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Recent Developments in Double Emulsions for Food Applications

Nissim Garti and Axel Benichou

The Hebrew University of Jerusalem, Jerusalem, Israel

I. OPENING REMARKS

Multiple emulsions are emulsion within emulsions. The major types are of water-in-oil-in-water (w/o/w) and oil-in-water-in-oil (o/w/o) double emulsions. These multicompartment liquid dispersions, at least in theory, have a significant potential in many food applications because the internal droplets can serve as an entrapping reservoir for active ingredients that can be released by a controlled transport mechanism. In practice, double emulsions consist of large and polydispersed droplets which are thermodynamically unstable with a strong tendency for coalescence, flocculation, and creaming.

Efforts have been made to improve the two major drawbacks of these systems related to the thermodynamic emulsion's instability and the uncontrolled release of active matter. Almost any possible blends of low-molecular-weight emulsifiers, oils, cosolvents, and coemulsifiers have been tested. The nonviscous fluid double emulsions were always unstable and the release of the active matter from the inner phase to the outer continuous phase remained difficult to control. Only semisolid double-emulsion, gelled or thickened systems have a long shelf life with prolonged stability. Biopolymers, synthetic graft and comb copolymers, and polymerizable emulsifiers that impart steric or mechanical stabilization exhibited improved stability and better controlled release. Macromolecular surfactants, naturally occurring or synthetic polymeric amphiphiles, will increase the viscosity of each of the phases, will complex with the oil or the emulsifiers, and will

be able to form systems that will behave much like microcapsules, microspheres, and/or mesophasic liquid crystals.

The chapter will stress the most recent findings that can enhance the stability of the double emulsions and/or will reduce droplets sizes for potential food applications. The achievements include (1) choice of food-grade emulsifiers to enhance emulsion stability at both inner and outer interfaces, (2) droplet size reduction by forming microemulsions (or liposomes) as the vehicles for the active matter in the internal phase, (3) use of different preparation techniques to enhance the monodispersibility of the droplets, and (4) use of various additives (carriers, complexing agents, natural polymeric emulsifiers) to control and modify the reverse micellar transport phenomena.

Double emulsions were recently used as intermediate structures in the preparation of microcapsules capable of protecting entrapped addenda and assisting in controlling delivery. The literature is ''flooded'' with tens of new examples every year, demonstrating release patterns and control of active ingredients using double emulsions. However, practically no double-emulsion-based products exist in the marketplace.

The chapter will critically review the relevant literature and will bring some new emerging improvements involving the stability and the control issues. Mechanistic considerations will be discussed and alternative ways to deal with the double emulsions concerns related to food applications will be evaluated.

II. 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 is separated (compartmentalized) from the outer liquid phase by a layer of another phase (1–8).

Several types of double emulsion have been documented. Some consist of a single, internal compartment, whereas others have many internal droplets and are known as ''multiple-compartment emulsions.'' The most common double emulsions are 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. Thus, more suitable and more accurate term for such systems should be ''emulsified emulsions.'' A schematic presentation of

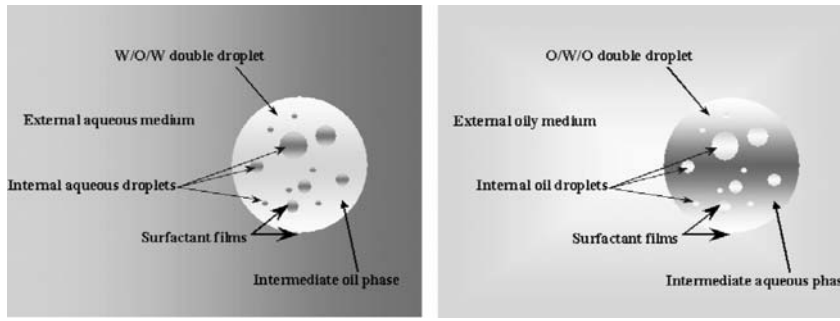

Figure 1 Schematic presentation of the two types of double-emulsion droplet. On the left is a typical w/o/w multiple droplet and on the right is a typical o/w/o multiple droplet.

the two types of double-emulsion droplets (w/o/w and o/w/o) is shown in Fig. 1.

Potential applications for double emulsions are well documented and many of these applications have been patented (9–15). In most cases, double emulsions are aimed for a 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 at removing 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 the protection of sensitive and active molecules from the external phase (antioxidation) have been recently mentioned (16–19). 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 the preparation of solid or semisolid microcapsules (20–22).

III. PREPARATION ROUTES: THE EMULSIFIERS

Double emulsions consist of two different interfaces that require two sets of different types of emulsifier. In o/w/o double emulsions, the first set of emulsifiers, for the internal interface, must be hydrophilic and the second set of emulsifiers, for the external interface, must be hydrophobic. For w/o/w double emulsions, the order of the emulsifiers is the opposite;

the inner emulsifiers are hydrophobic and 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 primarily w/o/w double emulsions, as most of the food 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 questionable in most cases. 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 was applied on the water which was added to the solution of the oil and the hydrophobic emulsifiers, to obtain stable w/o emulsion. In the second step, the w/o emulsion is gently added with stirring (not homogenization) to the water and hydrophilic emulsifier 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, of which two are interesting and worth being mentioned. The ''lamellar-phase dispersion process'' was reported by Vigie (23) (Fig. 3). The procedure is derived from the process employed to obtain liposomelike 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 some advantage because 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 a lamellar phase. When the lamellar mesophase exists, the hydrophile-lipophile balance (HLB) of the blend 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 10 wt%. Another drawback of this process is the weak control of the rate of encapsulation of the active substances.

Grossiord et al. (24) discussed an additional method termed the ''oily isotropic dispersion process'' by them. We prefer the more accurate term ''emulsified microemulsions.'' The idea is to disperse an oil phase within water by a surfactant and to form an L_2 phase (or water-in-oil microemulsion). 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 to multiple emulsions indeed. Moreover, there is no good evidence that the internal phase remains, after

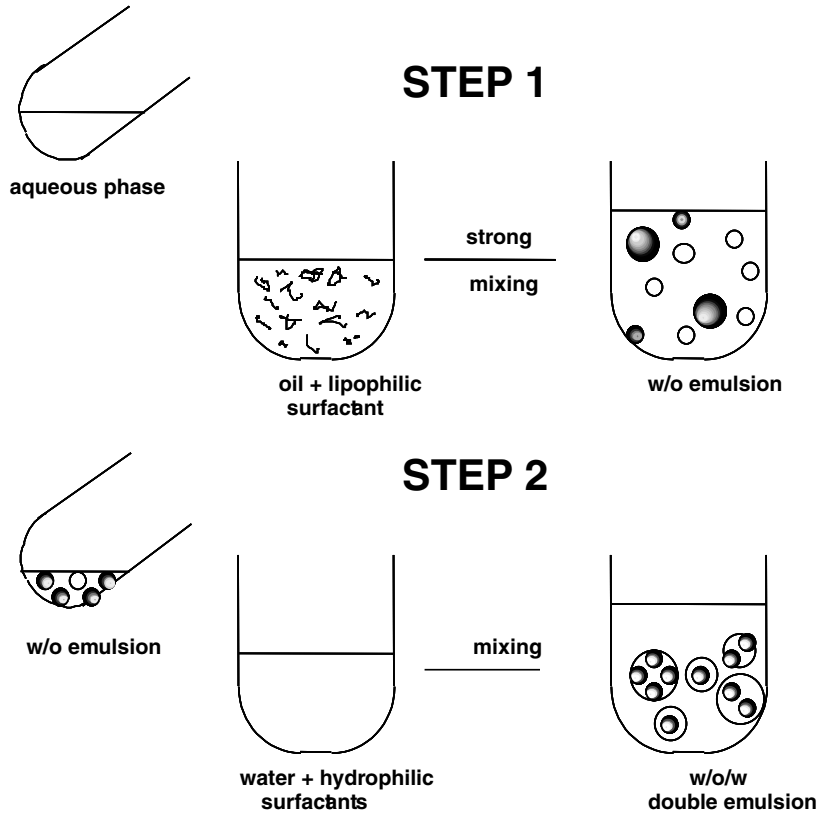

Figure 2 Schematic of a two-step process in the formation of a double emulsion.

the second emulsification process, a L_2 phase of a submicronal droplet in size. It seems that in some cases, the process is a well-characterized two-step emulsification that leads to relatively large double-emulsion droplets. The same concept of "emulsified microemulsion" was earlier reported by Pilman et al. (25) and also patented (10). This process is worth further investigation and should be more carefully evaluated. If one can prove that the internal compartmentalization is of a stable microemulsion, it might bring a breakthrough to this field because the sizes of the external droplets could be reduced to values below 1 μm. Such formulations will allow the formation of injectable double emulsions.

Gaonkar (10) claimed to have developed a completely new method in the preparation of double emulsions. This unique new type of preparation does not require any homogenization step and does not require the use of a

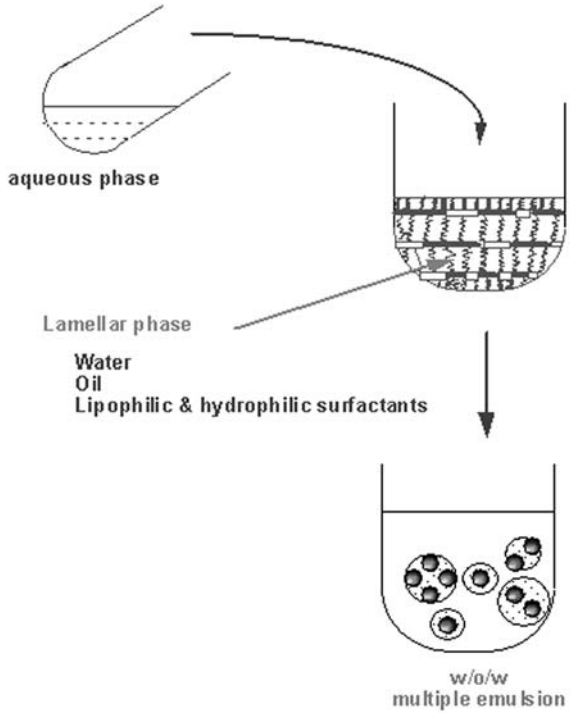

Figure 3 Preparation of water-in-oil-in-water multiple emulsion by lamellar-phase dispersion. (From Ref. 23.)

lipophilic emulsifier. In the method, a mixture of oil, water, a second alkyl containing a polar, protic solvent, such as methanol, ethanol, propanol, glycerol, propylene glycol, dodecanol, and their blend, and a hydrophilic emulsifier is prepared to form a o/w microemulsion. The o/w microemulsion is then diluted with sufficient water to cause destabilization of the microemulsion and to provide a w/o/w multiple emulsion.

Higashi et al. (26) described yet another 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 microdroplets 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, which is poured into the upper chamber of a special apparatus (Fig. 5). The external aqueous phase containing the hydrophilic emulsifier is continuously injected into the lower chamber to create a continuous flow. Nitrogen gas is fed into

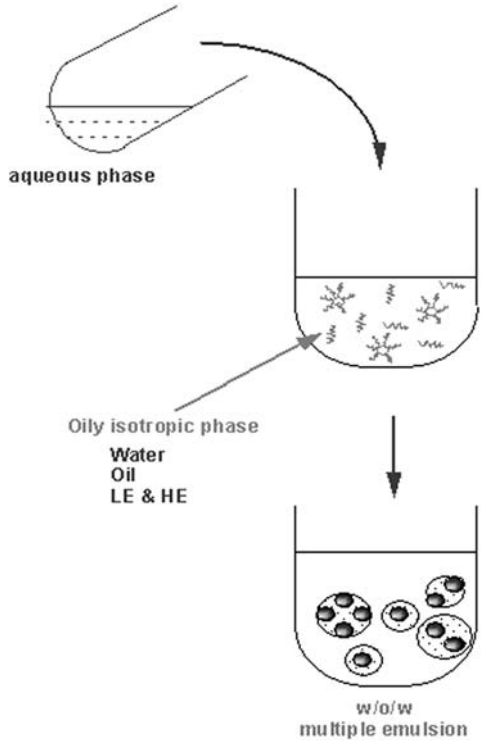

Figure 4 Preparation of water-in-oil-in-water multiple emulsion by "oily isotropic dispersion" (emulsified microemulsion). (From Ref. 24.)

the upper chamber, initiating a permeation of w/o through the controlled-pore glass membrane into the emulsifying chamber and forming a w/o/w multiple emulsion. The emulsion is progressively removed from the apparatus. This process was claimed to be used currently on an industrial scale.

It should be noted that low-molecular-weight emulsifiers migrate from the w/o interface to the oil phases and alter the required HLB of each of the phases. Most of the studies, in the years 1970–1985, searched for a proper monomeric emulsifier blend or combination (hydrophilic and hydrophobic) to be used at the two interfaces and the proper ratios between the two. Matsumoto and colleagues (1,27–33) established a minimum "magic" weight ratio of 10 of the internal hydrophobic to the external hydrophilic emulsifiers (Fig. 6). Garti and colleagues (34–39) proved that the free exchange between the internal and the external emulsifiers required a calculation of an "effective HLB value" of emulsifiers to optimize the stabilization of the emulsion. Complete parameterization work was done on almost

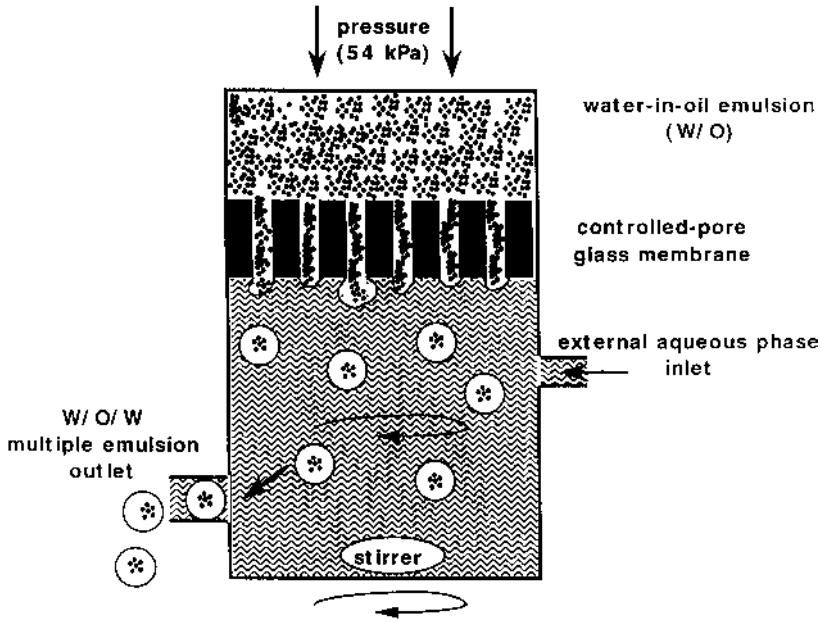

Figure 5 Preparation of water-in-oil-in-water multiple emulsion by a "membrane emulsification technique." (From Ref. 26.)

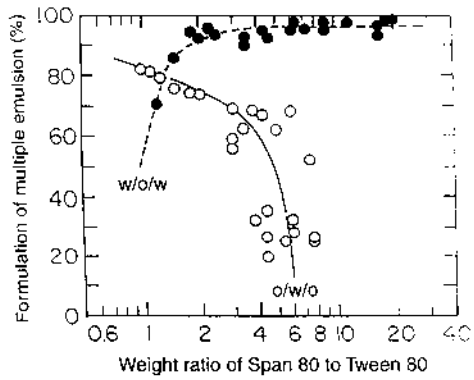

Figure 6 Yield of formation of double emulsions of w/o/w and o/w/o as a function of the weight/weight ratio of the internal hydrophobic emulsifier, Span 80, and the external hydrophilic emulsifier, Tween-80. (From Ref. 1.)

every possible variation in the ingredients and compositions (1–8). In most cases, the internal emulsifiers were used in great excess to the external emulsifiers. The nature of the emulsifiers also dictated 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 (40), modified or purified phospholipids (41), cholates, and so forth. The principles 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 stressed also that one must adjust the emulsifiers to the final application and must substitute one emulsifier by 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.) due 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 onto the interface, changing the delicate emulsifiers' HLB. The emulsifiers around the water or the oil droplets will not cover the droplets fully and the stability will be reduced.

IV. THE OIL PHASE

In food 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 (LCT), which are ''oxygen and hydrolysis sensitive'' by medium-chain triglyceride (MCT), which are fully saturated and thus oxidation resistant. Moreover, the MCT is easier to emulsify and requires less shear.

In cosmetic applications, the freedom to use 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 few examples. In addition, various waxes, sterols, and paraffin oils have been tried.

Several scientists 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.

V. STABILITY CONSIDERATIONS

Double emulsions made of low-molecular-weight emulsifiers (the so-called monomeric emulsifiers) are mostly unstable thermodynamically mainly because in the second stage of the emulsification, sever homogenization or shear are not recommended 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 droplets 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

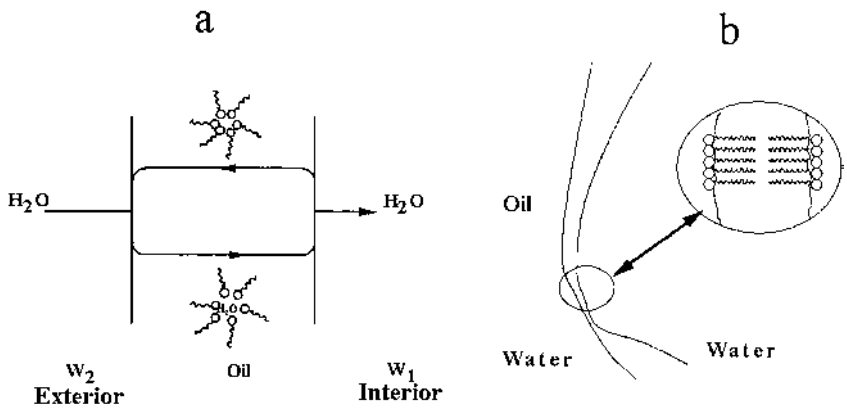

Figure 7 Schematic of two possible transport mechanisms: (a) reverse micellar and (b) lamellar thinning transport of a marker from the inner aqueous phase to the continuous aqueous phase.

of the emulsion worsens the situation and ended in a 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 engulfment of the internal droplets by the flow of water from the outer continuous phase to the inner droplets. Single- and multiple-compartment emulsions were prepared and evaluated in view of the enormous potential that these low-viscosity liquid systems have in the 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 parallel or simultaneously occurring phenomena of "reverse micellar transport" and coalescence.

All of 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 of the in-parallel pathways that take place in the double emulsions.

In a recent study, Ficheux et al. (42) identified two types of thermodynamic instability that are responsible for the evolution of double emulsions. Both mechanisms are in good agreement with the Bancroft rule (Fig. 8) but stress different aspects of the previously mentioned mechanisms. The mechanisms elucidated by the authors result in a 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" that is due to the rupture of the thin nonaqueous film that forms between the external continuous phase and the inner small water droplets. This instability irreversibly transforms a double droplet into a simple direct emulsion. Such a mechanism is suitable for delivery of entrapped water-soluble substances. The second mechanism was termed "coalescence between the small inner droplets within the oil globule." The first type of instability leads to a complete delivery of the small inner droplets toward the external phase, whereas the second one does not. The second mechanism leads to an increase of 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 due to the former instability is clearly related to the hydrophilic

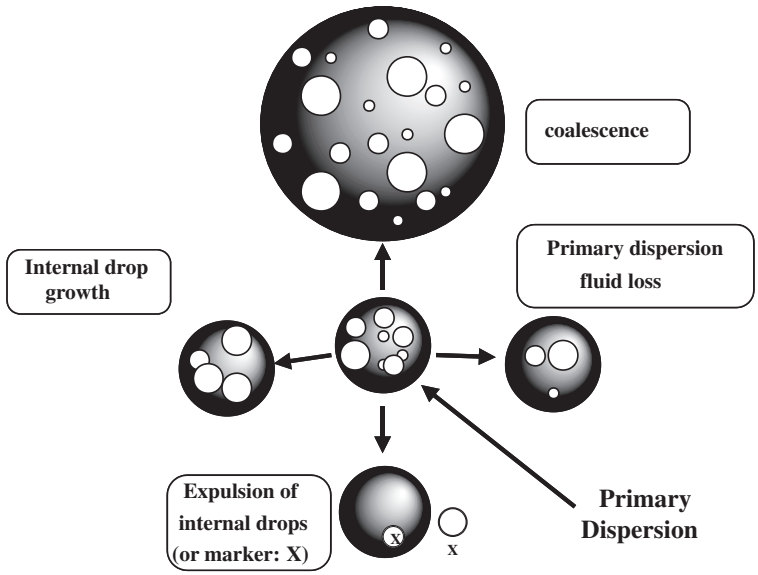

Figure 8 Schematic representation of the possible pathways for breakdown in multiple emulsions.

surfactant concentration in the external phase (Figs. 9a and 9b). Depending on the value of this concentration, double emulsions may be destabilized with a timescale ranging from several months to a few minutes.

Rosano et al. (43) 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 provided 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 correct salt concentration necessary to balance the osmotic pressure between the two water phases. In a set of experiments (Tables 1 and 2), they show a total of 15 formulations 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, and the others were made 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

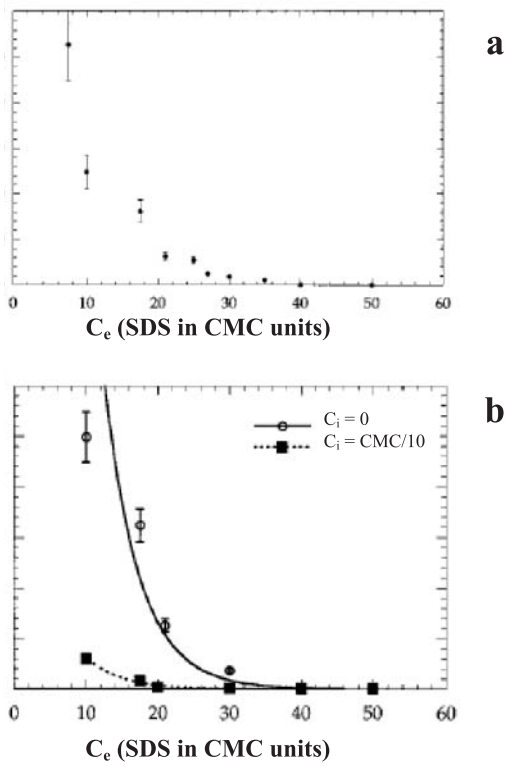

Figure 9 (a) Plot, at 20°C, of the lifetime τ, of internal droplets entrapped in the oil globules as a function of the external phase surfactant concentration C_e. The double emulsions are composed of 90% external phase and 10% double droplets. There is 10% water within the large double globules, 2% Span 80 was used within the oil, and SDS was used in the external water phase. (b) Influence of the internal surfactant concentration C_i on the $\tau = f(C_e)$ curve, at 20°C. System: Span 80/SDS as in (a). (After Ref. 42.)

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, the structure is still multiple, and a large increase in viscosity is observed (9500 cP for 0.07 wt% to 27600 cP for 0.4 wt% NaCl). The authors conclude that one must consider that three possible factors 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).

Table 1 The Role of the Concentration of Salt in the Inner W_1 Phase for Formation Made 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
Carbapol 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.

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

VI. RELEASE CONSIDERATIONS

Several attempts have been made to explain the transport phenomena of addenda and water from the inner to the outer phase of w/o/w double-emulsion droplets. It was demonstrated that for un-ionized lipid-soluble material dissolved in the oil phase, the release obeys first-order kinetics and is diffusion controlled with excellent accordance to Fick's law. However, ionized and un-ionized water-soluble materials (dissolved in the aqueous inner phase) can also be transported. Matsumoto et al. (27,31) have suggested two possible mechanisms for the permeation through the oil phase; the first being via the "reverse micellar transport" (Fig. 7a) and the second by "diffusion across a very thin lamellae" of the surfactant phase formed in areas where the oil layer is very thin (Fig. 7b).

Our studies (37–39) on the release of electrolytes in the presence of monomeric emulsifiers, have indicated that the transport takes place even if the droplets are very stable to coalescence and even when the osmotic pressure of the two phases has been equilibrated. The Higuchi approach and

Table 2 The Role of the Concentration of Salt in the Inner W_1 Water Phase for Formulations Made 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
Betaine	1	1	1	1	1	1	1	1	1
Carbapol 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.

model for the release of matter from polymeric matrices was adopted (44). A model was worked out and a modified release equation was offered and tested. The release factor B was plotted against the time, t, and reciprocal initial concentrations of the solute ($1/C_0$).

$$B = \frac{3}{2[1-(1-F)2/3]} - F = \frac{3D_e t}{r_0^2 C_0}$$

where D_e is the effective diffusion coefficient, r_0 is the radius of the outer phase droplets, F is the release fraction, and C_0 is the initial solute concentration.

The straight lines (with accordance with the above expression) and the excellent correlation coefficient obtained in these experiments indicate the existence of a ''diffusion-controlled release mechanism.'' It has been demonstrated that the water can flow into the inner w/o droplets if an osmotic pressure gradient is provided in the presence of hydrophobic emulsifiers present in the oil phase (32,33). It was also demonstrated that surfactant molecules, water molecules, and water-soluble addenda are transported at enhanced rates, even without the osmotic pressure gradient, in the presence of increasing concentrations of a lipophilic emulsifier added to the oil phase (37–39,44). Therefore, it has been concluded that the dominant diffusion-controlled mechanism is facilitated by the presence of reverse micelles formed at the oil phase. The mechanism was termed ''diffusion-controlled release mechanism of reverse micellar transport.'' Most of the release mechanistic studies in the literature involve double emulsions stabilized with synthetic polymeric amphiphiles and therefore, is of less interest for food applications. Nevertheless, these models of release patterns allow one to better understand the transport phenomena of entrapped addenda from the inner phase to the outer phase.

VII. STABILIZATION BY MACROMOLECULAR AMPHIPHILES

Macromolecules adsorb onto interfaces and facilitate more (or better) coverage than monomeric emulsifiers. The amphiphilic macromolecules form, in most cases, thick, flexible films which are strongly anchored into the dispersed and the dispersion phases. The adsorbed polymers are known to enhance steric stabilization mechanisms and were proved to be good emulsifiers in food colloids, primarily, in some food-grade oil-in-water emulsions. The use of macromolecular amphiphiles and stabilizers, such as proteins and polysaccharides, was long ago adopted by scientists exploring the stability of double emulsions. Gelatin (45,46), whey proteins

(47), bovine serum albumin (BSA) (48–51), human serum albumin (HSA), caseins, and other proteins were mentioned and evaluated. The proteins were used usually in combination with other monomeric emulsifiers (49). A significant improvement in the stability of the emulsions was shown when these macromolecules were encapsulated onto 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. Furthermore, from the release curves, it seems that the marker transport is more controlled. Dickinson et al. (50–53) concluded that proteins or other macromolecular stabilizers are unlikely to completely replace 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 substantial reduced.

The authors of this review (49) 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. 10). It was postulated that although BSA has no stability effect at the inner phase, it has a strong effect on the release of the markers (mechanical film barrier). On the other hand, BSA together with

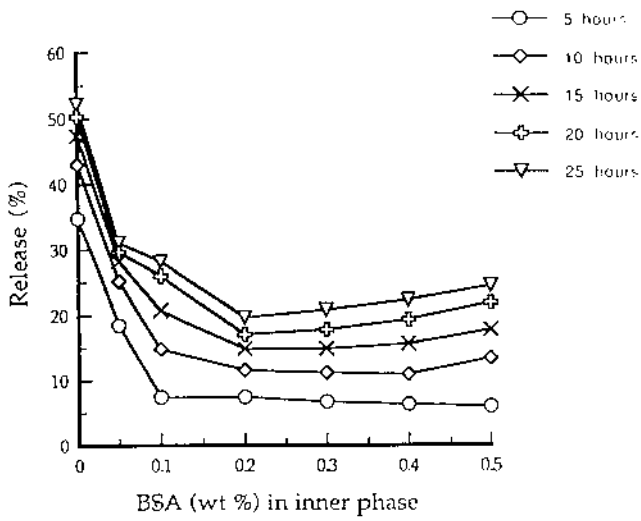

Figure 10 Percent release of NaCl with time from a double emulsion prepared with 10 wt% Span 80 and various BSA concentrations in the inner phase and 5 wt% Span 80 + Tween80 (1 : 9) in the outer aqueous phase. (From Ref. 49.)

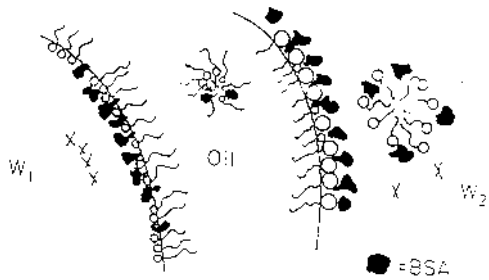

Figure 11 Schematic of a possible organization and stabilization mechanism of BSA and monomeric emulsifiers (Span 80) at the two interfaces of a double emulsion. (From Ref. 49.)

small amounts of monomeric emulsifiers (or hydrocolloids) serves as good steric stabilizers and improves stability and shelf life, and slows down the release of the markers. Therefore, BSA plays a double role in the emulsions: film former and barrier to the release of small molecules at the internal interface, and steric stabilizer at the external interface. The release mechanisms involving reverse micellar transport were also established (Fig. 11).

In more recent studies (54,55) 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 SDS 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 (55).

Cyclodextrins (α, β, and γ) were shown to be potential stabilizers for o/w/o emulsions (56). The advantages of the cyclodextrins are their ability to complex with certain oil components at the oil–water interface resulting in no need for additional surfactant. It appears that the stabilizer efficacy depends on the nature of the oil and the type of the 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 present at the interface, which changes the HLB and causes a destabilization effect. This elegant idea of interfacial complexation between the ''oil components'' and the surfactant cannot be a universal solution. The idea suffers from very severe intrinsic disadvantages once a 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 unfit.

VIII. STABILIZATION BY HYBRIDS OF BIOPOLYMERS

A. Protein–Polysaccharide Interactions in Aqueous Medium

Proteins, polysaccharides, and their blends, as examples of natural biopolymers, are surface-active materials. Under specific conditions (such as protein-to-polysaccharide ratio, pH, ionic strength, temperature, mixing processing), it has been stated that proteins and polysaccharides form hybrids (complexes) with enhanced functional properties in comparison to the proteins and polysaccharides alone. In the case of uncomplexed blends of biopolymers, competitive adsorption onto hydrophobic surfaces is generally reported. Conversely, electrostatic complexation between oppositely charged proteins and polysaccharides allows better anchoring of the newly formed macromolecular amphiphile onto oil–water interfaces (57).

Recently, the term ''water-in-water emulsion'' was employed to describe such dispersions when ''protein-rich aqueous aggregates'' are dispersed into a ''polysaccharide-rich aqueous medium'' (58). A mixture of gelatin and gum arabic solutions was reported (upon reducing the pH of the mixture) to separate in two phases (the upper phase very low in polymer content and the lower phase containing a highly concentrated precipitate of the two polymers). During the last century, many scientists developed models and theories to describe polymer interactions and phase separation (incompatibility) which occur when mixing two polymer solutions. The interactions between the polymers (protein–protein, protein–polysaccharide, or polysaccharide–polysaccharide) in solution and with the solvent (water in this case) will govern the solubility and cosolubility of the biopolymers, the viscoelastic properties of the final mixture, and even their behavior at different interfaces (solid–liquid or liquid–liquid).

Natural amphiphilic macromolecules or biopolymers are mainly based on proteins and polysaccharides. Attraction and repulsion are the two major types of interaction that occur between proteins and polysaccharides in solution and can result in complex formation or immiscibility of the two biopolymers (thermodynamic incompatibility). Owing to polyelectrolyte interactions in solution, these interactions and their consequences on the mixture will be strongly influenced by pH, ionic strength, conformation, charge density, and the concentration of the biopolymers.

In the aqueous solution, complex coacervation takes place between two oppositely charged polymers owing to electrostatic attraction. For instance, complexation between proteins and anionic polysaccharides occurs below the protein isoelectric point and at low ionic strengths (59). Factors that influence compatibility and complex formation are protein/polysaccharide ratio, pH, ionic strength (60–70), and the nature of the polymers (molecular weight, net charge, ternary structure, and flexibility

of chains) (70–75). Pretreatment of the polymer's solutions also enhances the complex formation. High-pressure (dynamic or hydrostatic) treatment as well as temperature have been reported to affect the stabilization of the newly formed complexes (64,76–79).

Phase diagrams could be built to describe the interactions for each biopolymer pair (Fig. 12, right). Two phase-separation phenomena can be observed, depending on the affinity between the biopolymers and the solvent. When the Flory–Huggins parameter, χ_{23}, which describes the protein–polysaccharide interactions, is positive, indicating a net repulsion between the two biopolymers, segregative phase separation (thermodynamic incompatibility) is generally observed. Solvent–biopolymer(s) interactions are favored to the detriment of biopolymer–biopolymer and solvent–solvent interactions, and the system demixes in two phases, each of which contains both of the two biopolymers. When the protein–protein or polysaccharide–polysaccharide interactions are sufficiently high in comparison to the polymer–solvent interactions (i.e., $\chi_{23} < 0$), congregative phase separation (or coacervation) is observed, leading to an upper solvent-rich phase and a lower biopolymer-rich phase forming the so-called "coacervate." This is possible at a pH higher than the isoelectric point (IEP) of the protein, when both the protein and the polysaccharide carry the same charge (in the case of a negatively charged polysaccharide). By reducing the pH of the mixture below to the IEP of the protein, the interactions between the two oppositely charged biopolymers are favored ($\chi_{23} < 0$) and complexation takes place. The newly formed protein–polysaccharide complex could be soluble or can lead to an aggregative phase separation (region II, Fig. 12). The Flory–Huggins theory presents some limits to the protein–polysaccharide interactions in aqueous medium-modified models have been established to describe these systems in the case of globular proteins and colloidal protein particles (80,81).

Complexation between two charged biopolymers is usually a reversible process depending on pH and ionic strength. At high ionic strength (0.2–0.3) or at pH values above the protein IEP, the complex will dissociate. However, electrostatic interactions between anionic polysaccharides and positively charged proteins can occur at a pH above the IEP of the protein.

B. Protein–Polysaccharide Interactions in Emulsion

Protein–polysaccharide interactions play a significant role in the structure and stability of many processed foods. The control of these macromolecular interactions is a key factor in the development of novel food processes and products as well as in the formulation of fabricated products. Much was written on the possible interactions between proteins and hydrocolloids in

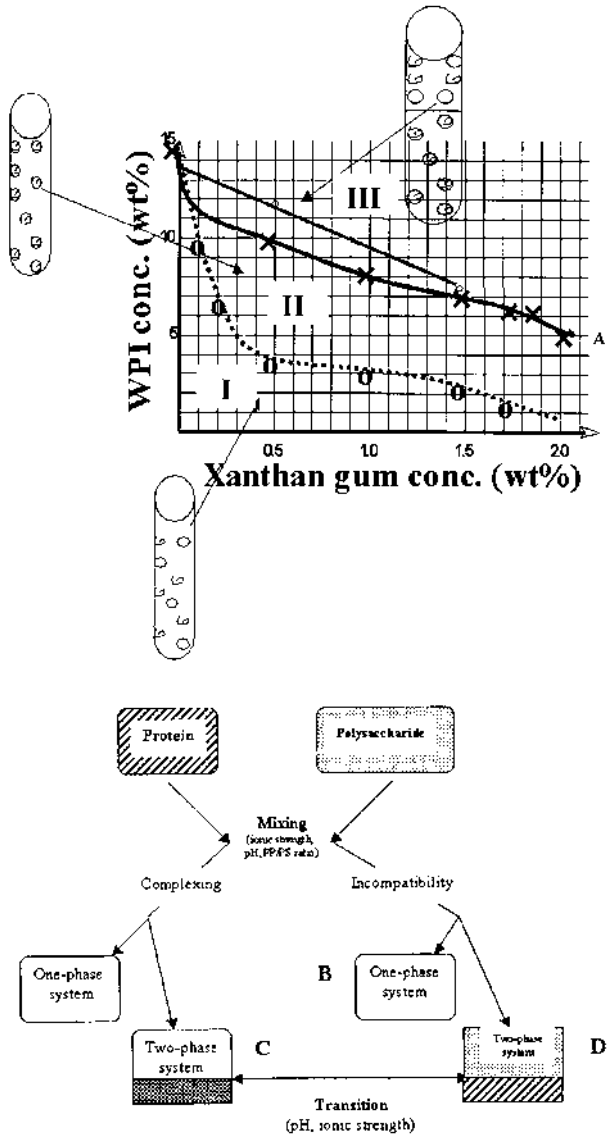

Figure 12 Phase diagram of water–whey protein isolate (WPI)–xanthan gum mixtures (top) which describes the different types of interaction between the protein and the polysaccharide in an aqueous medium (bottom). Binodal curves (solid line) delimit the boundaries between one phase region (below) and two separated phases region (above). Region II corresponds to protein–hydrocolloid hybrids formation.

model and real systems but very little advantage was provided by these studies in designing new amphiphilic molecules based on these hybrids. The chemically bound structures as well as the hydrogen-bound associates or even the ion–dipole or dipole–dipole associates form a well-balanced amphiphilic structures which can be utilized in fabricated emulsions and can replace the synthetic low-molecular-weight emulsifiers. These associates will not require any additional legislation (i.e., FDA) and will have textural and stability advantages. If one can add some nutritional or health benefits to it (and the hydrocolloids have shown such effects), the fabricated emulsions, food products, or food processes will become more attractive to the food producers.

Dickinson and colleagues claimed (82,83) that the best way to adsorb hydrocolloids onto interfaces is to link them to proteins. The proteins are surface-active materials consisting of flexible hydrophobic and hydrophilic moieties, preferentially adsorbing onto the interfaces and replacing the hydrocolloids from the surface. A hydrocolloid that is thermodynamically incompatible with an adsorbed protein (58,60) can be distributed at the interface only if it interacts somehow with the protein. Its distribution on the interface will depend on the nature of these interactions. Strong chemical bonds will cause a different surface distribution rather than weak associations between the two.

It was also recognized that polysaccharides could interact at the interface with other polymers (nonproteins, other polysaccharides) as well as with groups residing at the interface (protein–water, oil–water, or air–water) with the formation of an aqueous structured material with useful viscoelastic mechanical properties under conditions of low shear stress. Understanding the detailed chemistry of these polymer–polymer interactions and relating them to the observed rheology is a significant aspect of the study of hydrocolloids.

Complexation between proteins and polysaccharides at the emulsion droplet surface can improve steric stabilization. Droplet size can be smaller if the polysaccharide is present during homogenization, and, therefore, the rate of creaming may be reduced so long as there is no bridging flocculation. Covalent protein–polysaccharide complexation can also provide effective emulsion stabilization. The great improvement in stability arising from the presence of the polysaccharide during emulsification is attributable to the formation of a thicker, stronger steric-stabilizing layer around the droplets.

Tolstoguzov et al. (84) compared the surface properties of BSA and BSA–dextran complexes and the stability of *n*-decane-in-water emulsions prepared with them was evaluated by measuring the volume of the coexisting phases obtained after phase separation, under centrifugation (50 min at

23,000 *g*). Complete phase separation occurred for emulsions prepared with 0.2 wt% BSA. When BSA–dextran complexes were used (pH 6.0, 0.3 wt% biopolymer concentration), only 40% of the decane separated after centrifugation. Because the emulsification properties of the complexes were strongly dependent on pH and ionic strength, the authors attributed the emulsification power of BSA–dextran complexes to the electrostatic nature of the interactions. Even though both biopolymers carry a net negative charge at pH 7 (BSA–dextran sulfate, 1 : 3 by weight), a soluble ionic complex can be formed via local electrostatic interaction between the highly charged anionic polymer and positively charged patches on the globular protein. Surface shear viscosity measurements give independent evidence of an interfacial complex between BSA and dextran sulfate.

A similar synergistic effect of protein–polysaccharide complexes in stabilizing emulsions has been reported with corn oil–water emulsions prepared with casein–acidic polysaccharide complexes (85). For a pectin-to-total biopolymers ratio >0.2, an emulsion stability index of 100% was obtained corresponds to zero creaming of the emulsion after centrifugation for 30 min at 3000 *g*. Adsorbed complexes at the oil–water interface increased the interfacial viscosity, forming a gellike structure around the oil droplets. Moreover, these emulsions exhibited relatively high thermal stability at 95°C because of the gellike microstructures at the interface. Emulsions prepared with soy proteins–sodium alginate complexes at pH 7.0 and for 10 : 1 and 4 : 1 protein-to-polysaccharide ratios (86) exhibited improved thermal stability in comparison to soy protein-stabilized emulsion. The emulsion activity index (EAI) values were 0.801 and 0.953 for a control emulsion (standard emulsion stabilized with soy protein) and for an emulsion prepared with soy proteins–alginate complexes (protein-to-polysaccharide ratio of 4 : 1), respectively. The improvement of the emulsion stability and activity indices was attributed to the unfolding of the soy protein at the interface that enhances protein–polysaccharide interactions by exposing cationic groups and hydrophobic groups. The newly exposed hydrophobic groups could alter the complex surface properties by providing additional sites for binding lipids, and that allows better anchoring of the amphiphilic macromolecules into the oil phase, resulting in an enhanced emulsion activity and stability indices.

Nevertheless, under specific conditions of pH, ionic strength, and polysaccharide concentration, protein–polysaccharide complexes in emulsions could contribute, in some cases, to a decrease in the emulsion stability. Dickinson and Pawlowsky (87) envisaged the flocculation, creaming, and rheology properties of *n*-tetradecane-in-water emulsions stabilized with BSA–ι carrageenan complexes. The authors envisaged first the surface properties of soluble complexes by determining the evolution in time of the

surface tension [$\gamma(t)$] at different pH values (Fig. 13). BSA always provided lower γ values at any pH. The higher values of γ obtained with BSA–ι-carrageenan complexes have been attributed to a diminution of the number of free BSA molecules at the interface owing to complex coacervation, to a smaller diffusion coefficient as a result of complex sizes, and to increased viscosity of the solution. The electrostatic nature of BSA–ι-carrageenan complexes was clearly shown and could explain the dependence of the emulsion stability on pH and ionic strength. A relatively poor stability of the emulsion was described for polysaccharide concentrations above 0.01 wt% and was attributed to bridging flocculation of the oil droplets. At high carrageenan content (>0.1 wt%), stability against creaming of the emulsions was increased by the formation of a polysaccharide cross-linked network droplets with gellike rheological properties.

Covalently linked complexes have also shown emulsification capability (88). Because such complexes did not present much interest for food applications, we describe them succinctly. Oleic acid–water emulsions prepared with covalent linked β-lactoglobulin–carboxymethyl dextran conjugates were unaffected by heat treatment up to 80°C, demonstrating the heat stability of the complexes. Dickinson et al. (89) compared the influence of

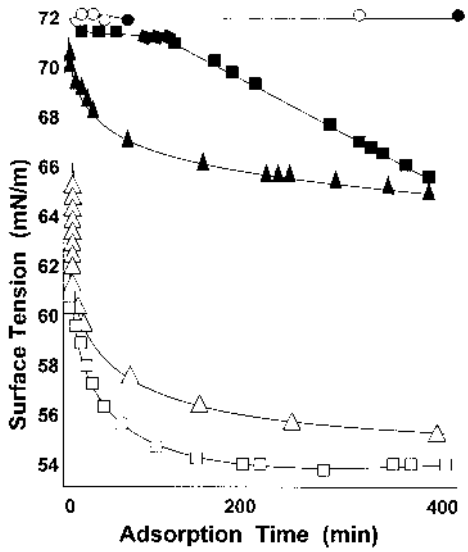

Figure 13 Time-dependent surface tension (γ) of biopolymer solution (5 mM imidazol, 25°C): ○ and ● pH = 8.0; △ and ▲ pH = 6.5; □ and ■ pH = 6.0. Open and closed symbols refer respectively to 10^{-3} wt% BSA and 10^{-3} wt% BSA + 4.10^{-3} wt% ι-carrageenan. (From Ref. 87).

ionic and covalent protein–polysaccharide interactions on 10 wt% *n*-hexane-in-water emulsions stabilized with BSA, nonionic dextran, and anionic dextran sulfate at neutral pH (Fig. 14). Both BSA–dextran conjugates (curve A) and BSA–dextran sulfate conjugates (curve C), yielded stable emulsions with droplet diameters that remained below 1.5 μm after 3 weeks standing at 25°C. Similar emulsion stability was obtained using a BSA–dextran sulfate mixture (curve D). On the other hand, emulsions prepared with a BSA–dextran mixture were unstable. Conversely conjugating or mixing BSA with amylopectin was not sufficient to stabilize emulsions (curves E and F).

Several scientists envisaged the preemulsification process influence on the emulsifying properties of protein–polysaccharides complexes (90–93). Hydrostatic pressures and preheating of the biopolymers suspensions have been often reported as a useful tool to modify and, to some extent, to enhance the emulsification power of these mixtures.

Heating was reported to increase the surface hydrophobicity of globular proteins such as ovalbumin, but a decrease in surface hydrophobicity was also demonstrated for BSA and β-lactoglobulin (88,91). Conversely, the hydrophobicity of 11S *Vicia faba* globulin was significantly increased by heating the native protein solution to 80°C for 2 min (93). When polysaccharide (i.e., ι-carrageenan) was added to untreated 11S *Vicia faba* globulin or heated at 80°C for 2 min, the surface hydrophobicity of the protein

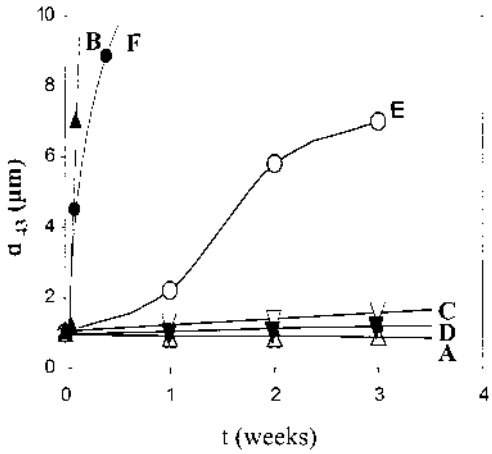

Figure 14 Average droplet diameter d_{43} as a function of time *t* of emulsions stored at 25°C and stabilized with: (△), BSA-dextran conjugate (curve A) (▲), BSA dextran mixture (curve B) (▽), BSA–dextran sulfate conjugate (curve C); (▼), BSA dextran sulfate mixture (curve D); (○), BSA-amylopectin conjugate (curve E); (●), BSA amylopectin mixture (curve F). (From Ref. 89).

remained unchanged. The temperature treatment of the protein solution induced extensive precipitation, whereas the mixed solution remained clear. It was concluded that ι-carrageenan inhibited the aggregation of the protein molecules by protecting protein-reactive sites.

The influence of temperature treatment on the emulsifying efficiency of 11S *Vicia faba* globulin pure or mixed with ι-carrageenan or κ-carrageenan in 20 wt% *n*-tetradecane-in-water emulsions was also studied (93). Heat treatment was reported to lower the emulsifying power of the protein. To the contrary, polysaccharide addition (protein to polysaccharide ratio of 3.3 to 1) leads to emulsions with smaller droplets at all temperatures. Mixed emulsions prepared with ι-carrageenan (high content of sulfate groups) have been found to be more stable against creaming than those prepared with κ-carrageenan (less sulfated). The average droplet diameters of emulsions were of 5.7 and 8.0 μm, respectively, for mixtures heated for 2 min at 80°C. High-pressure treatment affects significantly the surface hydrophobicity of proteins and protein–polysaccharide blends (90–93). High pressure induces subunit dissociation (quaternary structure) and, to some extent, unfolding of the globular subunits which result in the exposure of new hydrophobic groups within the protein molecules (93). Polysaccharide addition reduced the surface hydrophobicity of the pressurized protein by blocking the hydrophobic site onto the protein surface. In spite of reduced hydrophobicity, mixtures of pressurized κ-carrageenan–protein and ι-carrageenan–protein lead to the formation of stable emulsion with respect to creaming. The same trend as for temperature treatment was observed. The droplet sizes of emulsions prepared with the ι-carrageenan–protein mixture were smaller than those prepared with the κ-carrageenan–protein blend. Conversely, increasing the applied pressure on the protein solution yielded emulsions with larger droplets. This was attributed to protein aggregation by disulfide binding between free-cystein residues.

C. Double Emulsion Stabilized with Protein–Polysaccharide Hybrids

Recently, we have envisaged the stabilization of double emulsions with some ''molecular-recognition'' hybrid of whey protein isolate (WPI) and different charged and uncharged polysaccharides (7,94). The emulsifying power of WPI–xanthan gum hybrids was studied. It was found that WPI–xanthan ratio strongly influences the formation and stability of the droplets (Fig. 15). At low protein content (0.5–2 wt%), the droplet size decreases with time due to internal aqueous-phase expulsion. This was attributed to the weakness of the emulsifier film adsorbed onto the external oil–water interface. Microscopic observation revealed that for a WPI–xanthan gum

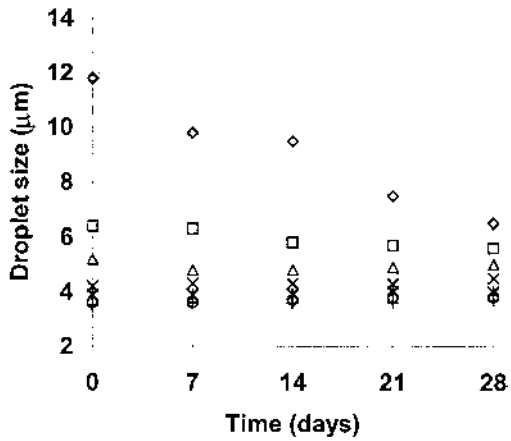

Figure 15 Double-emulsion droplet stability with time at room temperature prepared with 0.5 wt% xanthan gum and increasing amount of WPI as an external emulsifier: 0.5 wt%, ◇; 1 wt%,□; 2 wt%,△; 3 wt%, ×; 4 wt%, ∗; 5 wt%,○; 6 wt%,+.

ratio of 0.5/0.5 (w/w), double-emulsion droplets inverted to simple o/w droplets (empty of internal globules) after aging for 28 d at room temperature. At higher protein-to-polysaccharide ratios (3/0.5 to 6/0.5, w/w) double-emulsion droplets exhibited increased stability against creaming and coalescence. Double emulsions were prepared with different whey protein–xanthan ratios. Increasing the xanthan content to 1 wt% decreases the droplets diameter from 11.8 to 3.1 μm (Fig. 16a). It was found that by increasing the protein-to-polysaccharide ratio (region II of the phase diagram), double-emulsion droplets with reduced droplet size and increased stability are formed.

One can conclude that the binodal boundary line corresponds to the limit of complexation of the two biopolymers that will give optimum stabilization. At biopolymer content higher than the binodal composition, the addition of one of the two biopolymers will only slightly affect the double–emulsion droplets' stability and will only add a small depletion stabilizing effect to the emulsion droplets.

1. Rheology

Rheological measurements were performed on double-emulsion droplets. The phase angle, δ, was defined as $\arctan(G''/G')$, where G′ is the storage modulus, G'' is the loss modulus, and $\tan(\delta) = G''/G'$. It was found that at

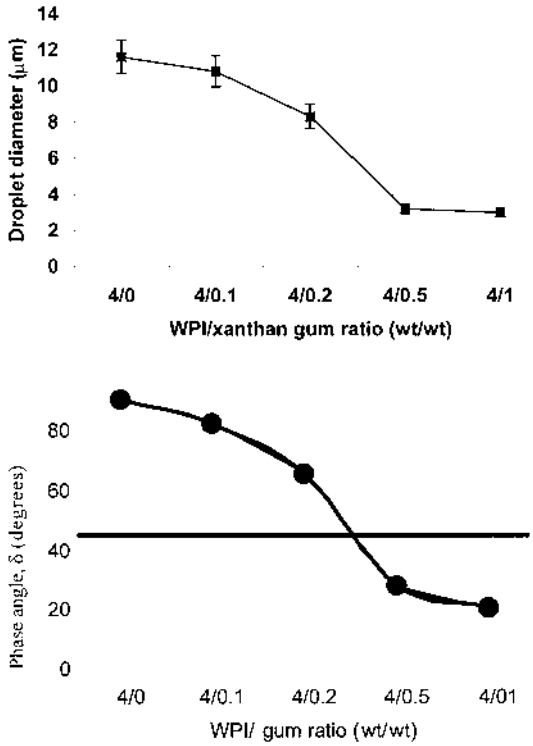

Figure 16 (a) WPI–xanthan gum ratio (w/w) influence on the droplet diameter (μm) of double-emulsion droplets, immediately after preparation. (b) WPI–xanthan gum ratio (w/w) influence on the phase angle δ (degrees) of double-emulsion droplets, immediately after preparation. The phase angle, δ, was defined as $\arctan(G''/G')$, where G' is the storage modulus, G″ is the loss modulus, and $\tan(\delta) = G''/G'$.

low levels of gum (0.1 wt%), the emulsions had high viscosity, with the phase angle (δ) close to 90°, indicating self-assembly of the two biopolymers onto the external oil–water interface. Therefore, the emulsions are stabilized mainly by steric interactions between the ''macromolecular-recognition hybrids'' adsorbed onto the oil. To the contrary, at a high level of gum (0.3–1 wt%), the emulsions exhibit more elasticity, regarded as a physical property derived from a depletion stabilization mechanism. The protein will preferably adsorb onto the oil–water external interface and the uncomplexed gum will migrate to the bulk and contribute to the stabilization by a depletion mechanism (Fig. 16b). The protein-to-gum ratio of 4/0.5, at which complexation between the protein and the gum takes place,

corresponds to an intermediary viscoelasticity of the system. Microscopic observations and zeta-potential measurements performed on the samples after rheological studies revealed that the emulsion droplets are still multiple droplets and that interactions between the biopolymeric molecules remained unchanged.

At a gum concentration of 0.5 wt%, the protein concentration does not affect the rheological behavior of the double emulsion that conserves its elasticity properties at all ratios with phase angle (δ) values around 25° at all protein contents. At the same time, the yield of preparation of double emulsions prepared with increasing amounts of protein and at 0.5 wt% xanthan gum was 64% for double emulsions stabilized with 6 wt% WPI and increased to 96% with the addition of 0.5 wt% xanthan gum.

2. Effect of pH on Double-Emulsion Stability

The pH is known to influence strongly the net harge of proteins. Therefore, it was important to qualify and to quantify its effect on the protein–polysaccharides interactions in solution and at the oil–water interface in double emulsions. The zeta potential of both solutions and emulsions was determined (Fig. 17). For the WPI–xanthan gum system, the pH affects the protein, the gum, and the combination of the two. The blends behave as the protein alone with very close isoelectric points to pure protein. Once the hybrid is adsorbed onto the oil droplets, the situation is significantly different. The isoelectric point of the protein-stabilized emulsion decreases, indicating unfolding of the protein and screening of its charged residues during

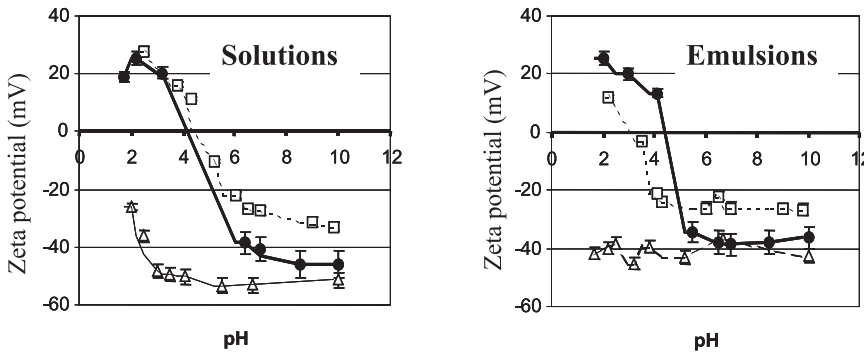

Figure 17 Zeta potential (mV) of solutions (left) and of multiple emulsions (right) prepared with 4 wt% WPI (□), 0.5 wt% xanthan gum (△), and the blend of the two at a weight ratio of 4/0.5 (○).

the emulsification process. At a low pH, one can conclude that the protein is dominant at the interface, and at a high pH, the gum is the dominant one at the interface. In other words, when the pH is reduced to below the isoelectric point of the blend, the steric stabilization is dominant, and when the pH increases, the system is mainly stabilized by a depletion mechanism. The pH also influences strongly the rheological behavior of double emulsions. At a pH below the isoelectric point of the blend [WPI–xanthan gum (4/0.5)], 4.8, the phase angle, δ, is higher than 45°, indicating that the emulsion is viscous and confirming steric stabilization mechanism for these systems. Conversely, at a higher pH (5–10), the system is predominantly elastic (δ close to 25° in all the pH range), indicating a depletion stabilization mechanism. Microscopy observations of emulsion stabilized with a WPI–xanthan gum mixture (4/0.5) at pH 7 reveals a weakness of the film around the oil globules, indicating the presence of uncomplexed biopolymers at the interface. It was concluded that at a pH below the isoelectric point of the blend, the two biopolymers strongly interact and yield fully covered double-emulsion droplets. When the pH of the external aqueous phase increases, at a constant WPI–xanthan gum ratio, the electrostatic interactions between the protein and the gum become weaker around the oil droplets and the ''biomolecular-recognition hybrid'' film becomes less rigid.

3. Entrapment in Double Emulsions (Vitamin B_1)

Much work was done on the entrapment of addenda in double emulsions, but only a few reports have shown promising results on real systems. In this subsection, we will bring a more recent example drawn from the authors' work.

The formulations stabilized with the mixture of WPI and xanthan gum were used for entrapping vitamin B_1 in the inner aqueous phase. A useful electrochemical method was developed to quantify the amount of vitamin B_1 present in the external aqueous phase. Differential pulse polarography based on the redox properties of the vitamin when it is present in the external aqueous phase allowed the determination of its release profiles under various conditions of pH, protein-to-polysaccharide ratio, and initial concentration of the entrapped addenda. The release profile, as a function of the pH of the external aqueous phase (Fig. 18) reveals that at pH 2, the external interface is better sealed against the release of the entrapped vitamin than at pH 4 and 7. It reconfirmed that there is an extra stability of these double emulsions derived from both electrostatic and steric mechanism stabilization of the external oil–water interface.

At pH 3.5, the increase in the protein-to-polysaccharide ratio also reduces the release rate. As was demonstrated earlier, at high hydrocolloid

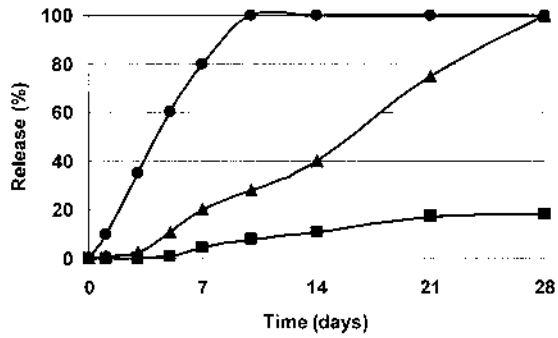

Figure 18 Effect of the external aqueous phase pH on the release profile of vitamin B_1 from multiple emulsions stabilized with WPI–xanthan gum (4/0.5) as the external emulsifier (pH 7,●; pH 4,▲; pH 2,■).

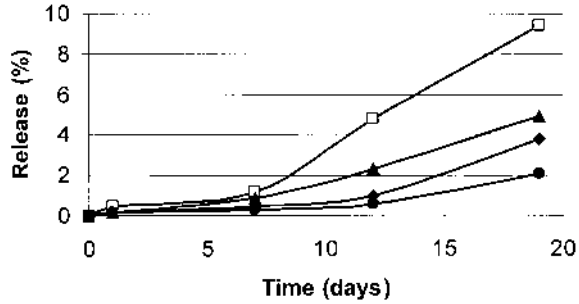

Figure 19 Release profile of vitamin B_1 from multiple emulsions at different WPI–xanthan ratios as the external emulsifier at pH 3.5 (4/0, □; 4/0.1, ▲; 4/0.2, ◆; 4/0.5, ●).

content, the system is stabilized by both electrostatic and steric mechanisms that improve the rigidity of the film around the double-emulsion globules and so delay the vitamin release (Fig. 19).

IX. STABILIZATION BY SOLID PARTICLES

To assist in the stabilization of emulsions, particles (such as fat crystals) must be adsorbed at the emulsion droplet interface, providing a physical barrier to coalescence. It is known that in many emulsified foods, solid particles are necessary for producing stabilization (e.g., ice crystals in ice cream, egg yolk particles in mayonnaise, and fat particles in whipping

cream). The key factors that will determine the influence of fat crystals on emulsion stabilization are (1) the wettability of the crystals at the interface, (2) interfacial film rheology, (3) particle microstructure (polymorphism and morphology), and (4) location of fat crystals [(in the dispersed (o/w emulsion) or continuous phase (w/o emulsion)] (95).

The wetting behavior of particles at the interface is described by contact angles, which are related to the surface tension of each of the three interfaces by Young's equation: $\gamma_{(o/w)}\cos\theta = \gamma_{(o/s)} - \gamma_{(w/s)}$, where θ is the contact angle measured through the water phase and $\gamma_{(o/w)}$, $\gamma_{(o/s)}$, and $\gamma_{(w/s)}$ are the surface tensions of the oil–water, oil–solid, and water–solid interfaces, respectively. $\cos\theta\,\gamma_{(o/w)}$ is also known as the adhesion tension. Modification of the contact angle (and, therefore, emulsion stability) can by achieved by a modification of the aqueous, oil, or solid phase so as to alter $\gamma_{(o/w)}$, $\gamma_{(o/s)}$, or $\gamma_{(w/s)}$.

In Fig. 20, where water acts as the continuous phase and oil as the dispersed phase, the colloidal particle is partially embedded within the interface at an equilibrium distance dictated by the interparticle forces. With a contact angle less than 90° at the solid–water–oil interface across the water phase, the particle will stabilize o/w emulsions. With contact angles greater

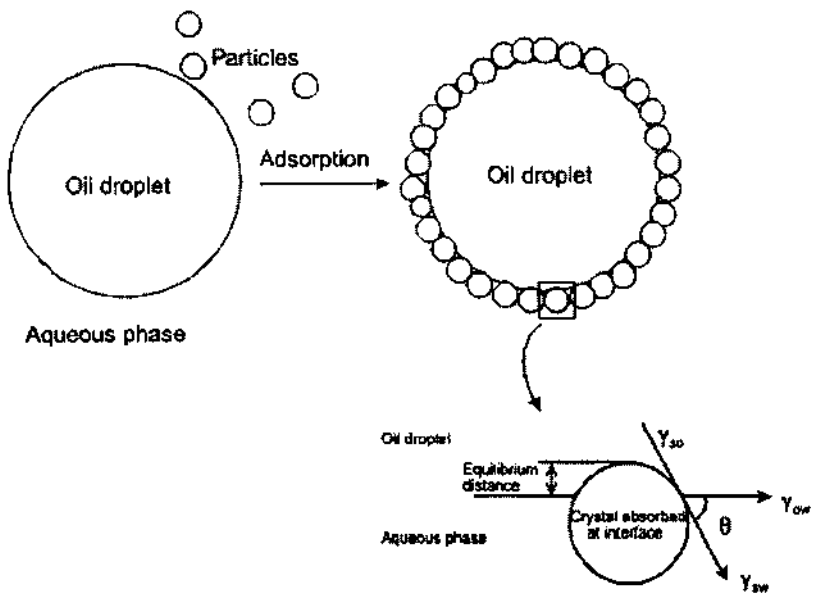

Figure 20 Adsorption and contact angles of fat crystals at the interface of an oil-in-water emulsion.

than 90°, the particles will stabilize a w/o emulsion. If the particles used are completely wetted by either the oil or water phase (and, therefore, the contact angle is 180°), they become fully dispersed in that phase and any emulsion-stabilizing effect is negated. A 90° contact angle means that a crystal is equally wetted by the oil and aqueous phases.

Stabilization of margarine and other similar food emulsions is achieved by partial adsorption of solid fat particles (β′-polymorphs) onto 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 recently reexamined and reconsidered by Bergenstähl (96,97) 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 (98), showing that colloidal microcrystalline cellulose (CMCC) particles can adsorb in a "solid form" onto oil droplets at the interface of water–oil emulsions and thus improve their stability by mechanical action. Oza and Frank (98) tried the mechanical stabilization concept on double emulsions. The 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 on double emulsions. In a most recent article, Khopade and Jain (99) 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 double 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.

Garti et al. (100) carried out several experiments with micronized particles of the α- and β′-polymorphs of tristearin fat together with polyglycerol–polyricinoleate (PGPR) as the internal emulsifiers in double emulsions. Solid fat particles did not sufficiently stabilize the water-in-oil emulsion, and, similarly, the PGPR (at the concentrations used in the formulation) did not provide good stability. However, it was shown that the use of the blend of the two components composed of a solid submicronal fat particles of α- and β′-polymorphs (which are more hydrophilic than the β-form and thus wet the oil–water interface better) "precipitate" onto 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 onto the water–oil interface (the w/o emulsion) (Fig. 21). The authors' interpretation of the results is that "the fat particles adsorb onto the hydrophobic emulsifier film and both, the solid particles and the emulsifier, wet the water and spread at the interface." The double emulsions

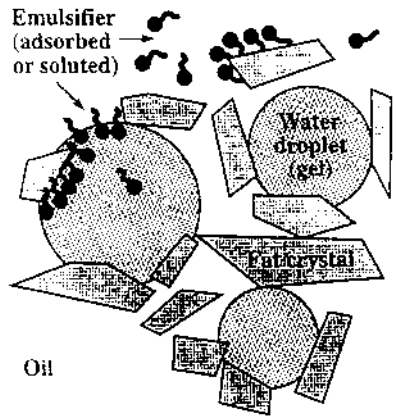

Figure 21 A Schematic of a colloidal margarine structure demonstrating the role of emulsifiers and fat crystals in stabilizing the (w/o) emulsion of margarine by colloidal fat crystals. (From Ref. 100.)

prepared by this technique were more stable than those prepared by monomeric emulsifiers.

Organophilic montmorillonite is an interesting clay that gained some interest in the 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 (101). The montmorillonite was added in the second step at the outer w/o/w interface. The droplets sizes decreased with the increase of the HCO-60 (0.1–3 wt%) concentration. The viscosity of the double emulsion increased as the concentration of the montmorillonite, and DIS-14 increased, indicating that the excess amount of the 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 stable o/w/o emulsions because the viscosity of the double emulsion is so high that the formulation becomes semisolid.

X. STABILIZATION BY INCREASED VISCOSITY

Most food emulsions are highly complex systems both in terms of composition and structure (102). To control the formation, the stability and rheology of food emulsions require an understanding of the interactions between the various elements present in the system. Recently, progress has been made to understand the interaction behavior at the o/w interface between

some of the components found in food emulsions, particularly between different proteins and surfactants, and, to a lesser extent, between proteins and fat crystals. Because fat crystal interactions at the interface are affected by protein, and protein interfacial behavior is affected by small-molecule surfactants, it is important to examine how all of these components interact. As with bulk emulsion properties, the interface can exhibit viscous, elastic, and viscoelastic properties. The ability of an emulsion to resist coalescence will largely depend on the properties of the interface. A highly viscous and rigid interfacial film will retard the rate of film drainage and resist rupture, thereby promoting stability (103). Hence, by controlling interface rheology, one can control the drainage of thin liquid films trapped between coalescing droplets, which may also affect the displacement of crystals away from the interface during droplet coalescence. Kiosseoglou (104) discussed the role of surface-active lipids at the o/w interface on the viscoelastic parameters of BSA, sodium caseinate, and egg yolk films during adsorption at the olive oil–protein solution interface. It was postulated that film viscoelasticity was the result of surface-active lipids interacting with folded and unfolded proteins at the interface.

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 phase through the oil membrane. Attempts were made to do the following: (1) 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 because the entrapment is not quantitative and the yields of entrapment are limited and are emulsifier dependent, (2) increase the viscosity of the oil phase (fatty acids salts); (3) increase the thickening or gelling of the external water by gums. This process is limited to cosmetic (or similar) applications in which semisolid emulsions are directly applied (Tables 3 and 4). Some of these examples are topical skin care products, creams, and body lotions (105,106).

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 sub-zero of ca $-20^{\circ}C$ to ca $40^{\circ}C$) during transport or storage. Clausse et al. (107,108) studied the phenomena in w/o/w emulsions by microcalorimetric [differential scanning calorimetry (DSC)] techniques. It was concluded that, because 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

Table 3 Example of w/o/w Multiple Emulsion Stabilized by a Calcium Alginate Gel Layer at the Internal Water Oil Interface

	Hexaglycerol mixed ester	1%
	CSL (calcium stearyl-2-lactylate)	0.75%
Oil phase	Soybean oil	23.25%
Internal aqueous phase	27% Solution 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 4 Composition for Low-Fat Spread Using a w/o/w Formulation

Outer aqueous phase	Salt	1%
	Gelatin	3%
	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.

w/o emulsion may shift toward the o/w emulsion and the double emulsion can invert. Therefore, it is not always obvious that increasing viscosity, gelation, or partial solidification improved the emulsion's stability.

XI. 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 of time 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 practicing such technology is the

difficulties arising in dispersing (and in keeping it stable) the micropraticle or nanoparticles in the continuous water phase. Microspheres and nanoparticles using solid encapsulation techniques were tested to replace the classical w/o emulsion. Only a few experiments were carried out showing that release can be slowed down, but the stability of these systems is very limited (109–123). 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.

XII. 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 miocrocapsules, (2) o/w/o double emulsions for improved solublization 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 along with the classical delivery applications in pharmaceuticals and food applications will be described that will emphasize the new emerging applications.

A. Controlled Delivery Applications

The intrinsic instability of the double emulsion caused difficulties for formulators and many of the investigators have decided to abandon this technology (6,7). However, one should bear in mind that the potential applications for double emulsions appear 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 corticosteroids, slow and targeted release of anticancer drugs), and (3) adsorption of toxic compounds. The technology has much promise also in nonpharmaceutical 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 (6,7). In fact, most of the applications of double emulsions for controlled delivery are in the field of pharmaceuticals and health.

Double emulsion may offer some advantages for food application mainly with relation to their capability to encapsulate (or entrap) in the internal compartments some water-soluble substances which 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 (124,125). On this basis, several new products have been patented in the form of w/o/w emulsions, such as salted creams (encapsulation of salt), aromatic mayonnaise, and so for in (126–129). 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) (124).

B. Double-Emulsions Solvent-Extraction Techniques for Preparation of Microspheres

Drugs, cosmetic ingredients, and foods additives are microencapsulated for variety of reasons, which include reducing local side effects, controlled release, site-specific (drug) delivery, and drug targeting. A tremendous amount of research work is done in a search of suitable methods to achieve a good 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 is one of the most useful methods for entrapping water-soluble compounds (109–123). Fig. 22 shows schematically the preparation technique. Over 100 articles and many patents have been published in the course of the last 5 years on the use of this technique. Most of the applications are rather related with pharmaceuticals and drug encapsulation than with foods. We have selected only some examples that are of a more significant value.

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

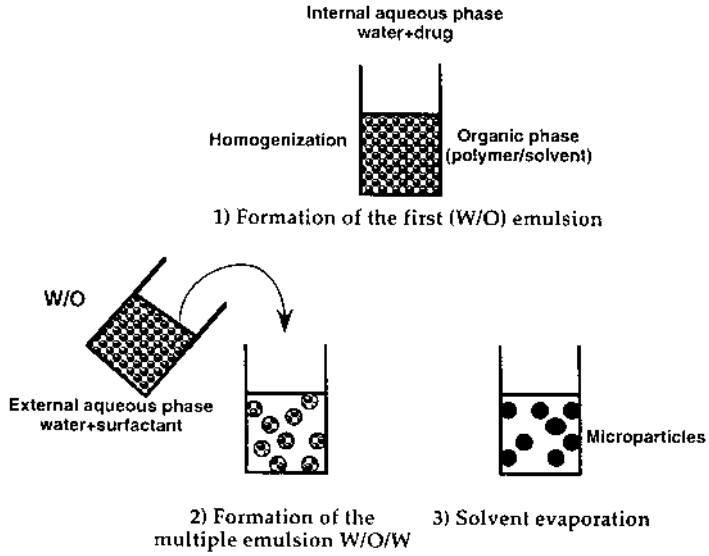

Figure 22 Preparation of microspheres by the multiple-emulsion solvent-evaporation technique.

The double-emulsion solvent-evaporation technique is commonly used to prepare biodegradable hydrophobic microspheres containing hydrophilic pharmaceuticals, proteins, and polypeptides for sustained release applications (111,115–123). In most cases, the microspheres are in the range size of 10–100 μm. However, recently, Blanco-Prieto et al. (116) managed to reduce the microcapsules sizes to less than 5 μm.

Couvreur et al. (115) reviewed the preparation and characterization of many of the different types of the solvent-evaporation microsphere and mostly discussed small poly(lactic-*co*-glycolic acid) microspheres (mean size lower than 10 μm) containing small peptides (Fig. 23). 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.

Much work was devoted in recent years to preparing microparticles of narrow size distribution with different biodegradable polymers. The sizes of common microcapsules are 40–50 μm (115–116,121–123).

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

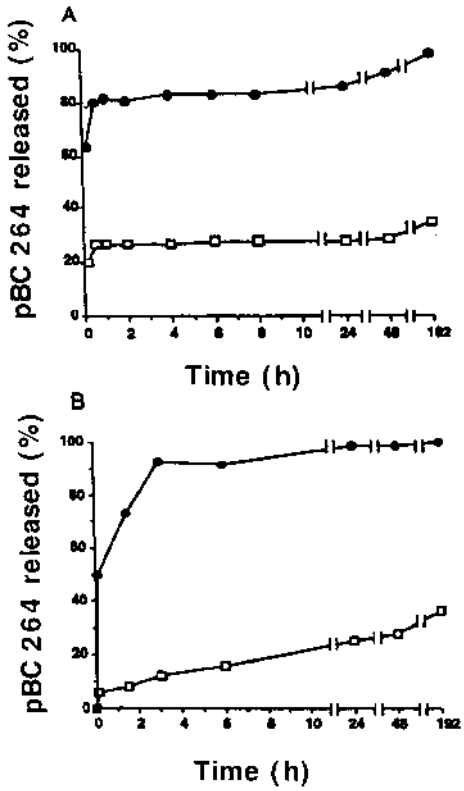

Figure 23 Release kinetic of pBC 264 in phosphate-buffered saline pH 7.4: (top) in vivo and (bottom) in brain tissue from PLGA microspheres prepared with either ovalbumin (●) or Pluronic F-68 (□). (From Ref. 115).

phase: the rotor/stator design was also investigated. All of 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 made of 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) (117). BSA was used as the model protein and poly(methyl methacrylate) (PMMA) was used as the model polymer. The droplets sizes of the w/o emulsion droplets were controlled using rotor/stator homogenization. The size of the microspheres thus prepared was found to increase with the increasing size of the protein powder in the s/o/w system but increase with decreasing size of the

liquid emulsion droplets in the w/o/w system. Empirical correlation can accurately predict the size of the microspheres. Protein loading in the microspheres decreased with respect to increase in w/o emulsion droplet size or in protein powder size.

In a most recent article (122), it was demonstrated that a milk model protein, β-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 BLG increases, the stability of the inner emulsion decreased and the entrapment was less efficient. Therefore, adjusting the combined pH effect 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, is the most significant 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 a strong adsorption of the protein on microsphere surface. Some more interesting articles on protein entrapments for various oral and other intakes can be found in the literature (117–120). Chitosan porous spherical particles were prepared from o/w/o double emulsions stabilized with a chitosan aqueous solution. The particulation was obtained by a simple evaporation technique (55).

XIII. 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 mentioned for o/w/o double emulsions which sound interesting and worth being mentioned.

Modulated release of triterpenic compounds from a o/w/o multiple emulsion formulated with dimethicones studied with infrared spectrophotometric and differential calorimetric approaches is one of these examples (130). 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 silicones decreased the oily touch of the final preparation and (2) due to the large range of viscosity, this excipient influenced the skin distribution of the active matter after the topical

application. The effects of different triterpenic compounds 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 silicone structure and the distribution of drugs in different skin levels or between the silicone structure and the percutaneous penetration. The incorporation 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: oil-in-water (o/w), water-in-oil (w/o), and oil-in-water-in-oil (o/w/o) (16). The stability in the o/w/o emulsion was the highest among the three types of emulsion. The remaining percentages, at 50°C after 4 weeks, were 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 the 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. An 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, the ratio of retinol in the inner oil phase to the total amount in the emulsion, increased with increasing oil fraction. The remaining percentage of retinol in the o/w/o emulsion was in excellent agreement with 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 improved 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 for stabilizing vitamin A.

Orange oil-in-water emulsions were encapsulated in another oil phase to form a double emulsion with orange oil inside its inner compartment (19). Although the yield was only 44.5%, it is a promising area for further research in the future in order to prevent air oxidation of the oil. Spray-drying of the double emulsion can provide a secondary coating to secure a maximum protection of orange oil and affords a free-flowing flavor powder. Spray-drying of the orange oil double emulsion had a very low destruction effect on its structure, as revealed by light microscopy. This method may have a potential application in different food or pharmaceutical products where maximum protection is required. A secondary coating was applied to the flavor oil already encapsulated in a double emulsion. The spray-drying technique includes spraying a flavor emulsion into a stream of hot air.

The water phase is then evaporated rapidly, leaving the flavor material locked in the carrier.

XIV. MULTIPLE-EMULSION 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 microstructure of the double emulsions. Some attention was given to this subject in recent years and the results are significant because they help to clarify certain aspects on the stability and release properties of the double emulsions (131). Few publications deal with w/o/w double emulsions and only one deals with the o/w/o emulsion. Most of the rheology work is old and do not contribute a new understanding. Therefore, we will bring only recent relevant work.

An interesting characterization of the mechanical properties of the oil membrane in w/o/w emulsions was done by an aspiration technique (132). It was adopted from techniques related to the evaluation of global or cell deformability. The deformability of an individual globule during a total or partial flow into a cylindrical glass tube calibrated under well-controlled conditions of aspiration was determined. An analysis of the behavior of the double emulsion by a migration of the lipophilic surfactant to the interface between the oily and the external aqueous phases was done. 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 et al. (131–133) applied linear shear flow on the w/o/w double emulsions that contained active matter, and from the rheological patterns, they learned about the bursting effect of the droplets with the release of entrapped substances and the composition of the system. The authors (133) described a set of two types of experiment: oscillatory dynamic tests and a 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, δ; the storage modulus G' and the loss modulus G'') provide a quantitative characterization of the balance between the viscous and elastic properties of the multiple emulsions. At lag phase, $\delta = 0^\circ$, and when $= 90^\circ$, the system is viscoelastic. The shear sweep and the temperature sweep characterize the multiple emulsion at rest. Fig. 24 describes a transition between an elastic behavior and a viscous

behavior, which occurs at critical stress values. The change in this parameters indicate a pronounced structural breakdown. The authors considered the influence of the formulation parameters on the swelling and release kinetics using the rheological properties. Parameters such as the oil nature, the width of the oil membrane, and the lipophilic and the hydrophilic nature of the surfactant have been evaluated. The two main parameters identified to affect the swelling/breakup kinetics were the difference in concentration in water soluble molecules between the internal and the external aqueous phase (Fig. 25), and the lipophilic surfactant concentration. It was also observed that the maximum viscosity values increase with the surfactant ratio (under hypo-osmotic conditions, Fig. 26). The same trend was observed when the release of water-soluble materials, $\beta(t)$, was followed (Fig. 27). The authors concluded that a progressive migration of the excess lipophilic surfactant in the oil phase toward the primary or the secondary interface occurs.

Stroeve and Varanasi (134) also examined the breakup of the multiple-mulsion globules in a simple shear flow and concluded from the critical Weber number $(We)_{cr}$ (Figs. 28 and 29) that the multiple emulsions exhibit behavior that is similar to that of simple emulsions. From Fig. 28, one can see, at least qualitatively, the evolution of $(We)_{cr}$ as a function of p

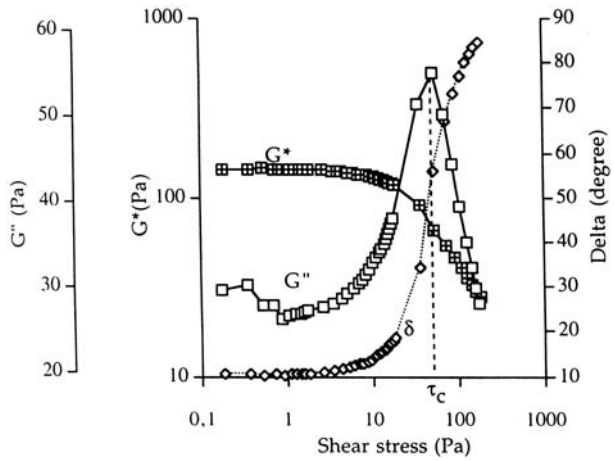

Figure 24 Changes in G^*, G'' and δ for increasing stress at fixed frequency in double emulsions. The double-emulsion composition of the water oil emulsion is 24% oil, various lipophilic surfactant concentrations and 0.7% $MgSO_4$. The o/w/o emulsion contain 80% water in oil, 2% hydrophilic surfactant, and demineralized water. (From Ref. 133.)

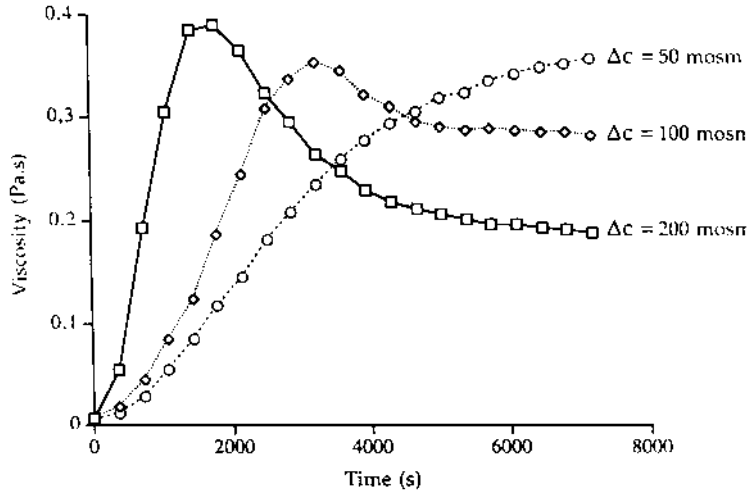

Figure 25 Change in viscosity versus time for different concentration gradients in the inner phase. (From Ref. 133.)

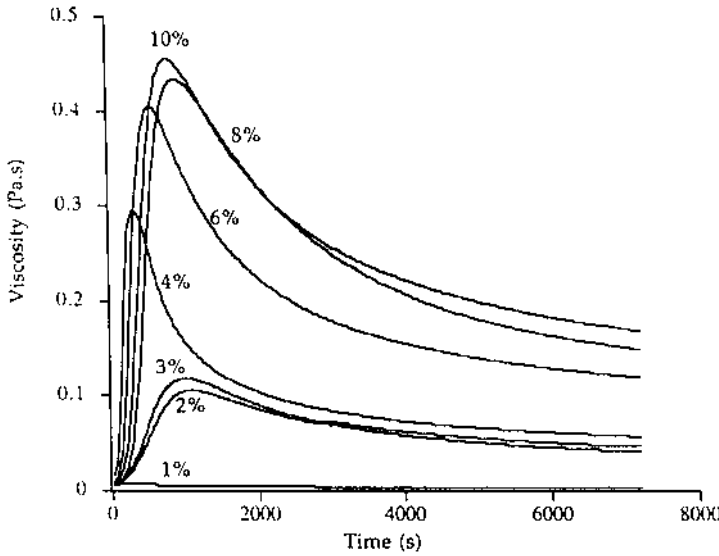

Figure 26 Change in viscosity versus time under hypo-osmotic conditions for different lipophilic surfactant concentrations. (From Ref. 133.)

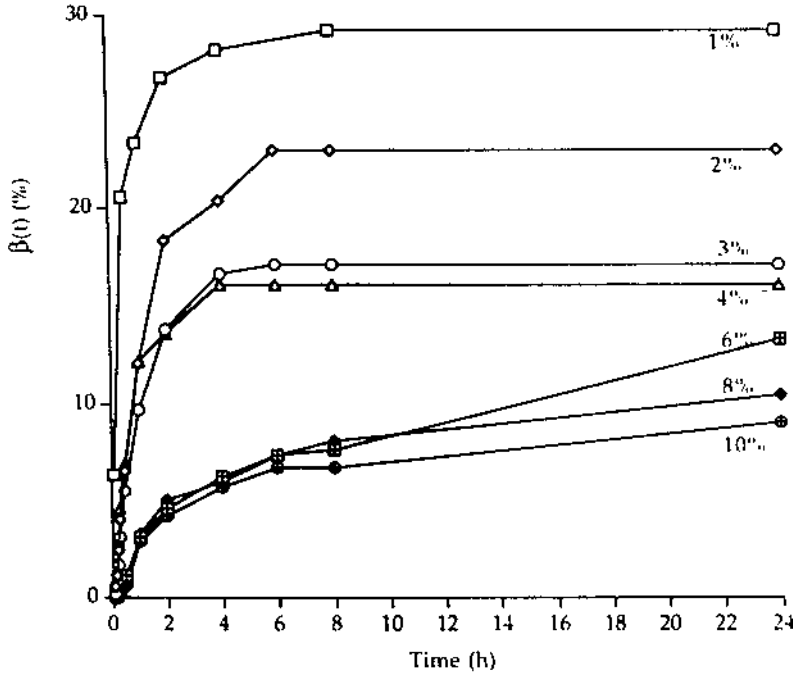

Figure 27 Change in released fraction β(t) versus time under hypo-osmotic conditions for different lipophilic surfactant concentrations. (From Ref. 133.)

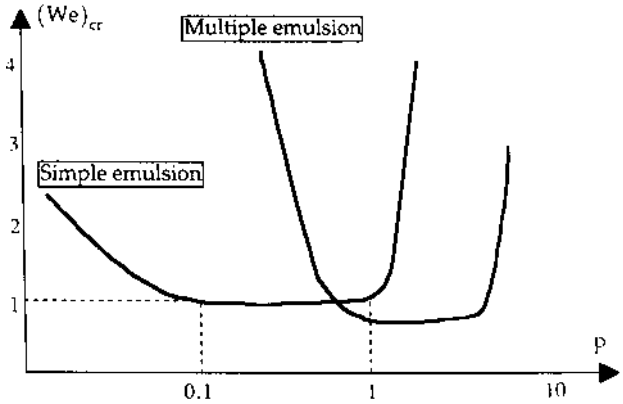

Figure 28 Changes in the critical Weber number $(We)_{cr}$ for simple- and multiple-emulsion disruptions as a function of the viscosity ratio dispersed to the continuous phase. (From Ref. 134.)

(the viscosity ratio between the drop and the continuous phase) for simple and multiple emulsions and that there are some differences between the two emulsions. The double emulsion has more heterogeneous characteristics. From Fig. 29, it is possible to obtain the minimum shear rate value which is able to produce the breakup of the oil globules and the release of water-soluble encapsulated molecules.

The studies showed also that the mechanisms taking place during the breakup were complex and did not always lead to a 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 lipophilic film were able to restrict the release.

De Cindio and Cacace (125) prepared food double emulsions and studied their rheological behavior by steady shear and oscillatory measurements. They have concluded that the w/o/w appeared to have rheological properties examined to those of a simple o/w emulsion having the same fraction of dispersed phase but lower oil content (Fig. 30). It was also demonstrated that the plot of both storage moduli G' and G'' versus oscillation frequency, W, are similar in all eight prepared emulsions, and the loss tangent is about 1, and both elastic and viscous contributions to the 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 the mixture of emulsifiers on the double-emulsion stability

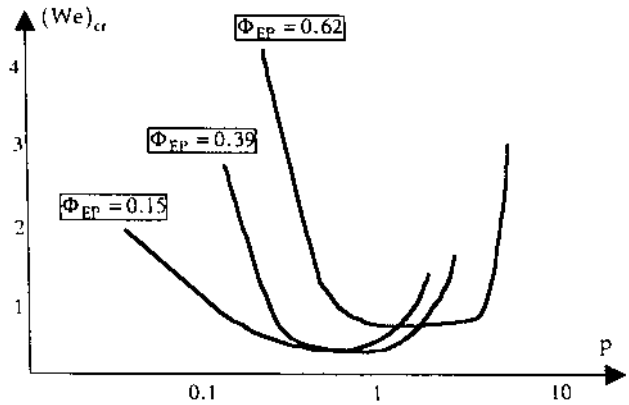

Figure 29 Changes in the critical Weber number $(We)_{cr}$ for multiple-emulsion disruption as a function of the viscosity ratio dispersed to continuous phase, for different primary emulsion volume fractions. (From Ref. 134.)

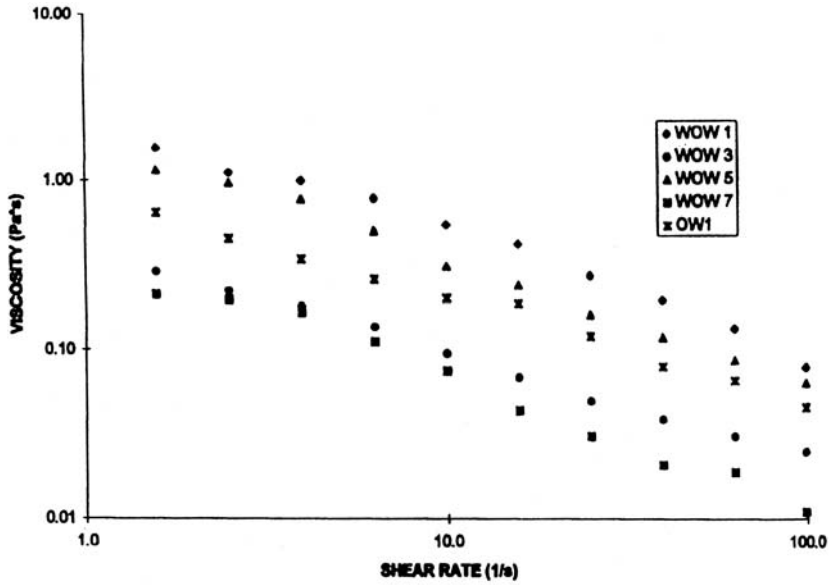

Figure 30 Apparent viscosity versus shear rate for both w/o/w and o/w systems at volume fraction of disperse phase of 0.3. (From Ref. 125).

was studied by an oscillatory ring-surface rheometer from which the interfacial elasticity at the oil–water interface can be evaluated.

Pal (135) 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 to be non-Newtonian above this volume fraction. All of the double o/w/o emulsions are highly non-Newtonian. The degree of shear thinning increases with the increase in primary o/w emulsion concentration (Fig. 31). 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. 32). 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 the w/o/w emulsion studied under a conic plate viscometer has shown a negative thixotropic flow pattern, mostly under a low shear rate. Upon raising the shear rate or the shear time, an increase of the shear stress was observed, which induced phase inversion to a w/o of a semisolid-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

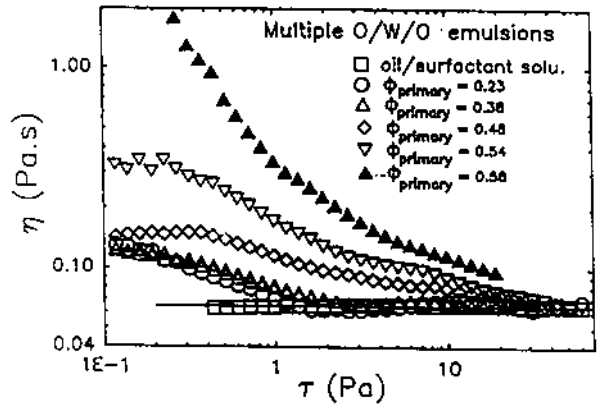

Figure 31 Apparent viscosity as a function of shear stress for multiple o/w/o emulsions. (From Ref. 135.)

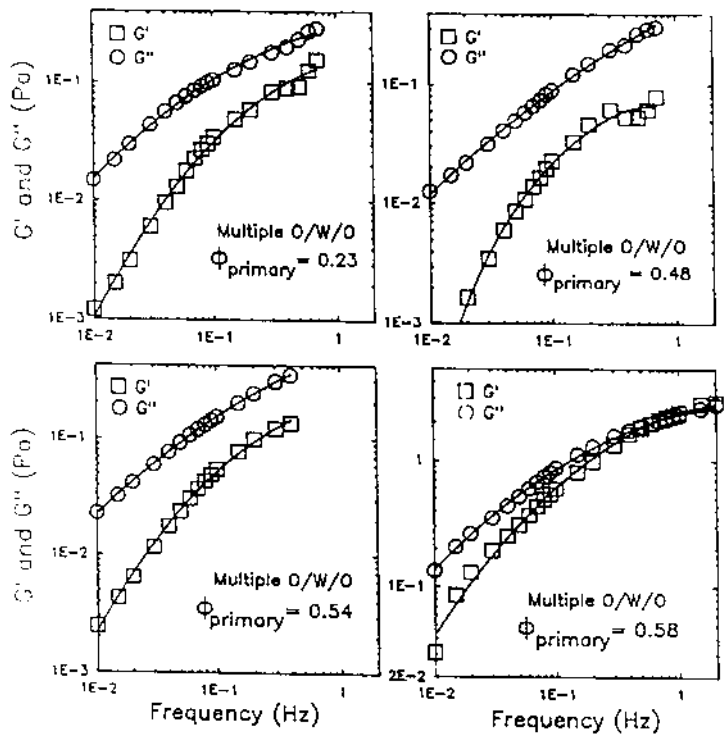

Figure 32 Storage and loss moduli for multiple o/w/o emulsions as functions of frequency. (From Ref. 135.)

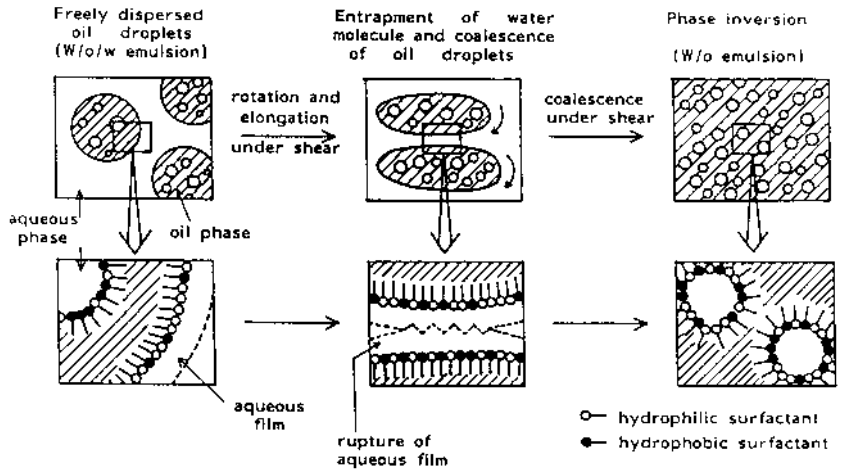

Figure 33 Proposed mechanism of phase inversion under shear. (From Ref. 136.)

such an inversion was suggested. Fig. 33 shows a proposed mechanism for phase inversion under the shear rate (136). Induced shear, causing phase inversion, could serve as a possible technique to release the drug.

XV. CONCLUDING REMARKS

Double emulsions are 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 in a controlled rate. However, the sizes of the droplets and the thermodynamic instability were a significant drawback of this technology.

The use of conventional low-molecular-weight emulsifiers did not solve these problems. However, much progress was made with the introduction of amphiphilic macromolecules as emulsifiers. This multianchoring of flexible macromolecules can improve the steric stabilization by forming, a thick, multilayered coating on the droplets. A variety of hybrids, complexes, adducts, and others between the amphiphiles and coemulsifiers or cosolvents has been suggested. These molecules improved significantly the stability 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 made also in identifying and characterizing the parameters and mechanisms involved in coalescence, aggregation, and rupture of the double-emulsion droplets. A good control of the rheological

parameters was achieved by a better understanding of their effect on the static and shear-induced stability.

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

The mains goals remain to obtain submicronal double-emulsion droplets with long-term stability (possible by emulsified microemulsions) and to trigger and control the release at will.

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

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