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Effect of Surfactant Phase in Perfluorocarbon Emulsification Efficiency

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The effect of preparation temperature on the emulsification efficiency of perfluoro-3-butyltetrahydrofuran (FC-75) was investigated. Polyoxyethylene (POE) oleyl ether surfactants were used as the emulsifier(s) in a range of HLB values of 7.5 to 9.5. The emulsions were prepared by paddle mixing as a method of low-shear emulsification. After centrifugation of the resulting O/W emulsions, the volume of FC-75 which separated was utilized as a measure of the emulsification efficiency. In general, emulsions prepared at temperatures where the surfactant was in a lamellar-to-isotropic surfactant solution transition, $L_\alpha \rightarrow L_3$, displayed a better emulsification efficiency than those prepared with other surfactant phases.

KEY WORDS: centrifugation; emulsification; perfluorocarbon; stability.

INTRODUCTION

In nonionic surfactant systems, dramatic differences in phase behavior have been shown to occur with changes in temperature (1–3). For example, in individual and mixtures of polyoxyethylene (POE)-type surfactants, several micellar formations, including lamellar (L_α), isotropic surfactant solution (L_3), and concentrated surfactant phase (L_2), have been investigated and documented by a variety of techniques (3–6). The presence of these and other surfactant phases in pharmaceutical emulsions has been described extensively in terms of phase diagrams for multiple-component systems (2).

The relationship between the surfactant phase present at emulsification and the effect on long-term stability has not been characterized completely. Emulsification by the phase inversion temperature (PIT) method is widely published, showing improved emulsion stability when prepared close to temperatures where an O/W emulsion inverts to a W/O system (7). Other studies published describe enhanced stability for emulsions prepared in the presence of the surfactant in a liquid crystal phase (8–10). In these studies, various concentrations of the different emulsifiers were required to obtain liquid crystalline phases. Related studies have shown differences in oil solubilization (5) and in oil/water interfacial film integrity (3) with the different surfactant phases.

The purpose of this investigation was to assess the effect of the surfactant phase at the time of emulsification, as controlled by sample temperature, on the emulsification efficiency of a perfluorocarbon liquid. It is postulated that a more efficient process should result in an emulsion with better stability. Perfluorocarbon liquids, when administered intravenously as O/W emulsions, have been shown to be effective as tissue oxygenators in medicine; however, these emulsions continue to present formulators with a wide variety of physical stability problems (11).

MATERIALS AND METHODS

Materials

Perfluoro-3-butyltetrahydrofuran (FC-75, 3M) was used as the nonaqueous phase of the emulsions. Polyoxyethylene-3 (POE-3) oleyl ether (Volpo 3, HLB 6.6)[4] and polyoxyethylene-5 (POE-5) oleyl ether (Volpo 5, HLB 8.8) were obtained from Croda, Inc.; polyoxyethylene-10 (POE-10) oleyl ether (Brij 96, HLB 12.4)[5] was obtained from Sigma Chemical Company. All materials were used as received from the manufacturer. Water used for sample preparation was deionized and filtered (Milli Q, Millipore).

Emulsion Preparation

For all of the samples, 20% (w/w) FC-75 was used with 5% (w/w) surfactant concentration. In order to obtain a range of hydrophile–lipophile balance (HLB) values, mixtures of the surfactants were utilized. (The HLB of surfactant mixtures was determined on a percentage by weight basis.) For each sample, the surfactant(s)/water mixture and FC-75 were heated separately to the desired temperature in a water bath (Neslab EX-210D); the aqueous phase was stirred during heating to effect a homogeneous dispersion in a 100-ml round-bottom flask. Temperatures were monitored with a digital thermometer (Fisher Scientific) and a YSI 400 series thermistor (NBS certified, Fisher Scientific). The emulsions were prepared by stirring for 20 min at 150 rpm in the round-bottom flask with a paddle mixer equipped with a digital controller (Hanson Research) at the desired emulsification temperature, followed by stirring to ambient temperature. All samples were prepared in duplicate and were stored for 24 hr prior to evaluation.

To assess the efficiency of the emulsification process, the emulsions were centrifuged at 25°C for two 5-min cycles at 515*g*, 3220*g*, and 12,900*g* (Beckman J2-21 centrifuge, JA20.1 rotor). The volume of FC-75 separated was measured after each centrifuge cycle and is reported as a volume percentage based on the total emulsion volume.

RESULTS

The results for the volume of FC-75 separated as a function of emulsification temperature for several HLB values are depicted in Figs. 1 through 4. For each figure, the values shown are the mean of two samples. The results for each sample are cumulative; after each 5-min centrifugation cycle, the volume of FC-75 separated was measured, and the

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[4] Volpo™ is a registered trademark of Croda, Inc.

[5] Brij is a registered trademark of ICI Americas, Inc.

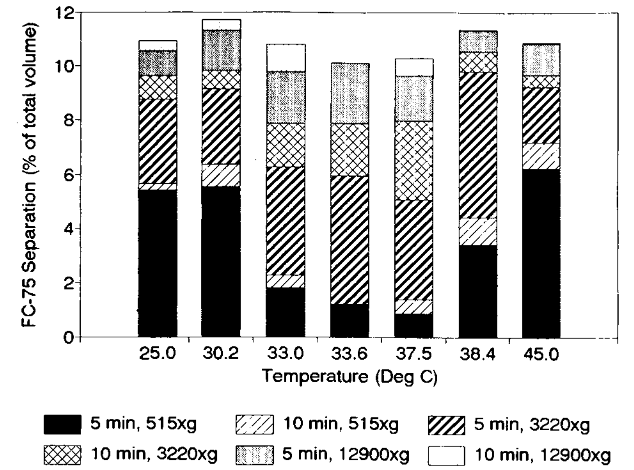

Fig. 1. FC-75 separation from 20% emulsions with 5% surfactant, HLB 8.0. Temperatures correspond to phase transitions identified in Table I.

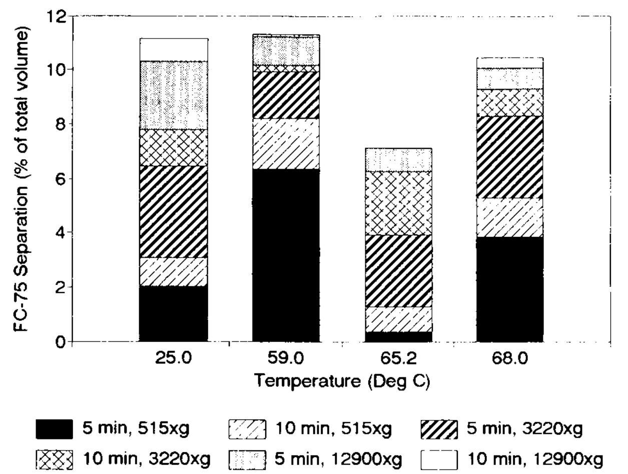

Fig. 3. FC-75 separation from 20% emulsions with 5% surfactant, HLB 9.5. Temperatures correspond to phase transitions identified in Table I.

centrifuge tube returned to the centrifuge for the next cycle (total of six cycles).

The emulsification temperatures for each HLB value were selected based on the results of previous conductivity–temperature profiles for 5% aqueous dispersions of the surfactant(s) (12). It was shown that dramatic changes in POE nonionic surfactant aggregation occur at specific temperatures; these transition temperatures were determined from electrical conductivity measurements of aqueous surfactant dispersions as a function of temperature. Several surfactant phases were of particular interest in studying the role of aggregate type in emulsification. Specifically, the lamellar (L_α) phase, isotropic solution phase (L_3), and regions around these events were of interest. Table I lists the emulsification temperatures investigated and the surfactant phase for the 5% surfactant dispersions at each temperature.

In general, an increase in centrifugation time and intensity resulted in an increase in FC-75 separation. The most dramatic differences in the amount of FC-75 separated as a function of emulsification temperature are evident in the first two centrifuge cycles (515*g*). Under more stressful conditions (12,900*g*), practically all of the FC-75 has separated. An approximate theoretical phase volume of 12.3% (v/v) can be used for comparison for complete coalescence to occur when the specific gravities of the components are taken into account. (Assuming volumes are additive, the following specific gravities were utilized: FC-75 1.76; POE-n oleyl ether surfactants 0.88.)

In Fig. 1, seven emulsification temperatures were investigated for HLB 8.0. Significantly (Duncan's multiple-range test, $P = 0.05$) smaller volumes of FC-75 separated at 33.0, 33.6, and 37.5°C for the first two centrifuge cycles when compared to the lower and higher temperatures for this HLB. Six emulsifications temperatures were studied for HLB 8.8 (Fig. 2). In these emulsions, no perfluorocarbon liquid separated in the sample prepared at 49.1°C until the fourth centrifuge cycle (10 min at 3220*g*). Similar differences were also apparent for the HLB 9.5 and 7.5 emulsions (Figs. 3 and 4, respectively).

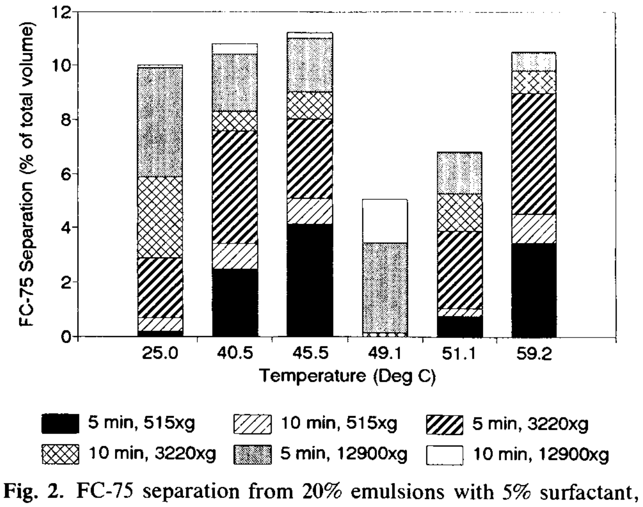

Fig. 2. FC-75 separation from 20% emulsions with 5% surfactant, HLB 8.8. Temperatures correspond to phase transitions identified in Table I.

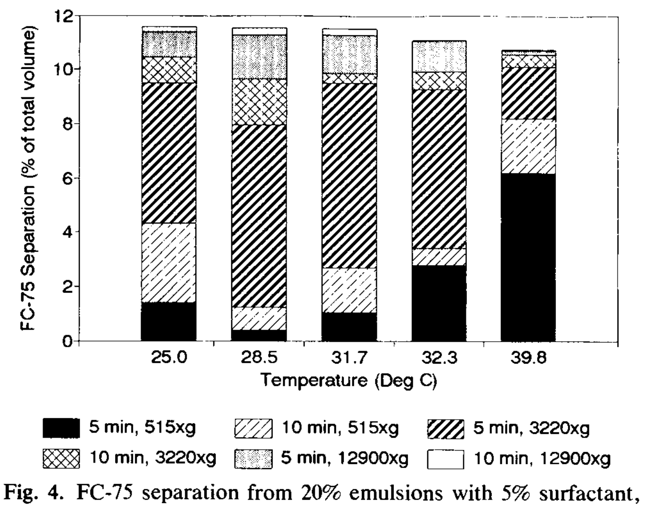

Fig. 4. FC-75 separation from 20% emulsions with 5% surfactant, HLB 7.5. Temperatures correspond to phase transitions identified in Table I.

Table I. Emulsification Temperatures for FC-75 Emulsions

HLB composition (%, w/w)	Emulsification temperature (°C)	Surfactant phase[a]
7.5 POE-3 oleyl ether/ POE-5 oleyl ether (59.1/40.9)	25.0	W + $L_{\alpha 1}$
	28.5	$L_{\alpha 1} + L_{\alpha 2} \rightarrow L_{\alpha 2} + L_3$
	31.7	$L_{\alpha 2} + L_3 \rightarrow L_3$
	32.3	L_3
	39.8	W + L_2
8.0 POE-3 oleyl ether/ POE-5 oleyl ether (36.4/63.6)	25.0	W + $L_{\alpha 1}$
	30.2	W + $L_{\alpha 1} \rightarrow L_{\alpha 1} + L_{\alpha 2}$
	33.0	$L_{\alpha 1} + L_{\alpha 2}$
	33.6	$L_{\alpha 1} + L_{\alpha 2} \rightarrow L_{\alpha 2} + L_3$
	37.5	L_3
	38.4	W + L_3
	45.0	W + L_2
8.8 POE-5 oleyl ether (100)	25.0	W + $L_{\alpha 1}$
	40.5	W + $L_{\alpha 1} \rightarrow L_{\alpha 1} + L_{\alpha 2}$
	45.5	$L_{\alpha 1} + L_{\alpha 2} \rightarrow L_{\alpha 2} + L_3$
	49.1	$L_{\alpha 2} + L_3 \rightarrow L_3$
	51.1	L_3
	59.2	W + L_2
9.5 POE-5 oleyl ether/ POE-10 oleyl ether (80.6/19.4)	25.0	W + $L_{\alpha 1}$
	59.0	W + $L_{\alpha 1} \rightarrow L_{\alpha 1} + L_{\alpha 2}$
	65.2	$L_{\alpha 1} + L_{\alpha 2} \rightarrow L_{\alpha 2} + L_3$
	68.0	W + L_3

[a] Phase notation from Ref. 12.

DISCUSSION

The results of this study illustrate significant differences in emulsification efficiency for each surfactant as a function of temperature. In this work for the temperatures investigated, it appears that the best emulsification occurred when the surfactant phase was approximately in the lamellar → isotropic solution ($L_{\alpha 2} + L_3 \rightarrow L_3$) transition as evidenced by the smallest amount of perfluorocarbon liquid separated.

Nonionic surfactant phase behavior and emulsification with respect to the isotropic solution phase (L_3) have been described by several authors (1,13,14). Shinoda *et al.* (7,14) describe this phase to occur at temperatures close to the cloud point of a nonionic surfactant and have shown a correlation between the temperatures around the cloud point in aqueous surfactant dispersions and the PIT in emulsified systems.

Several properties of the L_3 phase cited in the literature favor better emulsification. Increases in solubilization ability in the isotropic surfactant solution were found to occur in cyclohexane/polyoxyethylene nonylphenylether/water systems (14). The temperature region in which the isotropic solution phase of a surfactant occurs was considered to be the optimal temperature for maximum solubilization and formation of a "swollen micellar solution."

In addition to differences in solubilization capacity in the L_3 phase, comparison of the relative interfacial tensions between the surfactant:oil phase and surfactant:water phase is important (13,15). The cumulative interfacial tension between the three components dictates the curvature of the surfactant monolayer at the oil:water interface (3,13,15). In general, as the temperature is increased, the interfacial tension between the surfactant:oil phases decreases, while that between the surfactant:water phases increases (15). In the isotropic solution phase, it is believed that these interfacial tensions approach each other, creating an optimum balance and resulting in better emulsions.

The differences between the FC-75 emulsions reported here may also be evidence of differences in the flexibility of the resulting surfactant layer formed at the FC-75:water interface. Di Meglio *et al.* (3) reported the existence of flexibility transitions in aqueous POE-5 lauryl ether dispersions at temperatures where isotropic–lamellar transitions occur. A more flexible surfactant film may be formed at the FC-75:water interface during emulsification in the $L_{\alpha 2} \rightarrow L_3$ transition compared to that which would be formed by a rigid lamellar phase. A more flexible surfactant layer may be able to interact with the aqueous and nonaqueous phases in a more efficient manner.

As expected, the temperature and concentration range of the L_3 phase as well as all of the surfactant phases are dependent on the surfactant's chemical structure and electrolyte concentration, if present (13). For the POE-*n* oleyl ether surfactants used in this study, different individual phase diagrams would be expected due to differences in surfactant hydrophilicity. Specific phases, such as the L_3, may or may not be detectable in all of the individual components. For example, Mitchell *et al.* (13) report the existence of an L_3 temperature domain for POE-3 and POE-4 capryl (C_{10}) ethers but not for POE-5 or POE-6 capryl ether.

The size and type of the phases present for mixtures of POE nonionic surfactants have been suggested to be an equilibrium combination of the individual components (6,16). Nishikido (16) investigated the phase behavior of mixing POE-*n* lauryl ethers (n = 4, 5, 6) as a function of temperature and found the general phase diagrams of several mixtures to be intermediates between that of the individual components. Based on these observations and our previous work with the conductivity results for surfactant mixtures (12), it seemed logical to predict similar results for the emulsification behavior of surfactant mixtures, although some aberrations may be present.

In the emulsified systems in the present work, an interesting trend in the FC-75 separation volumes was apparent. As the HLB increased, the temperature range where the smallest volume of FC-75 separated shifted from the $L_{\alpha 2}$ region to the L_3 region. For example, after the first centrifugation cycle (black bars) in the HLB 7.5 emulsions (Fig. 4), the smallest volume of FC-75 separated in the sample prepared at 28.5°C ($L_{\alpha 2}$), whereas in the HLB 8.8 emulsion (Fig. 1), significantly less separation occurred in the 49.1°C ($L_{\alpha 2}$ + L_3) and 51.1°C (L_3) samples. As the HLB is increased, the more stable emulsions resulted from emulsification at $L_{\alpha 2}$ + L_3/L_3 phases. A similar result is seen in Fig. 3 for HLB 9.5; significantly less FC-75 separated in the emulsion prepared at 65.2°C, which correlates with the isotropic solution (L_3) phase of the surfactant.

These observations are supported by the phase diagrams reported by Mitchell *et al.* (13) for similar surfactants. In the phase diagrams reported for aqueous dispersions of POE-3, POE-4, POE-5, and POE-6 lauryl ether surfactants, the development of the L_3 phase is seen in the POE-4 and POE-5 lauryl ether systems. This phase is not present in the POE-3 and POE-6 diagrams.

In the present work, POE-3 (HLB 6.6) and POE-5 (HLB 8.8) oleyl ether surfactants were used to obtain HLB 7.5 and 8.0; for HLB 9.5, POE-5, and POE-10 (HLB 12.4) oleyl ether surfactants were combined. It appears that the domain of the L_3 phase is smaller for the HLB 7.5 emulsion compared to the HLB 8.8 system. In previous work (12), a more defined isotropic solution was seen in aqueous HLB 8.8 and 9.5 surfactant dispersions than was apparent in an HLB 7.5 dispersion.

CONCLUSION

From these experiments, it can be seen that for a series of POE-*n* oleyl ether surfactants ($n = 3, 5, 10$), the surfactant phase as controlled by emulsification temperature plays a significant role in the efficiency of the emulsification process. By measuring the volume of FC-75 separated from centrifuged emulsions, differences in emulsification efficiency were visible and served as a parameter for emulsification comparison. It is anticipated that the efficiency of the emulsification process will be related to the short-term stability; additional work which is in progress is required to identify this relationship.

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REFERENCES

1. D. Attwood and A. T. Florence. *Surfactant Systems, Their Chemistry, Pharmacy and Biology,* Chapman and Hall, London, 1983.
2. P. Becher. *Encyclopedia of Emulsion Technology, Vol. 1. Basic Theory,* Marcel Dekker, New York, 1983.
3. J. M. Di Meglio, L. Paz, M. Dvolaitzky, and C. Taupin. Local curvature in nonionic amphiphilic films. Effect of temperature at the cloud point and in the lamellar phases. *J. Phys. Chem.* 88:6036–6040 (1984).
4. T. A. Bostock, M. P. McDonald, and G. J. T. Tiddy. Phase studies and conductivity measurements in microemulsion-forming systems containing a nonionic surfactant. In K. L. Mittal and B. Lindman (eds.), *Surfactants in Solution, Vol. 3,* Plenum Press, New York, 1984, pp. 1805–1820.
5. N. Nishikido. Laplace pressure effect in micelles viewed from the temperature effect on saturation solubilization of alkanes. *J. Colloid Interface Sci.* 127:310–316 (1989).
6. S. A. Safran, P. Picus, and D. Andelman. Theory of spontaneous vesicle formation in surfactant mixtures. *Science* 248:354–356 (1990).
7. K. Shinoda and H. Saito. The stability of o/w type emulsions as functions of temperature and the HLB of emulsifiers: The emulsification by PIT-method. *J. Colloid Interface Sci.* 30:258–263 (1969).
8. T. Suzuki, H. Takei, and S. Yamazaki. Formation of fine three-phase emulsions by the liquid crystal emulsification method with arginine β-branched monoalkyl phosphate. *J. Colloid Interface Sci.* 129:491–500 (1989).
9. S. Friberg and L. Mandell. Influence of phase equilibria on properties of emulsions. *J. Pharm. Sci.* 59:1001–1004 (1970).
10. F. Fong-Spaven and R. G. Hollenbeck. Thermal rheological analysis of triethanolamine-stearate stabilized mineral oil in water emulsions. *Drug Dev. Indust. Pharm.* 12:289–302 (1986).
11. R. P. Geyer. Perfluorochemicals as oxygen transport vesicles. In T. M. S. Chang (ed.), *Biomaterials, Artificial Cells, and Artificial Organs, Vol. 16 (1–3),* Marcel Dekker, New York, 1988, pp. 31–49.
12. J. M. Pasternacki, R. L. Schnaare, and E. T. Sugita. Conductivity—temperature behavior of aqueous dispersions of nonionic surfactants (submitted for publication).
13. D. J. Mitchell, G. J. T. Tiddy, L. Waring, T. Bostock, and M. P. McDonald. Phase behaviour of polyoxyethylene surfactants in water. *J. Chem. Soc. Faraday Trans. I.* 79:975–1000 (1983).
14. K. Shinoda and H. Kunieda. Conditions to produce so-called microemulsions: Factors to increase the mutual solubility of oil and water by solubilizer. *J. Colloid Interface Sci.* 42:381–387 (1973).
15. K. Shinoda and H. Saito. The effect of temperature on the phase equilibria and the types of dispersions of the ternary system composed of water, cyclohexane, and nonionic surfactant. *J. Colloid Interface Sci.* 26:70–74 (1968).
16. N. Nishikido. Phase equilibria of nonionic surfactant-water systems as a function of temperature and pressure: Effect of mixing of surfactants. *J. Colloid Interface Sci.* 136:401–407 (1990).