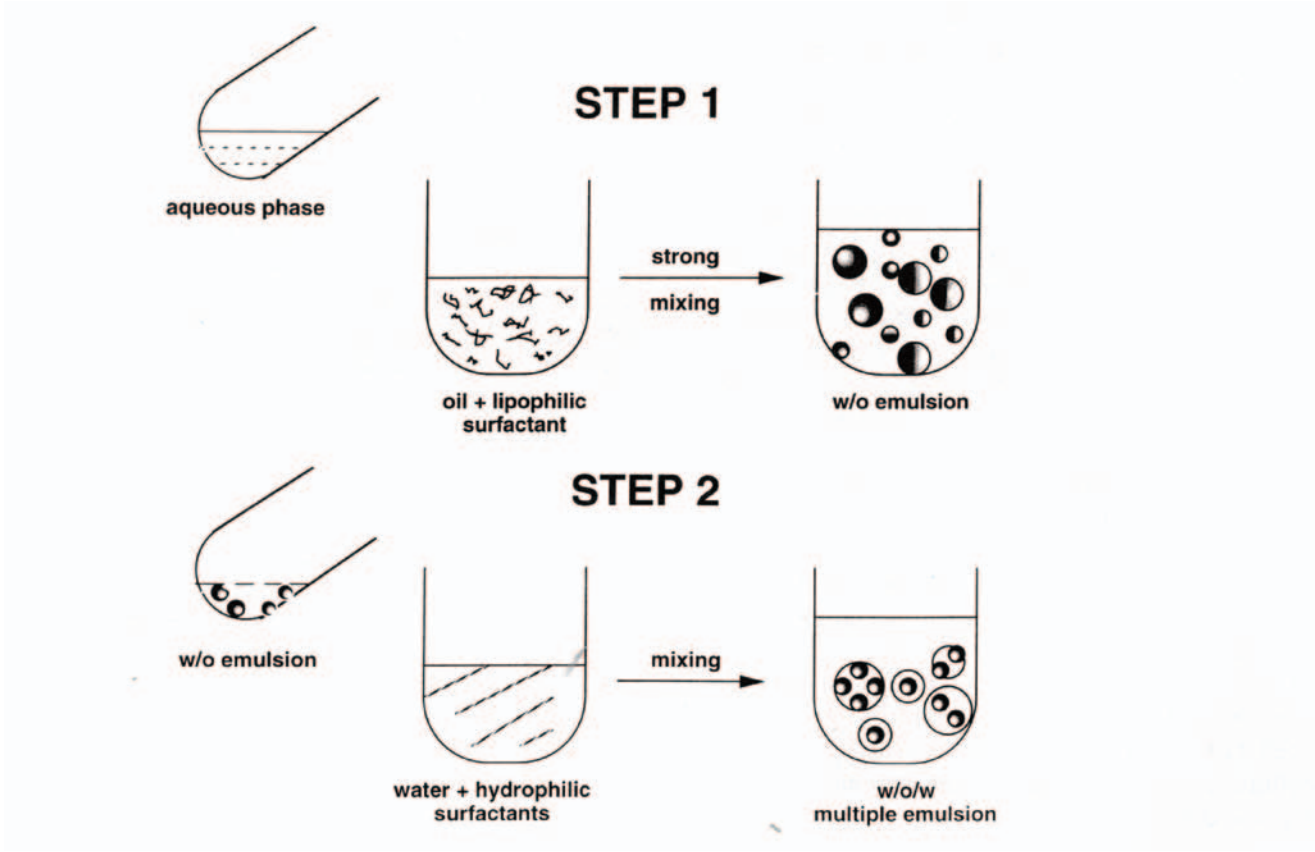


Figure 2 Schematic illustration of a two-step process in formation of a double emulsion.

process" was reported by Vigie (20) (Fig. 3). The procedure is derived from the process employed to obtain liposome-like vesicles with nonionic emulsifiers. This process can be used only when the constituents form a lamellar phase by mixing with water in definite proportions. This procedure offers advantage since it requires only a simple emulsification step. The mesophase formed by an ideal ratio of lipophilic emulsifiers in water is thermodynamically stable and can be obtained rapidly and easily. The method's main limitation is derived from the fact that most emulsifiers do not form lamellar phases. When the lamellar mesophase exists, the HLB of the mix of emulsifiers is often too high, which is disadvantageous for the stability of a multiple emulsion. In addition, the quantity of oil incorporated into the lamellar phase is always low, rarely higher than 10wt%. Another drawback of this process is the weak control of the rate of encapsulation of the active substances.

Grossiord et al. (21) discuss an additional method termed, by them, the "oily isotropic dispersion process." We prefer a more accurate terminology of "emulsified microemulsions." The idea is to disperse an oil phase within water by surfactant and to form an L_2 phase. This phase is further emulsified with water to form a double emulsion (Fig. 4). The problem is that there is no evidence that the formation of the microstructures by this method leads indeed to multiple emulsions. Moreover, there is no good evidence that the internal phase, an L_2 phase of submicrometer droplets, remains after the second emulsification process. It seems that in some cases the process is a well-characterized two-step emulsification that leads to relatively large double-emulsion droplets. This process is worth further investigation and should be more carefully evaluated. If one can prove that the internal com-partmentalization is of a stable microemulsion it might bring a breakthrough to this field since the sizes of the external droplets could be reduced to values below 1 urn. Such formulations will allow formation of indictable double emulsions.

Higashi et al. (22), described a new method of producing W/O/W multiple emulsions by a "membrane emulsification technique." This method permits the formation of monodispersed liquid microdroplets containing aqueous micro-

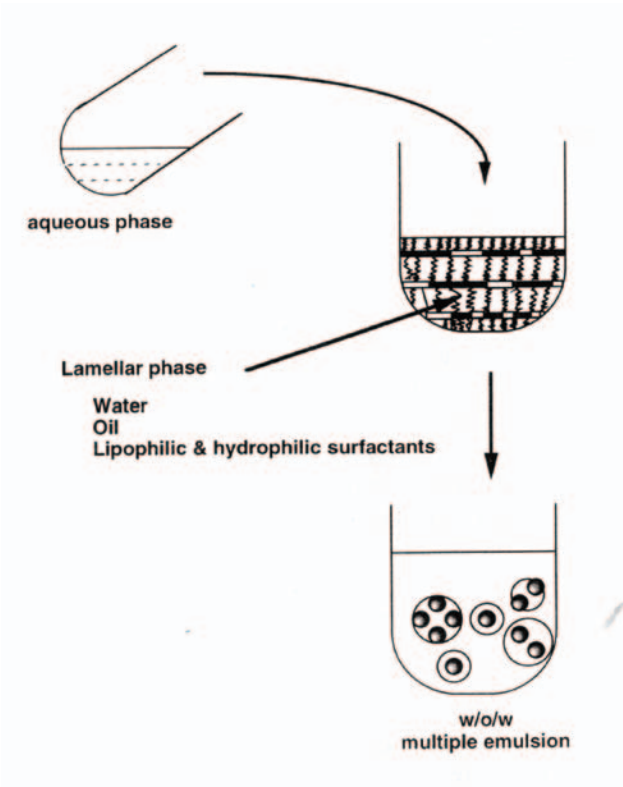


Figure 3 Preparation of W/O/W multiple emulsion by lamellar phase dispersion. (From Ref. 21.)

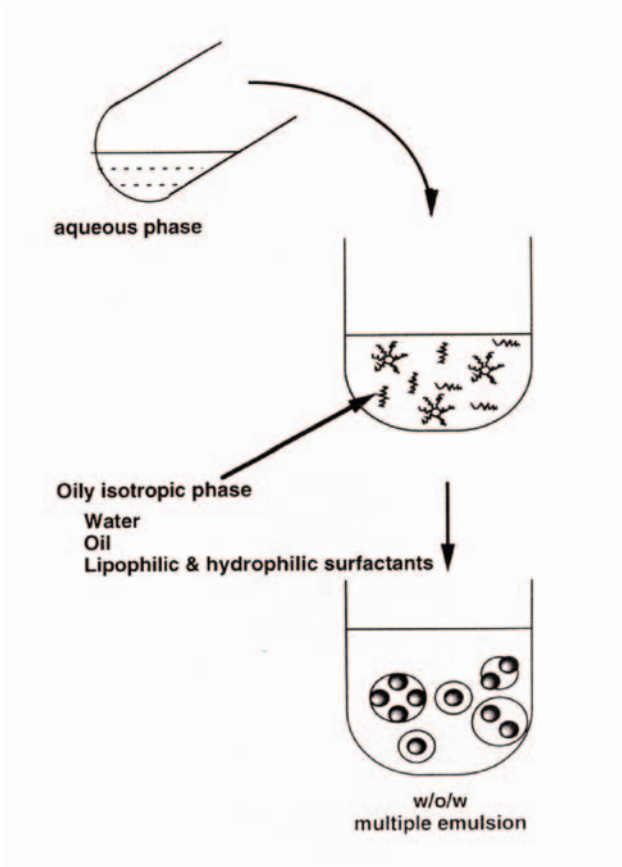


Figure 4 Preparation of W/O/W multiple emulsion by "oily isotropic dispersion" (emulsified microemulsion). (From Ref. 21.)

Table 1 W/O/W Double Emulsions (with 20 wt % W/O emulsion) Prepared with (A) 25 wt % Water, 1 wt % PGPR, 0.5 wt % Tristearin (TS), and 73.5 wt % Soybean Oil; and (B) 25 wt % Water, 1 wt % PGPR, 1 wt % TS and 73 wt % Soybean Oil[a]

	Tw-80 (wt %)	Sp-80 (wt %)	Size (W/O/W), $t = 0$ (μm)	Size (W/O/W), $t = 14$ days (μm)	Conductivity, $t = 0$ $(mS)^{-1}$	Conductivity, $t = 10$ days $(mS)^{-1}$
Set A	0.5	—	8	20	0.4	1.0
(1% PGPR,	1.0	—	7	22	0.8	1.4
0.5% TS)	2.0	—	6.5	25	1.2	1.4
$\varphi_1^{w/o} = 0.25$[b]	3.0	2.0	14	14	1.0	1.1
$\varphi_2^{w/o} = 0.2$[c]	6.0	4.0	18	18	1.8	2.2
Set B	0.5	—	11	11	1.1	0.8
(1% PGPR,	1.0	—	10	9	1.0	1.1
1% TS)	2.0	—	13	10	0.7	1.2
$\varphi_1^{w/o} = 0.25$	3.0	2.0	12			
$\varphi_2^{w/o} = 0.2$	6.0	4.0	13			

[a] The external phase contained various compositions of Tween 80 (Tw-80) and Span 80 (Sp-80). W/O/W, water-in-oil-in-water; W/O, water-in-oil.
[b] $\varphi_1^{w/o} = 0.25$, weight fraction of oil phase for W/O emulsion consisting of 25 wt % oil.
[c] $\varphi_2^{w/o} = 0.2$, weight fraction of W/O emulsion in the double W/O/W emulsion.

droplets to form a W/O/W system. In this method, the aqueous internal phase is mixed with an oil phase containing lipophilic emulsifier. The mixture is sonicated to form a W/O emulsion, and the upper chamber of a special apparatus (Fig. 5) is filled with the emulsion. The external aqueous phase containing the hydrophilic emulsifier is continuously injected into the lower chamber to create a continuous flow. Nitrogen gas fed into the upper chamber initiates permeation of the W/O droplets through the controlled-pore glass membrane into the emulsifying chamber, forming a W/O/W multiple emulsion. The emulsion is progressively removed from the apparatus. This process is claimed to be, at present, used on the industrial scale.

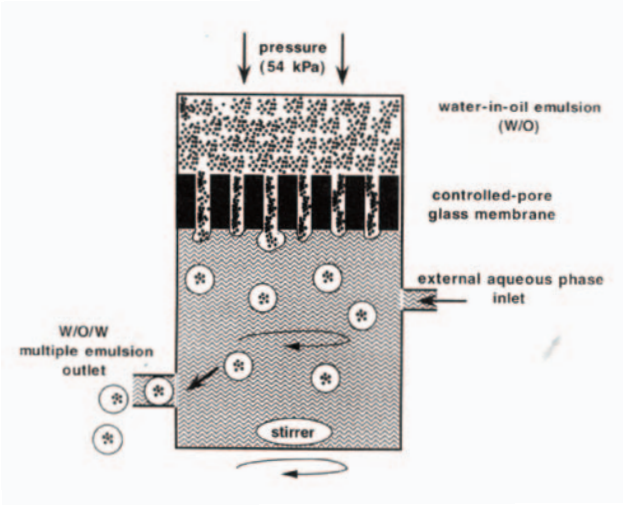


Figure 5 Preparation of W/O/W multiple emulsion by a "membrane emulsification technique." (From Ref. 22.)

It should be noted that low-molecular-weight emulsifiers migrate from the W/O interface to the oil phases and alter the required hydrophilic/hydrophobic balance of each of the phases. Most of the studies, in the years 1970 to 1985, searched for a proper monomeric emulsifier's blend or combination (hydrophilic and hydrophobic) to be used at the two interfaces and the proper ratios between the two. Matsumoto and coworkers (23—29) established a "magic" weight ratio of minimum 10 of the internal hydrophobic to the external hydrophilic emulsifiers (Fig. 6). Garti and coworkers (30-34), proved that the free exchange between the internal and the external emulsifiers required a calculation of an "effective HLB [hydrophilic—lipophilic balance] value" of emulsifiers to optimize the stabilization of the emulsion. Complete parametrization work was performed on almost every possible variation in the ingredients and compositions (1—9). In most cases the internal emulsifiers are used in great excess to the external emulsifiers. The nature of the emulsifiers also dictates the number of compartments and the internal volume that the inner phase occupies.

Many of the more recent studies explore various more sophisticated emulsifiers such as sphingomyelins (35), modified or purified phospholipids (36), cholates, etc. The

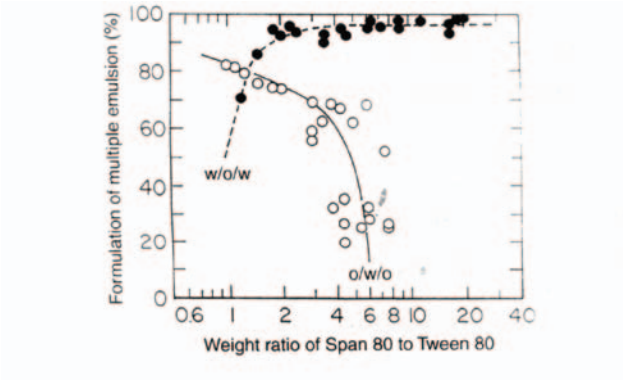


Figure 6 Yield of formation of double emulsions of W/O/W and O/W/O as a function of the w/w ratio of the internal hydrophobic emulsifier, Span 80, and the external hydrophilic emulsifier, Tween 80. (From Ref. 29.)

principals for selecting the proper emulsifiers are similar to those known for classical emulsions. Some of the emulsions might have better stability than others, but the general trend remains unchanged. It should be also stressed that one must adjust the emulsifiers to the application in mind and must substitute one emulsifier from the other, depending on the total composition of the system.

It must be also recognized that 'empty' double emulsions will behave differently from those containing active matter (electrolytes, biologically active materials, proteins, sugars, drugs, etc.) owing to osmotic pressure gradients (caused by the additives) between the outer and the inner phases. In addition, many of the active ingredients have some hydrophobicity and surface properties. Such molecules (peptides, drugs, pesticides) will migrate from the inner bulk and will adsorb on to the interface, changing the delicate emulsifiers' HLB. The emulsifiers around the water or the oil droplets will not fully cover the droplets, and the stability will be reduced. These phenomena are very frequently neglected or overlooked by many of us. The conclusions that are frequently derived from these oversimplified experiments and incorrectly structured emulsions can be misleading and incorrect. We will discuss further some of these aspects in this review.

III. THE OIL PHASE

In many food and pharmaceutical applications only a limited number of different "oil phases" (water-immiscible liquids) have been suggested and tried throughout years of research. In most applications the oil phase is based on vegetable or animal unsaturated triglycerides such as soya oil, cotton, canola, sunflower, and others. It was also suggested to replace the long-chain triglycerides (LCTs), which are "oxygen and hydrolysis sensitive," by medium-chain triglycerides (MCTs) that are fully saturated and thus oxidation resistant. The MCT is easier to emulsify and requires less shear.

In cosmetic applications the freedom in using different oils is greater. Long-chain fatty acids, fatty alcohols, and simple esters such as isopropyl myristate (IPM), jojoba oil, and essential oils are only a few of the examples. In addition, various waxes, sterols, and paraffin oils have been tried.

In agricultural applications, various organic solvents have been used. Solvents such as toluene and its derivatives, as well as chlorinated hydrocarbons, have been suggested.

Hydrocarbons, aromatic compounds, silicone oils, and fluorinated or halogenated hydrocarbons are only few of the main oils in use in industrial applications.

Several scientists have tried to correlate the nature of the oil phase and its volume fraction to the stability of the double emulsions. No unusual or surprising findings were observed. Double-emulsion interfaces behave very much like simple emulsions except for the severe limitations on sizes of the droplets and the internal distribution of the emulsifiers.

IV. STABILITY CONSIDERATIONS

Double emulsions consisting of low-molecular-weight emulsifiers (the so-called monomeric emulsifiers) are mostly unstable thermodynamically, mainly because in the second stage of the emulsification severe homo-genization or shear takes place and, as a result, large droplets are obtained. During years of research attempts have been made to find proper and more suitable combinations of emulsifiers to reduce droplet sizes and to improve the emulsion stability. Aggregation, flocculation, and coalescence (occurring in the inner phase and between the double-emulsion droplets) are major factors affecting the instability of the emulsions, resulting in rupture of droplets and separation of the phases.

Double emulsions are usually not empty. Water-soluble active materials are entrapped during the emulsification in the inner aqueous phase. It is well documented that, because of the difference in osmotic pressure through a diffusion-controlled mechanism, the active matter tends to

diffuse and migrate from the internal phase to the external interface, mostly through a mechanism known as "reverse micellar transport" (Fig. 7a). The dilemma that researchers were faced with was how to control the diffusion of water molecules as well as the emulsifier molecules, and mostly the active matter from the internal phase to the outer phase. It seemed almost impossible to retain the active material within the water phase upon prolonged storage. Attempts to increase the HLB of the external emulsifier or to increase its concentration in order to improve the stability of the emulsion worsened the situation and ended in faster release of the drug or electrolytes.

Much work was devoted to establish the effects of osmotic-pressure differences between the internal and the external phases on the stability of the emulsions, and on the release rates of the markers from the internal phase, and the

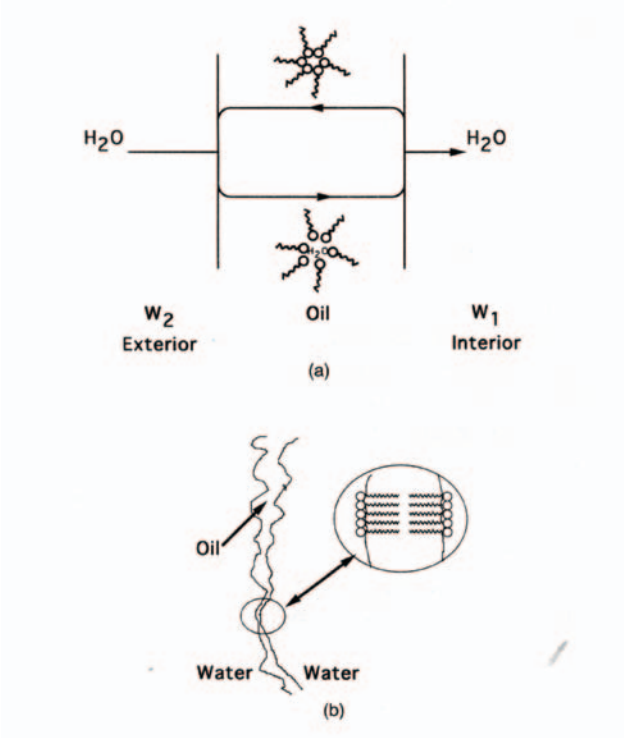


Figure 7 Schematic illustration of the two possible transport mechanisms: (a) reverse micellar; and (b) lamellar thinning transport of marker from the inner aqueous phase to the continuous aqueous phase.

engulfment of the internal droplets by the flow of water from the outer continuous phase to the inner droplets. Mono- and multiple-compartment emulsions were prepared and evaluated in view of the enormous potential that these low-viscosity liquid systems have in slow delivery of water-soluble drugs.

Additional instability mechanisms and release pathways have been demonstrated and discussed in detail by various authors. These mechanisms include "transport through thinned lamella" (Fig. 7b), transport of adducts or complexes that are formed in the oil phase, and other variations of these mechanisms. It seems, however, that the main instability and release mechanisms are the parallel or simultaneously occurring phenomena of "reverse micellar transport" and coalescence.

All the above mechanisms have been well established, but it seems that the stability and the release patterns of these complex double-emulsion systems depend on various parameters that simultaneously interplay and that a simplified or unique mechanism cannot explain all the in-parallel pathways that take place in the double emulsions.

In a recent study, Ficheux et al (37), identified two types of thermodynamic instability that are responsible for the evolution of double emulsions. Both mechanisms are within good agreement with the old Bancroft rule, but stress different aspects of the previously mentioned mechanisms. The mechanisms elucidated by the authors result in different behavior of the entrapped matter in the double emulsion. The first is a "coalescence of the small inner droplets with the outer droplets interface" which is due to the rupture of the thin nonaqueous film that forms between the external continuous phase and the inner small water droplets (Fig. 8). This instability irreversibly transforms a double droplet into a simple direct emulsion. Such a mechanism is suitable for delivery of water-soluble substances. The second mechanism is a "coalescence between the smaller inner droplets within the oil globule." The first type of instability leads to a complete delivering of the small inner droplets toward the external phase whereas the second one does not. The second mechanism leads to an increase in the average diameter of the internal droplets and a decrease in their number. The authors worked both with anionic (SDS, sodium dodecyl sulfate) and cationic (TTAB, tetradecyltrimethylamonium bromide). It was demonstrated that the kinetics associated with the release of the small inner droplets, resulting from the former instability, is clearly related to the hydrophilic surfactant concentration in the external phase (Fig. 9). Depending on the value of this concentration, double emulsions may be destabilised on a time scale ranging from several months to a few minutes.

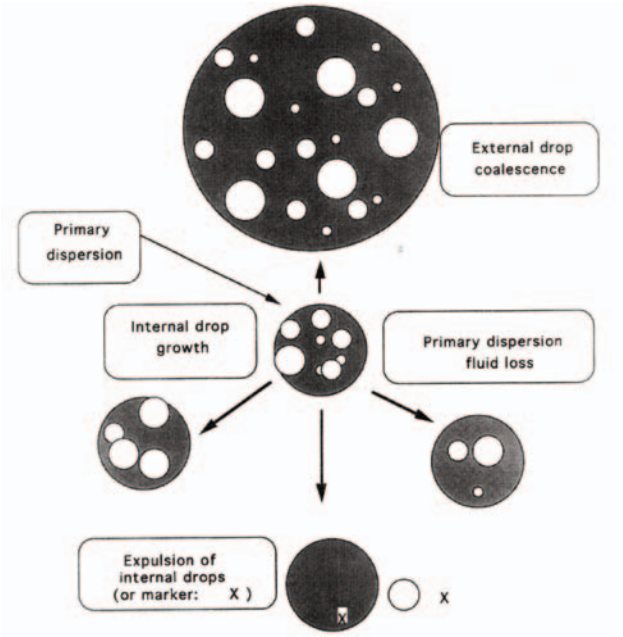
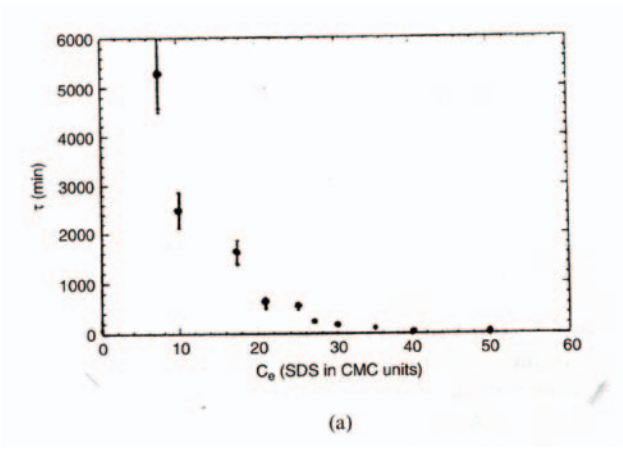


Figure 8 Schematic representation of the possible pathways for breakdown in multiple emulsions.

Rosano et al. (38) explored the influence of "ripening and interfacial interactions" on the stability of the W/O/W double emulsions. The oil-insoluble solute was shown to stabilize both the first W/O emulsion (of the inner water droplets) and the external O/W interface. The authors used a theoretical model and experimental results (video-microscope observations). It was shown that the presence of an electrolyte in the inner water phase is necessary for the stability of a multiple emulsion. The stability is achieved from the osmotic-pressure equalization derived from the differences (excess) in the Laplace pressure. This effect stabilizes the inner W/O emulsion. It is possible also to determine the right salt concentration necessary to balance the osmotic pressure between the two water phases. In a set of experiments (Tables 2 and 3) a total of 15 formulations are shown in which both the oil phase and the W^2 phase are constant, and the only parameter varied is the NaCl concentration in the W^1 phase. The first six formulations were prepared with betaine, whereas the others were prepared with SDS. In the case of betaine, without any salt in the inner phase, an unstable multiple emulsions is observed, and both Ostwald ripening and release of the W^1 phase into the W^2 phase occur. As the concentration of the salt is increased in the W^1 phase, the systems do not separate and the structure remains multiple, but a large increase in viscosity is observed (9500 cP for 0.07 wt % to 27,600 cP for 0.4 wt % NaCl). The authors conclude that one must consider three possible factors that influence the stability: the Laplace and osmotic pressure effects between the two aqueous phases, the interaction between the low and high HLB emulsifiers at the outer O/W interface, and the polymeric thickener-hydrophilic emulsifier interactions in the outer phase (W^2).



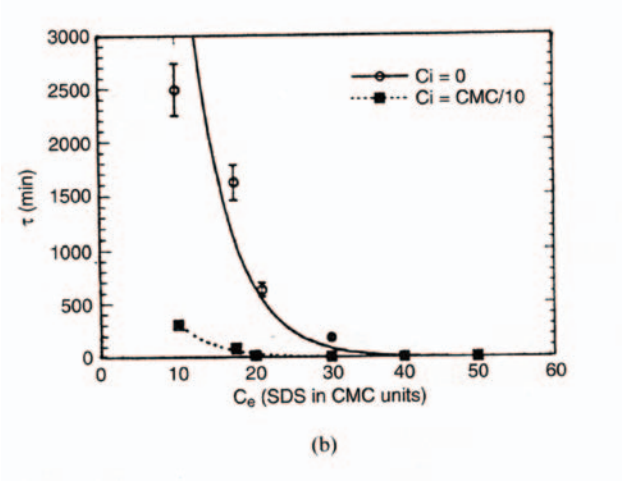


Figure 9 (a) Plot, at 20°C, of the life time τ, of internal droplets entrapped in the oil globules as a function of the external phase surfactant concentration C_e. The double emulsions are composed of 90% external phase and 10% double droplets. There is 10% water within the large double globules; 2% Span 80 was used within the oil, and SDS in the external water phase, (b) Influence of the internal surfactant concentration *C*i on the $\tau = f(Ce)$ curve, at 20°C. System: Span 80/SDS as in Figure 9a. The dashed and solid lines are only guides to the eyes (From Ref. 37.)

Table 2 Role of the Concentration of Salt in the Inner W_1 Phase for Formulations Prepared with Cocamidopropylbetaine

	Composition (wt %)					
Formulation number	1	2	3	4	5	6
W_1H_2O	27.5	27.43	27.35	27.2	27.1	27.2
NaCl	0	0.07	0.15	0.3	0.4	0.3
Light paraffin	21.5	21.5	21.5	21.5	21.5	21.5
Abil EM 90	1	1	1	1	1	1
W_2H_2O	48.9	48.9	48.9	48.9	48.9	48.9
Betaine	0.3	0.3	0.3	0.3	0.3	0.3
Carbopol 1342	0.1	0.1	0.1	0.1	0.1	0.05
Separation (%)	50 (20)[a]	0 (80)	0 (80)	0 (80)	0 (80)	0 (110)
Structure	Simple	Multiple	Multiple	Multiple	Multiple	Multiple

[a] Number of days.

Table 3 Role of the Concentration of Salt in the Inner Wj Water Phase for Formulation Prepared with SDS

	Composition (wt %)								
Formulation number	7	8	9	10	11	12	13	14	15
W_1H_2O	27.43	27.4	27.36	27.2	27.1	25.95	26.9	26.6	27.2
NaCl	0.07	0.1	0.14	0.3	0.4	0.45	0.6	0.9	0.3
Light paraffin	21.5	21.5	21.5	21.5	21.5	21.5	21.5	21.5	21.5
Abil EM 90	1	1	1	1	1	1	1	1	1
W_2H_2O	48.9	48.9	48.9	48.9	48.9	50	48.9	48.9	48.9
SDS	1	1	1	1	1	1	1	1	1
Carbopol 1342	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2
Separation (%)	4 (40)[a]	3 (40)	17 (40)	38 (40)	9 (40)	16 (40)	52 (90)	40 (40)	0 (20)
Structure	Multiple	Multiple	Multiple	Multiple	Multiple	Multiple	Multiple	Multiple	Multiple

[a] Number of days.

The variations between the different suggested mechanisms are not dramatic. Some authors tend to stress certain mechanistic aspects and to neglect others while other authors stress, in very specific formulations, the more relevant pathways. It seems that most suggested mechanisms are basically very similar.

V. STABILIZATION BY POLYMERIZABLE EMULSIFIERS

Florence and coworkers (39—41) were the first to recognize the need to improve the stability of double emulsions by improving the interfacial coverage. They suggested strengthening the "seal" of the external interface by nonconventional methods, using tailor-made monomeric emulsifiers. They made some attempts to use monomeric amphiphilic molecules with reactive functional group (serving as a ballast). The so-called "polymerizable emulsifiers" were adsorbed on to the external interface and were polymerized in situ. A cross-linked thick film of polymeric surfactant was formed at the interface. The procedure is tedious and very expensive since no commercial polymerizable and emulsifiers are easy to find. The results were somewhat disappointing. It is not clear if the relatively poor stability performance was derived from the uncontrolled or insufficient polymerization process or from the poor alignment of the polymerizable emulsifiers. No further attempts were made by other authors to explore this idea. We believe that the lack of further attempts by other investigators is mainly because the polymerizable surfactants are difficult to synthesize and to orient at the interface, and because there is only a slim chance that the polymerizable surfactants will be approved by any health authorities.

VI. STABILIZATION BY BIOPOLYMERS

Macromolecules adsorb on to interfaces and facilitate more (or better) coverage than monomeric emulsifiers. The amphiphilic macromolecules form, in most cases, thick and flexible films that are strongly anchored in the dispersed and the dispersion phases. The adsorbed polymers are known to enhance steric stabilization mechanisms and have proven to be good emulsifiers in food colloids, mainly, in some food-grade O/W emulsions. The use of macromolecular amphiphiles and stabilizers such as proteins and polysaccharides has long been adopted by scientists exploring the stability of double emulsions. Gelatin (42, 43), whey proteins (44), bovine serum albumin (BSA) (45—8), human serum albumin (HSA), caseins, and other proteins were mentioned and evaluated. The proteins were used usually in combination with other monomeric emulsifiers (46). A significant improvement in the stability of the emulsions was shown when these macromolecules were encapsulated on to the external interface. In most cases the macromolecule was used in low concentrations (maximum 0.2 wt %) and in combination with a large excess of nonionic monomeric emulsifiers. In some cases, casein alone served as the external emulsifier (46). Furthermore, from the release curves it seems that the marker transport is more controlled (Figs. 10-12). Dickinson and coworkers (47-50) concluded that proteins or other macromolecular stabilizers are unlikely to replace completely lipophilic monomeric emulsifiers in double emulsions. However, proteins in combination with stabilizers do have the capacity to confer some enhanced degree of stability on a multiple-emulsion system and, therefore, the lipophilic emulsifier concentration is substantially reduced.

The authors of this review (46) have used BSA along with monomeric emulsifiers, both in the inner and the outer interfaces (in low concentrations of up to 0.2 wt %), and found significant improvement both in the stability and in the release of markers as compared to the use of the protein in the external phase only (Fig. 13). It was postulated that while the BSA has no stability effect at the inner phase it has strong effect on the release of the markers (mechanical film barrier). On the other hand, BSA together with small amounts of monomeric emulsifiers (or hydrocolloids) serve as good steric stabilizers, improve stability and shelf-life, and slow down the release of the markers. The BSA plays, therefore, a double role in the emulsions: as film former and barrier to the release of small molecules at the internal interface, and as steric stabilizer at the external interface. The release mechanisms involving reverse micellar trans-

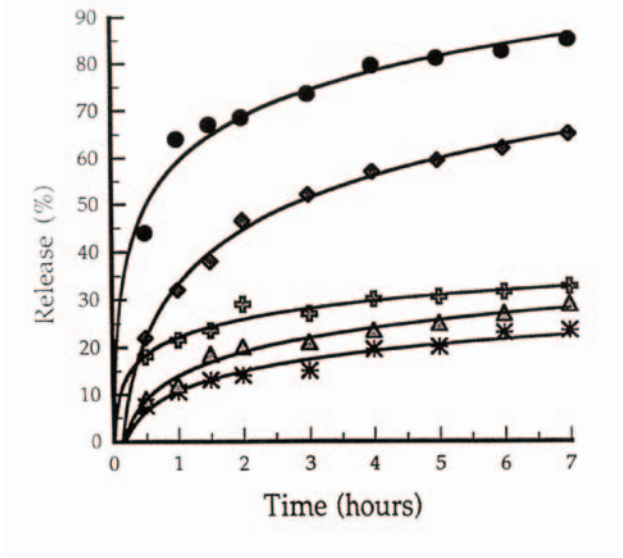


Figure 10 Release of methotrexate (MTX) from multiple W/O/W emulsions. The emulsions contained 2.5 wt % Span 80 and 0.2 wt% BSA as primary emulsifiers, with MTX (1 mg/ml) in the internal phase and the following oil phases: * — octane; **Δ** —dodecane; ✜ — hexadecane; ◇ — octadecane; and — isopropyl myristate (IPM). (From Ref. 40.)

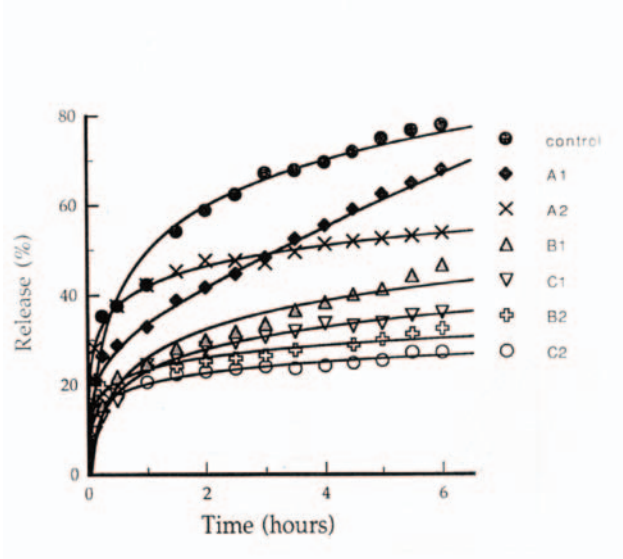


Figure 11 Profile of chloroquine phosphate release from W/ O/W multiple emulsions. Al: freshly prepared PVP emulsions; A2: PVP emulsions stored for 2 weeks; Bl: freshly prepared gelatin emulsions; B2: gelatin emulsions stored for 2 weeks; Cl: emulsion prepared with gum acacia; C2: acacia emulsion stored for 2 weeks. (From Ref. 41.)

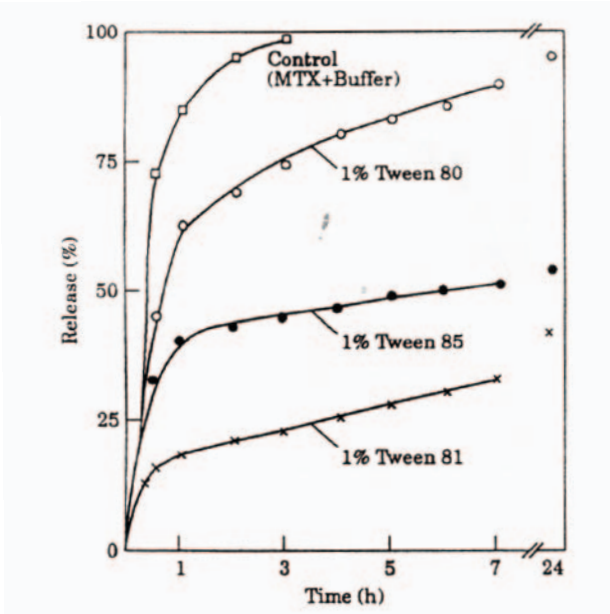


Figure 12 Effect of the secondary emulsifier (E2) on the relase of **MTX**from double emulsions prepared with 2.5% Span 80 and 0.2% **BSA** as primary emulsifiers with **IPM** (isopropyl myristate) as the oil phase. (From Ref. 3.)

port were also established (Fig. 14).

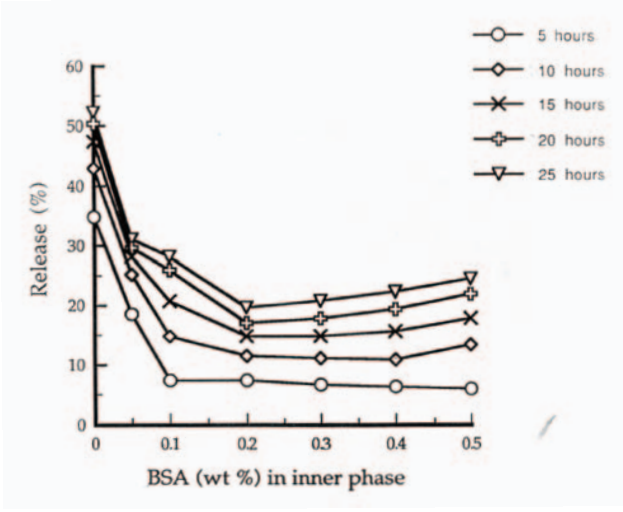


Figure 13 Percentage release of NaCl with time, from double emulsion prepared with 10 wt % Span 80 and various **BSA** concentrations in the inner phase and 5 wt % Span 80 + Tween 80 (1:9) in the outer aqueous phase.

In more recent studies (51, 52) the biopolymer chitosan was used as an emulsifier in food double emulsions. Chitosan has surface activity and seems to stabilize W/O/W emulsions. Chitosan reacts with anionic emulsifiers such as sodium dodecyl sulfate at certain ratios to form a water-insoluble complex that has strong emulsification capabilities. Chitosan solution was used to form double emulsions of O/W/O as intermediates from which by a simple procedure of stripping the water the authors formed interesting porous spherical particles of chitosan (52).

Cyclodextrins (α, β, and γ) were shown to be potential emulsifiers for O/W/O emulsions (53). The advantages of the cyclodextrins as emulsifiers are their ability to complex with the oil components at the oil/water interface which obviates the need for additional surfactant. It appears that the emulsifier efficacy depends on the nature of the oil and the type of cyclodextrin ($\alpha > \beta > \gamma$). The presence of any active matter in the inner phase (such as benzophenone) destabilized the emulsion. Only the α cyclodextrin yielded stable emulsions. The reason is an interfacial interaction between the components that are present at the interface, which changes the **HLB** and causes a destabilization effect. This elegant thought of interfacial complexation between the "oil components" and the surfactant cannot be a universal solution. The idea suffers from a very severe intrinsic disadvantages since once different additive is included in the emulsion. For every additive at any concentration an adjustment must be made and the given cyclodextrin or the complexing agent might be totally suited.

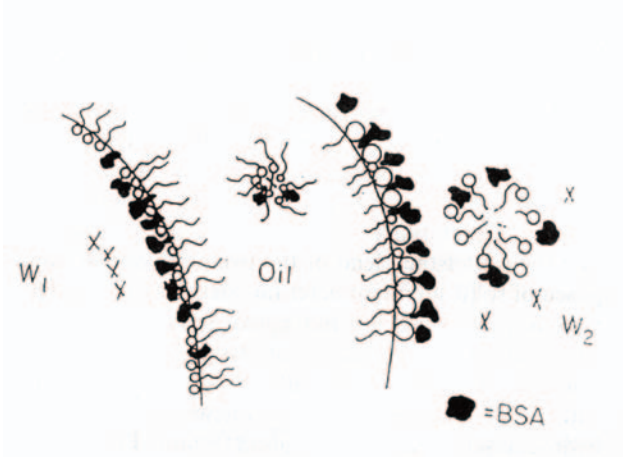


Figure 14 Schematic illustration of possible organization and stabilization mechanism of BSA and monomeric emulsifiers (Span 80) at the two interfaces of double emulsion.

VII. STABILIZATION BY SOLID PARTICLES

Stabilization of margarine and other similar food emulsions is achieved by partial adsorption of solid fat particles (β'-polymorphs) on to the water-oil interface, bridged by monomeric hydrophobic emulsifiers. The complex stabilization is achieved by "wetting" the oil phase by solid fat particles and emulsifiers (lecithins and monoglycerides of fatty acids). The concept was re-examined and reconsidered by Bergenstahl and coworkers (54, 55) and the mechanism was somewhat better elucidated.

The concept of stabilizing emulsions by solid particles (mechanical stabilization) was partially demonstrated, in an old and incomplete study (56), showing that colloidal microcrystalline cellulose (CMCC) particles can adsorb in a "solid form" on to oil droplets at the interface of a W/O emulsion and thus improve their stability by mechanical action. Oza and Frank (56) tried the mechanical stabilization concept on double emulsions. Their report shows some promise in improving stability and in slowing down the release of drug. This study was, for several years, the only example of applying the concept of mechanical stability to double emulsions. In a recent paper, Khopade and Jain (57) repeated the use of a similar process and managed to stabilize W/O/W emulsions by using MCC (microcrystalline colloidal cellulose) particles at both interfaces. The droplets were small and the yield of the multiple emulsion was fairly good. The increasing concentration of MCC in either the internal or external phase increased droplet sizes. These systems showed promise in tuberculosis therapy.

Recently, Garti et al. (58) carried out some experiments with micronized particles of the a- and /S'-poly-morphs of tristearin fat together with polyglycerol polyricinoleate (PGPR) as the internal emulsifiers in double emulsions. Solid fat particles did not sufficiently stabilize the W/O emulsion and similarly the PGPR (at the concentrations used in the formulation) did not provide good stability. It was, however, shown that the use of the blend of the two components composed of solid submicrometer fat particles of α- and β'-polymorphs (which are more hydrophilic than the β-form and thus wet better the oil-water interface) "precipitate" on to the water droplets and "cover" them. The fat particles bridge between the water droplets and sinter them only if a lipophilic surfactant (PGPR) was coadsorbed on to the water-oil interface (the W/O emulsion) (Fig. 15). The authors interpretation of the results is that "the fat particles adsorb on to the hydrophobic emulsifier film and both, the solid particles and the emulsifier, wet the water and spread at the interface." The double emulsions prepared by this technique were more stable than those prepared with

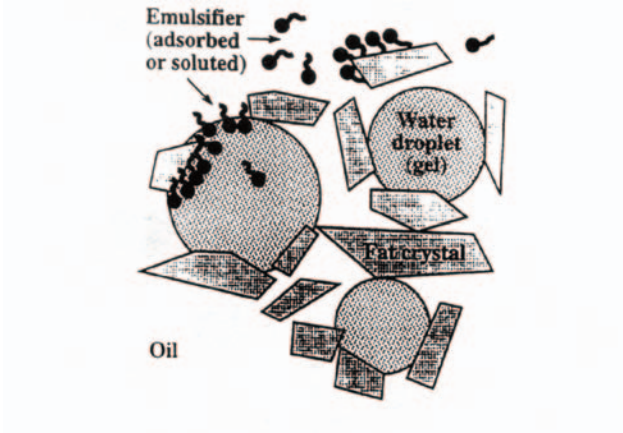


Figure 15 Schematic illustration of colloidal margarine structure demonstrating the role of emulsifiers and fat crystals in stabilizing W/O emulsion of margarine by colloidal fat crystals.

monomeric emulsifiers (Fig. 16). The release patterns were also examined.

Organophilic montmorillonite is an interesting clay that gained some interest in emulsion technology. Stable O/W/O emulsions with components consisting of hydrophilic nonionic surfactant (hydrogenated, ethoxylated castor oil, HCO-60), organophilic montmorillonite and commercial nonionic surfactant (DIS-14) were made (59). The montmorillonite was added in the second step at the outer W/O/W interface. The droplet sizes decreased with increase in the HCO-60 (0.1—3 wt %) concentration. The viscosity of the double emulsion increased as the concentration of the montomorillonite and DIS-14 increased, indicating that the excess amount of inner oil phase is adsorbed by the outer oil phase. The results indicate that the weight fraction of the inner oil phase should not exceed 0.3 wt % for a stable O/W/O emulsion since the viscosity of the double emulsion is so high that the formulation becomes semisolid.

VIII. STABILIZATION BY INCREASED VISCOSITY

It is obvious that restricting the mobility of the active matter in the different compartments of the double emulsion will slow down coalescence and creaming, as well as decrease the transport rates of the drug or the marker from the water

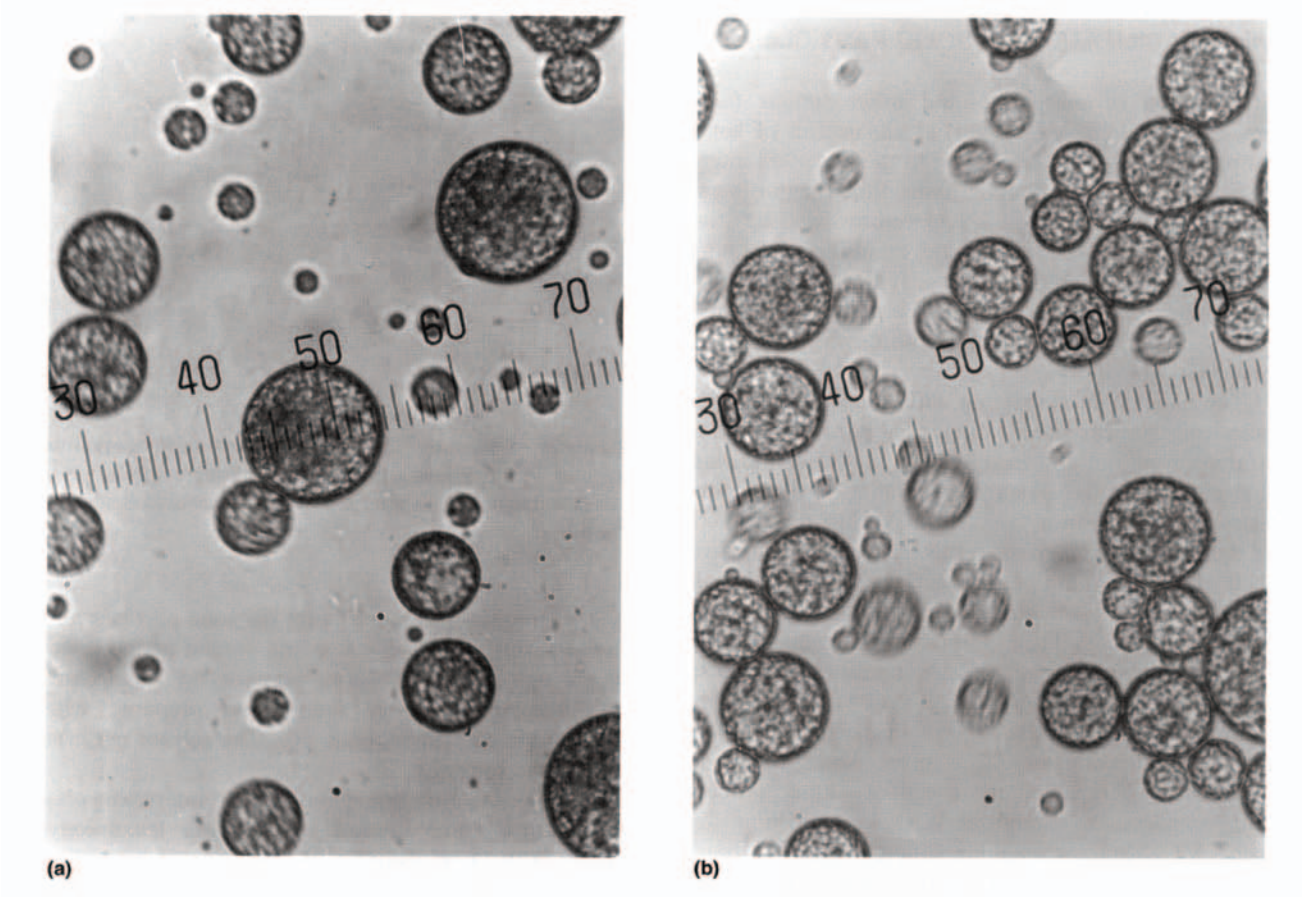


Figure 16 Photomicrographs of double emulsions (W/O/W) stabilized with fat crystals and PGPR (polyglycerol polyricinoleate) at the inner interface and Tween 80 at the outer interface: (a) after 24 h; and (b) after 3 weeks.

phase through the oil membrane. Attempts were made: (1) to increase the viscosity of the internal aqueous phase by adding gums/hydrocolloids to the inner water phase. Such a thickener may affect also the external continuous phase since the entrapment is not quantitative and the yields of entrapment are limited and emulsifier-dependent; (2) increase the viscosity of the oil phase (fatty acids salts); and (3) thickening or gelling the external water by gums is limited only to cosmetic or similar applications in which semisolid emulsions are directly applied (Tables 4 and 5). Some of these examples are topical skin-care products, creams, and body lotions (60, 61).

Double emulsions that were solidified after preparation may suffer from destabilization effects. This phenomenon is scarcely considered but in practice it can occur very often. The solidification occurs because of temperature changes [temperatures can fluctuate from subzero of (ca -20°C) to ca 40°C] during transport or storage. Clausse and coworkers (62, 63) studied the phenomenon in W/O/W emulsions by microcalorimetric (DSC) techniques. It was concluded that, out of thermodynamic equilibrium, double emulsions may suffer from water transfer during the solidification. This phenomenon occurs even if partial solidification takes place. In addition, a change in the size distribution of emulsion droplets is observed. The mean diameter of the droplets in the W/O emulsion may shift toward O/W emulsion and the double emulsion can invert. Therefore, it is not always obvious that increasing the viscosity, gelation, or partial solidification improves emulsion stability.

Table 4 Example of a W/O/W Multiple Emulsion Stabilized by a Calcium Alginate Gel Layer at the Internal Aqueous/Oil Interface

Oil Phase	Hexaglycerol mixed ester	1%
	CSL (calcium stearyl-2-lactylate)	0.75%
	Soybean oil	23.25%
Internal aqueous phase	2% Solution of SAlv (sodium alginate low viscosity)	25%
Outer aqueous phase	Polysorbate 20	0.36%
	Water	9.14%
	Vinegar	12%
	Sucrose	6%
	NaCl	2.5%
	Aqueous phase + 1% xanthan gum	20%

Table 5 Composition for Low-fat Spread Using a W/O/W Formulation

External aqueous phase	Salt	1%
	Gelatin	3% (270 bloom)
	Maltodextrin DE = 7[a]	10%
	Water	85%
	Sodium caseinate	1%
W/O emulsion	Water	57.9%
	Rapeseed oil	40%
	PGPR	2%
	Flavor	0.1%

[a]De: dextrose equivalent.

IX. MICROCAPSULES OR MICROSPHERES IN THE INTERNAL PHASE

Entrapping the active matter in solid or semisolid particles will dramatically decrease their release rates. Such double emulsions can be stored, before use, for prolonged periods without transporting the active matter to the outer interface. Upon use the double emulsion will be heated or sheared and the solid internal matrix will be ruptured and the active matter should be released. The major problem in practising such technology is the difficulties arising in dispersing (and in keeping them stable) the micro- or nano-particles in the continuous water phase. Using solid-encapsulation techniques, microspheres and nanoparticles, were tested as a replacement for the classical W/O emulsion. A few experiments were carried out showing that release can be slowed down, but the stability of these systems is very limited (64-78). Such methods are applicable only for emulsions that can be freshly prepared prior to their use. It is our hope that more efforts will be made in this direction.

X. VESICLES-IN-WATER-IN-OIL (V/W/O) EMULSIONS

There are some potential applications in which the external phase is nonaqueous. Florence and co-workers (79, 80) and Albert et al. (81) described systems in which the aqueous suspension of vesicles are dispersed in the continuous oil phase. The technique was exercised both for the study of its use in drug-delivery systems and as immunological adjuvants, as well as an intrinsically interesting colloidal system. The system is an emulsion prepared from a dispersion of niosomes in water, re-emulsified in an oil using a surfactant mixture of low HLB to achieve a stable W/O emul-

sion.

The product, which was termed by Florence and coworkers a V/W/O emulsion, is a close analog to an O/W/O emulsion. The authors have studied parameters such as the type of surfactant on the in-vivo release of a drug and have found that the degree of hydrophobicity of the Span surfactant had a significant influence on the release rate. Spans 60 and 40 released the drug at the lowest rate, while the more hydrophilic surfactants increased the release rates. The authors have interpreted the results in terms of gelation of the oil phase in the presence of the more hydrophobic surfactants (Fig. 17). Other factors such as the nature of the oil phase (octane, hexadecane, and ispropyl myr-istate), and the effect of temperature were also studied. The results were encouraging, but no dramatic improvement was made on the release and transport phenomena.

XI. EMULSIFIED MICROEMULSIONS (L_2/W)

In an attempt to reduce the sizes of the internal droplets it was suggested, by Pilman et al. (82), to replace the internal emulsion by a microemulsion or with thermodynamically swollen stable reverse micelles (an L_2 phase). In a short report they conclude that such an option is feasible. One can emulsify L_2 reverse micelles or microemulsions to form very small, external droplets (dictated by the size of the internal droplets). The paper does not give sufficient experimental details nor does it have much stability and structural data, but it serves as seeded idea to retry the concept. We (83) have prepared reverse microemulsions with Aerosol OT (AOT), in which small amounts of marker (2 wt % NaCl) were entrapped. The reverse micelles are thermodynamically stable. The L_2 phase was further added to a water solution containing various hydrophilic emulsifiers. The "emulsified microemulsion" has a stability similar to that of the classical monomeric emulsion, with better (slower) release rates. The problem is that since the internal droplets are so small it is impossible to detect them by classical optical methods, and it requires the use of advanced techniques such as cryo-TEM, SAXS (small angle x-ray scattering), and SANS (small angle neutron scattering) linked to self-diffusion NMR methods in order to evaluate the internal micro structure. The work is still in progress.

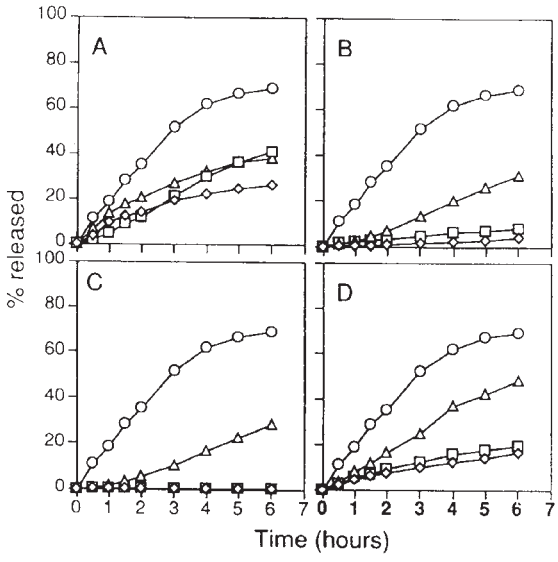


Figure 17 In-vitro release profile of 5(6)-carboxyfluorescein (CF) from various formulations prepared with (A) Span 20, (B) Span 40, (C) Span 60, and (D) Span 80; O: CF solution; □: vesicles, □: w/o emulsion; ◇: V/O/W emulsions. (From Ref. 80.)

XII. STABILIZATION BY POLYMERIC SYNTHETIC AMPHIPHILES

Stable double emulsions, based on various block copolymers of polyethylene oxides and polypropylene oxides known as Pluronics, have been used. In a recent example, Cole and Whateley (84) have used complexes of Pluronic F127:PAA (polyacrylic acid) in the internal aqueous phase. In the oil phase, Span 80 and Pluronic L101 (5 wt %) were used. The outer interface was stabilized by xanthan gum (0.25 wt %) and Tween 80 (1 wt %). Theophylline and ^{125}I-insulin (iodinated insulin) were incorporated in the internal aqueous phase of the stabilized multiple emulsion, and the release rates were studied. The release rates were found to be related to the droplet sizes of the emulsion which were dependent on the particle size of the pluronic F127:PAA complex in the internal aqueous phase and the type of the lipophilic surfactant in the oil phase. The authors have used the complex between the poloxamer surfactant and PAA that occurred at pH 2 and at low molar ratio as a barrier for the release of active matter from the inner to the outer phase.

The fact that poloxamers and proteins were excellent steric stabilizers for double emulsions encouraged scientists to try and design an "optimal synthetic polymeric emulsifier" to be adsorbed both at the internal and the external interfaces. However, only few commercial polymeric surfactants are available, many of which are designed for

other applications. It was, therefore, a challenging task for Garti et al. (85—87) to design and prepare a comb-like graft copolymer based on a modification made on the commercial polymer known as PHMS—PEG, which is a polyhydrogensilox-ane grafted with polythene glycol side chains. The modification added a flexible hydrophobic side chain (termed "spacer") to the backbone of the polymer on which the hydrophilic groups were grafted. A family of more than 16 products were synthesized and characterized. The synthesis opened an option of preparing several new families of emulsifiers with lipophilic and hydrophilic characteristics: (1) polymers with a wide range of grafting with various degrees of substitution (various DgS'); (2) polymers with different side ethy-lene glycol chain lengths [various EO repeating units $(EO)_n$]; and (3) various lengths of the backbone (various degrees of polymerization, DPs'). The surface properties of all the amphiphilic polymers (four hydro-phobic and 12 hydrophilic polymers) were measured. It was demonstrated that most of them could reduce the interfacial tensions of paraffin oils to very low values (below 10 mN/m). Emulsification was performed with various oils at various fractions. The emulsification did not require severe homogenization. The W/O droplets were stable and small and so also were the O/W emulsion droplets. In a two-stage emulsification process the water was added to the solution of the lipophilic emulsifiers and oil, and the hydrophilic emulsifiers were added to the external aqueous phase. The hydrophilic emulsifiers adsorbed on to the external interface. Excellent double emulsions were obtained, upon adding the W/O emulsion to the water phase without homogenization (Fig. 18), with small droplets and a narrow droplet size distribution. Excellent stability was achieved and the ability to keep the emulsions stable upon dilution was also remarkable. Microscopic observations indicated that the polymeric emulsifier had coated the interfaces with thick multilayer films.

The 'marker' (NaCl) was released extremely slowly (Fig. 19). When polymeric emulsifiers were used at both interfaces the release rates were extremely low. The authors also prepared emulsions with blends of the new polymeric emulsifiers and hydrophobic low-molecular-weight emulsifiers such as sorbitan esters (Span 80). It was found that the low-molecular-weight emulsifiers increased the release rates. The migration kinetics were strongly dependent on emulsifier concentration. The release rates were enhanced also when other emulsifiers, such as EDT-PGPR, were used in the internal interface. It was concluded that, while the polymeric emulsifiers, even if aggregating in the oil phase, are restricted in their solubilizing capacity as hydrophilic compounds, the hydrophobic monomeric emulsifiers (such as Span 80) can easily form, in the oil layer, reverse micelles that carry out the markers. Strong evidence for such a mechanism was found. Such a combination of monomeric and polymeric emulsifiers can serve as an efficient tool for controlling the release of markers from double emulsions.

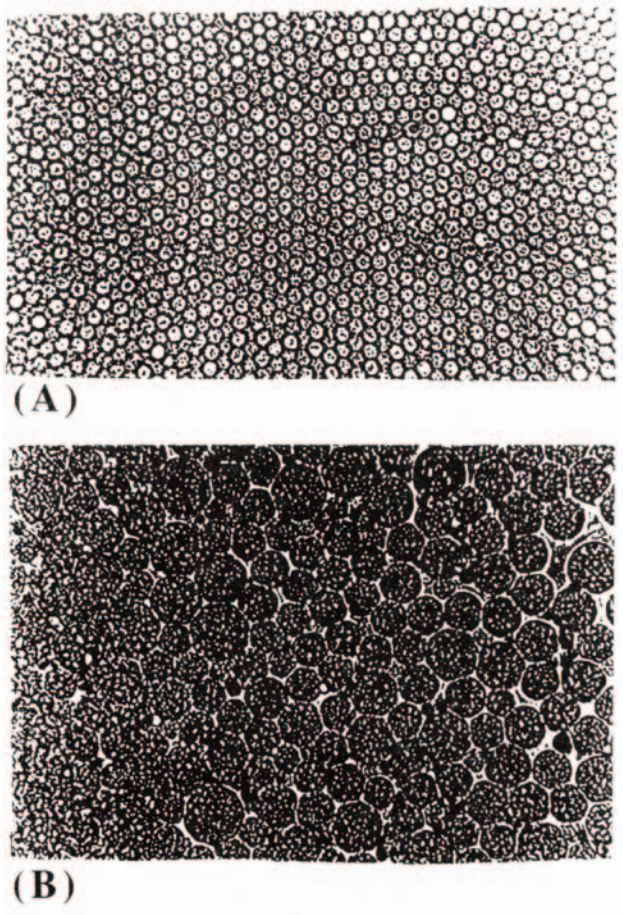


Figure 18 Photomicrograph of typical W/O/W double emulsion stabilized with tailor-made grafted PEG side chains on to poly(dimethylsiloxane) backbone (see text) after homogenization and centrifugation: (A) magnification x200; and (B) magnification x500

However, the most surprising phenomenon was the fact that the double emulsions were easily homogenized without "transporting" the markers into the aqueous solution during the emulsification process. The homogenization process yielded almost monodispersed double-emulsion droplets with very narrow size distribution ranging from 3 to 7 μm (Fig. 18).

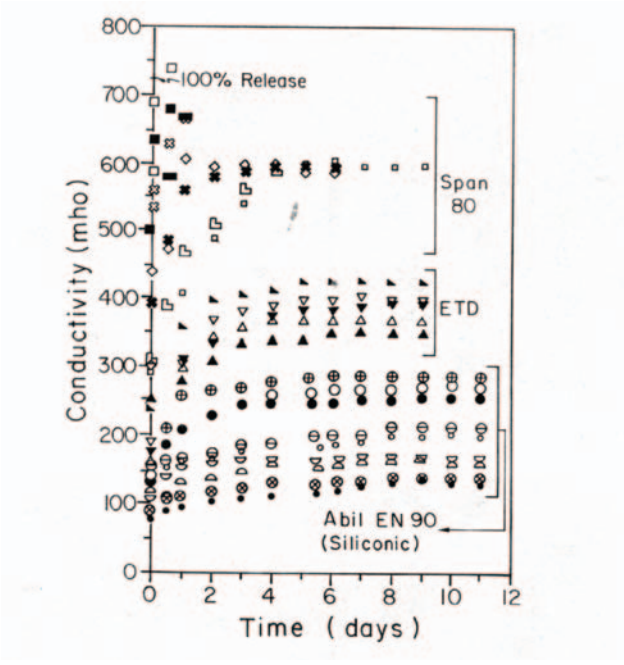


Figure 19 Plot of conductivity of the outer aqueous phase (reflecting the concentration of NaCl in the outer phase (% release) vs. time (days) in three sets of double emulsions. (1) All circles indicate use of Abil EM-90 as hydrophobic Emulsifier I. The lower curves (bull) indicate the most hydro-philic PHMS—PDMS—UPEG Emulsifier II, and the upper curves (O) in each set indicate the most hydrophobic PHMS-PDMS-UPEG with 52% substitution and 45 EO units. Each circle represents a different polymeric emulsifier. (2) All triangles represent use of polyglycerol polyricinoleate (ETD) as Emulsifier I, and the curves are arranged again with increasing hydrophobicity of Emulsifier II. The lower curve (A) represents the most hydrophilic emulsifier, and the upper curve in the set represents the most hydrophobic one (?). (3) All squares represent the use of Span 80 as Emulsifier I, and the curves are arranged with increasing hydrophobicity of Emulsifier II.

A close examination of the release profiles of mar-kers (NaCl or drug) from these double emulsions revealed that the release occurs in three stages. A long lag time (Stage A) in which almost no release takes place is observed immediately after preparation (Fig. 20, slope A). The lag time depends on the nature of the internal lipophilic polymeric emulsifier and its concentration ratio to the monomeric lipophilic emul sifier. In the second stage (Fig. 20, slope B) the release slope is gradual and diffusion controlled, and com plies with the Higuchi mechanism of release from a "slab into a sink" by a reverse micellar mechanism. In the third stage (Fig. 20, slope C) the release is very slow and levels off. The fact that the release mechanism is via reverse micelles has a significant advantage since it allows us to control the transport through the liquid membrane film by controlling the number of micelles present in the oil membrane. The polymeric emulsifiers that dissolve or aggregate in the oil phase do not have the characteristics of reverse spherical micelles. It is assumed that the polymers aggregate in rather "open" (unfolded) random coils. Therefore, such structures cannot solubilize much water and cannot transport the water molecules or the water-soluble marker. Emulsions prepared with polymeric emulsifiers alone practically do not release the marker upon storage. Very long lag times were detected and only minor quantities of marker were released at very slow rates. On the other hand, the use of variable concentrations of Span 80 (the lipo philic monomeric emulsifier) allowed one to control the lag time, the released amount of matter, and the rates. As one increases the amounts of Span 80 more marker is released. The lag time is shortened and the rates increase with the increase of added Span 80 (Fig. 21). One can add the monomeric lipophilic emulsifier either during emulsification (with given and calculated release kinetics) or, better, by adding it dropwise and/or "at need" after the emulsification or before using the double emulsion. Such "delayed addition" will allow one to store the emulsion for prolonged periods without transporting the active matter and to trigger its release at another release rate upon application.

The relevance of the control of the reverse micelles and dependence of the release kinetics on the reverse-micelles' concentration and size was demonstrated. The amount of solubilized water in the oil phase, as a function of Span concentration, was measured (after centrifugation). It was found that water is present only in very minor quantities when the siliconic emulsifier is employed by itself. The water concentration increased as the amounts of Span 80 were raised (87). These findings are also in good correlation with the release rates and the lag time.

It was, therefore, concluded that the internal polymeric emulsifier controls the release rates both by improving the film formation on the interface and by restricting the formation of reverse micelles in the oil phase. It is assumed that the presence of the two emul sifiers (Span 80 and silicone lipophilic emulsifier) form "reverse hemimicelles." These structures are capable of solubilizing less water and, therefore, less marker, a fact that leads to slower release and the ability to con trol better the release rates. Emulsions prepared from polymeric emulsifiers and without the lipophilic monomeric emulsifier remained stable on the shelf for over 6 months and did not show any leaking of the marker upon storage. Once the monomeric emulsifier was added drop-

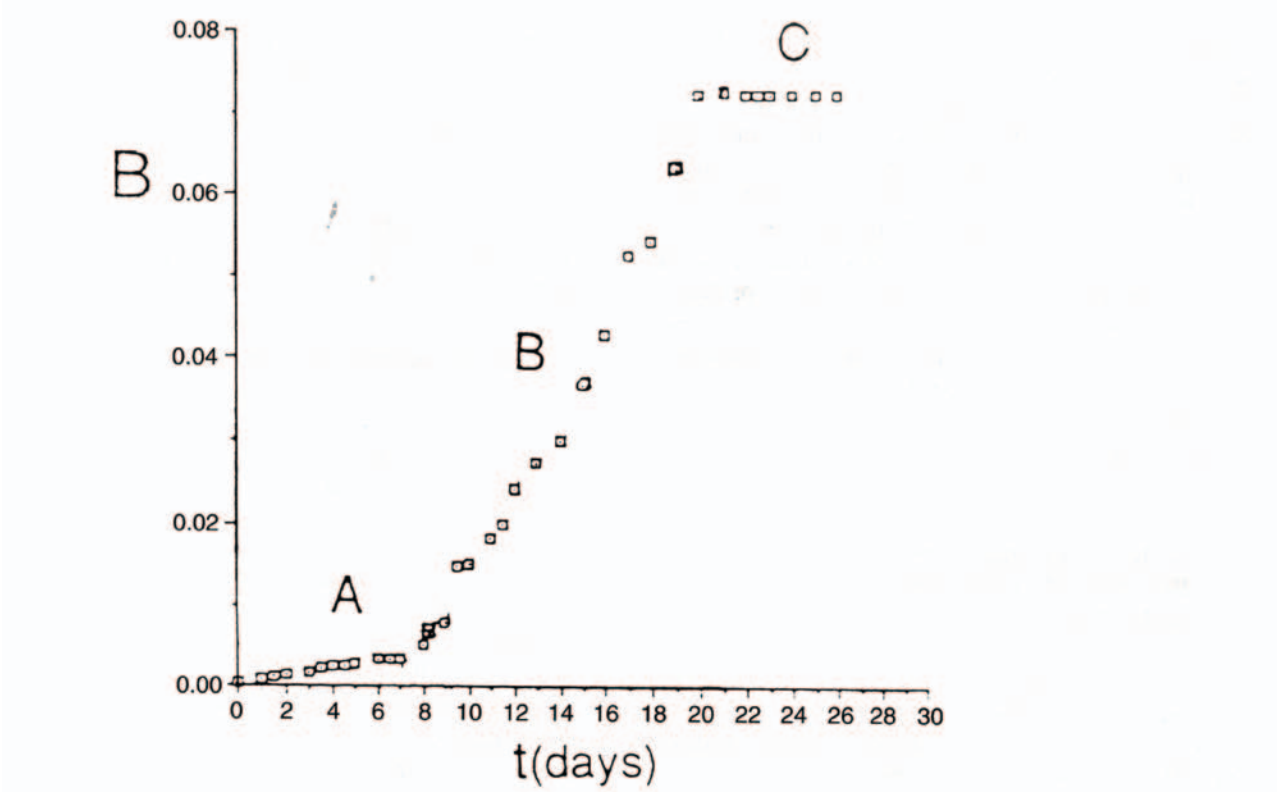


Figure 20 Plot of factor *B* (proportional to the release rate) as a function of time (*t*) for double emulsions stabilized with 5 wt % Abil EM-90 as the inner emulsifier and PHMS—PDMS—52.5% UPEG-45 (see Ref. 87) as the external emulsifier.

wise, at various concentration levels, the release started and was completely controllable by both the rate of addition of the monomeric emulsifier and by its concentration.

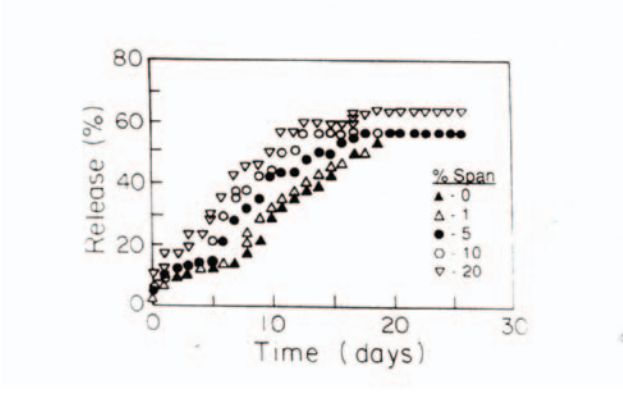


Figure 21 Release profiles of double emulsions stabilized with polymeric hydrophobic emulsifier at the internal interface (Abil-EM-90) and a combination of hydrophilic siliconic polymeric emulsifier (PHMS-PDMS-52% UPEG-45EO) with various concentrations of Span 80.

An interesting modification of using polymeric materials for stabilizing double emulsions was exercised by Khopade and Jain (88). "Stealth" multiple emulsions of small size, consisting of 6-mercaptopur-ine, were prepared by incorporation of sphingomye-lins (SMs) and monosialogangliosides [GM(1)] in the oil phase and by coating it with lipid-graft polyethylene glycol (PEG-PC). The three types of "stealth" double emulsions were characterized for size distribution, viscosity, encapsulation efficacy, drug release, and stability. The results suggest that PEG-PC-coated multiple emulsions are superior as prolonged release and extended blood-circulation carriers compared with double emulsions bearing either SM or GM(1). The authors (35) have used other drugs as well and included also a sonication step. The reported results on the anticancer activities of drugs entrapped in the double emulsions by this technique are quite encouraging. Similarly, the authors used also cocavalin-A and carbodiimide as ingredients for the formation of "lectin-functionalized double emulsions" (89). The anticancer activity was, similarly, superior to that of the noncoated double emulsions (Fig. 22).

In summary, all the recent improvements in the double-emulsion compositions (emulsifiers and oils), the improved evaluation related to the "weight" given to any of the possible instability mechanisms, and the better understanding of the instability factors that were achieved in the last 15 years of research work were supposed to solve most of the scientific problems of this technology. Yet, only very limited improvements in the stability of the emulsions and in extending their shelf-life have been recorded. There is practically very limited control of the release of the additives or the active matter.

XIII. DOUBLE EMULSIONS AS INTERMEDIATES FOR NANOPARTICULATION

Advanced double-emulsion formulations are no longer prepared for the purpose of simple delivery and release of active matter, but have changed application directions. Three main new directions can be clearly seen: (1) double emulsions as intermediates for the preparation of solid microspheres or microcapsules; (2) O/W/ O double emulsions for improved solubilization and chemical protection of water-insoluble active matter; and (3) double emulsions for selective adsorption of certain compounds for extraction and purification purposes.

Some major examples among the classical delivery applications in pharmaceuticals and food technology will be described that will emphasize the new emerging applications.

A. Controlled-delivery Applications

The intrinsic instability of the double emulsion caused difficulties to formulators and many of the investigators have decided to abandon this technology (8, 9). However, one should bear in mind that the potential applications for double emulsions appears to be enormous, mainly in the areas of food, cosmetics, medicine, and pharmaceutics. Throughout the years potential applications have been demonstrated in: (1) improved biological availability (parenteral nutrition, anticancer drugs); (2) delivery of drugs (sustained release of narcotic antagonistic drugs, prolonged release of

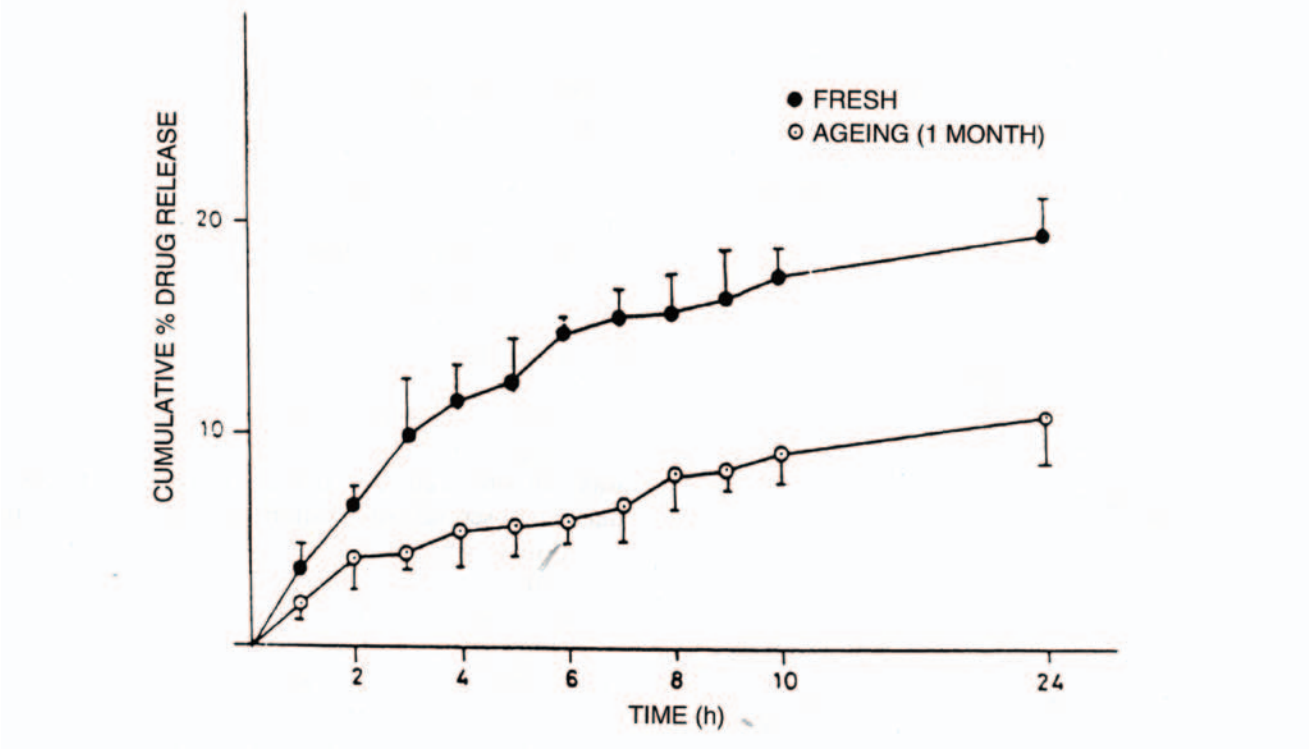


Figure 22 Drug-release profile of isoniazid from multiple emulsion based on uni/oligo/droplet internal phase. (From Ref. 57.)

corticos-teroids, slow release of Bleomycin, and targeted release of anticancer drugs); and (3) adsorption of toxic compounds. The technology has much promise also in non-pharmaceutical areas of slow and controlled release of materials such as fertilizers and pesticides for agricultural formulations as well as for controlled release for cosmetic, industrial, and food applications (8, 9).

A preparation of Ketamine [2-(Chlorophenyl)-2-(methylamino) cyclohexanone, $C_{13}H_{16}C1NO$, an anesthetic agent] in an O/W/O multiple emulsion for prolonged drug release was formulated and evaluated. Ketamine is used as a short-acting anesthetic in humans and in some animal species (90). Ketamine is poorly bound to plasma proteins and has a half-life of approximately 4 h following intravenous injection.

Ketamine leaves the blood very rapidly to be distributed into the tissues. The recommended dosage of intravenous Ketamine is 2.5—20 mg/kg. The objective of the study was to test the concept that a multiple emulsion could be formulated which has high porosity and lower viscosity at 37°C consistent with its intended use for sustained drug release and to prolong the half-life of the anesthesia. The results showed that 8.2% of the Ketamine was released (100 mg/ml in the inner phase) after 10 min, 67.0% at 30 min, and 95.5% at 60 min from the Ketamine/O/W multiple emulsion in a well-controlled manner (Figs. 23 and 24).

In another example, long-circulating multiple-emulsion systems for improved delivery of an anticancer agent of small droplet sizes, containing 6-mercaptopur-ine (88), were prepared by incorporation of sphingo-myelins (SMs) and monosialogangliosides (GMs) in the oil phase and by coating with lipid-grafted polyethylene glycol (PEG-PC). Three types of "stealth" multiple emulsions were characterized for size distribution, zeta potential, viscosity, encapsulation efficiency, drug release, and stability.

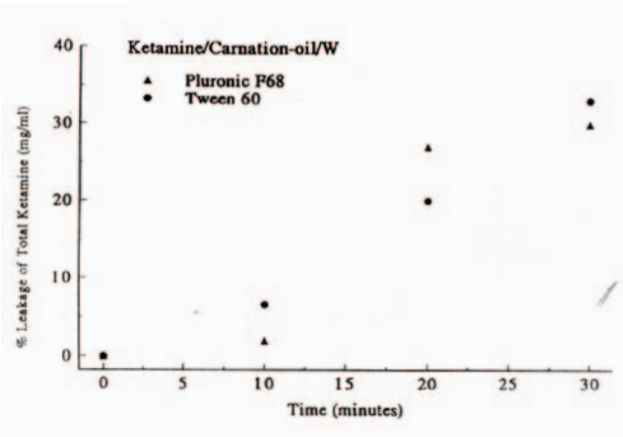


Figure 23 Effect of surfactant on Ketamine release from the multiple emulsion with shearing. (From Ref. 90.)

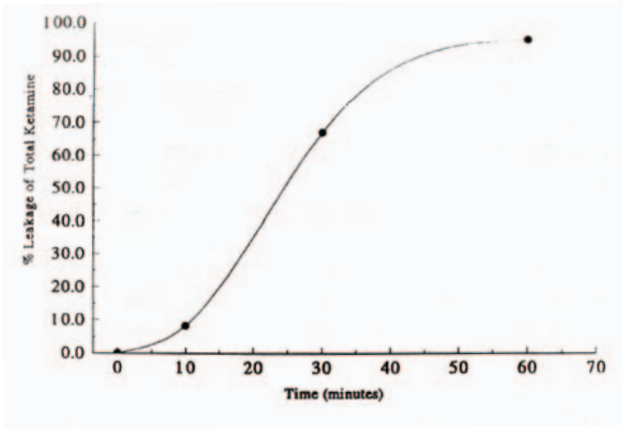


Figure 24 Ketamine release from the multiple emulsion with shearing. (From Ref. 90.)

Drug-disposition studies were performed with formulated multiple emulsions to assess "stealth" behavior. The results suggest that PEG-PC-coated multiple emulsions are superior as prolonged-release and extended blood circulating carriers compared to multiple emulsions bearing either SM or GM.

The insulin efficacy in double emulsions is an additional interesting example. The purpose of that study was to evaluate the hypoglycemic effects of W/O/W insulin emulsions containing lipoidal absorption after enteral administration to rats (91). The hypoglycemic effects of insulin were examined during an in-situ loop method in rats. The insulin emulsions prepared with soybean oil, triolein, or trilinolein slightly but significantly decreased the serum glucose levels compared to the insulin solution. By addition of 3 wt % limonene or 3 wt % menthol to the triolein emulsion, the hypoglycemic effect of insulin was promoted in the ileum but not in the colon. Strong hypoglycemic effects were observed with the triolein emulsion containing 2 wt % fatty acids such as oleic, linoleic, and linolenic acids. The remarkable enhancing effects occurred more predominantly in the colon than in the ileum. The effect of degree of unsaturation of the fatty acids was not observed. No tissue damage was noted by light-microscopic examination of both regions treated with triolein emulsion, or triolein emulsion containing menthol or oleic acid. W/O/W emulsions containing unsaturated fatty acids are able to enhance the ideal and colonic adsorption of insulin without tissue damage and may, therefore, be useful in dosage form in an enteral delivery system for insulin.

A stable multiple emulsion containing rifampicin (92) in the internal aqueous phase was prepared by the incorpo-

ration of additives in both the aqueous and oily phases. The formulation and process variables were optimized for primary and secondary emulsifica-tion. Drug-release studies were performed on selected multiple emulsions to observe the effect of dilution. The release data were fitted to the Higuchi equation for slab and spherical geometry, and effective diffusion coefficients were calculated. Stability studies undertaken for three months revealed good stability of the multiple emulsions with respect to creaming, phase separation, viscosity change, drug leakage, change in droplet size upon storage, and exposure to osmotic and shear stress. The in-vivo studies performed with stable multiple emulsions administered orally in human volunteers showed prolonged plasma drug levels. The multiple emulsions were found suitable for improved tuberculosis therapy.

Double emulsions may offer some advantages for food applications mainly with relation to their capability to encapsulate (or entrap) in the internal compartments some water-soluble substances that are then slowly released. The double emulsion can also be used in the food industry where an external water phase is more acceptable in terms of palatability than an oil one (93, 94). On this basis several new products have been patented in the form of W/O/W emulsions, as salted creams (encapsulation of salt), aromatic mayonnaise, etc. (95-98). Further food applications are related to the double-emulsion dielectric properties; for example: one can prepare a W/O/W system having the same volume fraction of the dispersed phase and the same texture as a simple emulsion, but with a lower oil content (due to the existence of the aqueous compartments in the food globules), i.e., low-calorie mayonnaise (93).

B. Double-emulsion-Solvent-evaporation Techniques for Preparation of Microspheres

Drugs, cosmetic ingredients, and food additives are microencapsulated for a variety of reasons, which include reducing local side-effects, controlled release, site-specific (drug) delivery, and drug targeting. A tremendous amount of research work has been done in a search for suitable methods to achieve an effective encapsulation of water-soluble active matter. The physical characteristics of the microspheres produced largely determine their suitability for use for different objectives. Microspheres are prepared from both natural and synthetic polymers.

Among microencapsulation techniques, the double emulsion-solvent-evaporation method, also known as "the in-water drying method," is one of the most useful methods for entrapping water-soluble compounds (64-78). Figure 25 shows schematically the preparation technique. Over 100 papers and many patents have been published in the course of the last five years on thee use of this technique. It is behind the scope of this review to screen or to categorize them. We have selected only some examples that are of a more significant value.

It is known that the preparation of microspheres by using O/W emulsions is not an efficient method for the entrapment of water-soluble drugs as the compounds rapidly dissolve in the aqueous continuous phase and are lost. It has been widely accepted that the problem of inefficient encapsulation of water-soluble drugs can be overcome by using the double-emulsion solvent-evaporation technique. Water-insoluble drugs are usually satisfactorily encapsulated by the O/W emulsion technique (64, 65).

The W/O/W emulsions are generally used for encapsulating proteins or peptides. These highly water-soluble molecules are quantitatively introduced into the internal aqueous phase of the multiple emulsions, which results in an increased encapsulation efficiency for the microcapsules in comparison with particles produced by the single-emulsion-solvent-evaporation method. The particular location of the proteins induces a stabilizing effect on the two emulsions which, in turn, contributes to a successful stabilization of the double emulsion and loading.

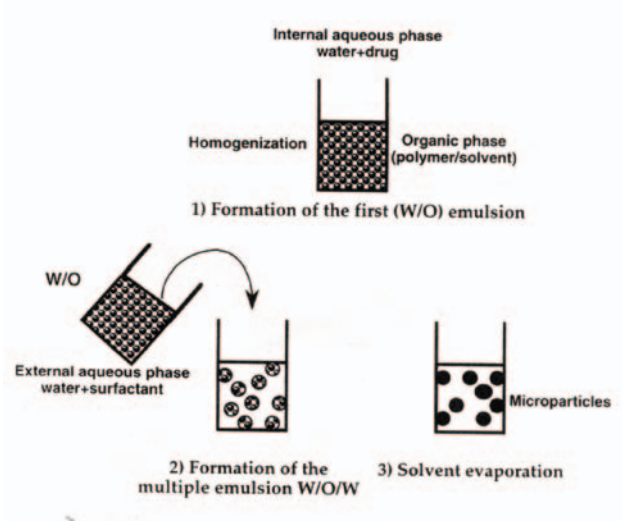


Figure 25 Preparation of microsphere-containing peptides by the multiple-emulsion solvent-evaporation technique.

The double-emulsion-solvent-evaporation technique is commonly used to prepare biodegradable hydro-phobic microspheres containing hydrophilic Pharmaceuticals, proteins, and polypeptides for sustained-release applications (66, 70-78). In most cases the microspheres are in the range size 10-100 μm. However, recently, Blanco-Prieto et al. (71) managed to reduce the microcapsule sizes to less than 5 μm.

The microspheres, consisting of poly(L)lactide, poly(DL)lactide, poly(DL)lactide-*co*-glycolide, polyhydroxybutyrates of various molecular weights, and poly(hydroxybutyrate-*co*-valerate), were characterized for their structure, size distribution, drug loading, release kinetics, surface morphology, and hydrophobi-city (69, 78) (Figs. 26—29). The influence of these properties on the dynamics of the immune response, following topical administration, was studied. The hydrophobic nature of polyhydroxybutyrate micro-spheres, compared with those formed using polylac-tides, was confirmed and the generation of a significant immune response was delayed using these preparation. Also, the time course of immune responses generated using a range of polyhydroxybutyrate molecular weights was examined.

Couvreur et al. (70) reviewed the preparation and characterization of many of the different types of solvent-evaporation microspheres and mostly discussed small poly(lactic-*co*-glycolic acid) microspheres (mean size lower than 10 μm) containing small peptides (Fig. 30). Three main evaporation strategies have been utilized in order to increase the encapsulation capacity: an interrupted process, a continuous process, and the rotary evaporation procedure.

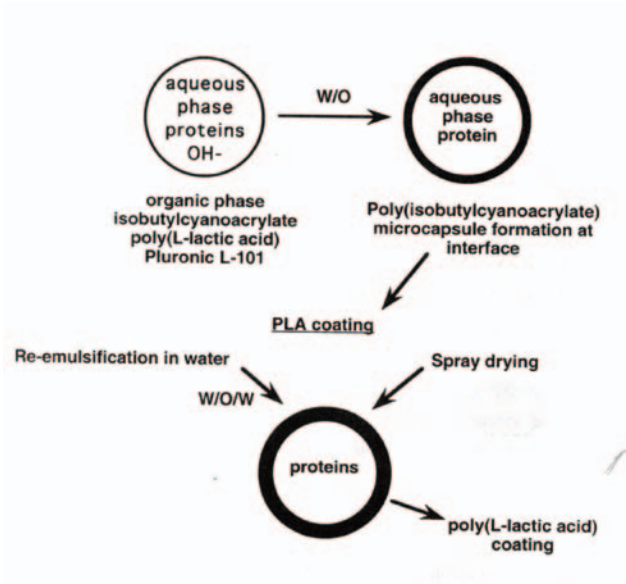


Figure 26 Schematic description of preparing PLA-coated PIBCA (polyisobutylcyanoacrylate) microcapsules that contain protein molecules. (From Ref. 78.)

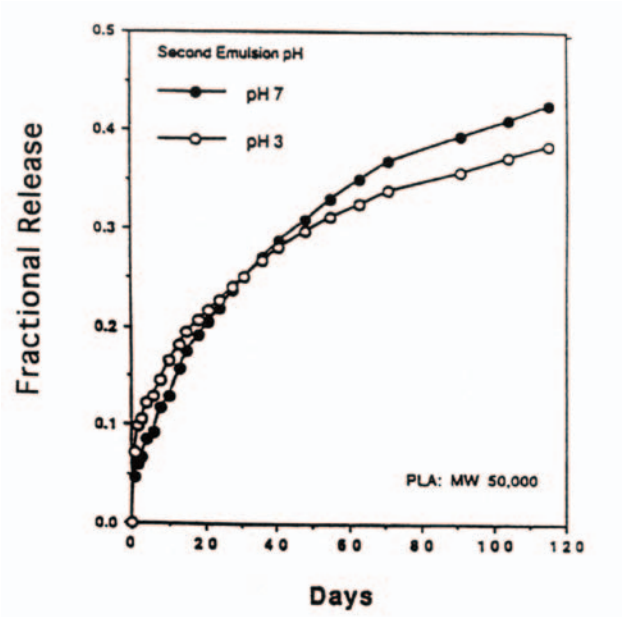


Figure 27 BSA release profiles from the PLA MW 50,000 coated PIBCA microcapsules prepared by the re-emulsifica-tion method at two different pH values. (From Ref. 78.)

Much work has been devoted in recent years to preparing microparticles of narrow size distribution, with different biodegradable polymers. The sizes of common microcapsules are 40 to 50 urn (70, 71, 76-78).

Liquid-liquid emulsification is a critical step in the double-emulsion microencapsulation process (W/O/W or O/W/O). It was found that the size of these droplets decreases with increasing homogenization intensity and duration. The emulsion droplet size depends as expected on viscosity, total volume size, and volume ratio of the continuous phase to the dispersed phase; the rotor/stator design was also investigated. All these physical parameters influence the structure of the microspheres obtained by this technique.

An interesting example is the incorporation of a protein-based drug in microspheres prepared from a hydrophobic polymer via double liquid-liquid emulsification (W/O/W) or by dispersing a powdered protein in a polymer solution followed by liquid-liquid emulsification (S/O/W) (72). Bovine serum albumin (BSA) was used as the model protein and poly(methyl metha-crylate) (PMMA) was used as

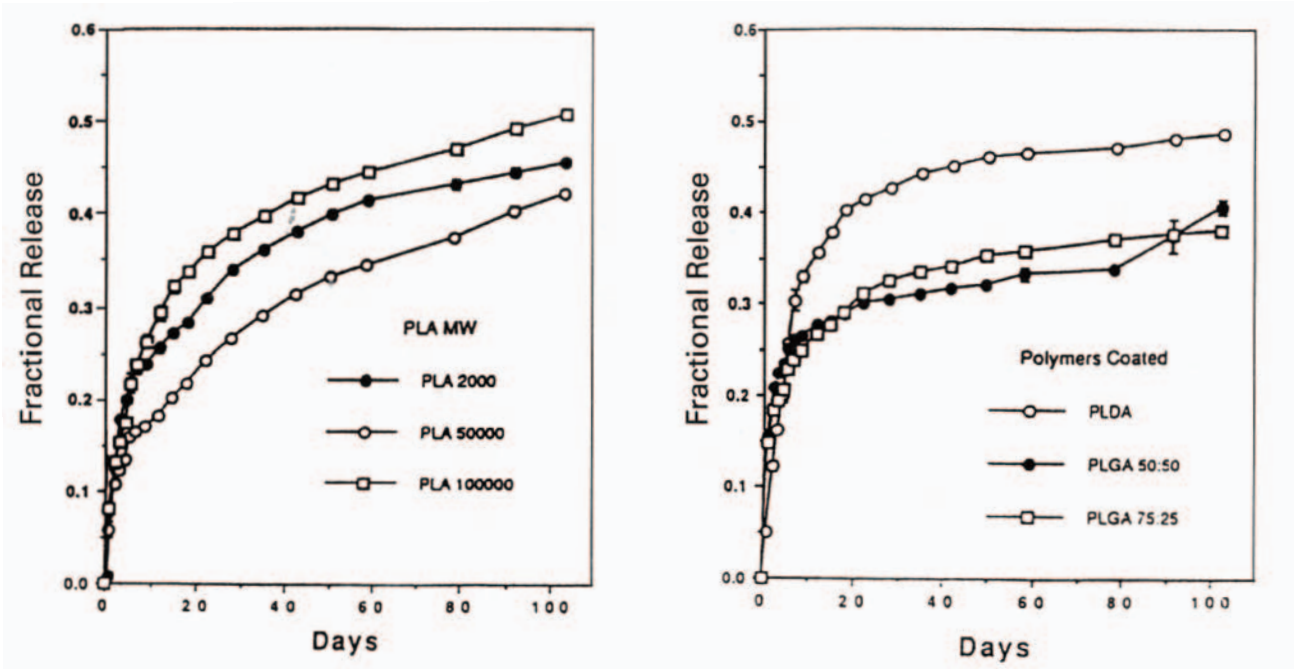


Figure 28 (A) BSA-release profiles from the PLA-coated PIBCA microcapsules prepared by spray-drying method; (B) BSA-release profiles from poly(DL-lactic acid) and poly(lactic-*co*-glycolic acid)-coated PIBCA microcapsules. (From Ref. 78.)

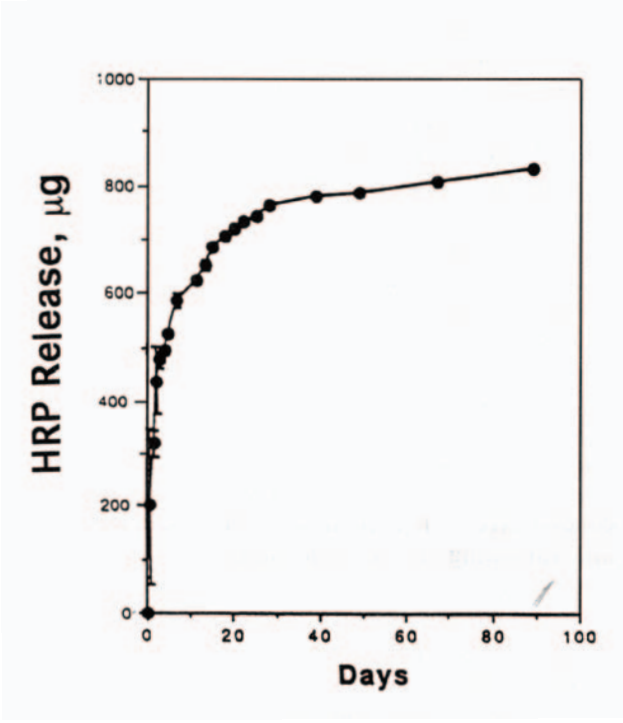


Figure 29 Horseradish peroxidase (HRP) release profiles from PLA (MW 2000) coated PIBCA microcapsules. (From Ref. 78.)

the model polymer. The droplet sizes of the W/O emulsion were controlled using rotor/stator homogenization. The S/O emulsion was characterized on the basis of protein powder size and shape, in both emulsification processes. The size of the micospheres thus prepared was found to increase with increasing size of the protein powder in the S/O/W system, but increased with decreasing size of the liquid emulsion droplets in the W/O/W system. Empirical correlation could accurately predict the size of the microspheres. Protein loading in the micro-spheres decreased with respect to increase in W/O emulsion droplet size or in protein powder size. These phenomena were attributed to two possible mechanisms: fragmentation along the weak routes in the W/O/W system and particle redistribution as the result of terminal velocity in the S/O/W system. The role of protein powder shape was not significant until the protein powder size exceeded 5 µm.

In a recent paper (77) it was demonstrated that a milk model protein, ß-lactoglobulin (BLG), was encapsulated into microspheres prepared by the solvent-evaporation technique. The effect of the pH of the outer aqueous phase on the protein encapsulation and release as well as on the microsphere morphology has been investigated. It was demonstrated that as the amount of the BLG increased the stability of the inner emulsion decreased and the entrapment was less efficient. Therefore, adjusting the combined

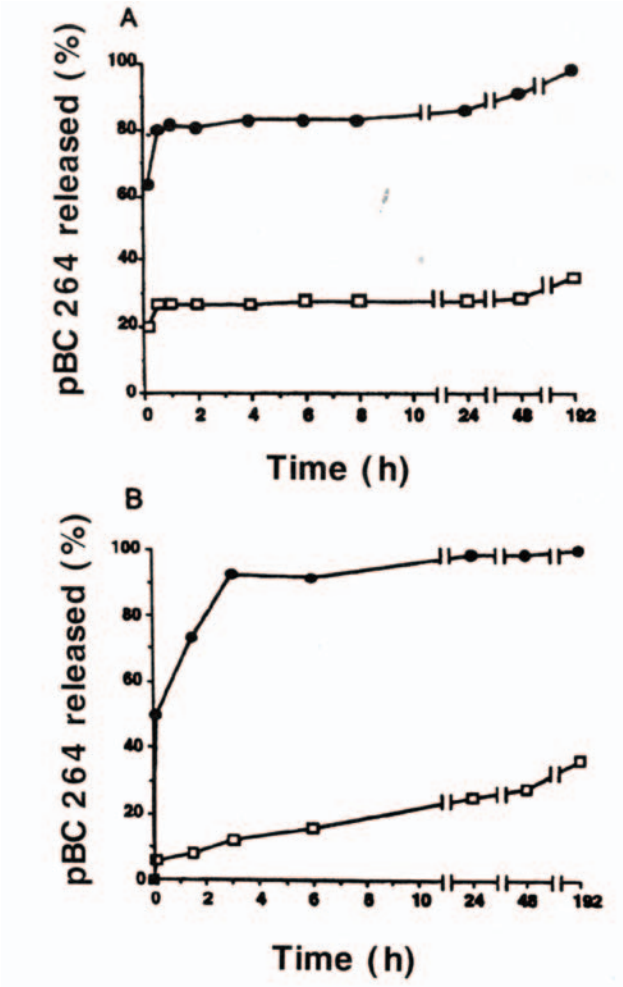


Figure 30 Release kinetic of pBC 264 in phosphate-buffered saline (pH 7.4) (A) and in vivo, in brain tissue (B) from PLGA microspheres prepared with either ovalbumin (bull) or Pluronic F-68 (?) (From Ref. 70.)

effect of pH and the stability of the inner emulsion may lead to better entrapment. As to the release, it was demonstrated that the "burst effect," attributed to a morphology change in the microcapsules characterized by the presence of pores or channels able to accelerate the release of the BLG, was the most signficant release factor. These pores were attributed to the presence of a large amount of BLG on the surface, which aggregates during microsphere formation at pH 5.2. It was concluded that it is beneficial to lower the solubility of the protein in the outer phase in order to improve the encapsulation efficiency, although this benefit is provided by strong adsorption of the protein on the microsphere surface.

Maa and Hsu (75) reported the formation of nano-particles by the double-emulsion method (W/O/W), using methylene chloride as an organic solvent and poly(vinyl alcohol) (PVA) or human serum albumin (HSA) as a surfactant. Experimental parameters such as the preparation temperature, the solvent-evaporation method, the internal aqueous phase volume, the surfactant concentration, and the polymer molecular weight were investigated for particle size, the zeta potential, the residual surfactant percentage, and the polydispersity index. Preparation parameters leading to particles with well-defined characteristics such as an average size around 200 nm and a polydispersity index lower than 0.1 were identified.

Some more interesting papers on protein entrap-ments for various oral and other intakes can be found in the literature (72—74).

Porous spherical particles of chitosan were prepared from O/W/O double emulsions stabilized with chitosan aqueous solution. The particulation was obtained by a simple evaporation technique (52).

XIV. O/W/O DOUBLE EMULSIONS

Oil-in-water double emulsions were considered to have less potential applications and therefore were less extensively studied. However, in more recent years several new applications have been reported for O/W/O double emulsions, which sound interesting and are worth being mentioned.

The modulated release of triterpenic compounds from an O/W/O multiple emulsion formulated with dimethicones, studied with infrared spectrophoto-metric and differential calorimetric approaches, is one of these examples (99). The authors explored the advantages in the release of triterpenic compounds from O/W/O emulsions. They found two principal advantages: (1) the use of low molecular weight sili-cones decreased the oily touch of the final preparation; and (2) owing to the large range of viscosity, these excipients influenced the skin distribution of the active matter after the topical application. The effects of different dimethicones incorporated within multiple emulsions were studied, through in-vitro penetration results. The residual film on the skin was also evaluated. Correlations were established between the sili-cones structure and the distribution of drugs at different skin levels or between the silicone structure and the percutaneous penetration. The in-

corporation of silicones within O/W/O multiple emulsions seems to be an efficient means of modulating the penetration and the distribution of drugs in the skin.

In another study the stability of retinol (vitamin A alcohol) was compared in three different emulsions: O/W, W/O, and O/W/O (15). The stability in the O/ W/O emulsion was the highest among the three types of emulsions. The remaining percentages, at 50°C after 4 weeks, were of 56.9, 45.7, and 32.3, in the O/W/O, W/O, and O/W emulsions, respectively. However, it was also reported that with increasing peroxide value of O/W and W/O emulsifiers, the remaining percentage of vitamin A palmitate and retinol in the emulsions increased significantly, indicating that peroxides in the formulas accelerate the decomposition of vitamin A. Organophilic clay mineral tan-oil gelling agent and a W/O emulsifier also affected the stability of retinol. The stability of retinol in the O/W/O emulsion increased with increasing inner oil phase ratio, whereas in O/W it was unaffected by the oil fraction. The encapsulation percentage of retinol in the O/W/O emulsion, and the ratio of retinol in the inner oil phase to the total amount in the emulsion, increased with increasing the oil fraction. The remaining percentage of retinol in the O/W/O emulsion was in excellent agreement with the encapsulation percentage, suggesting that retinol in the inner oil phase is more stable than that in the outer oil phase. Addition of antioxidants (*tert*-butylhydroxytoluene, sodium ascorbate, and EDTA) to the O/W/O emulsion improve the stability of retinol up to 77.1% at 50°C after 4 weeks. The authors concluded that the O/W/O emulsion is a useful formula to stabilize vitamin A.

XV. MULTIPLE EMULSIONS RHEOLOGY

The understanding of the rheological behavior of double emulsions is quite important in the formulation, handling, mixing, processing, storage, and pipeline transformation of such systems. Furthermore, rheological studies can provide useful information on the stability and internal micro structure of the double emulsions. Some attention has been given to this subject in recent years and the results are significant since they help to clarify certain aspects of stability and release properties of the double emulsions (100). Few publications deal with W/O/W double emulsions and only one deals with the O/W/O emulsion. Most of the work on rheology is old and does not contribute to new understanding. Therefore, we will discuss only recent relevant work.

An interesting characterization of the mechanical properties of the oil membrane in W/O/W emulsions was carried out by an aspiration technique (101). It was adapted from techniques related to the evaluation of globule or cell deformability. The deformability of an individual globule during total or partial flow into a cylindrical glass tube, calibrated under well-controlled conditions of aspiration, was determined. An analysis was performed on the behavior of the multiple emulsion by migration of the lipophilic surfactant to the interface between the oily and the external aqueous phases. It was shown that the elastic shear modulus and the interfacial tension of the oily membrane increased with the lipophilic surfactant concentration. This study also provides an explanation of the mechanism related to the swelling-breakdown process from physical and mechanical considerations.

Grossiord and coworkers (100, 102) applied linear shear flow to W/O/W double emulsions, which contained active matter, and from the rheological patterns they learned of the bursting effect of the droplets with the release of entrapped substances, and of the composition of the system. The authors (102) described a set of two types of experiment: oscillatory dynamic tests and steady-state analysis. They measured the stress and strain of the emulsions by applying sinusoidal shear. These parameters (shear or complex modulus G^*, the lag phase between stress and strain 8; the storage modulus G', and the loss modulus G") provide a quantitative characterization of the balance between the viscous and the elastic properties of the multiple emulsions. At the lag phase $\beta = 0°$ and when equal to 90° the system is viscoelastic. The shear sweep and the temperature sweep characterize the multiple emulsion at rest. Figure 31 describes a transition between elastic and viscous behavior, which occurs at critical stress values. The change in these parameters indicate a pronounced structural breakdown. The authors considered the influence of the formulation parameters on the swelling and release kinetics by using the rheological properties. Parameters such as the nature of the oil, the width of the oil membrane, and the lipophilic and hydrophilic nature of the surfactant have been evaluated. The two main parameters that were identified to affect the swelling/break-up kinetics were the difference in concentration in water-soluble molecules between the internal and the external aqueous phase (Fig. 32), and the lipophilic surfactant concentration. It was also observed that the maximum viscosity values increase with the surfactant ratio (under hypo-osmotic conditions, Fig. 33). The same trend was observed when the release of water-soluble materials, $B(t)$, was followed (Fig. 34). The authors concluded that a progressive migration of the excess of lipophilic surfactant in the oil phase toward the primary or the secondary interface occurs.

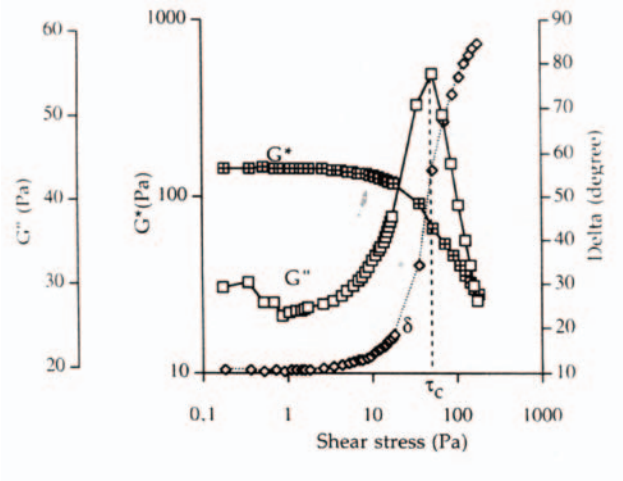


Figure 31 Changes in G^*, G", and S for increasing stress at fixed frequency in double emulsions. The double-emulsion composition of the W/O emulsion is 24% oil, various lipo-philic surfactant concentrations, and 0.7% $MgSO_4$. The O/ W/O emulsion contains 80% water in oil, 2% hydrophilic surfactant, and demineralized water. (From Ref. 102.)

Stroeve and Varanasi (103) examined also the break up of the multiple-emulsion globules in a simple shear flow and concluded from the critical Weber number $[(we)_{cr}]$ (Figs. 35 and 36) that the multiple emulsion exhibits behavior that is similar to that of simple emulsions. From Fig. 35 one can see at least qualitatively, from the evolution of $(We)_{cr}$ as a function of p (the viscosity ratio between the drop and the continuous phase) for a simple and multiple emulsion, that there are some differences between the two emulsions. The double emulsion has more heterogeneous characteristics. From Fig. 36 it is possible to obtain the minimum shear rate value that is able to produce the break up of the oil globules and the release of water-soluble encap-

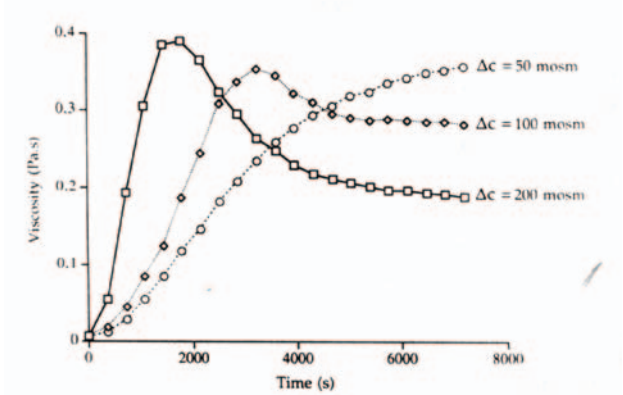


Figure 32 Change in viscosity vs. time for different concentration gradients in the inner phase. (From Ref. 102.)

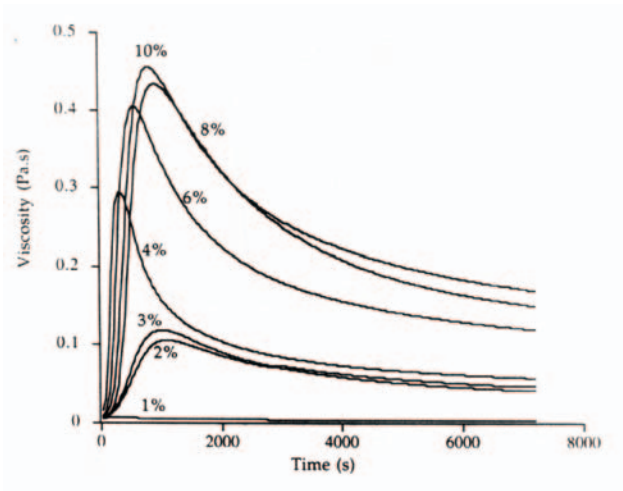


Figure 33 Change in viscosity vs. time under hypo-osmotic conditions for different lipophilic surfactant concentrations. (From Ref. 102.)

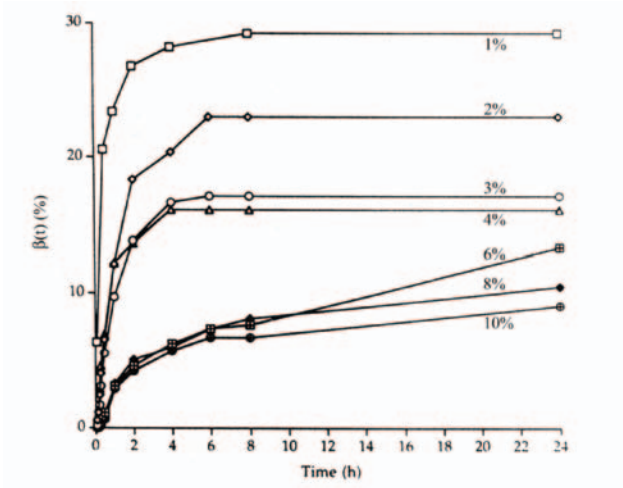


Figure 34 Change in released fraction vs. time under hypo-osmotic conditions for different lipophilic surfactant concentrations. (From Ref. 102.)

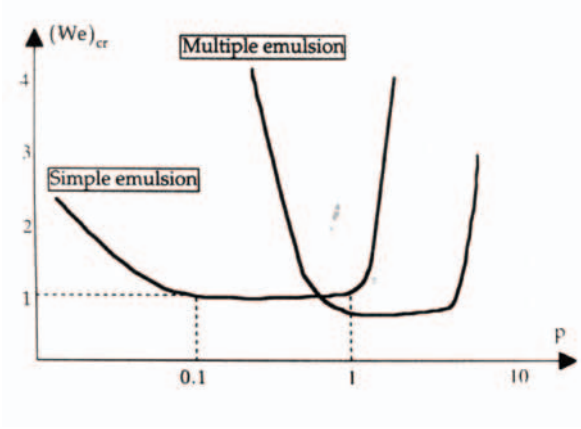


Figure 35 The critical Weber number for simple and multiple emulsions' disruption as a function of the viscosity ratio dispersed to continuous phase. (From Ref. 103.)

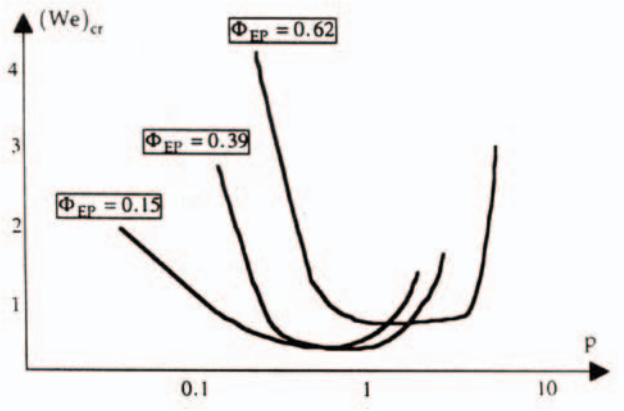


Figure 36 The critical Weber number for simple and multiple emulsions' disruption as a function of the viscosity ratio dispersed to continuous phase, for different primary emulsion volume fractions. (From Ref. 103.)

sulated molecules.

The studies showed also that the mechanisms taking place during the break up were complex and did not always lead to total release of the entrapped electrolyte. Some phenomena such as a partial leakage of the internal aqueous compartment or the expulsion of the aqueous microglobules covered by a residual liophilic film were able to restrict the release.

De Cindio et al. (94) prepared food double emulsions and studied their rheological behavior by steady shear and oscillatory measurements. They concluded that the W/O/W appeared to have rheological properties similar to those of a simple O/W emulsions having the same fraction of dispersed phase but lower oil content (Fig. 37). It was also

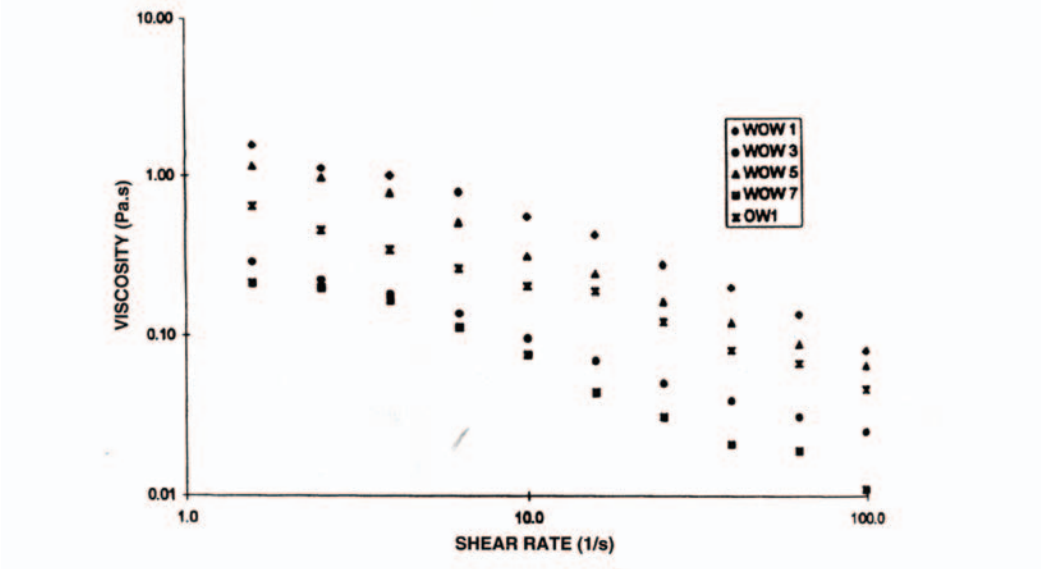


Figure 37 Apparent viscosity vs. shear rate for both W/O/W and O/W systems at a volume fraction of disperse phase = 0.3. (From Ref. 94.)

demonstrated that the plot of both storage modulus G' and G'' versus oscillation frequency, W, are similar in all eight prepared emulsions, the loss tangent is about 1, and both elastic and viscous contributions to viscoelastic behavior of double emulsions are of similar magnitude. The similarity of texture between simple and double emulsions is very encouraging, leading to some interesting conclusions and new perspectives. The influence of a mixture of emulsiners on the double-emulsion stability was studied by an oscillatory ring-surface rhe-ometer from which the interfacial elasticity at the oil-aqueous interface could be evaluated.

Pal (104) studied the rheology of O/W/O double emulsions. The simple O/W emulsions were found to be Newtonian up to a dispersed-phase concentration of 45% by volume and nonNewtonian above this volume fraction. All the double O/W/O emulsions are highly nonNewtonian. The degree of shear thinning increases with the increase in primary O/W emulsion concentration (Fig. 38). The oscillatory measurements indicate that the multiple emulsions are predominantly viscous in that the loss modulus falls above the storage modulus over the entire frequency range investigated (Fig. 39). Upon aging, the storage and loss moduli of the double emulsions show a significant increase. However, the increase in viscosity with aging is only marginal.

The rheological behavior of W/O/W emulsion studied with a cone-and-plate viscometer has shown a negative thixotropic flow pattern, mostly under low shear rate. Upon raising the shear rate or the shear time an increase in the shear stress was observed, which induced phase inversion

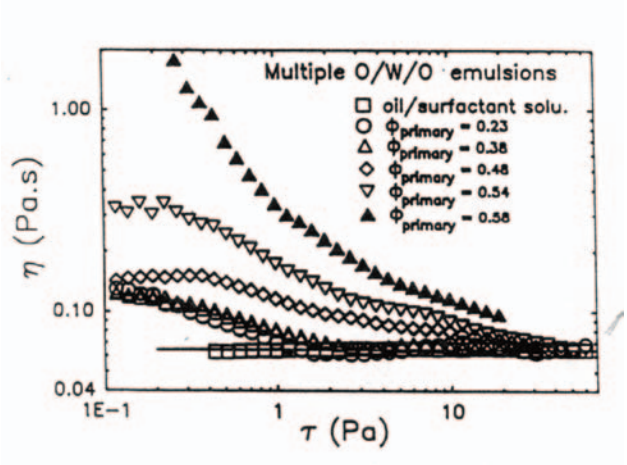


Figure 38 Apparent viscosity as a function of shear stress for multiple O/W/O emulsions. (From Ref. 104.)

to a W/O of a semi-solid type emulsion. The hydrodynamic parameters (dissipated energy, kinetic energy, and impulse applied to the emulsion by the rotating cone) causing the phase inversion were determined and a mechanism for such inversion was suggested. Figure 40 shows a proposed mechanism for phase inversion under shear rate (105). Induced shear, causing phase inversion, can serve as a possible technique for the release of drugs.

XVI. CONCLUDING REMARKS

Double emulsions have been known for over three decades and were extensively studied in the last 15 years. The internal phase is an excellent reservoir for active matter that needs protection and can be released at a controlled rate. However, the sizes of the droplets and the thermodynamic instability was a significant drawback of this technology.

The use of conventional low-molecular-weight emulsiners did not solve these problems. However, much progress has been made with the introduction of amphiphilic macromolecules as emulsifiers. These multianchoring flexible macromolecules can improve the steric stabilization by forming thick multilayered coating on the droplets. A variety of hybrids, complexes, adducts between the amphiphiles, and coemul-sifiers as cosolvents have been studied. These molecules improved the stability significantly and slowed the release rates.

Physical methods of separation, filtration, and extraction also had a positive effect on the release patterns of any drug or active matter. Progress was also made in the characterization of the parameters and mechanisms that are involved in the coalescence, aggregation, and rupture of double-emulsion droplets, and effective control of the rheological parameters was achieved by a better understanding of their effect on the static and shear-induced stability.

It seems that double-emulsion technology can now be applied in various areas, mainly in food, cosmetics, and pharmaceuticals (for nonintravenous applications).

The main goals remaining are: to obtain submic-rometer double-emulsion droplets with long-term stability (possibly with emulsified microemulsions) and to trigger and control the release at will.

Compatible blends of biopolymers (hydrocolloids and proteins) are excellent future amphiphilic candidates that under certain combinations will serve both as 'release controllers' and 'stability enhancers' for the future preparations of double emulsions.

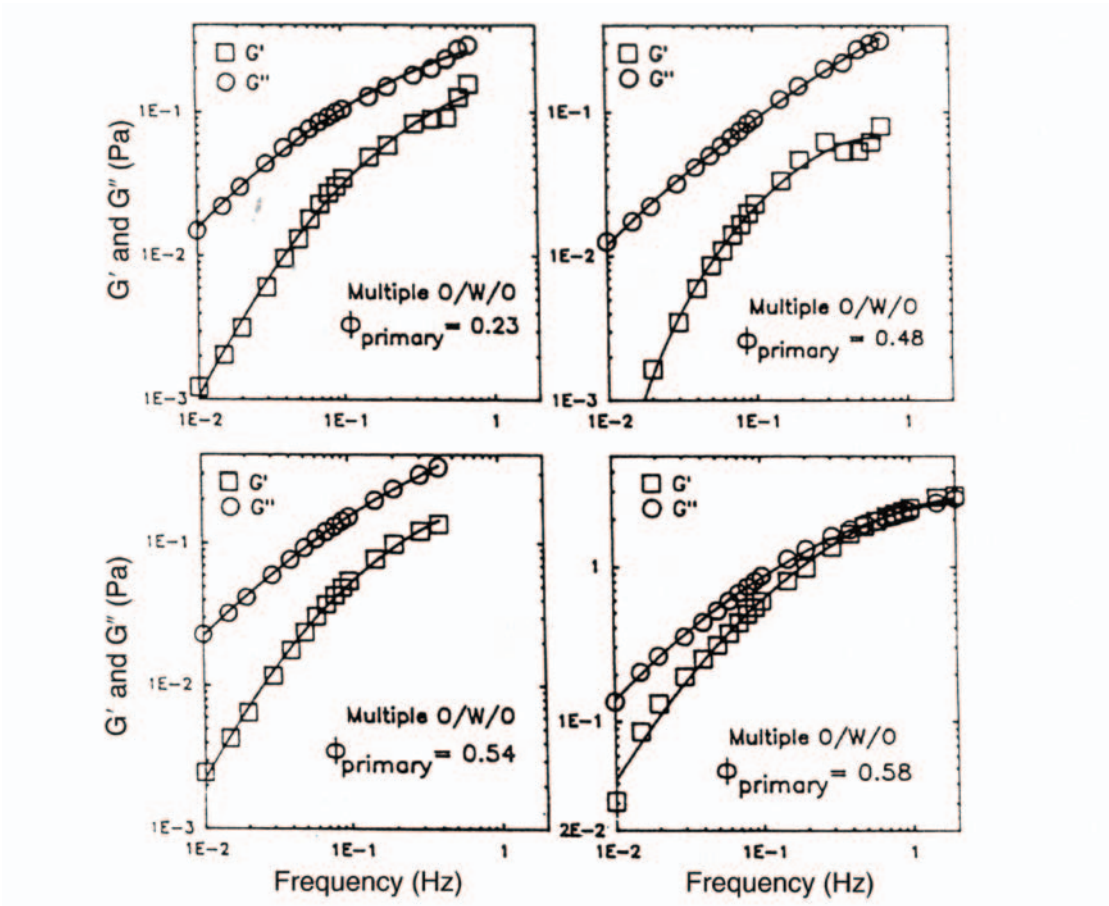


Figure 39 Storage and loss moduli for multiple O/W/O emulsions as functions of frequency. (From Ref. 104.)

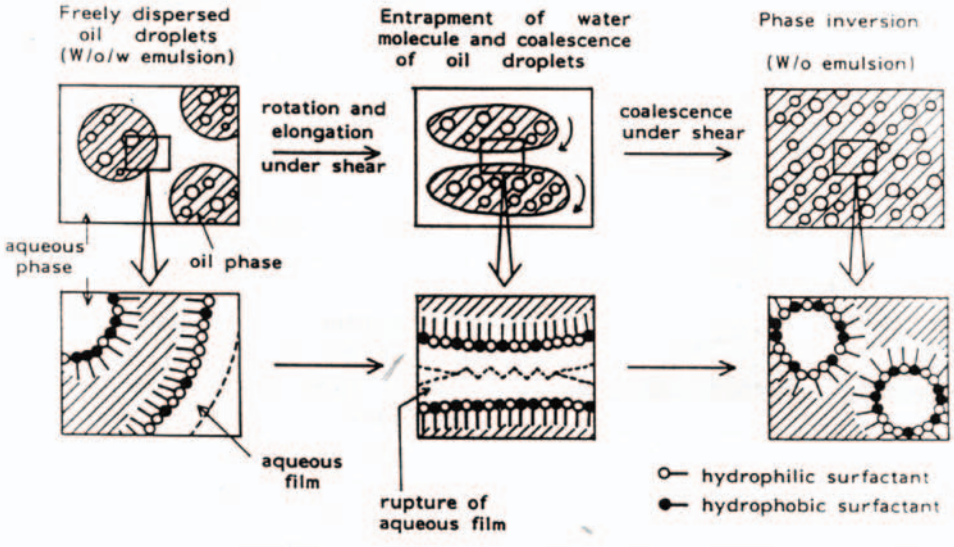


Figure 40 Proposed mechanism of phase inversion under shear. (From Ref. 105.)

ACKNOWLEDGMENTS

The authors are grateful to Dr Abraham Aserin for his valuable help and critical reading of the present manuscript.

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