

The nature of the oil phase and the release of solutes from multiple (w/o/w) emulsions

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The effect of the nature of the oil phase of w/o/w emulsions stabilized by interfacial complexation between span 80 (sorbitan mono-oleate) and albumin has been studied. The long-term stability of the systems has been assessed by photomicrography and by measuring the quantity of an internal marker (NaCl) remaining entrapped with time. The number of multiple oil drops and the diameters of the internal aqueous droplets were determined over 6 weeks, and the amounts of NaCl entrapped over the same period were followed. There were no significant changes in w/o/w emulsions prepared with a range of hydrocarbons (octane, dodecane, hexadecane, toluene and cyclohexane), indicating stable multiple emulsions. The release of NaCl and 5-fluorouracil (5-FU) separately entrapped in the internal aqueous phase of w/o/w emulsions was measured. Diffusion of the un-ionized species of 5-FU across the oil phase or through localized thin oil lamellae is the primary transport mechanism. In the presence of surface active agents, water is solubilized in inverse micelles which would possess the ability to solubilize other water-soluble components, such as NaCl and 5-FU. The mixed inverse micellar units of Span 80 and polysorbate (Tween) 80 therefore act as solute carriers across the liquid hydrocarbon membrane separating the two aqueous phases of the emulsions. The main factor in determining the differences in rates of release from the hydrocarbon emulsions appears to be the droplet size of the internal aqueous phase.

A number of factors have been identified as affecting the stability of w/o/w emulsions. These include the method of preparation, the nature of entrapped materials, particularly the effect of electrolytes, phase volumes, concentration and type of emulsifiers (Florence & Whitehill 1981, 1982). Although multiple emulsions have been noted as a novel system which can be used for the separation of hydrocarbons, the influence of the oil phase on the stability of the resulting multiple emulsion has been relatively unexplored (Li 1971).

For potential pharmaceutical purposes, the oils used have included refined hydrocarbons such as light liquid paraffin, esters of long chain fatty acids and vegetable oils (Florence & Whitehill 1985). Davis & Walker (1983), using a fluorescent tracer technique, measured the yield of multiple emulsion droplets made from mineral oils (liquid paraffin and squalene) and vegetable oils (sesame oil, maize oil and arachis oil). They found the yield to be dependent upon the nature of the oil phase. Frankenfeld et al (1976) used mixtures of 'solvent 100 neutral' and 'Norpar 13' to vary the viscosity of the oil phase in attempts to control the rate of release of solute across the oil membrane. It is reasonable to suppose that, as with conventional emulsions, the nature of the oil phase can markedly affect the behaviour of the

system, and the stability of the oil membrane against leakage of the entrapped material will depend, among other factors, on the nature of the oil used in preparing the emulsion.

During the search for stable w/o/w emulsions as vehicles for sustained release of drugs, we have made use of interfacial complexation between non-ionic surfactant and macromolecules (Law et al 1984; Florence et al 1985; Omotosho et al 1985). The formation of a complex interfacial membrane at the primary w/o interface was found to enhance the stability of the emulsions and to slow down the release of solute from the emulsion droplets. The long-term stability of the emulsions has been assessed using photomicrography and by measuring the quantity of an internal marker (NaCl) remaining entrapped with time.

The release of NaCl and 5-fluorouracil (5-FU) separately entrapped in the internal aqueous phase of w/o/w emulsions has been studied. Mechanisms of release are not fully understood; transport across the oil phase is clearly important and could involve un-ionized species, inverse micelles and/or swollen micelles of both hydrophilic and lipophilic surfactants. We provide here one explanation for the release of NaCl from w/o/w emulsions by considering the properties of the micellar system of Span 80 in the oil phases and by measuring the rate of mass transport of water through a thin layer of oil.

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MATERIALS AND METHODS

Bovine serum albumin (BSA) essentially globulin-free was obtained from Sigma Chemical Co. UK in a crystallized and lyophilized form. Water was freshly double distilled. Span 80 was from Koch-Light Laboratories, Colnbrook, Berks, UK. Polysorbate (Tween) 80, 5-fluorouracil (5-FU), isopropyl myristate (purity 98%), hexadecane (purity 99%), dodecane (purity 99%), octane (purity 99%), were obtained from Sigma Chemical Co., UK. Sodium chloride (Analar), sorbitol (GPR), toluene (Analar) and cyclohexane (Analar) were from BDH Ltd, UK.

Preparation of w/o/w emulsions

Multiple emulsions were prepared as described previously (Omotosho et al 1985) using bovine serum albumin (0.2%) in the internal aqueous phase, Span 80 (2.5%) in the oil phase and Tween 80 (1.0%) in the external aqueous phase with octane, dodecane, hexadecane, toluene, cyclohexane and isopropyl myristate as the oil phases. Either sodium chloride (1.25%) or 5-FU (1 mg ml⁻¹) was incorporated into the inner aqueous phase. The osmotic pressure created by the presence of sodium chloride in the internal aqueous phase of the w/o/w emulsion was balanced by adding sorbitol to the external aqueous phase, the appropriate concentration being calculated from its sodium chloride equivalent osmolarity (5.48% sorbitol = 0.9% NaCl).

Measurement of w/o/w emulsion stability

An indirect method of estimating the number of multiple drops in w/o/w emulsions is by incorporating an internal marker in the internal phase of the primary w/o emulsion. The quantity of sodium chloride entrapped in the internal phase of w/o/w emulsions was measured over a period of six weeks. Sodium chloride was used as the internal marker. The quantity of NaCl leaked to the external aqueous phase, which is assumed to arise from loss of the internal droplets due to rupture of the stabilizing surfactant layer, was assayed using a conductivity bridge as previously described (Omotosho et al 1986). In addition to determining the NaCl remaining entrapped with time, the stability of w/o/w emulsions was assessed by photomicrography by measuring the change in the mean droplet diameter and droplet size distributions of the internal phase and the change in number of multiple oil drops.

Release studies

Cuprophane tubing (size 5 20'/32' in diameter, Med-cell International, London), was washed several

times with double distilled water and left soaking in distilled water overnight before use. Immediately after preparation, 5 ml of the w/o/w emulsion containing 1.25% of sodium chloride or 1 mg ml⁻¹ of 5-FU was pipetted to a bag made of Cuprophane double tied at each end. The Cuprophane bag containing the emulsion was placed in 200 ml of dialysis medium at 25 °C with constant stirring. When sodium chloride was present in the internal phase of the emulsion, the conductivity of the dialysis medium (double distilled water) was monitored over a period by means of a conductivity dip cell connected to a conductivity bridge. Phosphate buffer at pH 7.4 served as the dialysis medium for w/o/w emulsions containing 5-FU. The amount of 5-FU released was determined by the absorbance at 265 nm. Controls were determined as described in results. Each release rate curve was drawn from the mean of at least 3 experiments.

Interfacial tension

Interfacial tension was measured by the pendant drop technique of Andreas et al (1938) at 20 °C. The toluene/water interfacial tension was 34.99 mN m⁻¹ compared with a literature value of 36.1 mN m⁻¹ (Andreas et al 1938). The octane-water and hexadecane-water interfacial tensions were 50.1 mN m⁻¹ (literature value: 50.2 mN m⁻¹, Donahue & Bartell 1952) and 53.32 mN m⁻¹ (literature value: 53.30 mN m⁻¹, Dickinson et al 1985), respectively.

RESULTS AND DISCUSSION

Micrographic observations of w/o/w emulsions

Some microscopic features of the w/o/w emulsions immediately after preparation were noteworthy: both the internal aqueous droplets and the multiple oil drops varied in size according to the nature of the oil used in preparing the emulsions (Table 1). The internal droplets of w/o/w emulsions of isopropyl myristate numbered between 20–50 with a mean number diameter of 2.1 µm. The w/o/w emulsion prepared with toluene as the oil phase and under

Table 1. Changes in the mean droplet diameter of internal aqueous droplets in w/o/w multiple emulsion.

Time of preparation	Mean droplet diameter (µm)				
	Octane	Dodecane	Hexadecane	Cyclohexane	Toluene
0	12.6	7.5	8.0	12.0	4.5
1 Week		8.1	8.0		
2 Weeks	13.4	8.1	8.1	12.6	4.6
4 Weeks	13.9	8.1	8.1	12.3	4.7
6 Weeks	13.4	8.2	8.1	12.3	4.9

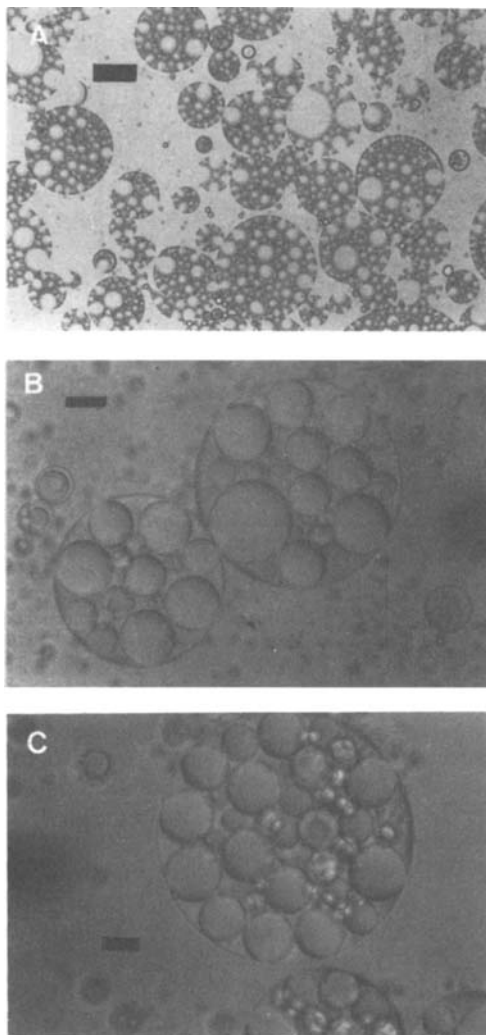


FIG. 1. Photomicrographs of w/o/w emulsions prepared with 2.5% Span 80 and 0.2% BSA as primary emulsifiers; 1% polysorbate 80 served as the secondary emulsifier with the following oil phases (Bar scale = 10 μm). A = toluene; B = octane; E = hexadecane.

similar processing conditions (Fig. 1A) possessed internal droplets which were apparently flocculated but which deflocculated on dilution with 1% polysorbate 80; these had a mean internal droplet diameter of 4.5 μm .

The internal droplets of w/o/w emulsions prepared with octane as the oil phase (Fig. 1B) had mean internal droplet diameters of 12.6 μm and those formulated with hexadecane (Fig. 1C) had a mean internal droplet diameter of 8.0 μm . The number of internal droplets of w/o/w emulsions prepared with

hydrocarbon were mostly between 8–30 with few droplets having more than 50 internal droplets.

There was a correlation between the interfacial tension at the oil–water interface and the droplet size. The interfacial tensions at the isopropyl myristate–water interface and toluene–water interface were 25.38 and 34.99 mN m^{-1} and the mean droplet diameters were 2.1 and 4.5 μm , respectively. For the octane–water interface and the hexadecane–water interface, interfacial tensions of 50.13 and 53.32 mN m^{-1} were measured, and these could be compared with an internal droplet size of 12.6 μm and 8.0 μm , respectively.

The relatively large mean internal droplet diameter of w/o/w emulsions prepared with octane and cyclohexane (interfacial tension at the cyclohexane–water interface was 50.24 mN m^{-1}) deserve mention. The oil phase of w/o/w emulsions prepared with octane, cyclohexane and toluene have higher water solubility than the other hydrocarbons used in this work (McAuliffe 1966); there is, therefore, greater tendency for water to pass from the external phase to the internal phase during secondary emulsification which could enhance the growth of the internal droplets. W/o/w emulsions prepared with toluene as the oil phase have more numerous internal droplets (Fig. 1A). Growth of the internal droplets under this diffusion-controlled process appear to be less significant compared with w/o/w emulsions of octane and cyclohexane which have fewer internal droplets.

Fig. 2 shows the change in number of multiple oil drops with time. The reduction in number of multiple oil drops on storage could be brought about by expulsion of the internal droplets arising from breakdown of the middle oil membrane phase. Coalescence between multiple and simple oil drops would also lead to a reduction in number of multiple

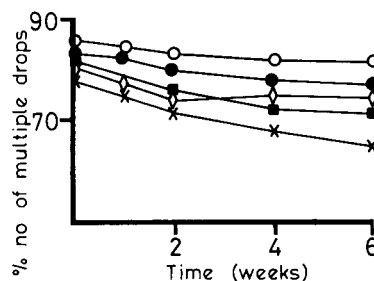


FIG. 2. Stability of multiple w/o/w emulsions prepared with hydrocarbon oils: (○) octane, (●) dodecane, (■) cyclohexane, (◇) hexadecane, (×) toluene. The composition of the emulsions is the same as in Fig. 1.

oil drops in the system. There is no significant change in the number of multiple oil drops prepared with hydrocarbon, indicating stability.

The percentage of NaCl entrapped in the internal phase of w/o/w emulsions prepared with octane, dodecane, hexadecane, cyclohexane and toluene is shown in Fig. 3. The quantity of NaCl entrapped appeared to parallel the number of multiple oil drops in the system. However, there was a marked difference in their entrapment efficiency; more than 90% of NaCl initially incorporated into the w/o/w

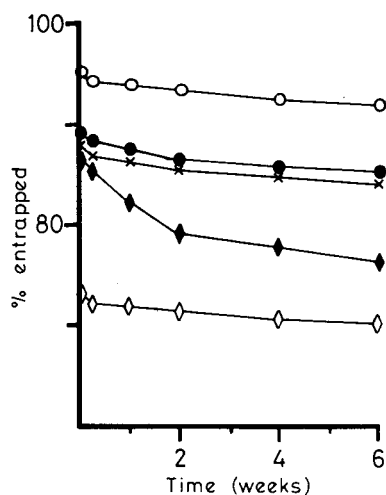


FIG. 3. The effect of oil phase on the quantity of NaCl entrapped in the internal aqueous phase of multiple w/o/w emulsion. The emulsions contained 2.5% Span 80 and 0.2% BSA as primary emulsifiers with 1.25% NaCl in the internal phase and different oil phases: (○) octane, (●) dodecane, (×) cyclohexane, (◇) hexadecane, (◆) toluene. 1% polysorbate 80 in 7.6% Sorbitol was the secondary emulsifier.

emulsion prepared with octane remains entrapped on storage over six weeks compared with 70% for w/o/w emulsions prepared with toluene within the same storage period. Collings (1971) suggested that the degree of drug leakage from the internal phase of multiple emulsions was greatest with systems of low particle size (large surface area). The variation in the internal droplet size of multiple emulsions prepared with different oil phases would seem to account for the marked differences in their NaCl entrapment efficiency.

Release studies

The results of studies of in-vitro release of NaCl from w/o/w emulsions prepared with octane, dodecane,

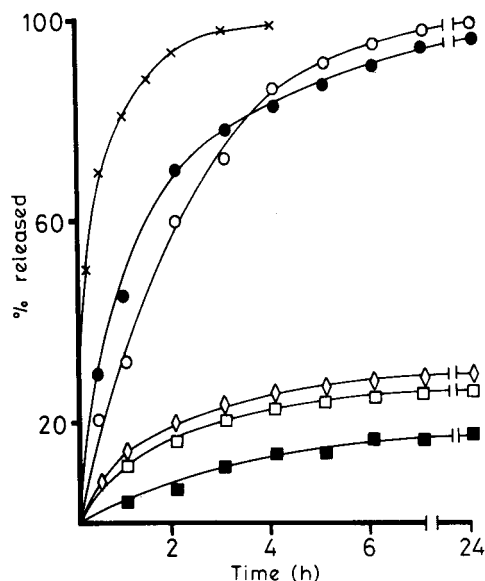


FIG. 4. Release of NaCl from w/o/w emulsions prepared as indicated in Fig. 3 and with the following oil phases: (×) isopropylmyristate, (●) hexadecane, (○) toluene, (◇) cyclohexane, (□) dodecane and (■) octane.

hexadecane, cyclohexane, toluene and isopropyl myristate are shown in Fig. 4. Solute is released faster from multiple emulsions formulated with isopropyl myristate (mean internal droplet diameter of 2.1 μm) and slowest from those prepared with octane (internal aqueous droplet diameter of 12.6 μm). Multiple emulsions prepared with octane, dodecane and cyclohexane sustained the release of NaCl with less than 30% of the entrapped NaCl released over a period of 24 h. For a given internal phase volume, a decrease in droplet size would increase the surface area exposed resulting in an increased release rate which would explain the large differences in rates of release of NaCl from w/o/w emulsions prepared with octane, hexadecane, toluene and isopropyl myristate.

Fig. 5 shows the release profile of 5-FU from w/o/w emulsions prepared with hydrocarbons. 5-FU is released at a faster rate than NaCl presumably because of its greater lipophilicity.

Effect of osmolarity differences on release

Addition of NaCl or sorbitol to the internal phase of w/o/w emulsions has been shown to prolong the release of naltrexone hydrochloride due to the salting out effect of NaCl at the inner w/o interface (Brodin et al 1978). However, the presence of such solutes in the internal phase would induce osmotic

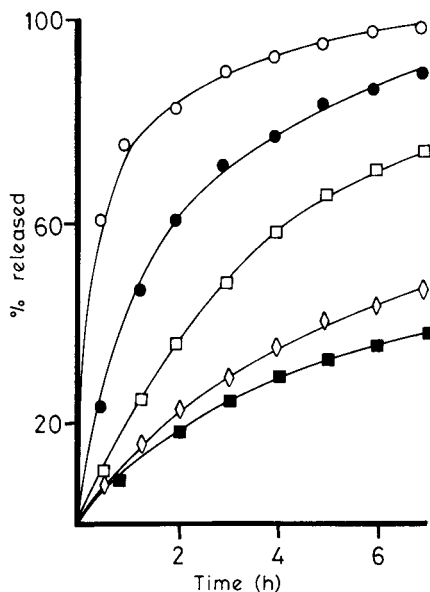


FIG. 5. Release of 5-FU from multiple w/o/w emulsions. The emulsions contained 2.5% Span 80 and 0.2% BSA as primary emulsifiers, with 5-FU (1 mg ml^{-1}) in the internal phase and the following oil phases: (■) octane, (◇) cyclohexane, (□) dodecane, (●) hexadecane, (○) toluene, 1% polysorbate 80 served as the secondary emulsifier.

flux of water from the external phase to the internal phase of the emulsion. This influx of water would induce thinning of the oil layer, which will affect the long-term stability of the system. Multiple emulsions have been prepared in this work with NaCl added to the external phase and 5-FU to the internal phase. The addition of NaCl to the external phase of the emulsion caused a prolongation of release of 5-FU from a w/o/w emulsion prepared with octane in as much as the osmotic imbalance is not so extreme as to cause rupture of the oil membrane (Fig. 6).

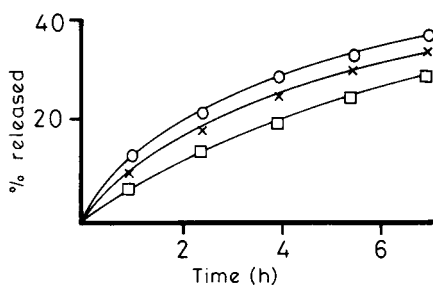


FIG. 6. Effect of osmolarity differences on release of 5-FU from w/o/w emulsion (see text for the preparation of the emulsions). The external phase of the emulsion contained: (○) control buffer pH 7.4, (×) 0.1 M NaCl, (□) 1 M NaCl.

A typical photomicrograph of a w/o/w emulsion prepared with octane after the release of a drug has occurred (Fig. 7) showed that the emulsion remained

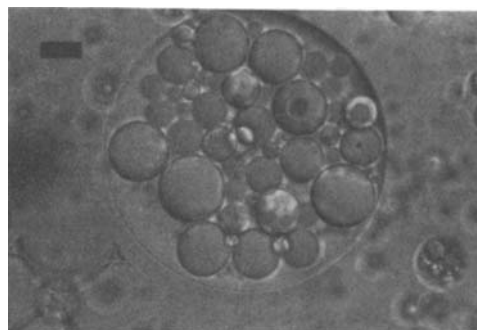


FIG. 7. Photomicrograph of w/o/w multiple emulsions prepared with octane after the release studies showing the presence of large and swollen internal droplets. (Bar scale = $10 \mu\text{m}$)

stable with large multiple oil drops remaining. In such stable systems, diffusion across the oil layer or through localized thin oil lamellae is the primary transport mechanism. Diffusion of un-ionized species of 5-FU across the oil phase is the most probable release mechanism of 5-FU entrapped in the internal phase of w/o/w emulsions. In an attempt to explain the release of NaCl from the emulsions we have considered the suggestion (Florence & Whitehill 1981, Kita et al 1978; Garti et al 1985) that water may be carried in inverse micelles across the oil layer between the two aqueous phases of w/o/w emulsions. The flux of water through the oil phase will affect the release of ionized species in w/o/w emulsions. To test this mechanism of micellar transport we have measured the rate of mass transport of water through a thin layer of oil following the procedure of Schatzberg (1965). Fig. 8A and B show the rate of mass transport of water through a thin layer of hexadecane and isopropyl myristate, respectively. As would be expected from micellar transport, the rate of mass transport of water increased on increasing the concentration of Span 80 in the oil phase. Also, the mass transport of water is higher in isopropyl myristate than the corresponding values for hexadecane. During the first emulsification step, and in the presence of Span 80 in the oil phase, water is taken up in the form of water swollen micelles, the formation of water swollen micelles and mixed inverse micelles being facilitated by the presence of secondary emulsifier (polysorbate 80). These swollen micelles and/or mixed inverse micelles would

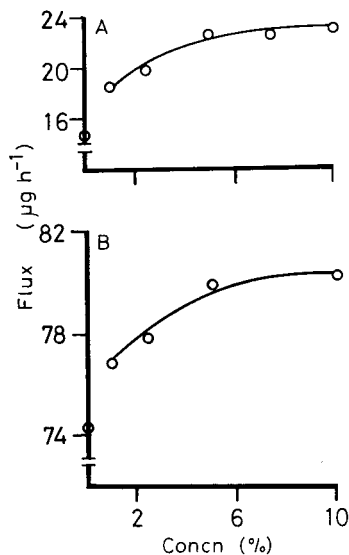


FIG. 8. A. Effect of Span 80 concentration on mass transport of water through hexadecane thin layer. B. Effect of Span 80 concentration on mass transport of water through isopropyl myristate thin layer.

possess the ability to solubilize a variety of water soluble components. Thus the large micellar units of both Span 80 and polysorbate 80 could act as a carrier of NaCl between the two aqueous phases of a w/o/w emulsion.

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