International Journal of Pharmaceutics, 59 (1990) 131–135
Elsevier

IJP 02002

Thermal stability of fluorocarbon emulsions that transport oxygen

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(Received 25 July 1989)
(Modified version received 7 September 1989)
(Accepted 21 September 1989)

Key words: Oxygen transport; Poloxamer; Cloud point; Emulsion stability; Fluorocarbon

Summary

Pluronic F-68 (Poloxamer 188) is a non-ionic block co-polymer which has been used to emulsify perfluorocarbons (PFCs) for use as oxygen transport fluids. The surfactant has been shown to be an effective stabiliser of perfluorodecalin emulsions.

Autoclaving is the most practical method of sterilising parenteral formulations, but PF-68 stabilized perfluorodecalin emulsions break down rapidly on autoclaving which prevents this technique being used. Presented here are results which suggest that the observed breakdown of the emulsions on autoclaving is related to the cloud point of the surfactant. This is further underlined by the effect of additives which elevate or depress the surfactant cloud point and change the stability of the corresponding emulsions. We have used such additives to prepare PF-68 stabilized perfluorodecalin emulsions which are stable to sterilization by autoclaving.

Introduction

Perfluorocarbons are totally fluorinated molecules, usually liquid at room temperature, which possess the ability to dissolve large amounts of the respiratory gases oxygen, carbon dioxide and nitrogen. They are also chemically inert because of the strength of the carbon–fluorine bonds. The combination of these two properties makes them useful candidates for biological oxygen transport media. Perfluorodecalin (FDC) belongs to this class of compounds, and emulsions of this material have been widely studied (e.g., see Clarke et al., 1975).

As liquid perfluorocarbons are immiscible with blood and other physiological fluids, they have to be administered as oil-in-water emulsions. An emulsion consists of two immiscible liquids, one dispersed in the other in form of droplets. It is a thermodynamically unstable system, and the tendency is for the water/oil interfacial area to decrease, thereby reducing the surface free energy of the system. This is achieved by several processes, including droplet coalescence or a diffusional process known as Ostwald ripening (Davis and Smith, 1976; Davis et al., 1981), both of which lead to the production of larger droplets of oil in the system, with a consequent risk of embolism on administration.

Pluronic F-68 (Poloxamer 188) is a non-ionic surfactant with an HLB value of 29, having the structure:

$$H\text{-}(O\text{-}CH_2\text{-}CH_2)_A\text{-}(O\text{-}CH(CH_3)\text{-}CH_2)_B\text{-}(O\text{-}CH_2\text{-}CH_2)_A\text{-}OH$$

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It has been used as an emulsifier in the formulation of commercial FDC emulsions such as Fluosol-DA (Green Cross Corp., Japan). Pluronic F-68 effectively reduces droplet coalescence and the addition of a high boiling point perfluorocarbon oil greatly reduces the residual emulsion instability due to Ostwald ripening (Sharma et al., 1986). It has been shown that the toxicity of the emulsion is directly related to the emulsion droplet diameter (Kazumasa et al., 1975); above 300 nm, the toxicity of the emulsion increases markedly. It is therefore vital to ensure that destabilisation of the emulsion is prevented or controlled as far as possible if the emulsion is destined for intravenous use.

An emulsion destined for physiological use must undergo sterilisation. The two most convenient options are autoclaving or filtration. Filtration through a membrane filter of 0.22 μm mesh is sufficient to remove pathogens; this is, however, not a practical method of sterilizing perfluorocarbon emulsions since the emulsion droplets, of diameter 200–300 nm, rapidly clog the pores of the filter. Filtration is also likely to alter the droplet distribution which may have an adverse effect on emulsion stability. The method of choice is therefore autoclaving at 121°C for 15 min. However, FDC emulsions stabilized by Pluronic F-68 coalesce during thermal sterilisation which prevents this approach being used.

In order to study the thermal degradation of FDC emulsions, we have measured their stability at temperatures up to 130°C. Since Pluronic polymers have a cloud point near 110°C, it seemed likely that this was implicated in the lack of stability to autoclaving. Consequently, we measured the cloud point of the Pluronic F-68 solution in the presence of fluorocarbons and additives such as soya oil and electrolytes, which are known to alter cloud point (Maclay, 1956). The effects of these additives on the stability of the emulsion have also been investigated. The results demonstrate that the emulsion stability decreases rapidly above the surfactant cloud point, and that a system stable to autoclaving can be produced by adding a material which raises the cloud point to above 121°C.

Materials and Methods

Perfluorodecalin (FDC) and perfluoroperhydrofluoranthene (FHF) were gifts from ISC Chemicals (Bristol); Pluronic F-68 was obtained from Atochem (France); soya oil was purchased from J. Sainsbury PLC; sodium chloride was GPR from BDH (Poole).

The cloud points of the following Pluronic F-68 solutions and mixtures were measured:

4% w/v PF68
4% w/v PF68, 20% FDC
4% w/v PF68, 20% FDC, 1% FHF
4% w/v PF68, 20% FDC, 2% soya oil
4% w/v PF68, 20% FDC, 1% FHF, 2% soya oil
4% w/v PF68, 4% NaCl.

In all cases the immiscible components were premixed to a coarse dispersion immediately prior to measurement. Emulsification of these systems resulted in turbid mixtures in which the cloud point could not be visually observed.

Emulsion formulations studied were (all % w/v, remainder distilled water):

20% FDC, 4% PF-68
20% FDC, 4% PF-68, 1% FHF
20% FDC, 4% PF-68, 1% FHF, 2% soya oil
20% FDC, 4% PF-68, 1% FHF, 4% NaCl.

Also, the dependence of the stability of FDC emulsion (4% PF68 emulsifier) on soya oil concentration in the range 0–5% was measured.

The fluorocarbon components were mixed and emulsified with the aqueous phase using a model 110 Microfluidiser (Microfluidics Inc; Newton, MA; 4 cycles at 40°C and 9000 p.s.i. pressure). NaCl was dissolved in the aqueous phase after emulsification. Soya oil was immiscible with both phases and so was added to the emulsion premixture immediately prior to emulsification.

Determination of polymer solution cloud point

The surfactant solutions, with or without additives, were placed in thick-walled glass tubes and flame-sealed. The tubes were then immersed in a liquid paraffin bath and heated with a stirrer hotplate. The temperature of the oil was measured with an immersed mercury-in-glass thermometer, and was increased at approx. 2°C per min to ensure that the temperature of the contents of the tube was close to that of the bath. On heating, the temperature at which clouding of the mixture began was noted, and that at which clouding disappeared on cooling was also recorded. A difference of about 2°C was observed between the two, which was presumed to be due to the temperature lag between bath and sample. The cloud point was taken as the mean value of both temperatures.

Determination of emulsion stability

The emulsion formulations were heated in a bench autoclave to temperatures from 50 to 130°C for 15 min. The emulsion droplet diameters were then determined by photon correlation spectroscopy (Malvern K7025 correlator, CBM 3032 computer, Siemens 40 mW He-Ne laser). The stability of the emulsions was expressed as the stability index D_t/D_0, where D_t is the mean diameter after 15 min at temperature t and D_0 is the initial mean diameter.

Results

The cloud points of the surfactant mixtures are listed in Table 1. The cloud point of Pluronic F-68 was raised from 115 to 128°C by 1% soya oil, depressed to 97°C by NaCl (4% = 0.68 M) and, within experimental error, remained unchanged by perfluorodecalin and perfluoroperhydrofluoranthene.

Fig. 1 shows the stability of FDC emulsion to a 15 min autoclave cycle at 121°C, and the con-

TABLE 1

Cloud point of Pluronic F68 solution in the presence of fluorocarbons, soya oil and electrolyte

Mixture composition	Cloud point (°C) [a]
4% w/v PF68	115
4% w/v PF68, 20% FDC	115
4% w/v PF68, 20% FDC, 1% FHF	115
4% w/v PF68, 20% FDC, 2% soya oil	128
4% w/v PF68, 20% FDC, 1% FHF, 2% soya oil	128
4% w/v PF68, 4% NaCl	97

[a] All temperatures ±1°C.

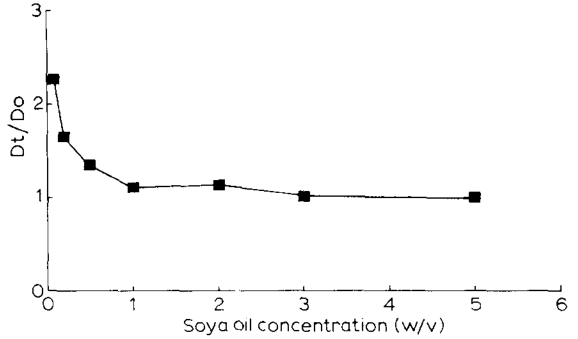

Fig. 1. Particle size growth of FDC emulsion during a standard autoclave cycle (15 min at 121°C) as a function of added soya oil concentration.

centration dependence of the stabilization by soya oil. A soya oil concentration of 2% w/v was sufficient to prevent measurable droplet growth during the autoclave cycle. This soya oil concentration was found to elevate the cloud point of the surfactant to 128°C (Table 1).

Fig. 2 depicts the thermal stability of the perfluorocarbon emulsion systems studied. The addition of 2% w/v soya oil elevated the cloud point sufficiently to enable sterilisation of the emulsion without significant emulsion destabilisation. Conversely, 0.68 M NaCl in the emulsion made the emulsion unstable above 100°C. The slope of the curve shows that there was a rapid rise in the

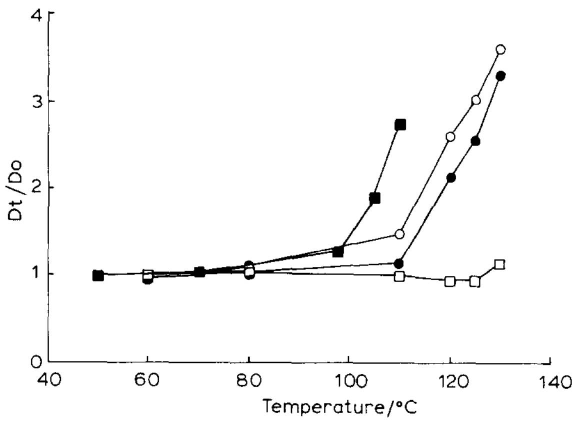

Fig. 2. Particle size growth of FDC emulsion formulations as a function of temperature (15 min autoclave cycle). (●) 20% FDC, 4% PF-68, (○) 20% FDC, 4% PF-68, 1% FHF, (□) 20% FDC, 4% PF-68, 1% FHF, 2% soya oil, (■) 20% FDC, 4% PF-68, 1% FHF, 4% NaCl.

droplet size of the emulsion on the attainment of a threshold temperature.

Discussion

The cloud point of non-ionic surfactants is the temperature at which the surfactant 'precipitates' out of the solvent medium. The surfactant molecule is kept in solution below the cloud point by hydrogen bonding between the hydrophilic moiety (polyoxyethylene in this case) and water. Hydrogen bonding is temperature sensitive, decreasing as temperature increases. At the cloud point, no affinity exists between the solvent and the surfactant and a further increase in temperature results in the separation of an aqueous phase and a surfactant phase. The cloud point remains constant over a wide range of surfactant concentrations (Schott and Han, 1977).

The cloud point determination carried out on mixtures of Pluronic F68 and various additives showed the ability of some of them to elevate or to depress the cloud point of the surfactant. The addition of soya oil causes an elevation of the cloud point. Conversely, NaCl depresses the cloud point. Maclay (1956) recorded the effects of the hydrophilic and hydrophobic nature of additives on polymer cloud point. The effect of electrolytes has also been extensively investigated (Schott and Han, 1977; Schott and Royce, 1984). Ionic additives can be divided into two groups: salting-in and salting-out electrolytes, which respectively elevate and depress the cloud points of non-ionic surfactants. NaCl belongs to the salting-out group of electrolytes, and is thought to exert its effect by polymer dehydration, i.e. the ions compete with the hydrophilic portion of the surfactant for water of hydration, leading to its precipitation.

The nature of the interaction between soya oil and Pluronic F68 is less well understood, but it is possible that the oil is incorporated into the centre of the surfactant micelle, stabilizing it at higher temperatures. The major barrier to understanding in this area is that, despite much study (e.g., see Schmolka, 1975; Attwood et al., 1984), the structure of Pluronic solutions is still not completely understood.

It is noteworthy that perfluorodecalin and perfluoroperhydrofluoranthene did not measurably influence the polymer cloud point. This may be an indication of the lack of interaction between the surfactant and the perfluorocarbon. The low affinity of Pluronic F-68 for the perfluorocarbons suggests that it is a poor stabilizer of the emulsions: this is supported by our observations (Johnson et al., in preparation) that the majority of the 4% surfactant used in a typical perfluorodecalin formulation is present in solution and little is adsorbed to the oil droplets. This large amount of free surfactant appears to be necessary in order to provide the driving force for the weakly adsorbed surfactant layer on the oil droplet. Alternatively, it is possible that exclusion stabilization by the polymer network is responsible for preventing droplet coalescence.

There is a strong correlation between the temperature at which the emulsions begin to coalesce and the cloud point of the corresponding surfactant mixtures. The threshold temperature for destabilization of the PFC emulsion was increased from 115 to 128°C by the addition of 2% soya oil, thus producing a formulation which is both stable to autoclave sterilization and composed of biologically acceptable materials. At present, it is not clear whether the soya oil is associated with the perfluorocarbon droplets as a 'secondary surfactant' or present as a separate emulsion phase. Further research is required to clarify the microscopic nature of this system.

Conclusions

There is a strong correlation between the cloud point of the surfactant and the temperature at which the perfluorodecalin emulsion begins to coalesce. Addition of soya oil elevates the surfactant cloud point sufficiently to enable effective thermal sterilisation while maintaining the desired physicochemical properties of the emulsion.

Acknowledgement

The authors would like to thank ISC Chemicals Ltd, Bristol, U.K., for financial support.

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