

Influence of Interfacial Rheological Properties of Mixed Emulsifier Films on the Stability of Water-in-Oil-in-Water Emulsions

FOYEKE O. OPAWALE AND DIANE J. BURGESS

Department of Pharmaceutical Sciences, School of Pharmacy, University of Connecticut, Storrs, CT 06269, USA

Abstract

The purpose of this study was to investigate the influence of mixtures of the emulsifiers Span 80, 83 and 85 and Tween 80 on multiple emulsion stability. An oscillatory ring-surface rheometer was used to measure interfacial elasticity at the oil–aqueous interface. Multiple emulsions were prepared via a two-step emulsification process and stability was evaluated by investigation of drug transport from freshly prepared and eight-day-old emulsions by use of a dialysis method. Photomicrography and droplet-size analysis of multiple emulsions were also conducted.

Spans 80 and 83 were appreciably elastic ($683.10 \pm 29.13 \text{ mNm}^{-1}$ and $1128.09 \pm 14.81 \text{ mNm}^{-1}$, respectively at 5% w/v) when present at the mineral oil–aqueous interface whereas Span 85 and Tween 80 were not ($11.10 \pm 3.88 \text{ mNm}^{-1}$ (5% w/v) and 0 (0.1–5% w/v) respectively). The interfacial elasticities of Spans 80 and 83 decreased in the presence of Tween 80 in the aqueous phase; this was attributed to co-adsorption of Tween 80 at the interface or aqueous-phase solubilization of the Spans within mixed micelles, or both. Drug-transport studies indicated that drug release on storage was lower from water-in-oil-in-water (w/o/w) emulsions prepared with 5% w/v Span 80 or 83 and 0.1% w/v Tween 80 than from emulsions prepared with 5% w/v Spans 80 or 83 and 1% w/v Tween 80. Photomicrography and droplet-size analysis indicated the same trend—emulsions containing a higher percentage of Tween 80 were less stable. The relatively stable w/o/w emulsions (e.g. 5% w/v Spans 80 or 83 and 0.1% w/v Tween 80) contained a large number of multiple droplets for up to eight weeks of storage whereas the relatively unstable w/o/w emulsions (e.g., 5% w/v Span 85 and 0.1% w/v Tween 80 and 5% w/v Spans and 1% w/v Tween 80) contained mostly simple droplets after only one week of storage. The mean volume/weight droplet size decreased on storage with breakdown of these w/o/w emulsions to simple oil-in-water emulsions.

There was a positive correlation between the interfacial elasticity and emulsion stability data. Mixed emulsifiers giving higher film strength, as quantified by interfacial elasticity measurements, resulted in more stable w/o/w emulsions.

Multiple emulsions are complex systems consisting simultaneously of both types of simple emulsion, water-in-oil (w/o) and oil-in-water (o/w) (Florence & Whitehill 1982). The dispersed-phase droplets contain smaller dispersed droplets of the same phase as the continuous phase, thus multiple emulsions have two interfaces. Although they have potential application as sustained-release drug-delivery systems (Fukushima et al 1983; Omotosho et al 1989; Nakhare & Vyas 1995), the development of multiple

emulsions for these and other applications is limited by their inherent instability (Florence & Whitehill 1982). Possible mechanisms of instability of water-in-oil-in-water (w/o/w) emulsions include coalescence of the internal aqueous droplets, coalescence of multiple oil droplets, rupture of the oil layer, and migration of surfactant from one interface to the other (Florence & Whitehill 1981; Magdassi et al 1984). Emulsion instability is a general problem which can lead to undesirable changes in drug-release rates (Davis 1987).

Emulsifiers stabilize simple emulsions by reduction of interfacial tension or formation of a

Correspondence: D. J. Burgess, Department of Pharmaceutical Sciences, School of Pharmacy, U-92, University of Connecticut, 372 Fairfield Road, Storrs, CT 06269, USA.

mechanical or electrical interfacial barrier (Florence & Rogers 1971), or both. Effective reduction of interfacial tension aids dispersibility and enhances the formation of smaller droplets. Smaller initial droplet size usually results in enhanced kinetic stability of simple emulsions to creaming (Rowe 1965). However, the strength of the mechanical barrier might be more important than interfacial tension to long-term emulsion stability (Myers 1988). The mechanical barrier or film strength can be quantified by interfacial rheology measurements (Warburton 1993). Several studies have reported the characterization of film strength particularly for water-soluble emulsifiers such as proteins (Halling 1981; Burgess & Sahin 1994, 1997a; Murray & Dickinson 1996). The elasticity of the protein film has been reported to positively correlate with o/w emulsion stability (Halling 1981; Burgess & Sahin 1997b).

Although attempts have been made to stabilize multiple emulsions (Law et al 1986; Sela et al 1995) using different emulsifier systems, the role emulsifiers play in multiple emulsion stability is still unclear. The formulation of multiple emulsions requires at least two surfactants, one capable of stabilizing w/o emulsions and one capable of stabilizing o/w emulsions (Matsumoto et al 1976). Examples of surfactants commonly used to prepare w/o and o/w emulsions are the lipophilic sorbitan fatty acid esters (Spans) and hydrophilic ethoxylated sorbitan fatty acid esters (Tweens), respectively. Multiple emulsions can be reproducibly prepared by employing Span and Tween surfactant pairs (Matsumoto et al 1976). These non-ionic surfactants substantially reduce interfacial tension (Wan & Lee 1973). It has been speculated that the film strength of both the primary and secondary interfaces is a major factor influencing multiple emulsion stability (Florence & Whitehill 1982). The primary interface is composed of lipophilic surfactants whereas both lipophilic and hydrophilic surfactants are present at the secondary interface. The high film-strength of Span surfactants, measured by interfacial elasticity at the mineral oil–water interface, has been reported to correlate positively with w/o emulsion stability (Opawale & Burgess 1998). Investigation of the interfacial film strength of mixed emulsifier films of Span and Tween surfactants (usually present at the secondary interface) in relation to multiple emulsion stability has not been reported previously. Interfacial interactions between these surfactants might affect w/o/w emulsion stability. Therefore, the purpose of this study was to investigate the interfacial elasticity of lipophilic surfactants (Spans 80, 83 and 85) in combination with the hydrophilic surfactant,

Tween 80 at the mineral oil–water interface and to determine any influence that these mixed-emulsifier systems might have on multiple emulsion stability. Information on surfactant interaction might enable a rational approach to multiple emulsion development.

Materials and Methods

Materials

Span 80 (sorbitan monooleate; hydrophilic-lyophilic balance (HLB) 4.3), Span 83 (sorbitan sesquileate; HLB 3.7), Span 85 (sorbitan trioleate; HLB 1.8), Tween 20 (polyoxyethylene-sorbitan monolaurate) and Tween 80 (polyoxyethylene-sorbitan monooleate; HLB 15) were obtained from Sigma, USA. Light mineral oil and hydrophilic Spectra/Por 6 dialysis membrane (molecular weight (MW) cut-off 8kD) were obtained from Fisher Scientific (Springfield, NJ). Sodium salicylate, U.S.P. was obtained from J. T. Baker (Phillipsburg, NJ). Double-distilled water was used in all experiments.

Interfacial rheology studies

A planar mineral oil–double distilled water interface was used as a simplified model to investigate Span–Tween interactions at the emulsion droplet interface. The lipophilic surfactants Spans 80, 83 and 85, (5% w/v) were dissolved in the oil phase and Tween 80 (0–5% w/v) was dissolved in the aqueous phase. The interface was investigated by use of a Surface Science Enterprises (UK) Mark II surface rheometer at $25 \pm 0.1^\circ\text{C}$. The experimental technique was similar to that employed previously (Opawale & Burgess 1998). All measurements were repeated in triplicate; the mean values and standard deviations are reported.

Preparation of water-in-oil-in-water emulsions

W/o/w emulsions were prepared by a two-step emulsification process. In the first step, w/o emulsions were prepared by adding the aqueous phase (0.5% w/v sodium salicylate, 12.5 mL) to an equal volume of mineral oil containing Span (5% w/v) while stirring with a Fisher Dyna mixer (600 rev min^{-1}) for 15 min. In the second step Tween 80 (0.1 or 1% w/v; 50 mL) was added to the primary emulsion which was then stirred for 5 min at the same speed to produce the multiple emulsion. Multiple-emulsion samples were prepared in triplicate.

Drug-transport studies

Drug-transport studies were conducted by placing multiple-emulsion samples (5 mL) containing the model drug (0.5% w/v sodium salicylate) in dialysis bags (MW cut-off 8kD). The bags were placed in vessels containing double-distilled water

(250 mL) at $37 \pm 0.1^\circ\text{C}$. The vessels were stirred with magnetic stirrers at 100 rev min^{-1} . Samples (5 mL) were withdrawn from the receiver compartment at intervals up to 7 h and replaced with the same volume of solvent to maintain volume and sink conditions in the vessel. Samples were analysed for drug-content by UV spectrophotometry at 293 nm. Drug-transport studies were performed on freshly prepared and eight-day-old emulsion samples. These experiments were repeated three times and the means and standard deviations calculated.

Photomicrography

The w/o/w emulsions were studied microscopically after preparation and after storage at room temperature, a Nikon Microscope being used to monitor emulsion breakdown. Photomicrographs were taken at $200 \times$ magnification.

Droplet-size analysis

Multiple-emulsion droplet size was determined both for freshly prepared samples and for samples which had been stored for 8 days, by means of a Particle Sizing Systems (Santa Barbara, CA) Accusizer Model 770 optical particle sizer (size range $1\text{--}500 \mu\text{m}$). The multiple emulsion ($5 \mu\text{L}$) was diluted with Tween 80 (0.1% w/v; 50 mL) before measurement. All droplet-size measurements were repeated twice per sample. Mean values and standard deviations were calculated for three repeat emulsion preparations, two samples per emulsion ($n = 3$).

Results and Discussion

Interfacial rheology

Spans 80 and 83 were appreciably elastic at the mineral oil–aqueous interface, probably because of hydrophobic interactions between monomers or inverse micelles, or both, and the build up of multilayers (Figure 1, Table 1). At the concentrations investigated the interfacial elasticity of Span 85 was relatively low and that of Tween 80 was zero (Table 1). These low interfacial elasticity data are considered to be because the presence of the three oleic acid groups (Span 85) and the bulky polyoxyethylene chains (Tween 80) on the sorbitan rings cause steric hindrance of intermolecular interactions and multilayer build-up.

The interfacial elasticities of the Spans were considerably lower when Tween 80 was added to the aqueous phase, and occasionally dropped to zero (e.g. 5% w/v Span 80 and 1% w/v Tween 80). The reduction in interfacial elasticity was concentration-dependent, the values dropping further with increasing concentrations of Tween 80 from

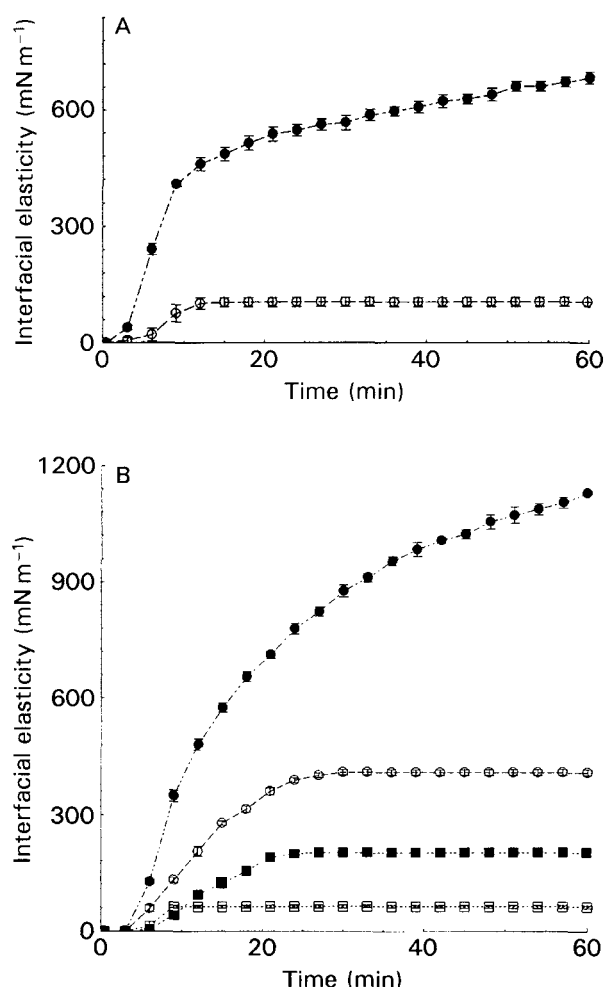


Figure 1. The effects of Tween surfactants on the interfacial elasticity of the mineral oil–water interface with time for (A) 5% w/v Span 80 (●, 5% w/v Span 80 alone; ○, 5% w/v Span 80 with 0.1% w/v Tween 80) and (B) 5% w/v Span 83 (●, 5% w/v Span 83 alone; ○, 5% w/v Span 83 with 0.1% w/v Tween 80; ■, 5% w/v Span 83 with 1% w/v Tween 80; □, 5% w/v Span 83 with 0.1% w/v Tween 20). Temperature $25 \pm 0.1^\circ\text{C}$. Values are means, $n = 3$; the error bars indicate \pm range.

0.1 to 1% w/v (Figure 1, Table 1). Two possible explanations for this reduction are competitive adsorption between the Tween 80 and the Span molecules and solubilization of the lipophilic Span

Table 1. Effect of Tween 80 on the interfacial elasticity of Spans 80, 83 and 85 (5% w/v) at the mineral oil–water interface.

Amount of Tween 80 (% w/v)	Interfacial elasticity ($\text{mN m}^{-1} \pm \text{s.d.}$)		
	Span 80	Span 83	Span 85
0	683.10 ± 29.13	1128.09 ± 14.81	11.10 ± 3.88
0.1	104.80 ± 23.81	409.67 ± 15.01	0
1	0	202.32 ± 5.42	0

Experiments were performed at 25°C for 60 min ($n = 3$).

surfactants in the aqueous phase as a result of the formation of mixed micelles. It has been speculated that this type of aqueous-phase solubilization is a result of reduced multiple emulsion yield with increasing concentration of the secondary hydrophilic surfactant (Matsumoto et al 1976; Matsumoto & Kang 1989). The kinetics of film formation differed in the presence of the Tweens (Figure 1). In the absence of the Tween surfactants the interfacial elasticities of the Spans increased rapidly initially, followed by a slower increase over the study period (Figure 1). The initial increase is attributed primarily to the adsorption of surfactant monomers or inverse micelles, or both, whereas the second slower phase is attributed to molecular interaction, rearrangement or possible multilayer build up, or all three. In the presence of the Tweens, a plateau (Figure 1) followed the initial rapid increase in interfacial elasticity. This plateau in interfacial elasticity indicates that the Tweens prevent further interaction, molecular organization or multilayer build-up of the Span molecules at the oil-water interface.

The elasticities of Span-Tween 80 surfactant mixtures were different at different Tween 80 concentrations, in the order Span 83 > Span 80 > Span 85 (Table 1). The higher interfacial elasticity values of Span 83 are attributed to the mixture of monooleate and dioleate components, which enhance intermolecular hydrophobic interactions (Opawale & Burgess 1998). The increased hydrophobicity of Span 83 is reflected in its HLB value (3.7). Span 80, which contains mainly monooleate, has an HLB of 4.3. The chain-length of the alkyl group of the hydrophilic surfactant was also found to affect interfacial elasticity. Lower interfacial elasticities were observed for Span-Tween 20 mixtures than for the equivalent Span-Tween 80 mixtures. For example, the interfacial elasticity of Span 83 (5% w/v) was $62.97 \pm 7.42 \text{ mNm}^{-1}$ in the presence of 0.1% w/v Tween 20 and $409.67 \pm 15.01 \text{ mNm}^{-1}$ in the presence of 0.1% w/v Tween 80 (Tables 1 and 2). The interfacial elasticity was zero for the other Span-Tween 20 mixtures (Table 2). Tween 20 has a shorter alkyl chain length (polyoxyethylene monolaurate) than Tween 80 (polyoxyethylene monooleate) but similar interfacial activity (apparent critical micellar concentration (CMC) and interfacial tension values are $1.47 \times 10^{-4} \text{ M}$ (7.7 dyne cm^{-1}) and $1.90 \times 10^{-4} \text{ M}$ (8.6 dyne cm^{-1}), respectively (calculated from the % w/v values given by Wan & Lee 1973)). Longer alkyl chains are likely to promote hydrophobic interactions and hence increase film strength. These data might explain the increase in multiple emulsion yield reported by Matsumoto et

al (1976) with increasing alkyl chain-length of the hydrophilic surfactant.

Drug-transport studies

The change in drug release from multiple emulsions on storage reflects changes in the emulsion system and is therefore an indication of stability. This parameter has been used to assess multiple-emulsion stability (Vaziri & Warburton 1994). Sustained-release behaviour was observed for the freshly prepared w/o/w emulsions (Figures 2 and 3) but the rates of release of sodium salicylate from the emulsions increased on storage, indicating that the emulsions were destabilizing with time (Table 3). This observed destabilization might be a consequence of unfavourable interactions between the Spans and Tween 80 resulting in weak mixed films at the secondary interface. This effect was more pronounced for 1% w/v Tween 80 than for 0.1% w/v. These data are in agreement with the interfacial elasticity data (Table 1). The change in drug release from the emulsions was dependent on the nature of the Span surfactant incorporated at the low Tween 80 concentration (0.1% w/v) and followed the order of the interfacial film strength, as measured by interfacial elasticity—Span 83 > Span 80 > Span 85. At the high Tween 80 concentration (1% w/v), the effect of the different Span surfactants seems to be masked.

Droplet-size analysis and photomicrography

A decrease in the mean volume-weight droplet diameter (a calculated size based on a volume/weight average) on storage was observed for all emulsions investigated except those prepared with 5% w/v Span 80 or 83 with 0.1% w/v Tween 80. The decrease in droplet diameter seems to indicate breakdown of the w/o/w emulsions to simple o/w emulsions as evidenced by optical microscopy (Figures 4 and 5). The data indicate that mixed emulsifiers with substantial interfacial elasticity at the planar mineral oil-water interface produced

Table 2. Effect of Tween 20 on the interfacial elasticity of Spans 80, 83 and 85 (5% w/v) at the mineral oil-water interface.

Amount of Tween 20 (% w/v)	Interfacial elasticity ($\text{mNm}^{-1} \pm \text{s.d.}$)		
	Span 80	Span 83	Span 85
0	683.10 ± 29.13	1128.09 ± 14.81	11.10 ± 3.88
0.1	0	62.97 ± 7.42	0
1	0	0	0

Experiments were performed at 25°C for 60 min (n = 3).

Table 3. Effect of Tween 80 (0.1 and 1% w/v) in the external aqueous phase on release (%) of sodium salicylate from freshly prepared (day 0) and 8-day-old water-in-oil-in-water emulsions.

Oil phase	0.1% w/v Tween 80			1% w/v Tween 80		
	Day 0	Day 8	Change (%)	Day 0	Day 8	Change (%)
Span 80	46.83 ± 6.10	63.92 ± 3.30	17	58.52 ± 0.38	92.58 ± 3.16	34
Span 83	57.90 ± 4.64	65.87 ± 1.54	8	50.88 ± 0.42	84.86 ± 0.53	34
Span 85	54.13 ± 3.65	93.12 ± 3.50	39	57.95 ± 2.09	95.95 ± 5.28	38

Experiments were performed at 37°C for 7h. Results are means ± s.d. (n = 3).

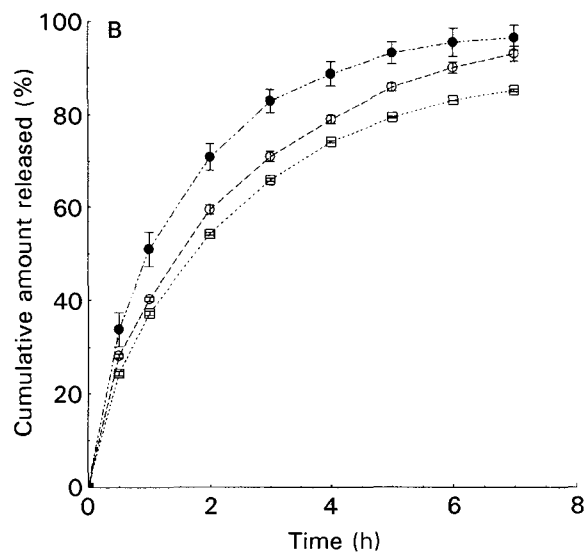
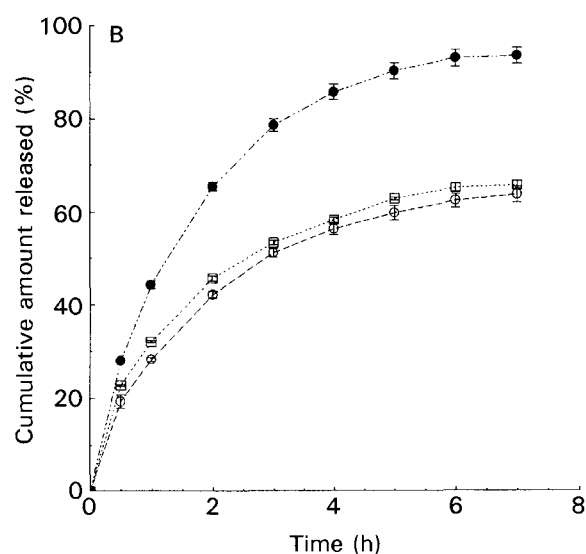
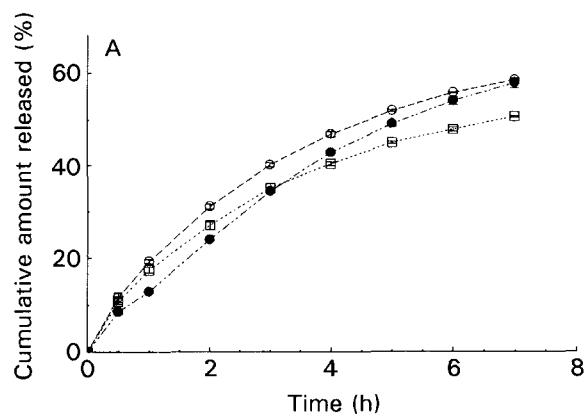
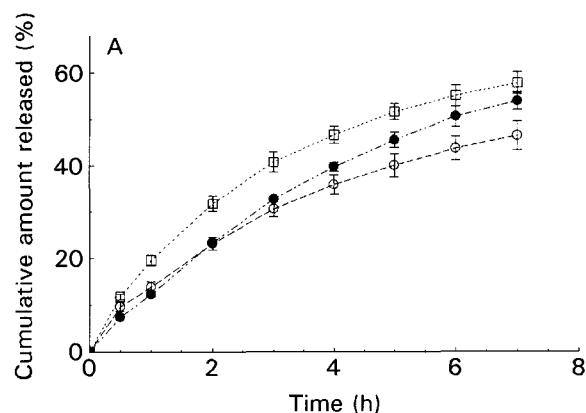


Figure 2. Percent release with time from (A) freshly prepared and (B) 8-day-old water-in-oil-in-water emulsion prepared with 5% w/v Spans (80, 83, 85) in the oil phase and 0.1% w/v Tween 80 in the external aqueous phase: ○, 5% w/v Span 80 with 0.1% w/v Tween 80; □, 5% w/v Span 83 with 0.1% w/v Tween 80; ●, 5% w/v Span 85 with 0.1% w/v Tween 80. Temperature 37 ± 0.1°C. Values are means, n = 3; the error bars indicate ± range.

Figure 3. Percent release with time from (A) freshly prepared and (B) 8-day-old water-in-oil-in-water emulsions prepared with 5% w/v Spans (80, 83, 85) in the oil phase and 1% w/v Tween 80 in the external aqueous phase: ○, 5% w/v Span 80 with 1% w/v Tween 80; □, 5% w/v Span 83 with 1% w/v Tween 80; ●, 5% w/v Span 85 with 1% w/v Tween 80. Temperature 37 ± 0.1°C. Values are means, n = 3; the error bars indicate ± range.

more stable w/o/w emulsions, indicating that film strength imparts stability against multiple droplet rupture. The relatively unstable w/o/w emulsions contained mainly simple oil droplets on the eighth day (Figures 4 and 5), whereas the relatively stable

emulsions, in which the droplet diameters did not decrease on storage (e.g. 5% w/v Span 80 or 83 with 0.1% w/v Tween 80), retained a considerable number of multiple droplets on days 8 and 56 (Figures 6 and 7).

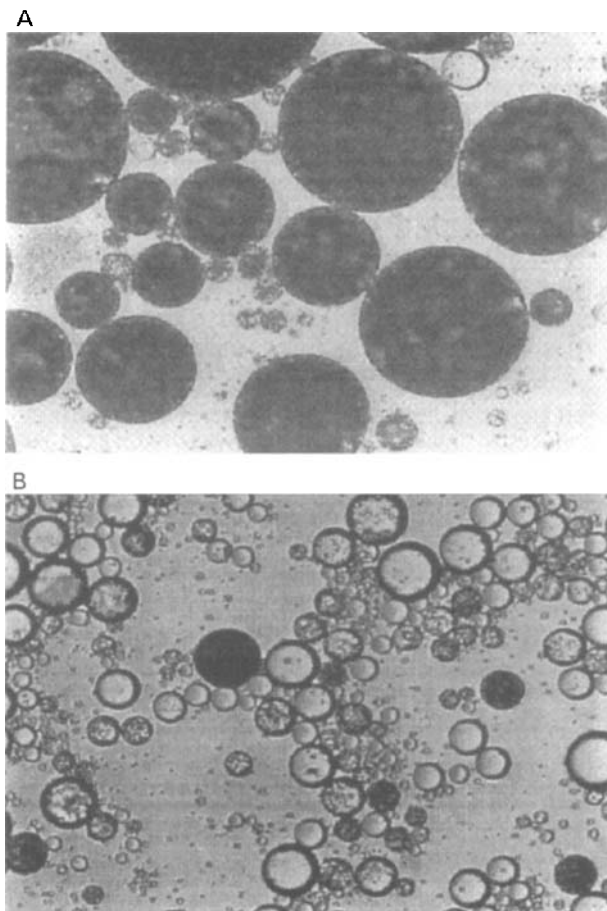


Figure 4. Photomicrographs of (A) freshly prepared and (B) 8-day-old water-in-oil-in-water emulsions prepared with 5% w/v Span 83 in the oil phase and 1% w/v Tween 80 in the external aqueous phase (magnification 200 \times).

The droplet size of Span 80 and Span 83 emulsions with 0.1% w/v Tween 80 increased on storage. The increase in size is possibly because of swelling, as a result of the osmotic gradient induced by the presence of the drug in the internal aqueous phase, or multiple droplet coalescence, or both. Despite their larger initial droplet size the w/o/w emulsions prepared from 5% w/v Spans 80 or 83 with 0.1% w/v Tween 80, were more stable than those prepared with 1% w/v Tween 80 (Table 4). This indicates that film strength, evidenced by higher interfacial elasticity, is more important than initial droplet size in improving multiple emulsion stability for the systems investigated.

The multiple emulsions prepared from 5% Span 80 and 0.1% w/v Tween 80 were compared with the multiple emulsions prepared from 5% Span 80 and 1% w/v Tween 80 with 0.2% w/v bovine serum albumen (BSA) in the internal aqueous phase as described by Omotosho et al (1986). Omotosho et al (1986) reported that addition of

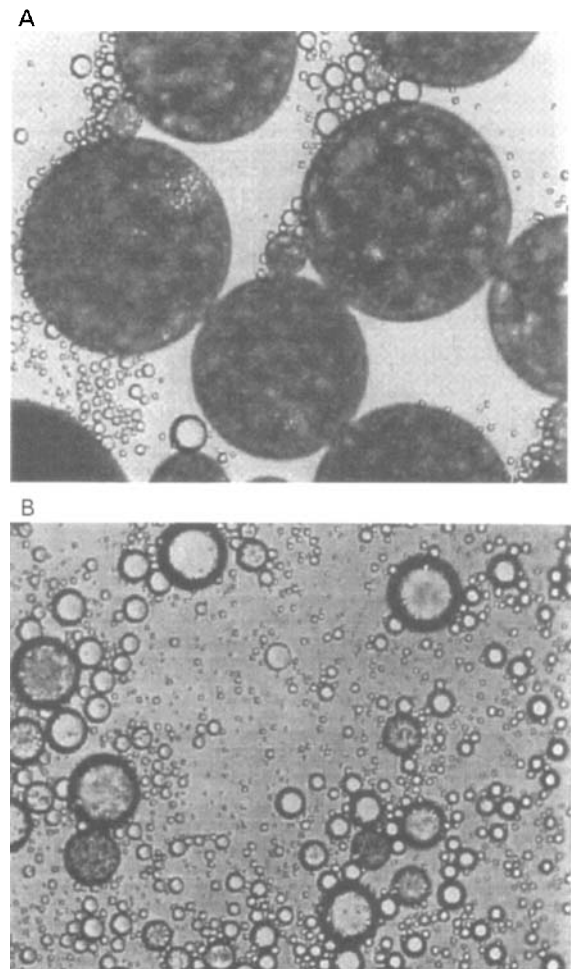


Figure 5. Photomicrographs of (A) freshly prepared and (B) 8-day-old water-in-oil-in-water emulsions prepared with 5% w/v Span 85 in the oil phase and 0.1% w/v Tween 80 in the external aqueous phase (magnification 200 \times).

BSA to the internal aqueous phase stabilized w/o/w multiple emulsions. The emulsion with the lower concentration of Tween 80 (0.1% w/v) and no additives in the internal aqueous phase was more stable than the emulsion prepared from higher concentrations of Tween 80 and BSA. This was apparent after storage for 13 days only, as evidenced by photomicrography (Figure 8). The stability of the emulsion prepared with BSA in the internal aqueous phase was no different from that of the emulsion prepared without BSA using the same concentration of surfactant (i.e. 5% w/v Span 80 with 1% w/v Tween 80) over the same storage time (Figure 8). These results indicate that the presence of BSA in the internal aqueous phase of multiple emulsions does not stabilize w/o/w emulsions effectively, because the high concentration of Tween 80 reduces the film strength at the secondary interface as indicated by the interfacial

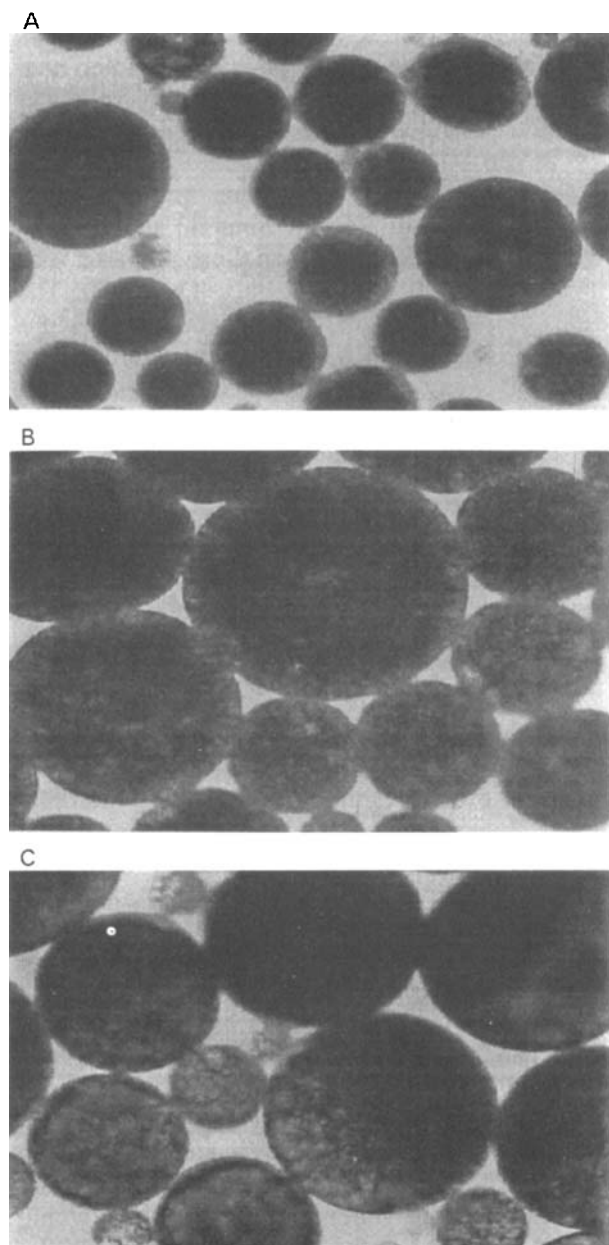


Figure 6. Photomicrographs of (A) freshly prepared, (B) 8-day-old and (C) 8 week old water-in-oil-in-water emulsions prepared with 5% w/v Span 80 in the oil phase and 0.1% w/v Tween 80 in the external aqueous phase (magnification 200 \times).

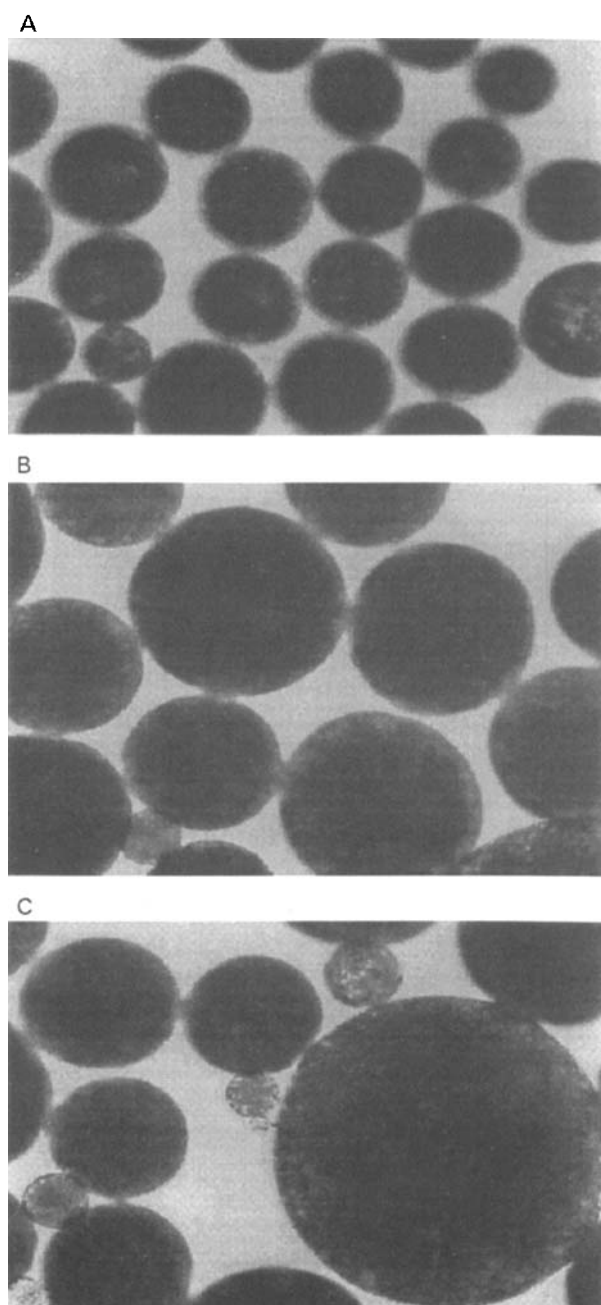


Figure 7. Photomicrographs of (A) freshly prepared, (B) 8-day-old and (C) 8 week old water-in-oil-in-water emulsions prepared with 5% w/v Span 83 in the oil phase and 0.1% w/v Tween 80 in the external aqueous phase (magnification 200 \times).

Table 4. Mean volume-weight diameters ($\mu\text{m} \pm \text{s.d.}$) of freshly prepared (day 0) and 8-day-old water-in-oil-in-water emulsions stored at 21 $^{\circ}\text{C}$.

Oil phase	0.1% w/v Tween 80		1% w/v Tween 80	
	Day 0	Day 8	Day 0	Day 8
Span 80	203.28 \pm 22.51	244.45 \pm 11.64	126.76 \pm 1.60	57.35 \pm 4.23
Span 83	213.81 \pm 8.78	266.70 \pm 4.20	96.13 \pm 3.09	61.01 \pm 2.07
Span 85	236.50 \pm 21.16	115.72 \pm 2.43	166.14 \pm 15.00	67.51 \pm 6.75

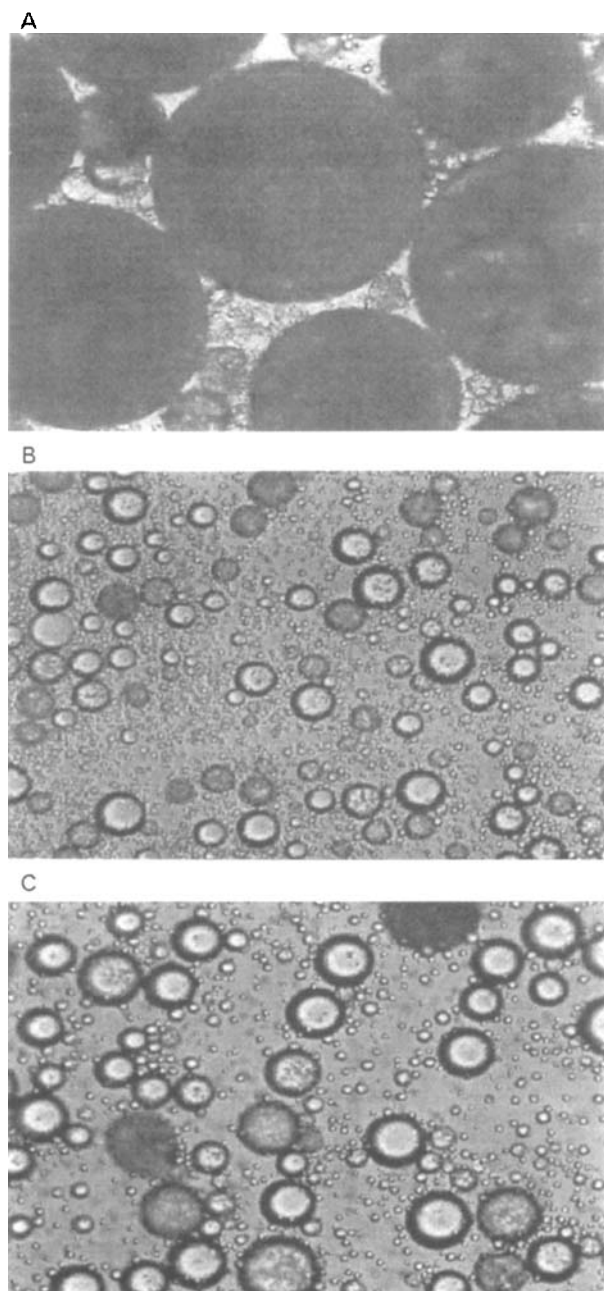


Figure 8. Photomicrographs of water-in-oil-in-water emulsions prepared with 5% w/v Span 80 in the oil phase: (A) no additives in the internal aqueous phase and 0.1% w/v Tween 80 in the external aqueous phase; (B) 0.2% BSA in the internal aqueous phase and 1% w/v Tween 80 in the external aqueous phase and (C) no additives in the internal aqueous phase and 1% w/v Tween 80 in the external aqueous phase (magnification 200 \times).

rheology and emulsion-stability studies described above.

Although the planar oil–water interface is the best model available for measurement of emulsifier interfacial elasticity, it does not necessarily reflect the surfactant interfacial concentrations and their relative ratios in the emulsion systems. This model

enables the determination of general trends only. For example, the effect of increasing concentration can be investigated. Although a higher interfacial elasticity was obtained by use of the model planar oil–water interface for the 5% w/v Span 83–1% w/v Tween 80 system than for the 5% w/v Span 80–0.1% w/v Tween 80 system, multiple emulsions prepared with 5% w/v Span 83 and 1% w/v Tween 80 were less stable than those prepared with 5% w/v Span 80 and 0.1% w/v Tween 80. The initial droplet-size analysis of these emulsions indicated that a much greater concentration of Tween 80 was adsorbed at the emulsion–droplet interface of the 5% w/v Span 83–1% w/v Tween 80 system. Increased bulk surfactant concentration leads to smaller initial mean droplet diameters as a result of a greater reduction in the interfacial free energy by increased surfactant adsorption (Rowe 1965). Consequently, the 5% w/v Span 83–1% w/v Tween 80 system is expected to have weaker interfacial film strength than the 5% w/v Span 80–0.1% w/v Tween 80 system.

Conclusions

Interfacial film strength at the secondary multiple-droplet interface, as measured by interfacial rheology, seems to have a significant effect on multiple emulsion stability. However, care should be taken when interpreting these data, because the planar oil–water interface is a model and might not reflect actual interfacial concentrations and ratios at the emulsion–droplet interfaces. Nevertheless, trends can be successfully determined using this model. In general, interfacial elasticity correlated with multiple emulsion stability, because surfactant systems with higher elasticity tended to result in more stable emulsions. Therefore, interfacial rheological investigation of mixed films would seem to be useful in the development of multiple-emulsion formulations. Unfavourable interactions between Tween 80 and the Span surfactants results in a considerable reduction in interfacial elasticity, which in turn reduces w/o/w emulsion stability. Improved stability was obtained with those mixed emulsifiers for which interfacial elasticities were relatively high, particularly at lower concentrations of Tween 80 (0.1% w/v). The inclusion of BSA in the internal aqueous phase of emulsions prepared from 5% w/v Span 80 with 1% w/v Tween 80 did not improve emulsion stability, confirming the importance of the secondary interface to multiple-emulsion stability. This study indicates the need to identify water-soluble emulsifiers with favourable interactions with Span surfactants, leading to strong interfacial films at the secondary interface, to

replace Tween 80 which has typically been used in multiple emulsion formulation.

Acknowledgement

The authors are grateful to the Upjohn Company for partial financial support of this study.

References

- Burgess, D. J., Sahin, N. O. (1994) Interfacial rheology of β -casein solutions. In: Herb, C. A., Prud'homme, R. K. (eds) *Structure and Flow in Surfactant Solutions*. American Chemical Society, Washington DC, pp 380–393
- Burgess, D. J., Sahin, N. O. (1997a) Interfacial rheological and tension properties of protein films. *J. Colloid Interface Sci.* 189: 74–82
- Burgess, D. J., Sahin, N. O. (1997b) Influence of protein emulsifier interfacial properties on oil-in-water emulsion stability. *Pharm. Dev. Tech.* 3: 21–29
- Davis, S. S. (1987) Physicochemical criteria for semi-solid dosage forms. In: Grimm, W. (ed.) *Stability Testing of Drug Products*. Germany, pp 40–56
- Florence, A. T., Rogers, J. A. (1971) Emulsion stabilization by non-ionic surfactants: experiment and theory. *J. Pharm. Pharmacol.* 23: 153–169
- Florence, A. T., Whitehill, D. (1981) Some features of breakdown in water-in-oil-in-water multiple emulsions. *J. Colloid Interface Sci.* 79: 243–256
- Florence, A. T., Whitehill, D. (1982) The formulation and stability of multiple emulsions. *Int. J. Pharm.* 11: 277–308
- Fukushima, S., Juni, K., Nakano, M. (1983) Preparation of and drug release from W/O/W type double emulsions containing anticancer agents. *Chem. Pharm. Bull.* 31: 4048–4056
- Halling, P. J. (1981) Protein-stabilized foams and emulsions. *Crit. Rev. Food Sci. Nutr.* 15: 155–203
- Law, T. K., Whateley, T. L., Florence, A. T. (1986) Stabilization of W/O/W multiple emulsion by interfacial complexation of macromolecules and nonionic surfactants. *J. Contr. Rel.* 3: 279–290
- Magdassi, S., Frenkel, M., Gartic, N., Kasan, R. (1984) Multiple emulsions II: HLB shift caused by emulsifier migration to external interface. *J. Colloid Interface Sci.* 97: 374–379
- Matsumoto, S., Kang, W. W. (1989) Formation and applications of multiple emulsions. *J. Disper. Sci. Tech.* 10: 455–482
- Matsumoto, S., Kita, Y., Yonezawa, D. (1976) An attempt at preparing water-in-oil-in-water multiple-phase emulsions. *J. Colloid Interface Sci.* 57: 353–361
- Murray, B. S., Dickinson, E. (1996) Interfacial rheology and the dynamic properties of adsorbed films of food proteins and surfactants. *Food Sci. Technol. Int.* 2: 131–145
- Myers, D. (1988) *Surfactant Science and Technology*. VCH, New York, pp 209–253
- Nakhare, S., Vyas, S. P. (1995) Prolonged release of rifampicin from multiple W/O/W emulsion systems. *J. Microencapsulation* 12: 409–415
- Omotosho, J. A., Law, T. K., Whateley, T. L., Florence, A. T. (1986) The stabilization of W/O/W emulsions by interfacial interaction between albumin and non-ionic surfactants. *Colloids Surfaces* 20: 133–144
- Omotosho, J. A., Whateley, T. L., Florence, A. T. (1989) Release of 5-fluorouracil from intramuscular W/O/W multiple emulsions. *Biopharm. Drug Dispos.* 10: 257–268
- Opawale, F. O., Burgess, D. J. (1998) Influence of interfacial properties of lipophilic surfactants on water-in-oil emulsion stability. *J. Colloid Interface Sci.* 197: 142–150
- Rowe, E. L. (1965) Effect of emulsifier concentration and type on the particle size distributions of emulsions. *J. Pharm. Sci.* 54: 260–264
- Sela, Y., Magdassi, S., Garti, N. (1995) Release of markers from the inner water phase of W/O/W emulsions stabilized by silicone-based polymeric surfactants. *J. Contr. Rel.* 33: 1–12
- Vaziri, A., Warburton, B. (1994) Slow release of chloroquine phosphate from multiple taste-masked W/O/W multiple emulsions. *J. Microencapsulation* 2: 641–648
- Wan, L. S. C., Lee, P. F. S. (1973) Influence of nonionic surfactants on the interfacial tension in an oil/water system. *Can. J. Pharm. Sci.* 8: 136–139
- Warburton, B. (1993) In: Collyer, A. A. (ed.) *Techniques in Rheological Measurements*. Chapman and Hall, London, p. 55