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Review Article

The formulation and stability of multiple emulsions

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Introduction

A large literature has accumulated on the theoretical and practical aspects of emulsions. However, of the many emulsion systems studied, little attention has been paid to multiple emulsions. These complex systems have shown promise in many technologies, particularly in pharmaceutics and in separation science. Their potential biopharmaceutical applications (Whitehill, 1980), a consequence of the dispersal of one phase inside droplets of another, include use as adjuvant vaccines (Taylor et al., 1969), as prolonged drug delivery systems (Elson et al., 1970; Collings, 1971; Benoy et al., 1972; Brodin et al., 1978), as sorbent reservoirs in drug overdosage treatments (Frankenfeld et al., 1976; Chiang et al., 1978) and in immobilization of enzymes (May and Li, 1974). The use of multiple emulsions in the separation field has included the separation of hydrocarbons (Li, 1968) and the removal of toxic materials from waste water (Li and Shrier, 1972). Multiple emulsions have also been formulated as cosmetics (British Patent, 1979) and as household products such as wax polish (Mackles, 1968). In some disciplines, certain multiple emulsions have been termed 'liquid membrane' systems, as the liquid film which separates the other liquid phases acts as a thin semi-permeable film through which solute must diffuse moving from one phase to another. There are, therefore, many potential practical applications of multiple emulsions. The production of stable, reproducible systems is therefore important, if the results of experiments with these systems are to be understood and are to be reliable. One of the main problems associated with the use of multiple emulsions to date is their inherent instability. If the mechanisms of instability were understood more clearly, then stable, commercially viable multiple emulsion systems could be prepared.

This review surveys the problems in the manufacture of complex emulsions, the mechanisms of breakdown and the factors which control their stability as well as the various methods which have been developed to assess stability.

Emulsions have been described as heterogeneous systems of one immiscible liquid dispersed in another in the form of droplets which usually have a diameter greater than 1 μm (Becher, 1965). These two liquids are also chemically unreactive and form systems characterized by a low thermodynamic stability. Simple emulsions are classified according to the nature of their continuous or dispersed phase, i.e. as either water-in-oil (w/o) or oil-in-water (o/w) emulsions. Emulsifier is present in each system to stabilize the emulsion and a variety of ionic and non-ionic surfactants are available for this purpose although few are acceptable for use in vivo. Lipophilic (oil-soluble, low HLB) surfactants are used to stabilize w/o emulsions whereas hydrophilic (water-soluble, high HLB) surfactants are used to stabilize o/w systems. Emulsifiers can be blended to give mixtures of intermediate HLB values providing systems with optimal stability.

Multiple emulsions are more complex systems as the drops of the dispersed phase themselves contain even smaller dispersed droplets, which normally consist of a liquid miscible and, in most cases identical with, the continuous phase. They are, therefore, emulsions of emulsions.

Multiple emulsions have a fairly long history. Many have observed multiple droplets in what were thought to be classical two-phase systems. Seifriz described in 1925 oil drops of an o/w emulsion containing droplets of water. He also noted tiny oil globules inside the water drops of a w/o emulsion. Seifriz termed these emulsions 'bimultiple' systems. He found that more complex 'trimultiple', 'quatremultiple' and even 'quinquemultiple' systems existed. These multiple drops persisted on standing for 3 h and on repeated shaking.

Although multiple systems have been known for such a long period of time, it is only in the past 15 years that they have been purposely prepared and studied. The two major types of multiple emulsion are the water-in-oil-in-water (w/o/w) emulsion in which internal and external aqueous phases are separated by an oil layer, and the oil-in-water-in-oil (o/w/o) emulsion where an aqueous phase separates internal and external oil phases. A schematic representation of a w/o/w multiple emulsion drop is shown in Fig. 1 and a photomicrograph in Fig. 2. The two dispersed phases can be clearly seen—the small internal aqueous droplets, surrounded by a surfactant-stabilizing layer, are dispersed in the oil phase which, in turn, is dispersed in the external aqueous phase and is also surrounded by a surfactant layer.

W/o/w emulsions possess many of the advantages of w/o emulsions but, in addition, have a low viscosity, due to the lower viscosity of the aqueous external phase, which makes them more convenient to handle and use, especially to inject.

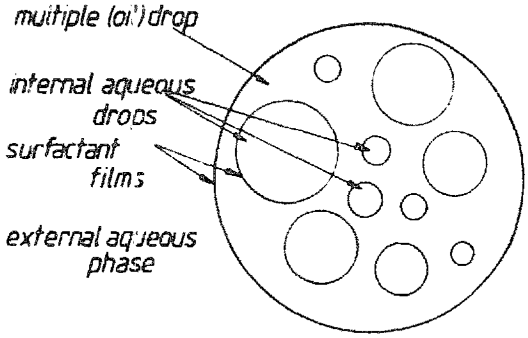

Fig. 1. Schematic representation of a w/o/w multiple emulsion drop.

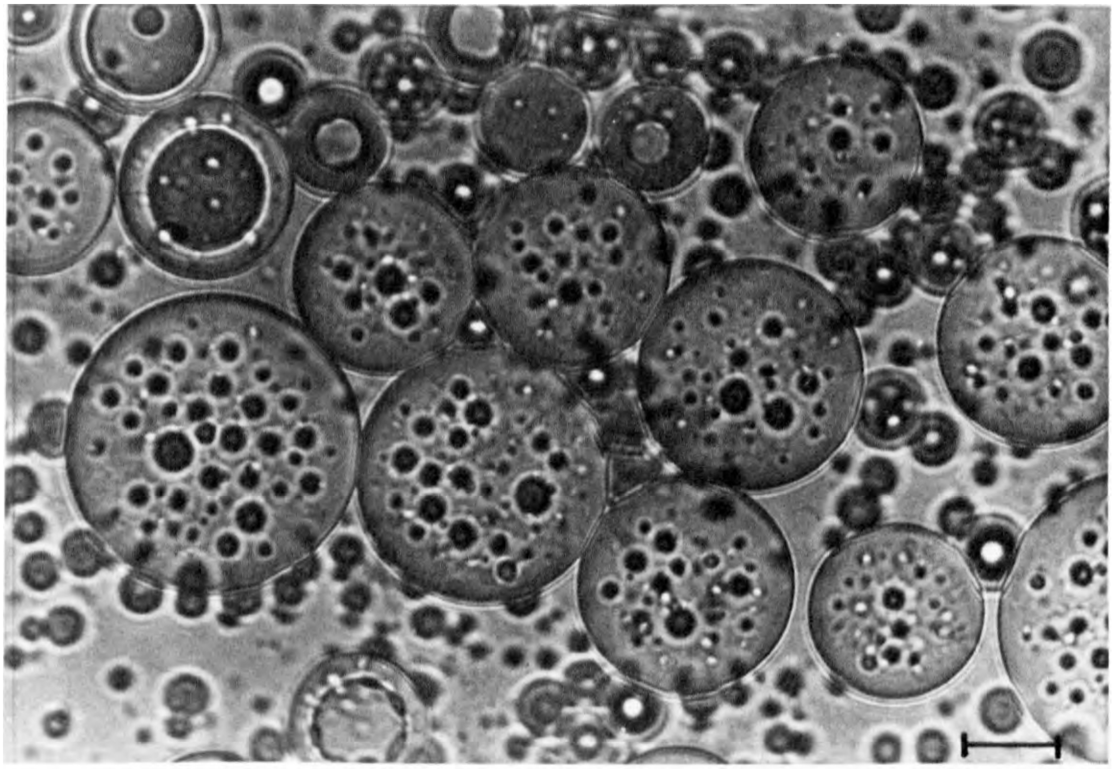

Fig. 2. Photomicrograph of a typical w/o/w emulsion. Bar marker = 25 μm.

Other variations on the theme are, of course, possible (e.g. gelatin microcapsule in oil-in-water dispersions) some of which will be discussed below.

Nomenclature

Due to the complexity of multiple emulsions, it is useful to have an unambiguous system of nomenclature. In a w/o/w emulsion, for example, the continuous oil phase of a primary (w/o) emulsion, used to prepare the multiple emulsion, becomes the disperse oil phase of the multiple emulsion. The surfactant or surfactant mixture used to prepare the primary emulsion is termed the primary emulsifier mixture and the volume fraction of water in the w/o emulsion ($\phi_{w/o}$) becomes the internal aqueous phase volume of the w/o/w emulsion. A useful subscript notation for complex emulsions was suggested by Sheppard and Tcheurekdijan (1977) to define the individual components and their sources in order to avoid ambiguity. For example, to describe a w/o/w emulsion, the aqueous dispersed phase, w_1, of the primary w_1/o emulsion is dispersed in the oil phase, o. The w_1/o emulsion is redispersed in the external, continuous phase of the multiple emulsion, w_2. Thus the system can be notated as $w_1/o/w_2$. In most cases, the two aqueous phases will be identical, and thus the system can be represented as $w_1/o/w_1$. Conversely, an oil/water/oil system can be written $o_1/w/o_2$.

Sheppard and Tcheurekdijan also suggested the use of an 'order' terminology, which may be of use in describing more complex systems. A $w_1/o/w_2$ or $o_1/w/o_2$ system would be described as a second-order emulsion (two liquid–liquid interfaces). More complex systems could be described as 3rd, 4th, etc., order emulsions; simple emulsions would thus be first-order emulsions. A $w_1/o/w_1$ emulsion is a two-component, second-order emulsion, and an $o_1/w/o_2$ emulsion is a three-component second-order emulsion.

Mulley and Marland (1970) have studied multiple drop formation in systems containing 3 different immiscible liquid phases. They discuss the many possible forms such systems could take, depending on conditions; for example $o_1/w/o_2$; $o_2/w/o_1$; $o_1/w/o_1$; $o_2/w/o_2$; $w/o_1/w$; $w/o_2/w$; $o_1/o_2/w$; $o_2/o_1/w$; $w/o_2/o_1$; $w/o_1/o_2$. The authors point out that some of these combinations may coexist in a particular system. Even more complex situations can be envisaged, e.g. $o_1/w/o_1/w/o_2$ or $o_2/w_3/o_1/w/o_2$ etc.

Formation of multiple emulsions

There have been many reports in the literature of the unexpected formation of multiple emulsions during normal emulsification procedures. There is usually little reproducibility in these 'accidental' preparations and, in most cases, the content of multiple drops is quite low and the products are of poor stability. Multiple structures probably form in many liquid–liquid interactions but are so unstable that they disappear before being observed.

In emulsions with high disperse phase volumes (> 0.74) the drops may assume a complex structure and often multiple drops will be observed (Sherman, 1968). Microscopic examination of a w/o emulsion, for example, containing high volume concentrations of water, often reveals smaller droplets of oil within the dispersed water drops. Many workers have noted the appearance of multiple globules during emulsion inversion (Becher, 1965; Seifriz, 1925; Lin et al., 1975). This appears to result from partitioning of the surfactants between the two phases. Lin et al. (1975) noted that when a hydrophilic surfactant was initially placed in the oil phase a transient w/o/w emulsion was formed in the process of phase inversion from w/o to o/w emulsion type (see Fig. 3). A portion of the aqueous phase, added during the emulsification process, will be emulsified in the oil phase forming a primary w/o emulsion. If the conditions favour o/w emulsification, i.e. if the HLB of the surfactant is high this initial w/o emulsion will be unstable and on further agitation the w/o emulsion is mixed into the excess water to form a w/o/w emulsion. As surfactant migrates to the outer aqueous phase, the unstable, larger globules of water readily coalesce with smaller droplets to form a final o/w emulsion.

This is not found if the hydrophilic surfactant is initially placed in the aqueous phase. Becher (1958) reported the inversion of o/w and w/o emulsions to multiple systems followed later by reversal to the opposite type. It appears that, under certain conditions, when inversion takes place, some of the original structure becomes trapped in the final emulsion.

Kessler and York (1970) studied the hydrodynamic formation of multiple-drop structures by using a macroscopic approach. Using a liquid-injection technique with immiscible liquids such as carbon tetrachloride into glycerine, they analyzed jets of liquid by high-speed photography. They observed that, as a ligament of liquid was

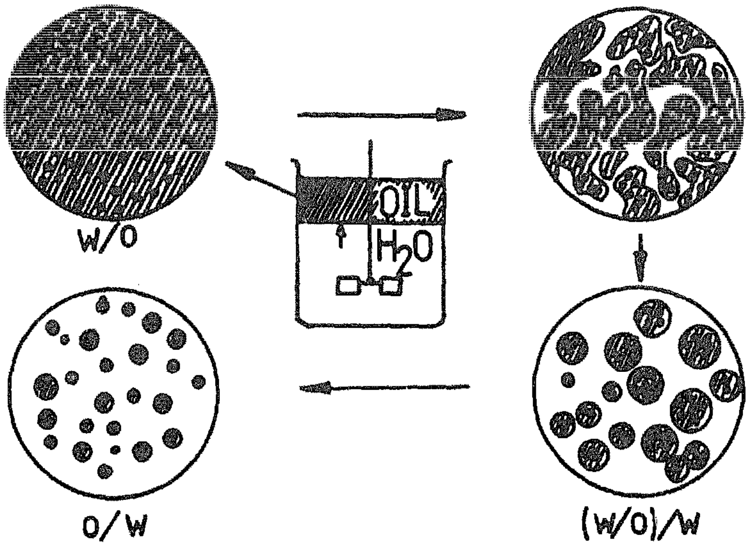

Fig. 3. Possible sequence of events leading to the final formation of an o/w emulsion via a transient (w/o)/w emulsion, when hydrophilic surfactant is initially located in the oil phase, after Lin et al. (1975).

drawn out from the orifice, it did not break off but re-coalesced with itself, trapping some external phase in the process. This elongated form then collapsed to form a drop containing the external liquid phase. The number of entrapped internal droplets and the probability of entrapment was proportional to the relative volume of the multiple drop.

Multiple emulsions which will possess some degree of stability may be prepared by using pairs of surfactants, one of which will stabilize a w/o emulsion (lipophilic) and one of which is capable of stabilizing an o/w emulsion (hydrophilic) and this forms the basis for the intentional preparation of multiple systems.

Manufacture

Multiple emulsions may be prepared in the laboratory by the re-emulsification of a primary emulsion. A two-stage procedure is, therefore, necessary (Fig. 4). The first stage involves the preparation of the primary emulsion which, in the preparation of a w/o/w emulsion, is a w/o emulsion. In the second step, the primary emulsion is further emulsified in water to form the multiple emulsion.

The primary emulsion may be prepared in the usual manner; for example, with a laboratory mixer, by ultrasonication, etc. In this case, a lipophilic surfactant is used to promote the formation of a w/o emulsion. This emulsion is then poured into a solution or dispersion of the secondary surfactant in water. The secondary surfactant, in this case, is hydrophilic to promote o/w emulsification in which the 'oil' phase is the w/o emulsion. The second emulsification step is critical, as excess mixing can fracture the drops, resulting in a simple oil-in-water emulsion. The small internal water droplets are lost too and mix with the external aqueous phase as the oil drops are torn apart (Fig. 5). For this reason, high-shear mixers and sonication

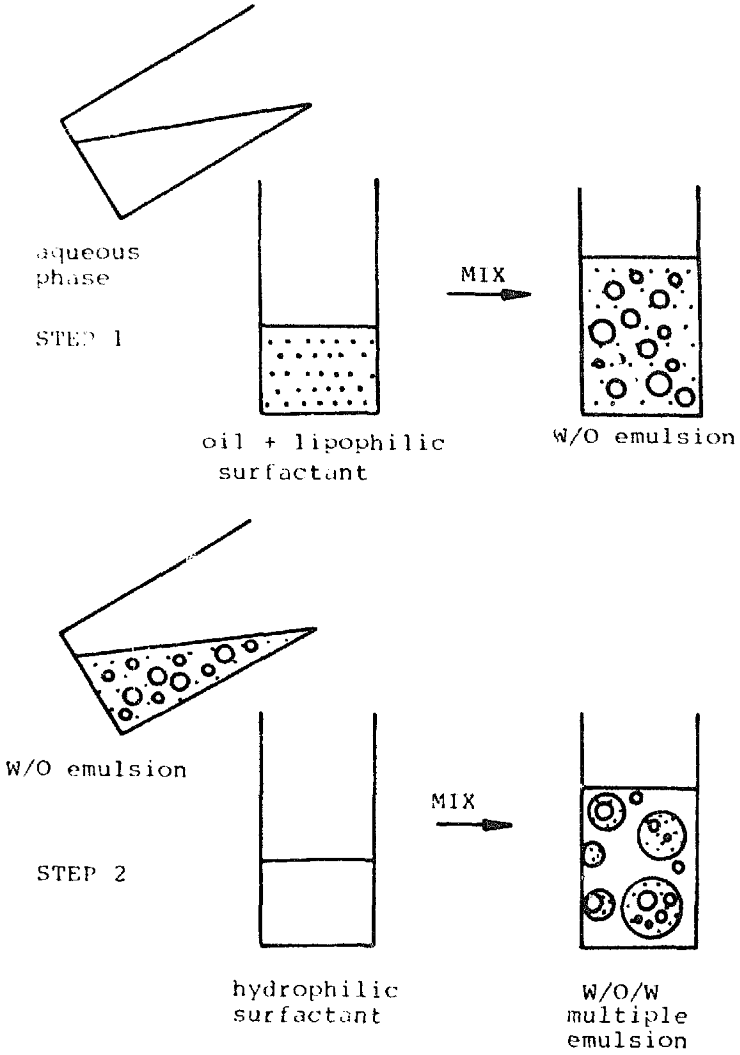

Fig. 4. Two-stage preparation of a w/o/w multiple emulsion.

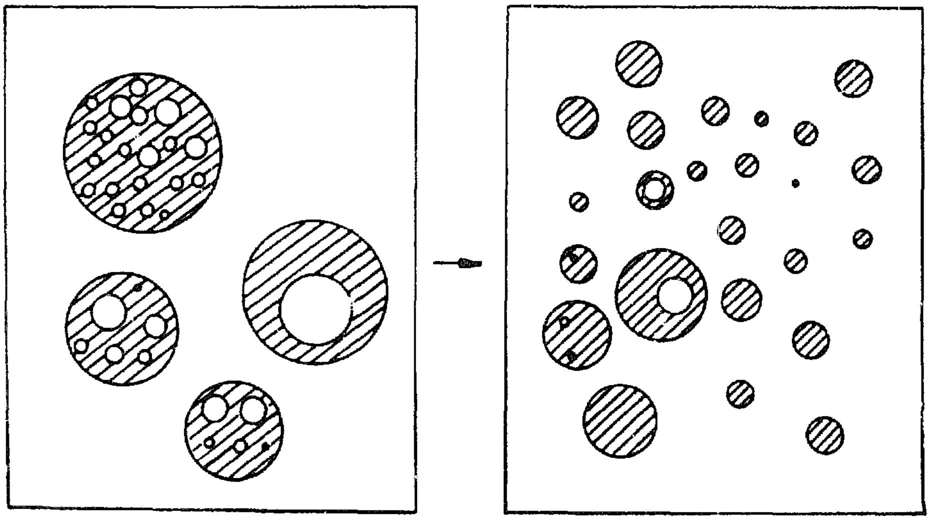

Fig. 5. Diagrammatic representation of multiple drop breakup due to excessive shearing.

are usually unsuitable methods of preparation. Davis and Burbage (1978) found that, while stable multiple (w/o/w) drops could be prepared with 10-s bursts of ultrasound, emulsions sonicated for 30 s were devoid of multiple drops after 28 days. A small, low-shear mixer may be employed or the mixture may be shaken by hand. However, no matter what emulsification method is used for the second step, some of the internal aqueous phase is unavoidably lost to the external aqueous phase.

A typical formulation might be as follows. The primary (water-in-oil) emulsion could be formed from isopropyl myristate (47.5%), sorbitan mono-oleate (Span 80) lipophilic surfactant (2.5%) and distilled water to 100%. The multiple (water-in-oil-in-water) emulsion may then be prepared by adding the primary (w/o) emulsion (50%) into a polyoxyethylene sorbitan mono-oleate (Tween 80)-hydrophilic surfactant (2% w/v) solution in water. In a similar fashion, the reverse procedure would be followed to prepare an o/w/o emulsion. The primary o/w emulsion would be re-emulsified, using a lipophilic surfactant, in oil. Using this procedure, multiple emulsions may be easily prepared with high reproducibility.

The preparation of 'liquid membrane' emulsions, used to separate hydrocarbons, is essentially similar, but no secondary surfactant is used so that the primary emulsion separates from the external aqueous phase under gravity. The liquid membrane phase refers to the middle phase between the internal and external phases. Various additives may also be present to control the stability, permeability and selectivity of the 'membrane'. The hydrocarbon mixture to be separated is first emulsified in an aqueous medium containing surfactant. The hydrocarbon/water emulsion is then mixed with a selected solvent, forming an o/w/o system. The more permeable hydrocarbons permeate the aqueous phase and pass into the solvent phase, the less permeable components remaining in the internal oil phase. The multiple system is then allowed to cream and the components are separated (Li, 1968). Liquid membranes of the w/o/w type have been used in the treatment of waste water (Li and Shrier, 1972), in which the aqueous reagent phase is emulsified in oil and the w/o emulsion mixed with the waste water (the external aqueous phase). Contaminants pass from the waste water to the internal aqueous phase where they become trapped.

Composition of multiple emulsion systems

Oils

The nature of the oil can markedly affect the behaviour of the system, although most oils will form multiple emulsions if the correct manufacturing conditions are applied. For pharmaceutical uses, oils used have included the refined hydrocarbon oils such as light liquid paraffin and esters of long-chain fatty acids including vegetable oils, for example, ethyl oleate and isopropyl myristate. Other oils used have included olive oil, arachis oil and sesame oil. Mixtures of oils may also be used, for example mixtures of hydrocarbons have been emulsified and separated by the liquid membrane technique (Li, 1968). Frankenfeld et al. (1976) used mixtures of 'Solvent 100 Neutral' (an isoparaffinic, dewaxed oil of high viscosity) and 'Norpar 13' (a non-viscous, normal paraffinic solvent) to vary the viscosity as the oil phase in attempts to control the transfer rates of solutes across the oil membrane.

Surfactants

Ionic or non-ionic surfactants may be used although the type selected would depend on the use to which the multiple emulsion was to be put. In food, drug and cosmetic fields the toxicity of the compounds must be considered. Non-ionic emulsifiers are preferred for pharmaceutical use, not only because of their lower toxicity but also because they are less likely to interact with ionic compounds. Matsumoto et al. (1976) have found that non-ionic surfactants gave better yields of multiple drops than did ionic surfactants.

It is necessary to use at least two surfactants, one for the primary emulsion and the other for the dispersion of this emulsion to form the multiple system. The optimum surfactant to emulsify a given oil can be determined by use of the hydrophile–lipophile balance (HLB) system (Griffin, 1949; Morris, 1965). The present authors have carried out an investigation into the optimal HLB required for both primary and secondary emulsification steps in the formulation of a water/isopropyl myristate/water emulsion. Estimates of stability were carried out by visual observation of a series of emulsions prepared with blends of surfactants having different HLB values. A series of w/o emulsions contained 47.5% oil and 2.5% surfactant over an HLB range of 2–8; the amount of oil phase which separated with time was plotted (Fig. 6). The system which separates least is the optimal one. The procedure to determine the optimal HLB value of secondary surfactant is similar but employs surfactants with HLB values in the range 6–16 (Fig. 7). The w/o/w emulsion was prepared using the optimal primary surfactant and mixed with 50% water containing blends of more hydrophilic surfactants.

The use of the HLB system is limited as it is based on the observation of creaming of the emulsions as an index of instability. Creaming may, however, be affected by

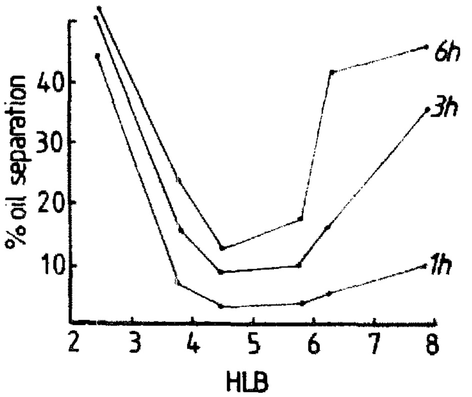

Fig. 6. Volume separation of oil phase of water/isopropylmyristate emulsions with HLB value of surfactant blend at various times after preparation. Surfactants used—Span 85 (HLB 1.8) and Span 20 (HLB 8.6).

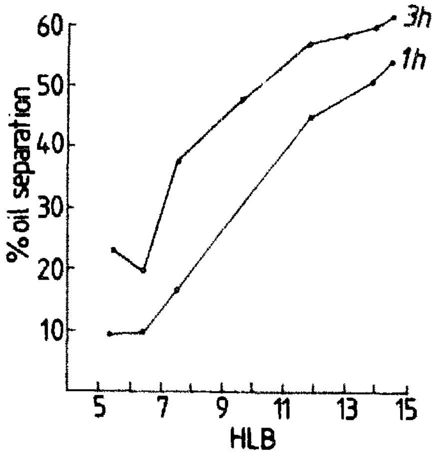

Fig. 7. Volume separation of aqueous phase of water/isopropyl myristate/water emulsions with HLB of the secondary surfactant blend at various times after preparation. Surfactants used—Span 80 (HLB 4.3) and Tween 80 (HLB 15.0).

other factors, such as the viscosity of the continuous phase. The HLB system also neglects the effects of surfactant concentration on stability (Florence and Rogers, 1971b). Nevertheless, it provides a useful approach to the choice of the optimal surfactant system. In general, in a w/o/w emulsion, the optimal HLB value of the primary surfactant will be in the range 2–7 and in the range 6–16 for the secondary surfactant. Equilibration of the systems after mixing will undoubtedly result in the transfer of surfactant between the aqueous and nonaqueous components. Saturation of the phases with the two surfactants used should prevent instability during this equilibration.

There will be an optimal concentration of surfactant required to stabilize the system; low concentrations may not be sufficient to stabilize the emulsion and may result in the rapid degradation of the emulsion. High concentrations may serve to increase the viscosity of the system but may be objectionable from the point of view of toxicity. The general range is between 1 and 10%, but Matsumoto et al. (1976) suggest that concentrations of greater than 30% by weight of Span 80 in the oil phase are required to obtain yields of more than 90% of multiple drops. They also found that yields of w/o/w emulsion fell markedly when the concentration of secondary emulsifier (Tween 20) in the external aqueous phase was increased. Matsumoto et al. considered that the ratio of concentration of primary to secondary surfactant was significant in this particular system, and they suggested that more than 10 times as much Span 80 as Tween 20 was required to obtain 90% or higher yields of w/o/w emulsion. This is thought to be due to the solubilization of molecules of the primary (lipophilic) surfactant in the outer aqueous phase when the concentration of the secondary (hydrophilic) surfactant exceeds the critical micelle concentration. As the concentration of secondary surfactant increases, more of the primary surfactant may be incorporated into the secondary surfactant micelles, reducing the concentration of primary surfactant in the oil phase able to stabilize the w/o system. This would lead, if the concentration of primary surfactants at the interface falls, to the rupture of the oil layer resulting in the loss of the internal aqueous drops. This would explain the significance of the ratio of surfactant concentration on the yield of multiple drops.

Phase volumes

Matsumoto et al. (1976) found that the internal phase volume ($\phi_{w/o}$) had no significant effect on the yield of w/o/w emulsion under the experimental conditions studied. It would appear that w/o/w emulsions can be prepared using a wide range of internal phase volumes. Collings (1971) quotes a range of 5–75% water-in-oil, but the optimal range is 25–50%. He found, however, that the internal phase volume influenced the release of materials from the internal aqueous phase. Interesting results by Matsumoto et al. (1976) suggested that the secondary phase volume ($\phi_{w/o/w}$) influences the yield of multiple drops over a range of low volume fractions. When $\phi_{w/o/w}$ exceeded about 0.4 there was no significant effect.

Nature of entrapped materials

The nature of entrapped materials may have a bearing on the stability of the system. Due to the nature of the multiple emulsion, the middle phase may act as a membrane and osmotic effects may become significant. These effects are discussed in more detail later. Entrapped solutes may interact with the surfactant or in the case of surface active drugs be adsorbed at the interface, both potentially resulting in decreased stability.

Multiple emulsion types

The structure of w/o/w multiple globules is dependent on the nature of the secondary surfactant. Whitehill and Florence (1979) have shown that it is possible to prepare 3 different types of w/o/w emulsion (Fig. 8). Three different water-isopropyl myristate-water emulsions have been prepared using various non-ionic surfactants, each consisting of a primary water-isopropyl myristate emulsion stabilized with 2.5% sorbitan mono-oleate and containing 50% water. The multiple emulsions were then prepared by re-dispersing the primary w/o system in an equal volume of water containing 2% hydrophilic surfactants; Brij 30 (System A), Triton X-165 (System B) and Span 80: Tween 80 (3:1) (System C). The character of each multiple emulsion was significantly different. Type A emulsions were composed of small multiple drops (mean diameter 8.6 μm) 82% of which contained only one internal aqueous droplet (mean diameter 3.3 μm). Type B systems consisted of larger multiple drops (mean diameter 19 μm) containing smaller, but more numerous internal aqueous droplets (mean diameter 2.2 μm), whereas type C emulsions were composed of very large multiple drops (mean diameter 25 μm) which contained very large numbers of internal droplets which were difficult to resolve. In type C emulsions, it is thought that the primary (encapsulated) system is a flocculated w/o emulsion. The structure of the multiple drop probably depends on the efficiency of the second emulsification step.

In addition to the structures described, more complex multiple drops can also exist, where up to 5 phases may be present, e.g. w/o/w/o/w emulsions. In each system, however, simple oil drops are also present. All of these structures may coexist to some extent, although the emulsions we have described (Whitehill and Florence, 1979) consist predominantly of either type A, B or C drops.

Typical drop size distributions of w/o/w (multiple) drops and internal aqueous droplets of a type B emulsion are shown in Fig. 9. The difference in size of the two types of drop can be clearly seen. The presence of simple oil drops (i.e. containing no internal aqueous droplets) complicates matters further as it gives rise to 3 size distributions within a particular system. The w/o/w emulsions prepared by Matsumoto et al. (1976) appear to be similar to the type A drops described above. They claim to have produced a system where each single aqueous droplet is surrounded by a thin oil layer, in turn dispersed in water, although they were not able to observe this directly. They estimated the mean thickness of the oil layer on the surface of the internal aqueous droplets, $\bar{\Delta}$, (assuming that the oil phase is uniformly distributed over the surface of each aqueous droplet) from the mean diameter of the drops, a, and the volume fraction of the drops in the w/o emulsion ($\phi_{w/o}$) as follows:

$$\bar{\Delta} = \frac{1}{2}\left[\left(a^3/\phi_{w/o}\right)^{1/3} - a\right] \tag{1}$$

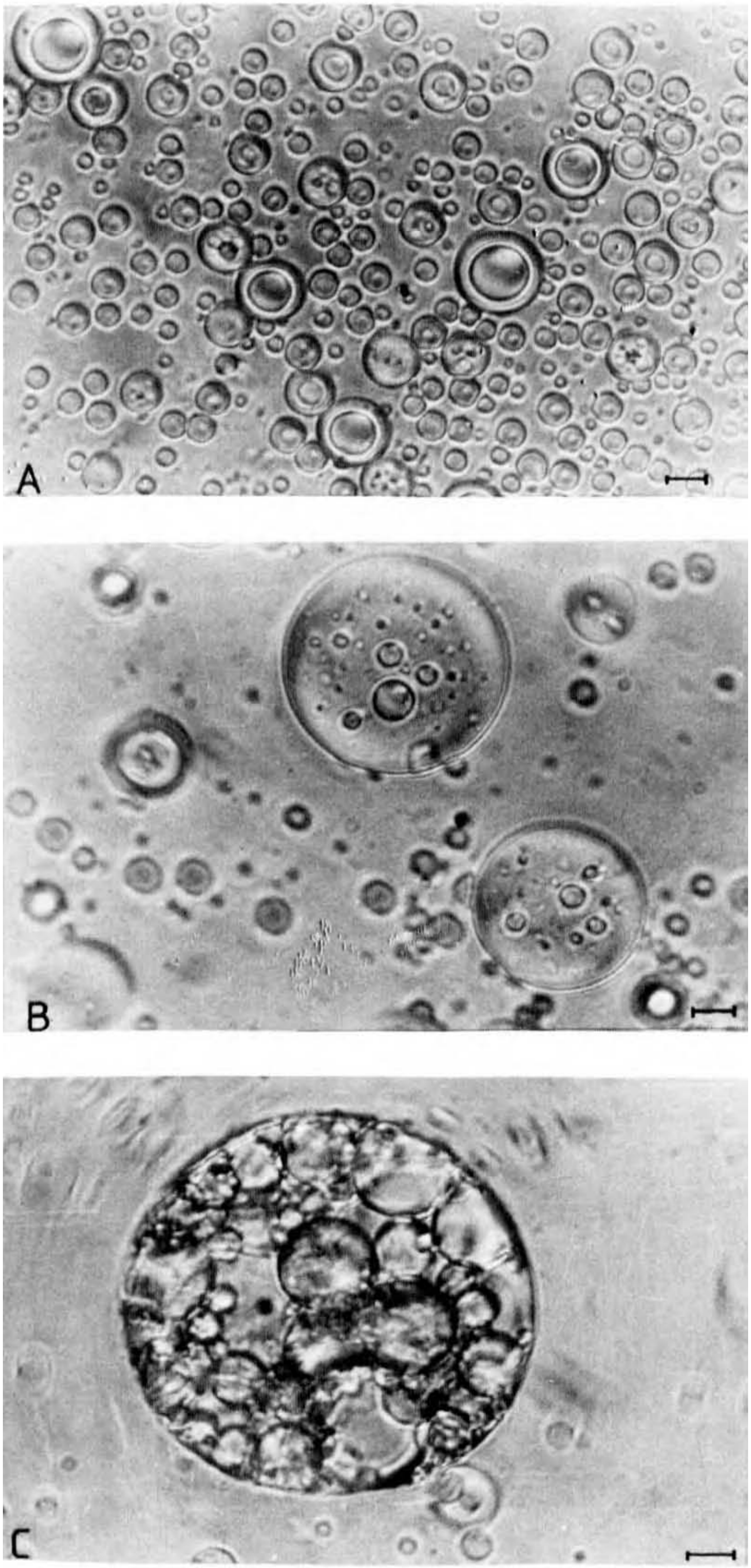

Fig. 8. Photomicrographs of three different water-isopropyl myristate-water emulsions A, B and C as designated in the text. Bar = 10 μm.

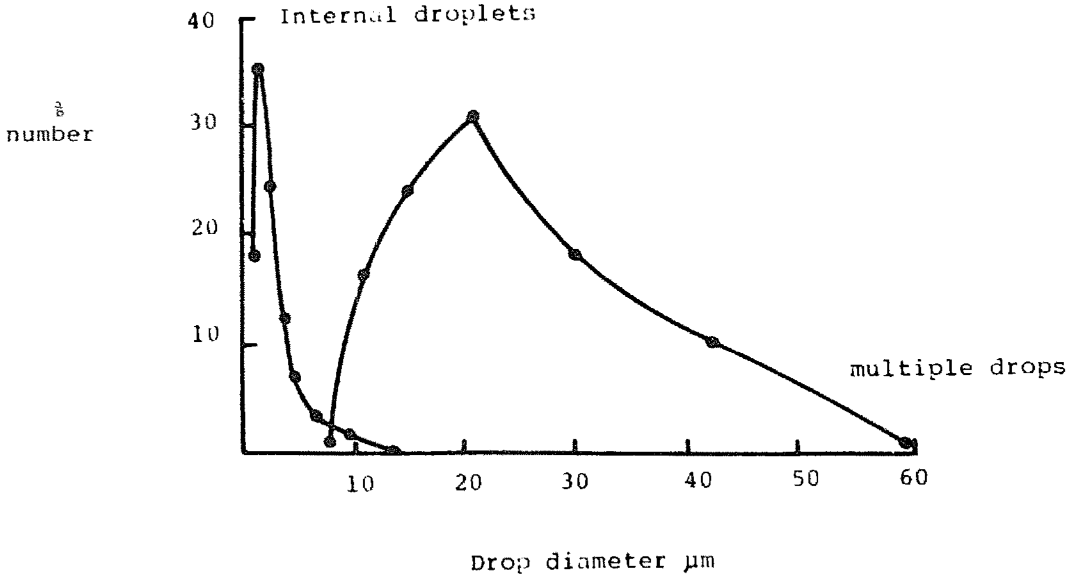

Fig. 9. Droplet size distribution of the internal aqueous and multiple drops of a type B multiple emulsion.

Assessment of stability of multiple emulsion systems

The complex nature of multiple emulsion systems has hindered the study of their stability. Over the past few years, a number of new techniques have been developed to detect and quantify breakdown. This section describes some of these techniques and discusses some of the advantages and problems associated with the various methods.

Measurement of the number and size of the multiple drops over a period of time gives a good indication of stability. The simplest and most direct method of achieving this is by photomicrography. There are many problems associated with this method, not the least being the tedium of the process which involves the observation of random samples under the microscope and comparing the sizes of the observed multiple, internal and simple drops with standard size circles on an eyepiece graticule. In may cases, the number of internal droplets entrapped can also be monitored.

As 3 different size distributions exist, it may be difficult to determine if the drops contain internal droplets, particularly if they are very small (< 1 μm) or if they take up a large proportion of the multiple drop and the internal droplet interfaces therefore become indistinct. Small simple drops may pass below large simple drops giving a false impression of multiple drops. Matsumoto et al. (1976) report the preparation of w/o/w emulsions in which the internal droplets could no be seen under the microscope because of reflected light from the surface of the oil drops. Another problem is that possible increases in droplet diameter due to coalescence may be offset by decreases in droplet size due to shrinkage or loss of internal droplets from multiple drops. The usual problems associated with photomicrography, of 'freezing' the motion of small droplets in Brownian motion particularly at higher magnifications (where longer exposures are required) also exist. Despite these problems this method provides useful information on stability and has been used by Davis and Burbage (1978) and by Whitehill and Florence (1979) and Florence and Whitehill (1981) in a study of breakdown mechanisms. The present authors have studied the change in the ratio of multiple to simple oil drops, the change in the number and size of the internal drops and changes in the diameters of the multiple drops over a period of time in 3 different w/o/w systems. Observation of these changes enabled the authors to build up a picture of breakdown pathways in multiple drops of different structures.

Davis and Burbage avoided the problems associated with very small internal droplets by using a freeze-etching electron microscope technique (Davis and Burbage, 1977) which is not applicable to routine size analysis. Samples of water/light liquid paraffin/water emulsions were prepared using Arlacel 83 (10%) as primary surfactant and Tween 80 as secondary surfactant. The samples were freeze-etched and examined using an electron microscope. From the electron micrographs, the internal water droplets were clearly seen and could be sized. Disadvantages with this method are the time and expense involved and the possibility of missing internal droplets not 'caught' within the frozen section.

A method of analysis based on the semi-permeable nature of the oil film between the two aqueous phases of a w/o/w emulsion was developed by Davis et al. (1976). The emulsion was exposed to an osmotic gradient provided by electrolyte in the external phase. Droplet shrinkage resulted from the transport of water from the internal to external aqueous phase as a consequence of the osmotic gradient. The rate and amount of shrinkage, related to surface area and the volume of the internal aqueous phase, was measured by Coulter Counter which, however, is not able to discern whether a drop is multiple or simple. Using a graphical inflection technique, Davis and Burbage (1978) found that, initially, during storage the mean diameter of the multiple drops fell as internal water was lost but later increased due to droplet coalescence.

An indirect method for estimating the number of multiple drops in w/o/w emulsions was described by Matsumoto et al. (1976). The primary, w/o, emulsion was prepared containing a small amount of a marker material (glucose) in the aqueous phase. Immediately after preparation the w/o/w emulsion was dialyzed against a certain volume of distilled water and the quantity which had migrated to the external aqueous phase was determined by microanalysis. The yield of multiple drops was obtained as follows:

$$\text{Yield}\ (\%) = 100 - 100a/(C/V_1 + V_2 + V_3) \qquad (2)$$

where a is the quantity of migrated glucose in g/ml of distilled water, C is the original weight of glucose in the inner aqueous phase and V_1, V_2 and V_3 are the volumes of the inner aqueous phase, external aqueous phase and distilled water, respectively. During the 20-hour period of dialysis some deterioration in the system may take place.

A similar method involved measurement of the passage of ions into the external aqueous phase due to rupture of the oil phase (Kita et al., 1977a). The w/o/w emulsion, containing a small quantity of sodium chloride in the internal aqueous phase, was placed behind a dialysis membrane and ion-sensitive electrodes used to detect the quantity of migrated ions. The multiple drop yield was calculated as:

$$\text{Yield}\,(\%) = 100 - 100x/V_i \tag{3}$$

where

$$x\,(\text{ml}) = A(V_0 + V_d)/(B - A) \tag{4}$$

and A and B are the concentrations of the particular ion in the distilled water and aqueous phase, respectively, x (ml) is the volume of leaked internal aqueous phase, and V_i, V_0 and V_d are the volumes of the internal aqueous phase, external phase and distilled water, respectively. As water is lost from the internal to external aqueous phase in a w/o/w emulsion the viscosity of the external phase should decrease somewhat and, as the overall viscosity of the emulsion is dependent on continuous phase viscosity, this should be reflected in the rheological properties of the w/o/w system. In view of this, Kita et al. (1977b) attempted to estimate the stability of w/o/w emulsions by following viscosity changes with time.

The viscosity of a disperse system in the Newtonian flow range is given by Mooney's equation as:

$$\ln \eta_{\text{rel}} = a\phi(1 - \lambda\phi) \tag{5}$$

where η_{rel} is the relative viscosity, a is a shape factor, λ is the crowding factor and ϕ is the volume fraction of dispersed phase. This equation can be written as:

$$\ln \eta_{\text{rel}} = a(\phi_{\text{wi}} + \phi_0)/\{1 - \lambda(\phi_{\text{wi}} + \phi_0)\} \tag{6}$$

where the dispersed phase volume fraction is represented by $(\phi_{\text{wi}} + \phi_0)$ and where ϕ_{wi} and ϕ_0 are the volume fractions of the internal aqueous phase and oil phase, respectively (which effectively make up the dispersed phase in the multiple system). The equation can be modified to read:

$$\phi_{wi} = a[\log \eta_{rel} \cdot \{(2.303/a) - (2.303\lambda\phi_0/a)\} - \phi_0]/(a + 2.303\lambda \log \eta_{rel}) \tag{7}$$

which shows the relationship between the volume fraction of the internal aqueous phase and the relative viscosity of the system. ϕ_0 remains constant on rupture of the oil layer but ϕ_{wi} decreases with increasing ruptures of the oil layer as the internal water mixes with the external aqueous phase. Kita et al. (1977b) were able to use the viscometric method to estimate stability of w/o/w emulsions which had a relatively low volume fraction of internal aqueous phase (< 0.2). Emulsions with volume fractions above this do not exhibit Newtonian flow at low shearing rates. Ingredients such as glucose, bovine serum albumin and electrolytes altered the dispersion state of the drops and resulted in difficulties in evaluating the decreases in the amount of the internal aqueous phase.

The rate of swelling or shrinkage of the internal aqueous phase on ageing can be estimated from the rate change of viscosity:

$$\phi_{wi} = \left[(1 - \lambda\phi_0)\ln \eta_{rel} \cdot - \alpha\phi_0\right] / (\alpha + \lambda \ln \eta_{rel}) \tag{8}$$

where α and λ are constants as follows:

$$\alpha = (\eta_0 + 2.5\eta')/(\eta_0 + \eta') \tag{9}$$

$$\lambda = \exp(0.076 + 0.01003/D + 0.0029/D^2) \tag{10}$$

and η_0 and η' are the viscosities of the continous and dispersed phases, respectively, and D is the diameter of the drops (Matsumoto and Khoda, 1980). The rate of swelling or shrinkage can thus be calculated. The rate of change of viscosity in the initial stages of ageing was apparently proportional to the osmotic pressure difference across the oil phase.

Matsumoto and Khoda (1980) estimated the flux of water across the oil layer from changes in viscosity in the initial stages of ageing by using the modified form of Mooney's equation (Eqn. 7) which correlates the volume fraction of the internal aqueous phase and the viscosity of he system. This enabled them to estimate a water permeation coefficient (P_0) for the oil layer which they found to be in the range 10^{-4} to 10^{-5} cm · s^{-1} at 25°C.

The rate of swelling or shrinkage was calculated by subtracting the quantity of water taken up into the oil layer by solubilizing micelles from the total water flux.

$$(d\phi_w/dt)_{osmo.} = d\phi_w/dt - (d\phi_w/dt)_{solub.} \tag{11}$$

The value of $(d\phi_w/dt)_{solub.}$ was calculated from the initial slope of the plot of ϕ_{wi} versus time for samples in which the aqueous phases were isotonic. When glucose or sodium chloride was present in the internal aqueous phase the viscosity of the emulsions was found to increase initially and later decreased. This was explained by the migration of water from outer to the internal aqueous phase to satisfy the osmotic gradient caused by the glucose or sodium chloride in the internal phase. This would lead to swelling of the internal droplets leaving less water in the external phase resulting in an increase in viscosity. As the droplets continue to swell, however, the oil layer would eventually rupture causing the internal water to be released into the external phase again resulting in a decreased viscosity. The authors have used a cinemicrography technique (Florence and Whitehill, 1981) to evaluate water transfer rates from single drops in a w/o/w system. The lives of single drops in an osmotic gradient were recorded on film so that changes in drop size could be measured by frame-to-frame analysis on a film analyzer. From the volume flux of water, we were able to calculate an approximate diffusion coefficient for water in the oil layer due to the osmotic gradient

$$\phi_w = \frac{D_w A}{\Delta x} \cdot \frac{\Delta\pi}{RT} \tag{12}$$

where ϕ_w = volume flux of water, D_w = diffusion coefficient of water in oil phase, Δx = diffusion layer thickness, A = surface area of multiple drop, and $\Delta\pi$ = osmotic gradient. The osmotic permeation coefficient is given by (Davis and Burbage, 1978)

$$P_0 = D_w/\Delta x \tag{13}$$

When the internal aqueous phase contains electrolyte, the osmotic gradient created is:

$$\pi = \nu\,RT\,\xi\,m \tag{14}$$

where ν is the number of ionic species produced by the solute, ξ is the osmotic coefficient of the electrolyte, m is the molality of the electrolyte, R is the gas constant, and T is the absolute temperature. Using a calculated value of P_0 of 0.63×10^{-4} cm · s^{-1}, we calculated a value of 5.15×10^{-8} $cm^2 \cdot s^{-1}$ for the diffusion coefficient of water in the isopropyl myristate phase of a w/o/w emulsion in an osmotic gradient created by sodium chloride (0.2 M solution) in the external aqueous phase. The diffusion coefficient of inverse mixed non-ionic surfactant micelles in dodecane (η = 1.35 cP at 20°C) has recently been measured by photon correlation spectroscopy to be 2.7×10^{-7} $cm^2 \cdot s^{-1}$ (Al-Saden et al., 1982). The viscosity of isopropyl myristate is 4.1 times that of dodecane, and thus the same micellar unit might be expected to have a diffusion coefficient of approximately 6.5×10^{-8} $cm^2 \cdot s^{-1}$ (assuming similar micellar characteristics), close to the value obtained by cinemicrographic studies. There is some evidence, therefore, to suggest that the transport of water through the oil layer in w/o/w emulsions is by way of inverse micellar species.

The cinemicrography method described by Florence and Whitehill (1981) is useful for the examination of multiple emulsions under various conditions. The apparatus used was a modified time-lapse cinemicrography unit developed by Dawson (1963) for the study of cells in tissue culture. Droplet movements and breakdown mechanisms could be studied over various periods of time and recorded on film by using time-lapse and normal speed filming. Work has also begun using high-speed cinemicrography to record the rupture and coalescence phenomena in w/o/w systems.

A photodensitometric method to follow the breakdown of multiple emulsions has been suggested by Davis (1976). This is based on the change in the appearance of the multiple drops from dark through grey to clear on storage as the internal phase is lost to the external medium. This may be a useful method for studying changes in type C systems.

Stability and release mechanisms

The mechanisms of instability in multiple emulsions are complex and difficult to study. Florence and Whitehill (1981) have attempted an analysis of the possible mechanisms of instability. Theoretical predictions appear to agree with experimental results. W/o/w multiple drops, for example, may coalesce with other oil drops (simple or multiple) or they may lose their internal droplets by rupture of the oil layer on the surface of the internal droplets, leaving simple oil drops. Under the influence of an osmotic gradient, the middle oil phase acts as a 'semi-permeable' membrane resulting in the passage of water across the oil phase. This leads to either swelling or shrinkage of the internal droplets, depending on the direction of the osmotic gradient. Another possible breakdown mechanism may be coalescence of the internal aqueous droplets within the oil phase. A combination of these mechanisms may take place and the exact breakdown mechanisms remain unclear. The likelihood of events taking place may be predicted by analysis of the Van der Waals attractive forces and free-energy changes in these systems (Florence and Whitehill, 1981).

The ability of multiple emulsions to entrap materials is one of their most useful assets and so the passage of materials from the internal to external phase, across the middle phase, is important. This is particularly so in pharmaceutical systems where multiple emulsions are envisaged as possible controlled-release drug delivery systems. The stability and release characteristics of emulsion systems are influenced by a number of factors such as the composition of the emulsion, droplet size, viscosity, phase volumes, pH, etc. This becomes even more complex in multiple systems because there are two dispersed phases, two phase volumes, at least two surfactants, and 3 different droplet size distributions.

The release of materials from complex systems will be influenced by the factors controlling the stability and properties of simple emulsion systems but also by a number of factors specifc to multiple emulsion systems.

Specific factors controlling the stability of multiple emulsions

Effect of electrolytes

Electrolyte presence appears to be one of the most important factors in determining the stability and release of materials from the internal droplets. The effects of electrolytes are two-fold: (a) osmotic and (b) interfacial, the former being peculiar to multiple systems. The effects of electrolytes on electrical double layers, etc., have not been considered as they are not specific to multiple systems.

(a) Osmotic effects

Collings (1971) found that w/o/w emulsions broke down rapidly in vivo close to the site of injection, with the consequence that no significant delay in response to the entrapped drug was obtained compared to aqueous solutions of the drug. It was found that the premature breakdown of the emulsions, in vivo, was due to the fact that the middle oil phase was acting as a semipermeable membrane between the two aqueous phases. The osmotic pressure in the external environment (body fluids) was higher than the internal phase leading to shrinkage of the internal aqueous droplets, and/or rupture of the oil layer.

Thus water molecules may pass from one aqueous phase to the other. If the osmotic pressure is higher in the internal aqueous phase, water may pass to this phase resulting in swelling of the internal droplets which eventually burst, releasing the contents. The reverse is true if the osmotic pressure is higher in the external aqueous phase resulting in transfer of water from the internal to external aqueous phase causing shrinkage of the internal droplets. If the osmotic pressure difference across the oil layer is extreme, then passage of water is so rapid that almost immediate rupture of the oil drops occurs with expulsion of the internal droplets. This appears to occur frequently where the oil layer is thin, for example in type A and type C drops, but also occurs to some extent in type B drops. Materials other than electrolytes, such as proteins and sugars and of course drugs, in either aqueous phase can also exert this effect. Collings (1971) partially solved the problem by the addition of small amounts of sodium chloride to the internal aqueous phase so that this phase was isotonic with the final external phase but this appraoch can lead to inequality of pressure on storage which is unsatisfactory. The osmolarity may also be adjusted by the addition of other materials such as glucose or glycerol.

When the oil layer ruptures the inner aqueous phase in the droplet disappears instantaneously followed by mixing of the internal aqueous phase with the external aqueous medium, leaving a simple oil drop.

Davis and Burbage (1978) investigating the effects of sodium chloride on the size of multiple drops found that a threshold concentration of electrolyte exists below which little or no change in size occurs. This appeared to be related to drop diameter – larger multiple drops required a higher concentration of sodium chloride to effect shrinkage. Electrolytes, then, may be used to control the rate and amount of materials transferred from one phase to the other.

Matsumoto and Khoda (1980) measured the water permeation coefficient of the oil layer indirectly by measuring the change in viscosity of the w/o/w emulsion to determine change in globule diameter and using the following equation:

$$\phi_w = -P_0 A(g_2 c_2 - g_1 c_1) \tag{15}$$

where P_0 is the osmotic permeability coefficient of the oil 'membrane' ϕ_w is the trans-layer flux of water in moles per unit time, A is the area of the 'membrane', g is the osmotic coefficient and c, the solute concentration. The trans-layer flux of water can be replaced by $(d\phi_w/dt)_{osmo.}(cm^3 \cdot s^{-1})$ because ϕ_w is the volume of the inner aqueous phase in unit volume of the system, while the area can be replaced by the total surface area of the dispersed drops in unit volume of freshly prepared emulsion. The results of Matsumoto and Khoda are shown in Table 1. We have obtained a similar estimate of P_0 using a more direct cinemicrography technique (Florence and Whitehill, 1981).

TABLE 1

ESTIMATES OF THE WATER PERMEATION COEFFICIENT (P_o) OF THE OIL LAYER OF w/o/w EMULSIONS UNDER VARIOUS OSMOTIC PRESSURE GRADIENTS AT 25°C (DATA FROM MATSUMOTO AND KHODA, 1980)

Volume fraction of internal aqueous phase $\phi_{w/o}$	Volume fraction of primary emulsion $\phi_{w/o/w}$	Additives		$(d\phi_w/dt)_{solub.}$ (10^{-5} $cm^3 \cdot s^{-1}$) water solubilized surfactants in oil phase	$(d\phi_w/dt)_{osmo.}$ (10^{-5} $cm^3 \cdot s^{-1}$) total water passing across oil layer	P_o (10^{-4} $cm \cdot s^{-1}$) water permeation coefficient
		Internal aqueous phase	External aqueous phase			
0.5	0.05	None	None	2.86	0	0
0.5	0.05	17 mM NaCl	None	2.86	3.80	0.31
0.5	0.05	14 mM glucose	None	2.86	7.72	1.53
0.5	0.05	28 mM glucose	None	2.86	9.38	0.93
0.5	0.05	56 mM glucose	None	2.86	15.83	0.78
0.5	0.10	None	None	10.80	0	0
0.5	0.10	None	28 mM glucose	10.80	6.25	3.10
0.5	0.10	None	14 mM glucose	10.80	10.24	1.02

Using Eqn. 15, values of P_0 in the range -0.018×10^{-4} to -0.582×10^{-4} (mean value $-0.116 \pm 0.071 \times 10^{-4}$) $cm \cdot s^{-1}$ were found for passage of water from the internal to external aqueous phase in an osmotic gradient created by 3 M sodium chloride, and in the range -0.042×10^{-4} to -0.438×10^{-4} $cm \cdot s^{-1}$ (mean value $-0.162 \pm 0.15 \times 10^{-4}$) $cm \cdot s^{-1}$ for the flux of water in the opposite direction under the same conditions (Whitehill, 1981). The osmotic permeability coefficient is used to calculate the diffusion coefficient for water in the oil layer and so is useful in determining the mode of water transport under an osmotic gradient.

(b) Interfacial effects

The release of the narcotic antagonist, naltrexone hydrochloride, from the internal aqueous phase of a w/o/w emulsion was found (Brodin et al., 1978) to decrease initially with increasing sodium chloride concentration in the internal aqueous phase. A decrease in the diffusion coefficient of the drug of 73% was obtained with 9% w/v sodium chloride dissolved in the internal aqueous phase (Fig. 10). Sorbitol also caused a decrease in the diffusion coefficient but at an equivalent sodium chloride concentration of about 6% w/v. These results indicate that factors other than osmotic gradients are affecting passage of the drug. Brodin et al. (1978) suggested that sodium chloride competes with surfactant for water molecules at the inner w-o interface, which would result in a rigid interfacial layer which would be a more effective mechanical barrier to drug transfer.

Mechanism of passage of materials across the 'membrane' phase

There are several possible mechanisms by which materials may be transferred across the oil layer in a w/o/w system. The most obvious is by diffusion for unionized lipid-soluble materials. This will be dependent on the nature of the material (including its dissociation constant) and the oil and the pH of the aqueous

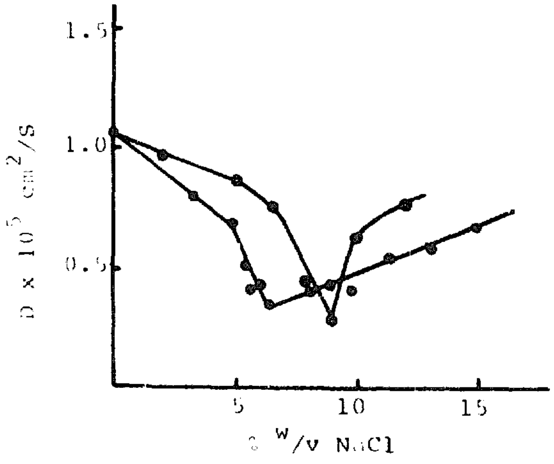

Fig. 10. Effective diffusion coefficients of naltrexone hydrochloride in w/o/w emulsions containing sodium chloride (●) or sorbitol (expressed as sodium chloride equivalents (○)) in the internal aqueous phase. From Brodin et al. (1978).

phase. In this respect, liquid membranes have been proposed as possible treatments for drug overdosage (Chiang et al., 1978). The system is shown systematically in Fig. 11. This system could be used, for example, in the treatment of overdosage of acidic drugs such as aspirin or barbiturates. The emulsion would be administered orally and the stomach environment (about pH 2) thus becomes the external aqueous phase. At low pH values the barbiturate would exist almost exclusively as the unionized form and so would be readily soluble in the oil phase. The drug could therefore easily pass across the oil layer to the internal aqueous phase in the direction of the concentration gradient. The internal aqueous phase is formulated to contain a basic buffer which would ionize the barbiturate. This serves two purposes —first, the concentration gradient of unionized drug is maintained, and secondly, the ionized form of the drug is virtually insoluble in the oil layer and so would become trapped within the internal aqueous phase and carried out with the emulsion as it is voided from the gastro-intestinal tract. The effect of extreme pH and other factors, such as peristalsis, on the stability of these systems, in vivo, is not, however, considered by the authors and the practical application of these systems would appear to be something for the future. It is obvious that the stability is of paramount importance in this case. However, as more than 90% of phenobarbitone and secobarbitone were removed within 10 min, in vitro, these systems are certainly worth further consideration.

Drug transport in vitro has been found to follow first-order kinetics, Fick's law

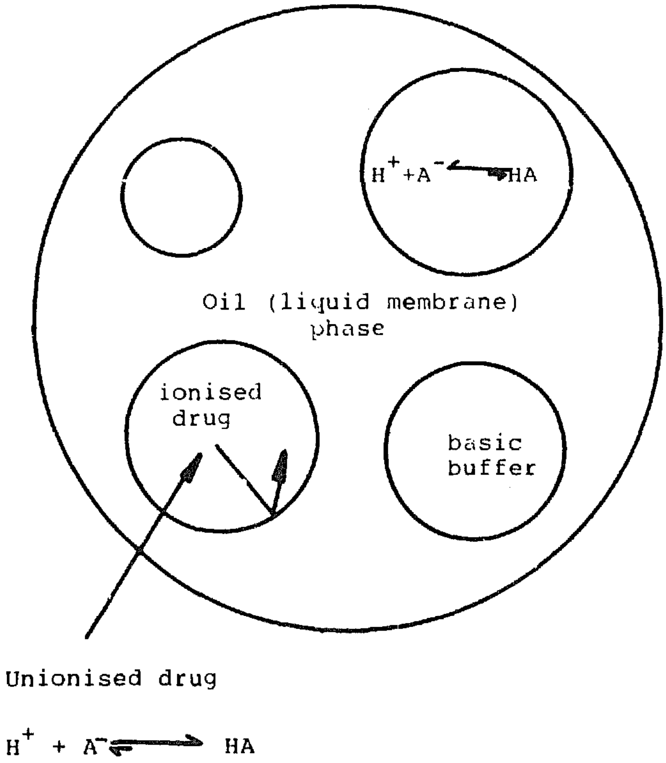

Fig. 11. w/o/w 'liquid membrane' system for removal of acidic drugs from an aqueous system.

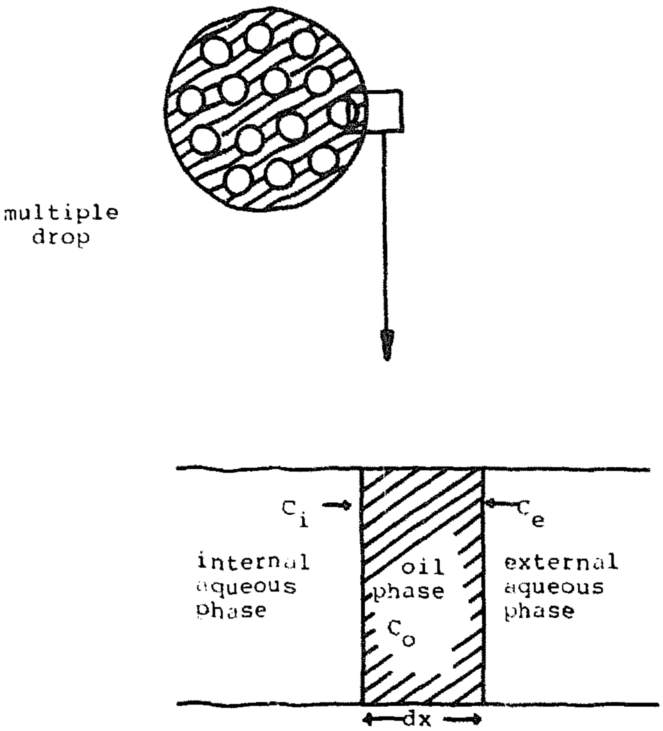

Fig. 12. Two-dimensonal model for diffusion controlled transport of unionized materials from the external aqueous phase to the internal aqueous droplets in a w/o/w system.

being obeyed (See Fig. 12)

$$\frac{dC_0}{dt} = \frac{-DA\Delta C}{dx} = \frac{-DA}{dx}(C_0 - C_i) \tag{16}$$

$$\text{Since } C_e = C_0/P \tag{17}$$

then

$$\frac{dC_e}{dt} = \frac{1}{P} \cdot \frac{dC_0}{dt} = \frac{DA}{dx}\frac{1}{P}(PC_e - C_i) \tag{18}$$

where C_0 = concentration of drug in the oil phase, C_i = concentration of drug in the internal aqueous phase, C_e = concentration of drug in the external aqueous phase, D = diffusion coefficient of drug in the oil phase, dx = diffusion layer thickness, P = partition coefficient of drug between the oil and external aqueous phase, and t = time. When a trapping agent is used in the internal aqueous phase, the material becomes ionized and so C_i is negligible; Eqn. 18 can be written as:

$$\frac{dC_e}{dt} = \frac{-DAC_e}{dx} = -kC_e \tag{19}$$

and so by integration.

$$C_e = C_e e^{-kt} \tag{20}$$

The rate constant, k, is a function of the diffusion coefficient of the drug, the area between the external phase and the liquid membrane, and the membrane thickness.

Frankenfeld et al. (1976) have also used such systems to remove phenobarbitone and aspirin from pH 2 solutions in vitro. Up to 95% of the drug could be removed in a few minutes. They also found that drug removal was diffusion-limited and followed first-order kinetics. Their results indicated that the capacity of the system was limited by the efficiency of the trapping agent but the rate of drug removal was dependent on diffusion rate. The permeation rate was affected by temperature, oil-layer thickness and viscosity, and the diffusion and partition coefficients of the material.

Brodin and Frank (1978) have investigated the release of naltrexone from peanut oil/water/peanut oil emulsions and found a complex release pattern, particularly in systems containing higher concentrations of the drug. It was found that the drug was released in two distinct phases, which they termed the α and β phases. The quick-onset α-phase corresponded to release from an oil suspension, which possibly reflected presence of drug particles in the external oil phase due to loss from the internal oil phase during manufacture of the emulsions. This was confirmed by the fact that emulsions prepared by sonication (giving rise to higher concentrations of drug in the external phase) exhibited a retarded β-phase. The release during the β-phase was much slower and appeared to be due to release from the internal oil phase. Emulsions containing lower concentrations of drug were found to release drug mainly from the internal oil phase. The situation was complicated, however, by the presence of both solid and dissolved naltrexone and the authors pointed out that, since the distribution of solid and dissolved drug was not known a quantitative discussion of the α- and β-phases was not possible.

Yang and Rhodes (1980) also found a two-phase mechanism for the transport of acidic acid solutes from the external aqueous phase to the internal aqueous phase. Assuming that no oil membrane rupture occurs the transport process may be described by equation 21,

$$C_e^t = Ae^{-\alpha t} + Be^{\beta t} + C_e^s \tag{21}$$

where C_e^t is the concentration of solute in the external aqueous phase at any time t, A and B are pre-exponential terms, α and β are the first-order rate constants and C_e^s represents the concentration of solute in the external aqueous phase when steady-state is reached. Chilamkurti and Rhodes (1980) went on to evaluate first-order micro-rate constants according to the equation

$$C_e \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} C_{LM} \underset{k_{32}}{\overset{k_{23}}{\rightleftharpoons}} C_i \tag{22}$$

where C_e, C_{LM} and C_i represent the concentrations of solute in the external aqueous,

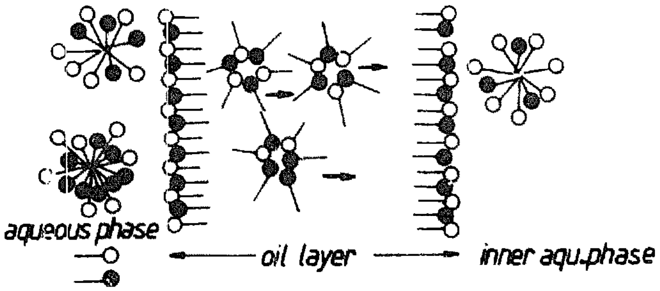

Fig. 13. Schematic illustration of a model for micellar transport of water from the outer aqueous phase to the inner aqueous phase through the oil layer in w/o/w emulsions, after Kita et al. (1978).

liquid membrane and internal aqueous phase, respectively, and k_{12}, k_{21}, k_{23} and k_{32} are the first-order rate constants.

Brodin and Frank (1978) also investigated the effect of changing the hydrophobicity of the drug on the release from o/w/o emulsions. It was found that the diffusion coefficient of thymol (which has a high partition coefficient in vegetable oils) in the o/w/o emulsions was similar to the diffusion coefficient in oil containing 'Span 80' and the primary o/w emulsion, which suggests that diffusion in the oil was the rate-limiting step in the o/w/o and o/w emulsions. The water membrane cannot be the rate-limiting step as the diffusion coefficient of thymol in water is relatively high.

o/w/o emulsion systems, as liquid membranes, have been used to separate hydrocarbons (Li, 1968). This technique involves selective permeation of the hydrocarbons through an aqueous 'membrane' phase. The solubility of the hydrocarbons in this aqueous phase is the determining factor for selective permeation, and so the compound which has the greatest solubility in water will be the preferentially diffusing compound. The diffusion coefficient of the permeating compound in the aqueous layer is also of importance.

Although diffusion seems to be the most important mechanism of transport in

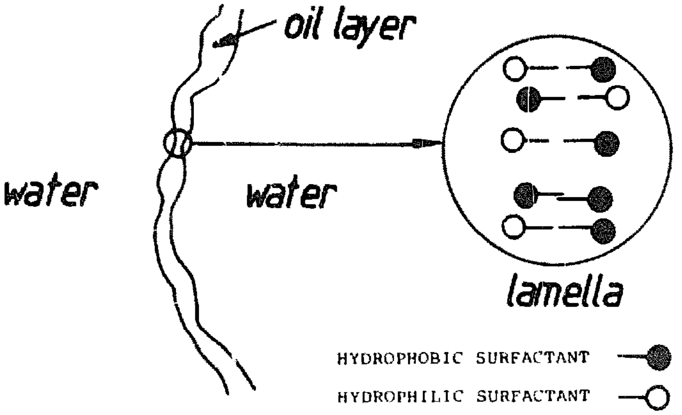

Fig. 14. Schematic illustration of a model for water transport through thin lamellae of surfactant due to fluctuations in the thickness of the oil layer, after Kita et al., (1978).

multiple systems, there is now some evidence to suggest that ionized materials may pass across the oil layer in the w/o/w emulsions. Kita et al. (1978) have suggested two possible mechanisms for the permeation of water through the oil phase in w/o/w emulsions. The first of these is by the carriage of water in mixed, inverse micelles of hydrophobic and hydrophilic surfactants, evidence of which has been found by Florence and Whitehill (1981). Alternatively, water molecules may diffuse across very thin lamellae of surfactants formed where the oil layer is very thin (Fig. 14). Experiments carried out by Kita et al. (1978) suggested that these mechanisms probably come into play when there is an osmotic pressure difference between the two aqueous phases. A w/o emulsion was placed within a semi-permeable membrane and the quantity of water migrating to the external aqueous phase was determined by measuring small quantities of glucose (originally in the internal aqueous phase) in the external aqueous phase. The same workers have also used a similar method in which the migration of ionic materials was measured (Kita et al., 1977a).

The third possible transport mechanism is carrier-mediated transport. This involves either incorporation of some material into the internal aqueous phase or membrane which reacts with the permeating compound to render it liposoluble. The carrier compounds effectively 'pump' the permeating compound across the membrane. An example of carrier compounds which 'pump' is discussed by Kopp et al. (1978). The example quoted is that of stearic acid (soluble in oil but not in water) used as a carrier for copper ions according to the following mechanism:

$$Cu^{2+} + 2HSt \rightleftharpoons 2H^{+} + CuSt_2$$

The copper ion reacts with two stearic acid molecules in the oil membrane, releasing two protons, and is transferred as copper stearate across the oil phase and exchanged against two protons at the inner interface. The protons are then transported as stearic acid back across the oil layer and exchanged against the copper ion at the outer interface. Using such a system, copper ions can be transported against a concentration gradient. Kopp et al. (1978) also discuss the mathematical treatment of permeation in liquid membrane with and without carrier compounds. The multiple drop may be considered as a sphere with a large number of immobile reaction sites (internal aqueous droplets) within. In the case of carrier-free diffusion, the transferred material is considered to react reversibly with the immobile sites but irreversibly when transport is carrier-mediated. This particular model is more useful in discussing carrier-mediated transport rather than the 'hollow sphere' model discussed by several other authors (Hochauser and Cussler, 1975; Matulevicius and Li, 1975; Cahn and Li, 1976; Kopp et al. 1978). Carrier-mediated transport is especially useful in the removal of contaminants from waste-water, in particular, heavy metals.

A fourth, and probably much less important transport mechanism is solubilization of minute amounts of the internal phase in the membrane phase resulting in the passage of very small quantities of materials.

Properties of the 'membrane' phase

The properties of the membrane phase will be of prime importance in determining the stability of the system and mass transfer rates. Of these properties, the most important is viscosity. Frankenfeld et al. (1976) prepared different w/o/w systems using a mixture of two oils—'Solvent 100 Neutral' (a high viscosity isoparaffinic oil) and 'Norpar 13' (a normal paraffinic oil). The relative proportions of each oil determined the overall viscosity of the oil (membrane) phase. The higher the concentration of 'Norpar 13', the lower the viscosity of the membrane, and the greater the rate of transfer of material from the external to the internal phase. The stability of the membrane toward rupture and leakage of entrapped materials, however, descreased with decreasing viscosity. In fact, formulations containing greater than 50% 'Norpar 13' were found to be too unstable for practical use. The presence of surfactants in the membrane may also alter its viscosity.

The thickness of the membrane phase and the type of surfactant used may also be important in determining stability and transport rates. Li (1971) found that ionic surfactants gave a much higher transport rate of toluene through an aqueous membrane. The diffusion rate of toluene also varied with the hydrophilic chain length of non-ionic surfactants. The concentration of emulsifier may also be an important factor. Collings (1971) investigated the effect of the concentration of primary surfactant on the release of sodium chloride from the internal aqueous phase of w/o/w emulsions and the results are shown in Table 2.

Properties of the interfacial films

In a second-order multiple emulsion system, there are two separate interfacial films to be considered. The nature of each film will depend on the nature of the primary and secondary surfactants, used to prepare the primary and multiple emulsions, respectively, and also on the presence of materials in the internal and continuous phases. The viscosity and elasticity of the o–w and w–o interfacial films are particularly important in determining the stability of both the primary and

TABLE 2

EFFECT OF PRIMARY SURFACTANT ('ARLACEL 83') CONCENTRATION ON THE RELEASE OF SODIUM CHLORIDE FROM THE INTERNAL AQUEOUS PHASE OF w/o/w EMULSIONS (FROM COLLINGS, 1971)

Concentration (%)	% release of sodium chloride/min
2	0.0584
4	0.0231
5	0.0148
6	0.0325
7	0.0347
8	0.0358
9	0.0687
10	0.0400

secondary emulsions by, for example, hindering the close approach of drops (by surfactant chain effects) and preventing coalescence (by acting as a mechanical barrier). They will also determine the transport of materials across the interface and through the different phases of the emulsion. The effects of salt on these films has already been mentioned. The extent to which these films retain their original characteristics on ageing is not known. Since the o–w and w–o interfaces are in close proximity to each other, diffusion of the emulsifier between the interfacial films may take place, thereby changing the composition and thickness of the adsorbed multilayer, presumably until an equilibrium is reached.

The presence of liquid crystal structures at both the w–o and o–w interfaces in multiple emulsions has been investigated by Kavaliunas and Frank (1978). Microscopic examination of w/o/w emulsions between crossed polarizers revealed the presence of liquid crystal phases at both inner (w–o) and outer (o–w) interfaces in a w/o/w system composed of water, *p*-xylene and nonylphenol diethylene glycol ether. Liquid crystalline phases were also detected in o/w/o emulsions at both interfaces. The presence of these liquid crystal structures was found to improve the stability of the emulsions markedly.

Properties of the primary emulsion

Instability of the primary emulsion will result in coalescence of the internal droplets within the membrane phase resulting in increased breakup of the multiple drop due to rupture. The internal aqueous phase volume ($\phi_{w/o}$) may influence the release of materials from the internal aqueous phase. Some results obtained by Collings (1971) are shown in Table 3.

Theoretical analysis of instability in multiple emulsions

Some of the possible instability mechanisms which are possible in a w/o/w system are shown in Fig. 15. The multiple (oil) drop may coalesce with other oil drops, simple or multiple (a); the internal aqueous droplets may be expelled individually (b, c, d, e) or more than one may be expelled (f); or they may be less

TABLE 3

EFFECT OF THE INTERNAL PHASE VOLUME ($\phi_{w/o}$) ON THE RELEASE OF SODIUM CHLORIDE FROM THE INTERNAL AQUEOUS PHASE OF w/o/w EMULSIONS (FROM COLLINGS, 1971)

Internal phase volume $\phi_{w/o}$	% release of sodium chloride/min
10	0.0174
20	0.00832
30	0.0143
40	0.0427
50	0.0400

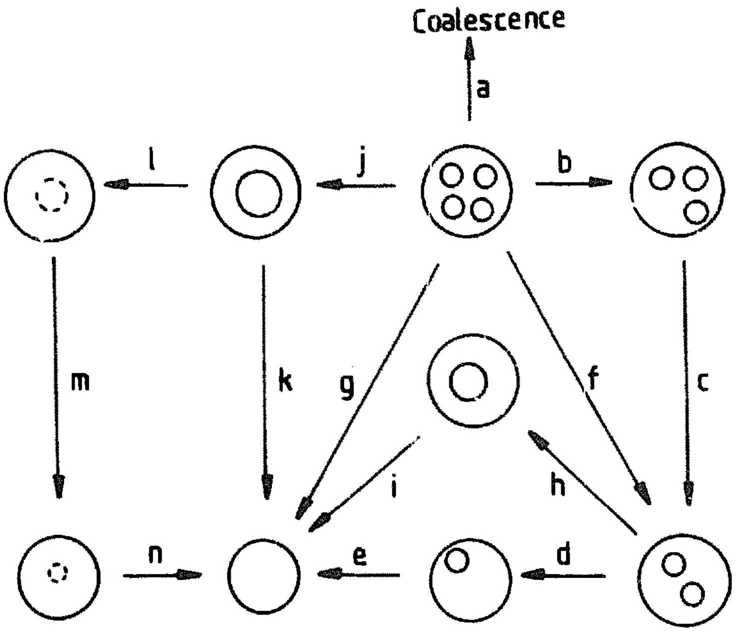

Fig. 15. Representation of the possible breakdown pathways which may occur in w/o/w or o/w/o systems. See text for explanation.

frequently expelled in one step (g); the internal droplets may coalesce before being expelled (h, i), (j, k); or water may pass by diffusion through the oil phase gradually resulting in shrinkage of the internal droplet (l, m, n). Whether all these mechanisms occur in all systems is not clear; neither is the relative importance of each mechanism in different w/o/w systems. On the other hand, this may be oversimplified and a combination of the above events may take place.

Calculation of the change in the free-energy of the system associated with each step in a particular breakdown pathway may allow a prediction of which pathway is likely to be followed. Coalescence of the multiple drops, for example, would result in a relatively large change in the free-energy brought about by the reduction in interfacial area. Coalescence between the small internal aqueous droplets, on the other hand, would not be expected to be a major route of breakdown. These predictions generally agree with experimental evidence (Florence and Whitehill, 1981). Some progress toward an understanding of these systems is also possible by considering the influence of the presence of water within the oil drops on the interaction between the oil drops and by consideration of the influence of the size of the internal water droplets on their internal stability and on the possibility of coalescence with the external aqueous phase. It is premature to consider all this in detail as the application of colloid stability theory to simpler emulsions has not been particularly successful (Florence and Rogers, 1971a). For type A w/o/w emulsions, the approach of Vold (1961) may perhaps be used if the oil layer is thought of as the homogeneous 'adsorbed' layer. Alternatively the effect of the internal phase on the size of the oil droplet is perhaps worth considering. The Hamaker constant for the oil phase may be determined from refractive index measurements at different wavelengths by the use of the Pulfrich refractometer (Whitehill, 1981) and then following the method of Gregory (1969). In the first instance interactions between identical type A multiple drops are considered for convenience.

For two spherical particles of radius $r_1 < r_2$ of composition p in a medium m with a separation Δ between the surfaces, the energy of attraction, V, is given by:

$$-12V = \left(A_p^{1/2} - A_m^{1/2}\right)^2 H\left(\frac{\Delta}{2r_1}, \frac{r_2}{r_1}\right) \tag{23}$$

where the quantities A_p and A_m are constants depending on the composition of particle and medium, and the function H(x, y) is positive, decreasing from its limiting value for $x \ll 1$ to 0 as x becomes very large,

$$\underset{x\to 0}{\overset{\lim}{\leftarrow}}(x, y) = \frac{y}{x(1+y)} \tag{24}$$

The nett interaction for two particles covered with an adsorbed layer is given by:

$$V = V_f - 2V_D \tag{25}$$

where V_f is the sum of the interaction of two solvated particles in contact and the interaction energy of two imaginary particles with the composition of the medium, and V_D is the interaction of an imaginary particle and a real solvated particle in contact.

Modifying Vold's approach to type A multiple drops, the total interaction energy controlling flocculation is given by the equation

$$\begin{aligned} -12V = {} & \left(A_w^{1/2} - A_o^{1/2}\right)^2 H_o + \left(A_o^{1/2} - A_{wi}^{1/2}\right)^2 H_w \\ & + 2\left(A_w^{1/2} - A_o^{1/2}\right)\left(A_o^{1/2} - A_{wi}^{1/2}\right) H_{wo} \end{aligned} \tag{26}$$

where the subscripts w_i, o and w refer to the internal aqueous phase, oil phase and external aqueous phase respectively. Since the internal and external phases are both water, this simplifies to

$$\begin{aligned} V = -\tfrac{1}{12}\Big[& \left(A_w^{1/2} - A_o^{1/2}\right)^2 H_o + \left(A_o^{1/2} - A_w^{1/2}\right)^2 H_w \\ & + 2\left(A_w^{1/2} - A_o^{1/2}\right)\left(A_o^{1/2} - A_w^{1/2}\right) H_{wo}\Big] \end{aligned} \tag{27}$$

The symbol H_o represents two drops of radius $(r + \delta)$ and separation Δ. H_{wo} represents the H function for a sphere of radius r and one of radius $(r + \delta)$ separated by a distance $(\delta + \Delta)$. H_w represents the function for two spheres of radius r separated by a distance $(\Delta + 2\delta)$. δ is the thickness of the oil layer.

If the Hamaker constant for the oil phase is close to that of water (as was the case for the water/isopropyl myristate/water systems investigated by the authors (Florence and Whitehill, 1981) the effect of replacing part of the oil drop with water is not great; in fact in a typical type A drop the effect of large internal droplet on attractive energies is found to be insignificant. Only when the internal droplet almost fills the whole diameter of the multiple drop is the influence of the internal phase noticeable. A more significant influence on Van der Waals forces of attraction between multiple drops appears to reside in the reduction in size which follows from expulsion of the internal droplets. The resultant reduction in diameter leads to a reduction in the force of attraction directly as V_A is related to globule radius, r, by

$$V_A = -\frac{(A_o^{1/2} - A_w^{1/2})^2 r}{\Delta} \tag{28}$$

A full analysis of interactions in multiple emulsions would obviously have to take account of forces of repulsion. The systems are too complex to allow any reasonable estimate of repulsive forces at this stage, although simplified models are being developed to allow an approach along this route.

Attempts to improve the stability of multiple emulsions

It will be apparent from the evidence so far presented, that multiple emulsions—whilst having many potential uses—are complex, inherently unstable systems. They are unlikely to be commercially acceptable until problems with their stability in vitro and in vivo are solved. Despite this, there are few reports in the literature, regarding attempts to improve their stability.

A major problem associated with w/o/w emulsions is creaming, which is probably due to the large size of the multiple drops. Creaming may be reduced by increasing the concentration of secondary surfactant but drug release may not be significantly retarded and, in any case, high surfactant concentrations are not desirable in pharmaceutical systems from the point of view of toxicity. The use of a thickening agent (e.g. polyvinylalcohol) in the external aqueous phase may also reduce creaming but one must be certain that the emulsions retain the characteristics of pourability. Substituting an oil which solidifies at room temperature but melts around body temperature should result in a stable system capable of being stored at room temperature but allowing release of the drug after administration to the body. Limited studies have been carried out with octadecane which melts at a temperature of 30°C (Whitehill, 1981). This has been shown to be of limited use.

Florence and Whitehill (1980, 1982) have investigated the possibility of stabilizing w/o/w systems by forming polymeric gels in the internal or external aqueous phases. Irradiation of hydrophilic poloxamer surfactants (used to stabilize the multiple emulsion) in the external aqueous phase and at the multiple drop interface results in polymerization of the poloxamer and the multiple globules become linked together by the crosslinked surfactant molecules. These semi-solid emulsion systems do not cream on storage but release the multiple drops when mixed with water. The authors have also been able to form water-oil-polyacrylamide gel and polyacrylamide-oil-water systems. One drawback of these approaches is the possible adverse effects of γ-irradiation on any entrapped drugs. It is possible, however, that the oil may exert a protective effect on labile drugs but this has still to be investigated.

Further work is still required on the colloidal aspects of stability and on the improvement of stability, for example, by interfacial polymerization. Once the problems of stability are overcome, w/o/w emulsions may show their true potential, providing systems with many biopharmaceutical advantages.

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