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Formation and stabilization of perfluorocarbon emulsions

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Summary

The formation and stabilization of perfluorocarbon emulsions with pluronic F-68 and soya lecithin were investigated. Greater ease of formation and stability were achieved with the soya lecithin. It was found that, in general, smaller droplet size was obtained with the emulsions prepared with lecithin, at all dispersed phase fractions. Zeta potential measurements indicated that the emulsions are stabilized via an electrostatic mechanism; the zeta potential increased with increasing lecithin concentration, up to a constant high value, and decreased with increasing dispersed phase fraction.

Introduction

Perfluorocarbon emulsions have been tested as potential blood substitutes during the last twenty years. This type of emulsions, first prepared by Sloviter and Komimoto (1967), are composed of various perfluorocarbons which are emulsified in an aqueous phase by using either BSA, egg lecithin or pluronic F-68. At present, there are at least three emulsions which have been developed on a large scale: Fluosol DA (Japan), Ftorosan (U.S.S.R.) and Emulsion II (China) (Naito and Yokoyama, 1978; Xiong et al., 1981; Beloyarster et al., 1983).

The most widely tested emulsion is the Fluosol DA, which is composed of perfluorodecalin and perfluorotributylamine as the dispersed phase, and pluronic F-68 and yolk phospholipids as emulsifiers. This emulsion has been employed in human studies, and appears to be a good blood substitute, although suffering from low stability (Riess and Le Blanc, 1982). Several attempts to overcome the instability of these emulsions were based on the use of either fluorinated surfactants, or new perfluorocarbons as the dispersed phase.

The perfluorocarbon emulsions were also proved to be effective in oxygen supply to immobilized cells (Aldercreutz and Mattiasson, 1982), and were recently suggested for use in a unique drug delivery system (Caizza et al., 1987).

The large-scale application of such emulsions is strongly dependent on the ability to solve the formulation problems of these systems. Therefore, it is essential to evaluate the factors which contribute to the formation and stabilization of these emulsions.

In this paper, we have attempted to evaluate the formulation of a model PFC emulsion, com-

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posed of perfluorodecalin as the dispersed phase and the monomeric and polymeric surfactants, soya lecithin and pluronic F-68.

Materials and Methods

Perfluorodecalin (Aldrich), the polymeric surfactant pluronic F-68 (BASF), and oil-free soya lecithin, Centrolex P (Central Soya) were used without further purification. According to the manufacturer, Centrolex P contains 23% w/w phosphatidylcholine, 20% w/w phosphatidylethanolamine, 14% w/w phosphatidylinositol, 8% w/w phosphatidic acid, 15% w/w glycolipids, and 8% w/w complexed sugars.

The emulsions were prepared as follows:

(a) Homogenization (2 min duration) of a concentrated lecithin solution with water, to yield final concentrations varying from 0.2 to 3% w/w. The initial concentrated lecithin solution was obtained by sonication of 10% w/w lecithin in water for 0.5 h in an ice bath (Fisher-Sonic Dismembrator, model 300). Homogenization was carried out using a Silverson Homogenizer (Silverson Machines).

(b) Addition of the perfluorodecalin (5–40% w/w), and homogenization for 5 min. The resulting emulsions were stored at 5 °C.

The droplet size distribution of each emulsion was measured on a Coulter counter model TA II (Coulter Electronics).

The zeta potential of the various emulsions was measured in emulsion samples which were diluted (1 : 30) in filtered demineralized water (0.2 μm Millipore filter). The zeta potential of each emulsion was based on measurements of the electrophoretic mobility of at least 60 droplets, by a Zeta-meter (Zeta Meter, Inc.). The zeta potential was calculated by using the Smoluchowski equation.

Interfacial tension measurements were conducted by a Lauda tensiometer, with a platinum iridium ring. The measurements were performed 10 min after formation of the interface between the lecithin solution and the perfluorodecalin (PFC).

Results and Discussion

Ease of formation and stability are the most important parameters in emulsion formation. These parameters indicate the initial droplet size, for a given preparation method, and the shelf-life of the emulsion.

The initial mean droplet size was measured as a function of emulsifier concentration, for two series of emulsions which were prepared with lecithin or pluronic F-68 and 10% w/w PFC. As shown in Fig. 1, the initial droplet size decreased with increasing emulsifier concentration, for both series, until a constant value was reached at concentrations above 0.5% w/w. It also appears that the lecithin is more effective than F-68 in reduction of the initial droplet size. The same effect was also observed for emulsions which were prepared at 5% w/w PFC: the mean droplet size was 5.6 and 9.2 μm for emulsions which were prepared with 1% w/w lecithin and 1% w/w pluronic, respectively. This order of effectiveness is in agreement with the results reported by Davis et al. (1976), for preparation of submicron perfluorodecalin emulsions, although they used a purified egg lecithin whereas soya lecithin was employed in the present study. The greater ease of formation achieved with lecithin probably results from the higher degree of reduction of the interfacial tension by lecithin than by pluronic F-68; the interfacial tension between perfluorodecalin and water decreased from 51 to 13.4 dyn/cm upon addition of 1% w/w lecithin to the water phase, compared to the value

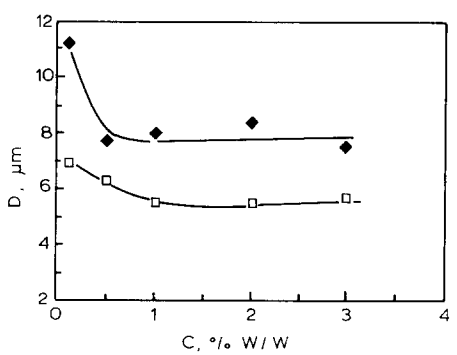

Fig. 1. Initial droplet size of emulsions prepared with various concentrations of lecithin or pluronic F-68 (10% w/w PFC).

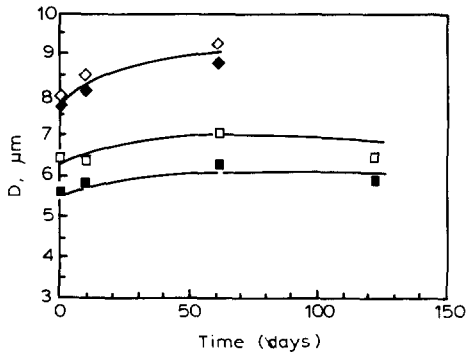

Fig. 2. The changes in droplet size in time, in emulsions prepared using 1% w/w (◇) and 0.5% w/w (◆) pluronic F-68, or 0.5% w/w (□) and 1% w/w (□) lecithin (10% w/w PFC).

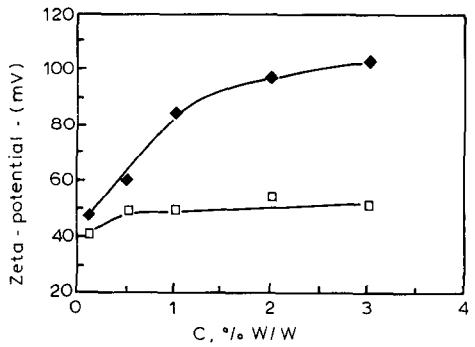

Fig. 3. Zeta potential of emulsions prepared with various concentrations of pluronic F-68 (□) or lecithin (◆) (10% w/w PFC).

of 23.9 dyn/cm reported for 1% w/w pluronic F-68 solution (Kaelble and Moacanin, 1979).

Once the emulsions are formed, their stability is dependent on the nature of the interfacial film which is formed around the droplets. In general, it was found that pluronic F-68 led to less stable emulsions than did the lecithin at all concentrations studied. The changes in droplet size with time are demonstrated in Fig. 2, for emulsions which were prepared using 0.5 and 1% w/w emulsifiers (10% w/w PFC). It is clear that no significant changes occurred in droplet size for emulsions prepared using lecithin as compared to those in the case of F-68. It should be noted that measurements were discontinued for emulsions stabilized by F-68 beyond 60 days, due to significant oil separation.

The enhanced stability of the emulsions which were prepared by use of lecithin can be attributed to electrostatic stabilization of the droplets. The mechanism of electrostatic stabilization was evaluated by zeta potential measurements which were conducted for the two series of emulsions containing various emulsifier concentrations (10% w/w PFC).

As could be expected, higher values of zeta potential were obtained for lecithin-stabilized emulsions compared to those in the case of pluronic F-68, at all emulsifier concentrations (Fig. 3). The increase in lecithin concentration led to an increase in the zeta potential, until a constant value was reached (~ − 100 mV), compared to a fixed, low zeta potential observed for F-68-stabilized emulsions.

The oxygen capacity of PFC emulsions is dependent on the fraction of the dispersed phase. Therefore, it was of interest to evaluate the formation and stability of emulsions prepared for various fractions of PFC. It was found that increasing the dispersed phase fraction led to an increase in the initial droplet size, at all surfactant concentrations. For example, increasing the PFC fraction from 5 to 40% w/w (Fig. 4), resulted in the initial droplet size increasing from 5.8 to 11 μm, respectively (1% w/w lecithin). This effect could result from a deficiency in emulsifier and could lead to decreased stability, as indeed was observed (Table 1).

Emulsions containing 10% w/w PFC were very stable even at emulsifier concentration as low as

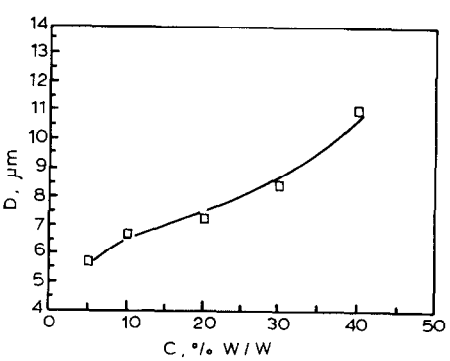

Fig. 4. Initial droplet size of emulsions prepared with 1% w/w lecithin, at various dispersed phase concentrations.

TABLE 1

Shelf life of PFC emulsions at various lecithin and PFC concentrations, defined as the minimal time required for oil separation (days)

% w/w lecithin	% w/w PFC		
	40	20	10
0.1	0.08	20	> 120
0.5	4	> 60	> 120
1	20	> 60	> 120
2	> 60	> 60	> 120
3	> 60	> 60	> 120

0.5% w/w (no phase separation, 120 days), while oil separation was observed already 4 days after preparation in emulsions containing 40% w/w PFC. Since the stabilization of these emulsions is mainly attributed to an electrostatic mechanism, it could be expected that the zeta potential would decrease with increasing PFC fraction, for any given emulsifier concentration, as confirmed in the results shown in Fig. 5.

The zeta potentials of the emulsions which were stabilized by pluronic F-68 are similar to those reported for emulsions stabilized by nonionic surfactants such as Spans and Tweens (Becher and Trifiletti, 1976). However, the finding that lecithin yields more stable emulsions than pluronic F-68 conflicts with the results of Davis et al. (1976). The difference probably results from the use of purified egg lecithin as compared to the soya

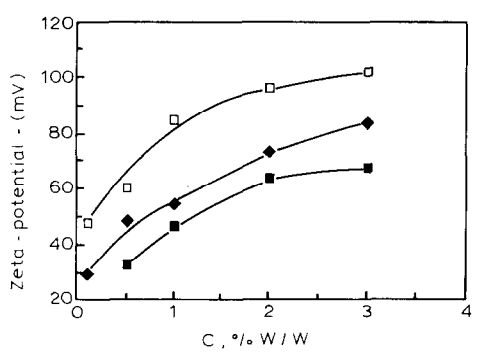

Fig. 5. Zeta potential of PFC emulsions prepared with various concentrations of PFC and lecithin: (□) 10% PFC, (◆) 20% PFC, (□) 40% PFC.

lecithin which contains phospholipids which are negatively charged at neutral pH (Sabet et al., 1982). As shown by Rydhag and Wilton (1981), the presence of negatively charged phospholipids is essential for stabilization of soybean oil in water emulsions. Preliminary results obtained for emulsions which were prepared by using purified phosphatidylcholine confirmed that the negatively charged phospholipids are also essential for stabilization of emulsions of perfluorodecalin in water.

References

Aldercreutz, P. and Mattiasson, B., Oxygen supply to immobilized cells. *Eur. J. Appl. Microbiol. Biotechnol.*, 16 (1982) 165–170.

Becher, P. and Trifiletti, S.E., Emulsion stabilized by nonionic surface active agents: effect of electrolyte. In Smith, A.L. (Ed.), *Theory and Practice of Emulsion Technology*, Academic Press, London, 1976, pp. 271–280.

Beloyarster, F.F., Mayersky, E.I. and Islamor, B.I., *Ftorosan-Oxygen Carrying Perfluorochemical Plasma Substitute*, Acad. Sci. USSR, Pushchino, 1983.

Caiazza, S., Fanizza, C. and Ferrari, M., Possible role of perfluoro chemical particles as drug delivery agents in liver. *Adv. Biomaterials*, 7 (1987) 655–660.

Davis, S.S., Purewal, T.S. and Buscall, R., The stability of perfluorocarbon emulsions: the effect of the chemical nature of the oil phase. In Kerker, M. (Ed.), *Colloid Interface Sci. Ser.* 2, Academic Press, London, 1976, pp. 265–288.

Kaelble, D.H. and Moacanin, J., Surface energetics analysis of artificial blood substitutes. *Med. Biol. Eng. Comput.*, 17 (1979) 593–601.

Naito, R. and Yokoyama, K., Perfluoro chemical blood substitutes. *Technical Information Series No. 5, 7*, Green Cross, Osaka, 1978, 1981.

Riess, J.G. and Le Blanc, M., Solubility and transport phenomena in perfluoro chemicals relevant to blood substitution and other biomedical applications. *Pure Appl. Chem.*, 54 (1982) 2383–2406.

Rydhag, L. and Wilton, I., The function of phospholipids of soybean lecithin in emulsions. *J. Am. Oil. Chem. Soc.*, 58 (1981) 830–837.

Sabet, V.M., Zourab, S.M. and Said, W., Interaction of surface active agents with lecithin at the xylene water interface and its effect on the stability of the resulting emulsion. *Colloids Surfaces*, 4 (1982) 359–366.

Sloviter, H. and Komimoto, T., Erythrocyte substitute for perfusion of brain. *Nature*, 216 (1967) 458–460.

Xiong, R., Zhang, R., Chen, H., Huang, W., Luo, C. and Cao, W., A clinical application of fluorocarbon artificial blood in 10 cases. *Chin. J. Surg.*, 19 (1981) 213–216.