IJP 02134

Long-term stability studies of fluorocarbon oxygen transport emulsions

O.L. Johnson, C. Washington and S.S. Davis

Department of Pharmaceutical Sciences, University of Nottingham, University Park, Nottingham NG7 2RD (U.K.)

(Received 8 February 1990)
(Accepted 10 March 1990)

Key words: Emulsion stability; Fluorocarbon; Perfluorodecalin; Ostwald ripening

Summary

We have studied the stability of a range of oxygen transport emulsions made from perfluorodecalin, stabilised by both a polymeric stabiliser (Pluronic F68) and an electrostatic stabiliser (lecithin) at a range of temperatures. The results demonstrate that the Pluronic-stabilised emulsions are significantly more stable than those stabilised by lecithin. The lecithin-stabilised emulsions showed significant coalescence over a storage period of 120 days. The effects of the stabilising additives soya oil and perfluoroperhydrofluoranthene were also examined; both were found to improve the long-term stability of the Pluronic-stabilised emulsions. Soya oil was essential to stabilise the emulsions containing Pluronic F-68 at the elevated temperatures required for autoclave sterilisation, while perfluoroperhydrofluoranthene did not itself stabilize the emulsions during autoclaving.

Introduction

For several years there has been interest in the production of artificial media to transport oxygen in vivo. The importance of such materials has been increased recently, mainly due to the attention being paid to the problem of infection from blood products. Several candidate oxygen transport systems have been studied, of which some of the most important are those based on perfluorinated molecules, with perfluorodecalin being one of the most widely studied (Clarke et al., 1975). These materials have a considerable capacity to dissolve oxygen, but cannot be injected directly into the circulation due to their immiscibility with water or body fluids. Consequently they are normally formulated as emulsions with a submicron particle size distribution. The emulsions can circulate freely in the body for an extended period before being trapped by the liver and eliminated, usually via the pulmonary route.

Only a small range of surface active materials are acceptable for the production of large volume parenteral emulsions, the most common being lecithin. This naturally derived material has been used for some time to stabilise parenteral feeding emulsions and its properties are reasonably well understood. The initial formulations of perfluorocarbon emulsions described in the literature were stabilised by Pluronic F-68, a block copolymer with a mean molecular weight of 8500 and an HLB number of 29. Early formulations stabilised by this material showed a range of undesirable

Correspondence: C. Washington, Department of Pharmaceutical Sciences, University of Nottingham, University Park, Nottingham NG7 2RD, U.K.

0378-5173/90/$03.50 © 1990 Elsevier Science Publishers B.V. (Biomedical Division)

effects in vivo, particularly transient pulmonary dysfunction due to complement activation (Vercellotti et al., 1982). These effects were initially ascribed to the Pluronic stabiliser, but recent work in our laboratories has suggested that impurities in the surfactants used are likely to be the cause, and that Pluronic F-68 itself is of remarkably low toxicity (Bentley et al., 1989). However, concern with this problem has led to a decline in interest in the use of Pluronics as emulsifiers in large volume parenterals. Our initial experiments (Bentley et al., 1989) have identified ultraviolet-absorbing impurities, reducing activity, and fluorescent impurities. This is consistent with the presence of short-chain aldehydes from the ethylene oxide polymerisation and cresol stabilising agents added to commercial grades of Pluronic. We are currently attempting to isolate and identify these impurities.

The in vivo behaviour of injected disperse systems is highly dependent on their particle or droplet diameter. Particulates larger than approx. 5 μm diameter are trapped in the lungs; any significant deposition could lead to severe clinical consequences. Intravenous emulsions are thus subject to a high degree of quality control and must display an extremely low coalescence rate. This is achieved routinely in the preparation of intravenous fat emulsions, which have a shelf life of 1–2 years; an intravenous fluorocarbon emulsion would need to display a similar stability in order to be commercially viable. The most widely known fluorocarbon emulsion, Fluosol-DA, must be stored deep-frozen in order to prevent large droplet accumulation.

Studies from this laboratory (Davis et al., 1981) have suggested that an important mechanism by which the droplet diameter of perfluorodecalin emulsions can increase is Ostwald ripening. We have proposed the use of a perfluorinated additive, perfluoroperhydrofluoranthene, to reduce the rate of this process significantly. We have also demonstrated that perfluorodecalin emulsions stabilised by Pluronic F-68 become unstable above the polymer cloud point of approx. 110°C and consequently cannot be sterilised by autoclaving (Johnson et al., 1990). The addition of soya oil was found to increase the cloud point of the polymer, and resulted in emulsions which could be autoclaved without major coalescence occurring.

We have studied the long-term stability of a range of 20% perfluorodecalin emulsions, stabilised by either Pluronic F-68 or lecithin, at various storage temperatures. The emulsions were stabilised both by addition of a high boiling point perfluorocarbon or a triglyceride oil. The most stable of the formulations were studied for 350 days.

Materials and Methods

Perfluorodecalin (FDC, 99% pure) and perfluoroperhydrofluoranthene (PPF, 99% pure) were kindly donated by ISC Chemicals, Avonmouth, and were used as received. Pluronic F-68 (PF68; Atochem batch no. G10T5N50) was purified using the method described previously (Bentley et al., 1989). The lecithin used was intravenous grade Lipoid E80, and was kindly donated by Lipoid KG (Ludwigshafen, F.R.G.). Soya oil was purchased from J. Sainsbury PLC.

Seven formulations were prepared. All contained 20% w/v FDC, with emulsifiers and additives as shown in Table 1.

Emulsions were prepared using a laboratory Microfluidizer (model 110T; Microfluidics, Newton, MA) and were processed for 4 cycles at 10000 psi. They were then sealed in glass vaccine bottles and autoclaved ($F_0 = 15$, 121°C). Samples were stored at 4, 25 and 37°C.

The droplet mean diameter was measured by photon correlation spectroscopy (Malvern 7025). Since this technique is not sensitive to the forma-

TABLE 1

Composition of perfluorocarbon emulsions

Formulation no.	% FDC	% PF68	% PPF	% Lecithin	% Soya oil
1	20	4	1		
2	20	4			2
3	20	4	1		2
4	20			4	
5	20			4	2
6	20		1	4	
7	20		1	4	2

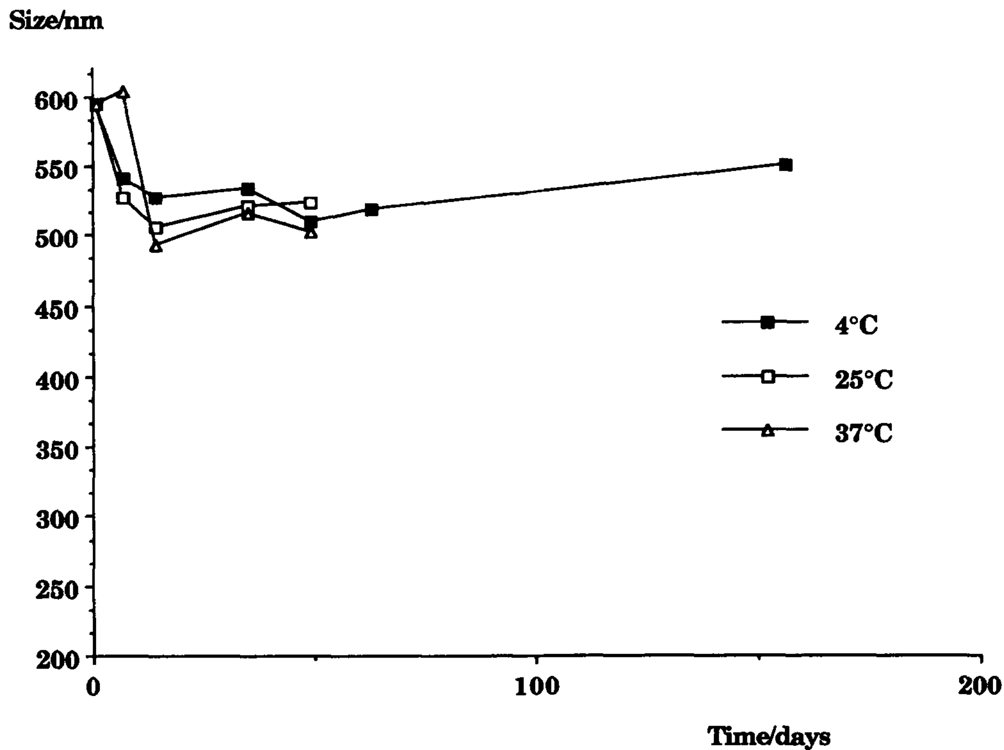

Fig. 1. PCS diameter as a function of time for formulation 1.

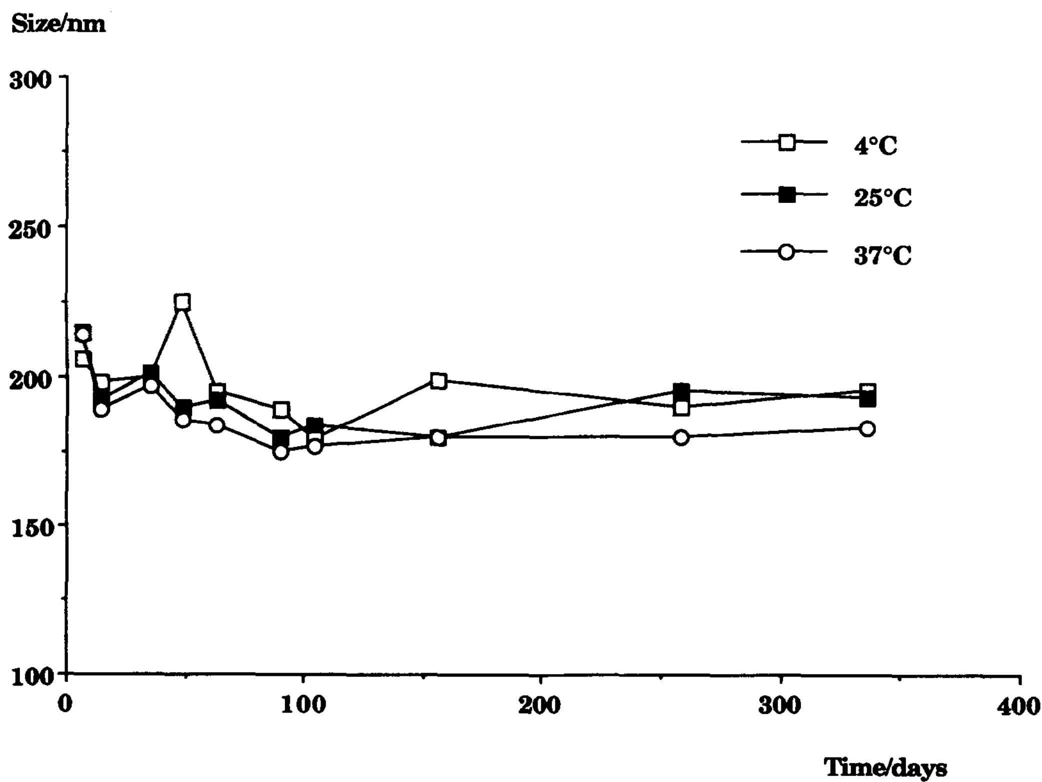

Fig. 2. PCS diameter as a function of time for formulation 2.

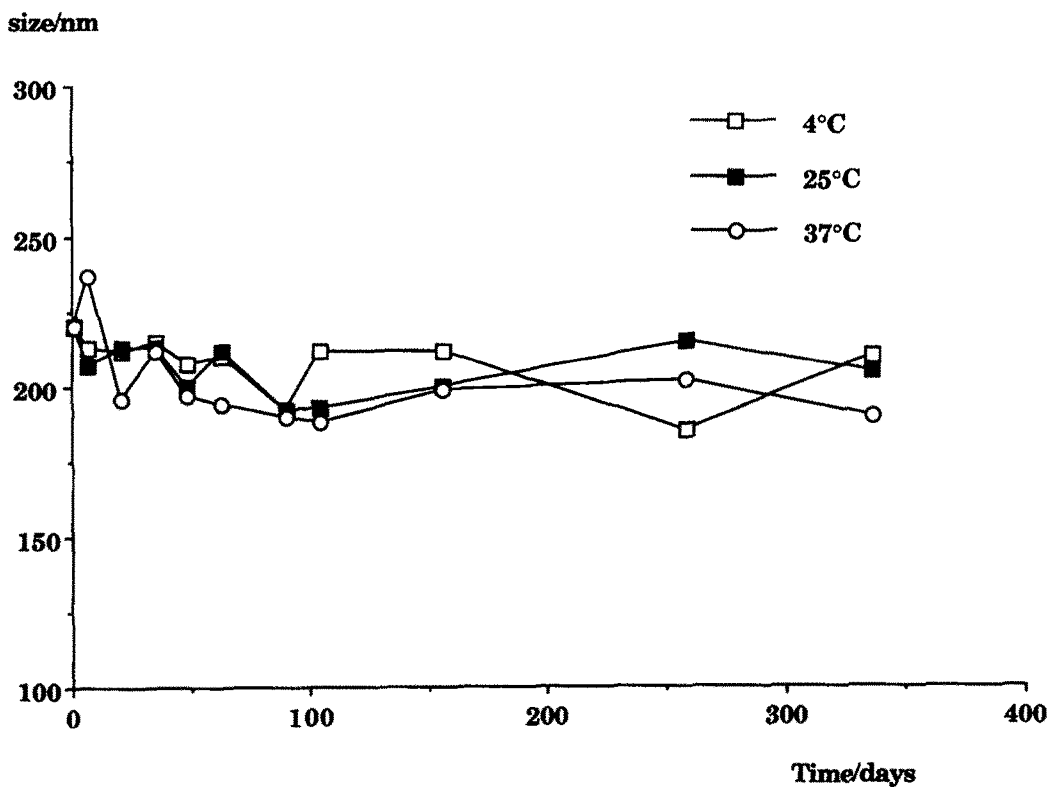

Fig. 3. PCS diameter as a function of time for formulation 3.

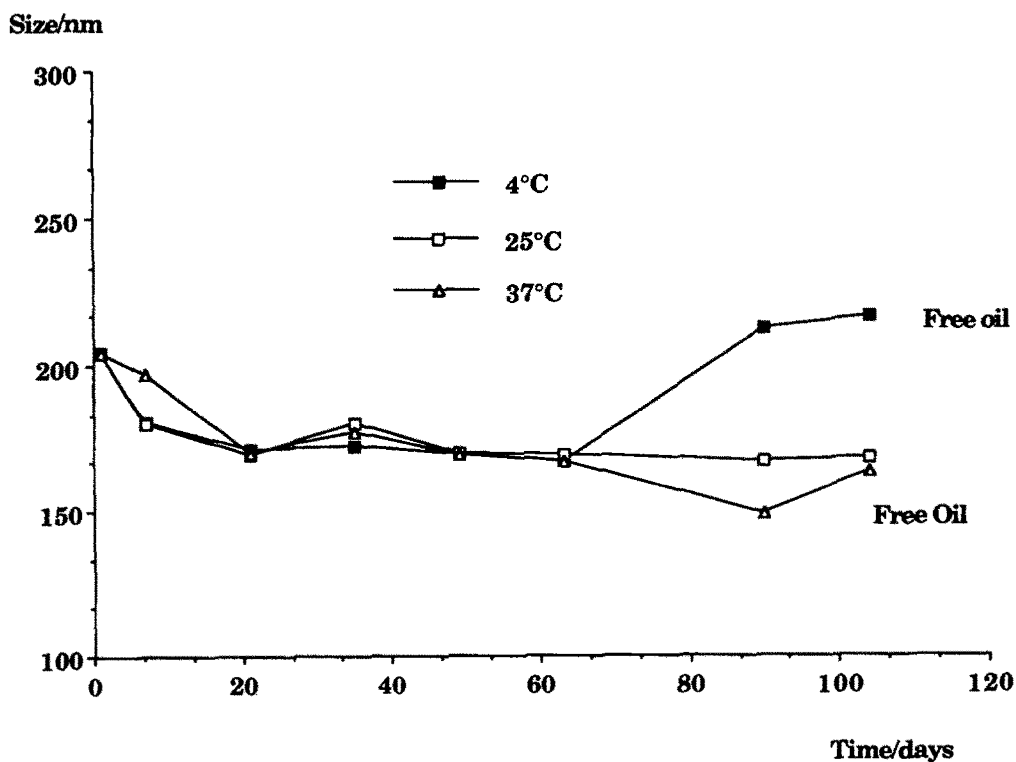

Fig. 4. PCS diameter as a function of time for formulation 4.

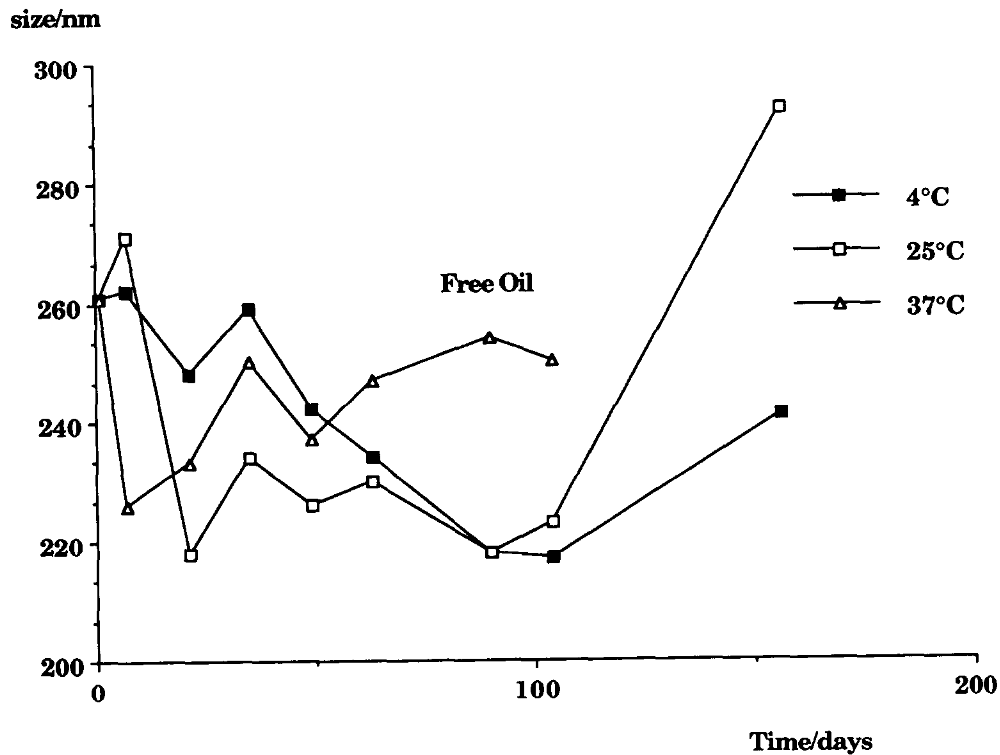

Fig. 5. PCS diameter as a function of time for formulation 5.

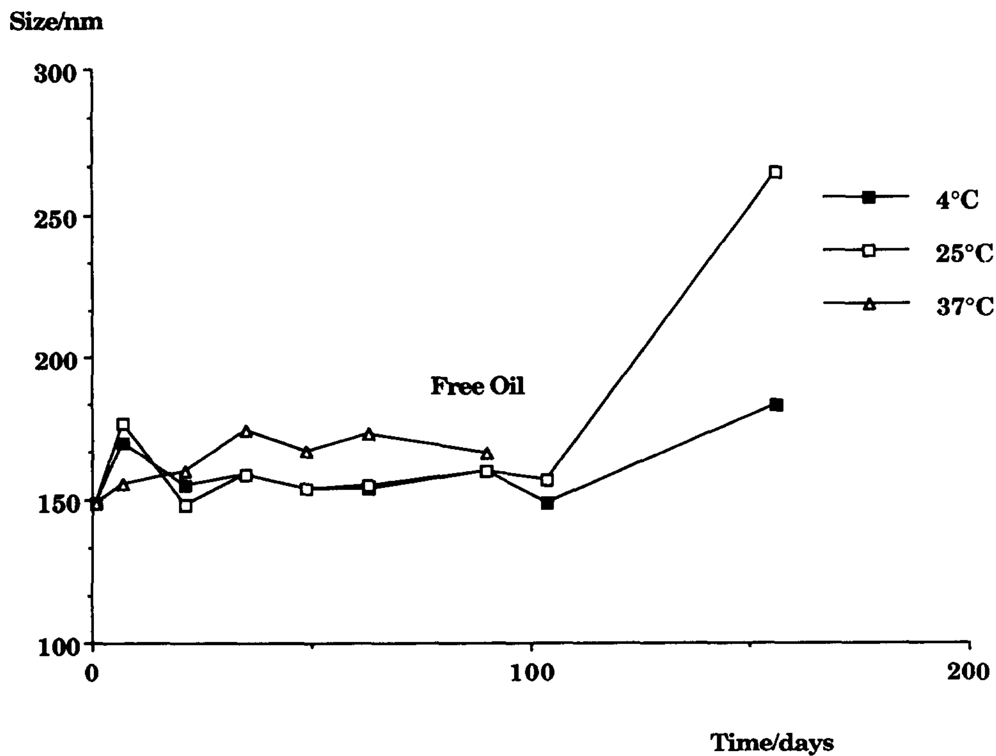

Fig. 6. PCS diameter as a function of time for formulation 6.

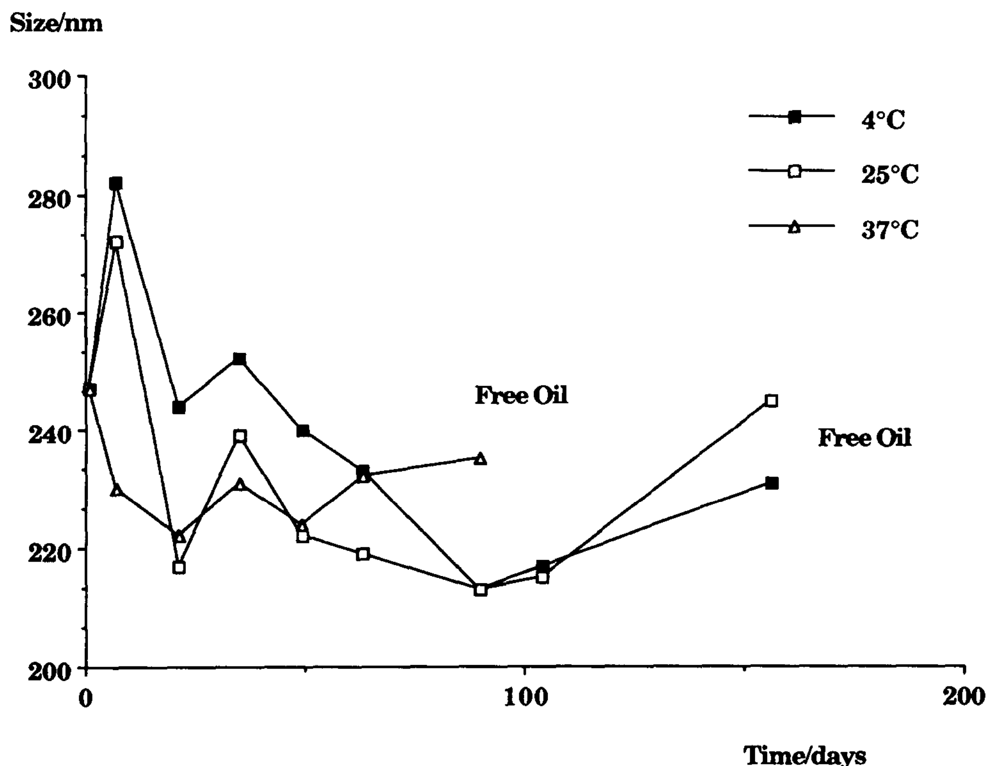

Fig. 7. PCS diameter as a function of time for formulation 7.

tion of very large (> 2 μm) droplets of oil, the emulsions were also observed visually to check for free oil separation. The extremely close refractive index matching between the perfluorodecalin and water precluded the use of laser diffraction to study the droplet size distribution in the 1–100 μm range. Zeta potentials of the formulations were measured with a Malvern Zetasizer 2 in freshly distilled water (pH 6.0).

Results

The PCS mean diameter of formulations 1–7 is shown in Figs 1–7, respectively, as a function of time and storage temperature. Formulation 1 was unstable to autoclaving and showed a large consequent increase in droplet diameter. Formulations 2 and 3 retained their initial diameter of 200 ± 10 nm throughout the duration of the experiment at all storage temperatures. These formulations slowly sedimented their fluorocarbon droplets over a period of several days to form a gelatinous layer which was, however, easily redispersible without measurable increase in droplet diameter. Formulation 4 retained its PCS diameter but showed free oil at 25 and 37°C after 100 days storage. Formulations 5–7 showed increased PCS diameter after

TABLE 2

Zeta potentials of 20% perfluorodecalin emulsions

Formulation no.	Zeta potential (mV)
1	−1.5
2	−4.8
3	−4.5
4	−24.0
5	−19.7
6	−24.7
7	−22.1

For comparison: zeta potential of Intralipid 20%: −45 mV. All zeta potentials ±2 mV S.D.

100 days at 4 and 25°C, and free oil was visible after 100 days in all three formulations at 37°C. The two lecithin-stabilised formulations which contained soya oil (formulations 5 and 7) had a significantly larger droplet diameter than those which did not (formulations 4 and 6).

All the formulations stabilised by Pluronic F-68 were colourless; formulation 1 was nearly transparent but became significantly more cloudy after autoclaving. Formulations 2 and 3, containing soya oil, were more turbid. All the formulations stabilised by lecithin were pale cream on manufacture, but darkened with age, the greatest darkening being seen in the emulsions stored at 37°C.

The zeta potentials of the emulsions are listed in Table 2. All the pluronic-stabilized emulsions had a zeta potential below −5 mV, whereas the lecithin-stabilized systems had zeta potentials in the range −20 to −25 mV.

Discussion

Formulation 1 is the only Pluronic F-68-stabilised system which did not contain soya oil. As we have shown recently (Johnson et al., 1990), Pluronic F-68-stabilised emulsions are unstable to autoclave sterilisation due to the fact that the cloud point of the polymer solution is 110°C, below the normal autoclave temperature of 121°C. The addition of 2% soya oil raised the cloud point above the autoclave temperature to 128°C and thus allowed the emulsion to be autoclaved without major coalescence. Formulation 1 was not so stabilized and its droplet diameter increased to approx. 600 nm on autoclaving. The increased droplet diameter in the system caused a fraction of the perfluorocarbon oil to form a white sediment.

Formulations 2 and 3, containing soya oil with or without PPF, were stable for the duration of the experiment. We have previously described the use of high-boiling perfluorinated hydrocarbons as inhibitors of Ostwald ripening (Davis et al., 1981) and demonstrated that they improve the stability of perfluorodecalin emulsions. The method by which soya oil improves the storage stability is unclear since it is not significantly miscible with perfluorocarbons; we suggest that a fraction of the soya oil is present in the interfacial film between the aqueous and fluorocarbon phases. The partition of soya oil between the droplet interface, the polymer micelles, and the bulk phase (excess droplets of soya oil) has not yet been measured. Our preliminary experiments (with fluorescently labelled soya oils) indicate that the majority of the soya oil is present as stabilized droplets, not unlike those present in a parenteral nutrition emulsion such as Intralipid. A small amount of soya oil is also present at the fluorocarbon-water interface.

All of the emulsions stabilized by lecithin showed an increase in droplet diameter with increasing storage time. Even the emulsions stored at 4°C showed noticeable sedimentation of fluorocarbon droplets after 100 days, visible as a coarse white sediment which resettled rapidly after agitation. This is in contrast to the Pluronic formulations 2 and 3, which only slowly sedimented fluorocarbon droplets. The high density of perfluorodecalin (1.9 g/cm^3) caused it to sediment even in stable formulations; interestingly, in the PF68-stabilized formulations, the droplets sedimented as a clear gel which could be redispersed with vigorous shaking, without apparent increase in droplet diameter while sedimented.

The lecithin-stabilized formulations were, not surprisingly, stable to autoclaving at 121°C. Lecithin-stabilised fat emulsions are also terminally sterilised at elevated temperatures without significant coalescence. The lecithin used in the present work was an unsaturated material similar to that used in the preparation of parenteral fat emulsions, and did not undergo a significant phase transition on heating, since its characteristic transition temperature was below 0°C. However, none of the lecithin-stabilized formulations were sufficiently stable to provide an extended shelf life.

The zeta potentials of the emulsions stabilized by Pluronic F-68 were low, since this material confers stability by steric means rather than by electrostatic repulsion. Addition of soya oil increased the zeta potential by approx. 3 mV, probably due to the presence of charged impurities such as fatty acids or soya lecithin residues. The zeta potentials of the emulsions stabilised by lecithin were considerably higher. However, it

would appear that they are not sufficiently high to produce a formulation with extended stability.

Parenteral grades of lecithin normally lead to surface potentials of −40 to −45 mV when used in the emulsification of vegetable triglycerides for intravenous feeding; these emulsions have a shelf life of several years. The zeta potential is primarily due to the acidic lipids phosphatidylserine and phosphatidylglycerol which are minor (2–5%) components. The majority of the emulsifier consists of uncharged phospholipids (phosphatidylcholine and phosphatidylethanolamine, which probably contribute to the structural rigidity of the interfacial film. We suggest that the zeta potential is anomalously low in the fluorocarbon emulsions studied here, since the lecithin only adsorbs weakly to the perfluorocarbon. The surface charge density is therefore low which leads to a low interfacial potential. Trial formulation experiments (Davis et al., unpublished work) have demonstrated that 4% lecithin is necessary to produce the emulsion stability observed in these experiments; this is in marked contrast to parenteral fat emulsions, which only normally contain 1.2% lecithin and display superior stability. Both emulsions have similar droplet diameters (200–300 nm) and so the variations in emulsifier requirement probably reflect variation in adsorption strength to the droplet surface.

It may be possible to increase the zeta potential of the lecithin-stabilized systems by using a lecithin with a higher level of charged impurities. Additionally, a small amount (< 0.1%) of oleic acid is often added to parenteral feeding emulsions to improve their stability, although data concerning the real effect of this additive is sparse. However it would appear that at present, Pluronic F-68 could still be the emulsifier of choice for the production of perfluorodecalin emulsions for clinical use.

Conclusions

Simple 20% perfluorodecalin formulations stabilized by Pluronic F-68 require the addition of a high boiling fluorocarbon oil to stabilize against Ostwald ripening, and a triglyceride oil to raise the cloud point above the autoclave temperature. The emulsions stabilized by lecithin are less stable than those emulsified with Pluronic F68, possibly due to their relatively low zeta potential. Experiments are in progress to stabilize these systems by increasing the zeta potential.

Acknowledgements

The authors would like to thank Rhône-Poulenc Chemicals, ISC Division, Avonmouth, for financial support.

References

Bentley, P.K., Davis, S.S., Johnson, O.L., Lowe, K.C. and Washington, C., Purification of Pluronic F-68 for perfluorochemical emulsification. *J. Pharm. Pharmacol.*, 41 (1989) 661–663.

Clarke, L. Jr, Wesseles, E.P., Kaplan, S., Miller, M.L., Becker, C., Emery, C., Stanley, L., Becattini, F. and Obrock, V., Emulsions of perfluorinated solvents for intravascular gas transport. *Fed. Proc.*, 34 (1975) 1468–1477.

Davis, S.S., Round, H.P and Purewal, T.S., Ostwald ripening and the stability of emulsion systems: an explanation for the effect of an added third component. *J. Colloid Interface Sci.*, 80 (1981) 508–511.

Johnson, O.L., Washington, C. and Davis, S.S., The thermal stability of fluorocarbon emulsions that transport oxygen. *Int. J. Pharm.*, 59 (1990) 131–135.

Vercellotti, G.M., Hammerschmidt, D.E., Craddock, P.R. and Jacob, H.S., Activation of plasma complement by perfluorocarbon artificial blood: Probable mechanism of adverse reactions in treated patients and rationale for corticosteroid prophylaxis. *Blood*, 59 (1982) 1299–1304.