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EMULSIFICATION METHODS FOR PERFLUOROCHEMICALS

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A comparison of emulsification methods for novel perfluorochemical (PFC) emulsions for biological uses related to oxygen transport has been made. The emulsions were based on perfluorodecalin (FDC) and contained small quantities of polycyclic, perfluorinated, higher boiling point oil (HBPO) additives to stabilise against the process of molecular diffusion known as Ostwald ripening. Emulsification methods studied include sonication and homogenization which produced emulsions having similar particle size distributions. However, sonication generated fluoride (F^-) ions which may have adverse physiological effects.

INTRODUCTION

There is growing interest in the potential value of emulsified PFCs as physiological oxygen-transport fluids.[1,2] A commercial preparation, Fluosol-DA 20% (F-DA; Green Cross, Japan), has already been tested in clinical trials in several countries.[3-6] More recent attention has focussed on the development and assessment of novel compositions of emulsified PFCs having improved stability characteristics.[7,8] These novel preparations were based on FDC and contained small quantities of HBPO additives to stabilize against the process of molecular diffusion known as Ostwald ripening. PFC emulsions are normally prepared either by sonication or, in the case of F-DA, by high-pressure

homogenization using a Manton-Gaulin apparatus.[9,10] However, sonication can be associated with PFC degradation and release of potentially toxic F- ions.[10] The present work was therefore undertaken to examine and ccmpare different emulsification methods for preparation of novel PFC emulsions. Assessment of particle size and polydispersity together with F- generation has been made.

MATERIALS AND METHODS

FDC (ISC Chemicals Ltd., Avonmouth) was emulsified with 1-4% Pluronic F-68 (ICI Ltd., Macclesfield) in an aqueous phase using one of the following methods: (1) sonication (Dawe Automatic 753A Soniprobe; Dawe Instruments Ltd., London); (2) homogenization (Manton-Gaulin 15M-8TA homogenizer; APV Gaulin, Boston, U.S.A.); and (3) homogenization (M-110 Microfluidizer; Microfluidics Corp., Newton, U.S.A.). Emulsions were prepared as 10%, 20% or 25% (w/v) preparations and additionally contained up to 2% of a C-16 HBPO additive, perfluoroperhydrofluoranthrene (ISC Chemicals Ltd., Avonmouth), to enhance stability; control emulsions contained no HBPO.[7,8] Mean emulsion particle size and distribution (polydispersity) were assessed on preparation and during subsequent storage using Photon Correlation Spectroscopy (PCS; Malvern Instruments Ltd., Malvern); emulsions were stored at 4°C or 37°C.

F- ions liberated during emulsification were measured using an ion selective electrode (Orion Research Inc., Boston, U.S.A.). Test emulsions were also subjected to ion-exchange and resin dialysis (IED) and their F- content re-measured.[11]

RESULTS

Selected data on mean particle size and polydispersity for emulsions containing 20% (w/v) FDC with 4% Pluronic and 1% of the C-16 HBPO additive, prepared by the different emulsification methods under optimum conditions, are given below in Tables I and II.

TABLE I

Particle Size Analysis of Emulsions Prepared by Sonication

Emulsifier concentration (w/v)	Sonication time (min)	Mean particle size (nm)	Poly-dispersity
4.0	30	257	0.031

TABLE II

Particle Size Analysis of Emulsions Prepared Using Either The Manton-Gaulin Homogenizer or Microfluidizer

Emulsifier concentration (w/v)	Pressure (psi)	No. cycles (vol/s)	Mean particle size (nm)	Poly-dispersity
a) Manton-Gaulin homogenizer				
4.0	7500	5	277	0.063
		10	270	0.058
		15	294	0.094
	9000	5	278	0.071
		10	284	0.081
		15	294	0.078
b) Microfluidizer				
4.0	9000	5	305	0.090
		10	277	0.050
		15	270	0.070

Particle size analysis revealed that there were only minor differences in emulsion characteristics using the three emulsification methods. However, in the case of emulsions prepared using either the Manton-Gaulin homogenizer or Microfluidizer, a pre-requisite was a pre-mix of emulsion constituents; the most effective technique was the HSl Hydroshear (APV Schroder, Luebeck, The Netherlands) for ca. 1 min. (2 cycles).

An additional finding was that in emulsions prepared using the Dawe Soniprobe, F^- contaminants were generated within the emulsion: F^- liberation was greater as the sonication time was increased from 10-30 mins and the maximum F^- concentration measured was 53 ppm. Increasing emulsifier concentration from 1-4% also enhanced F^- generation during sonication. F^- could be reduced to <2 ppm by subsequent IED and this was similar to the F^- content of emulsions prepared by either the Manton-Gaulin homogenizer or Microfluidizer, irrespective of emulsion composition.

CONCLUSIONS

These results show that, under optimum conditions, all three methods tested are suitable for emulsification of PFCs. On the basis of particle size analysis alone, no significant differences were observed between the methods. However, while sonication has the advantages of being convenient and requiring only small sample volumes, a major disadvantage is the liberation of potentially toxic F^- ions. F^- liberation was greater as sonication time was increased, reflecting greater total energy input. The additional finding that F^- liberation increased with emulsifier concentration is best explained as an increased surface area effect. Although the F^- content of sonicated emulsions could be readily reduced to <2 ppm by IED, we are nevertheless aware that other contaminants, formed as a result of fission of the C-F bond, would remain and these may be potentially toxic in biological systems.[12]

The problem of F- liberation was not encountered when either the Manton-Gaulin or Microfluidizer homogenization techniques were used and therefore, emulsions prepared using these methods are more acceptable for potential physiological uses. Moreover, emulsification time is shorter using these techniques. It is noteworthy, however, that the principal disadvantages of these homogenization methods are: (1) the requirement for adequate pre-mixing; (2) large sample volumes (ca. 500 ml minimum) for the Manton-Gaulin apparatus; and (3) a "dead-space" effect which may result in reduced emulsion yield.

We therefore conclude that homogenization methods are preferable for emulsification of PFCs for biological uses.

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