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FLUOROCARBONS ENCAPSULATED IN PHOSPHOLIPID VESICLES

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Dedicated to Prof. Dr. A. Haas in view of his 60th birthday.

SUMMARY

Conventional fluorocarbon emulsions are compared to phospholipid vesicles encapsulating fluorocarbons (PFC's) in cages. Such spherical aggregates behave differently from emulsion droplets. A few outstanding properties such as stability and viscosity are discussed in terms of vesicle structure. Nmr data of such PFC-dispersions are reported.

INTRODUCTION

Fluorocarbon-based gas carrying emulsions have attracted much interest in medicine and biology [1]. In the course of our research programme we produced a first generation emulsion consisting of perfluorodecalin (PFD) and F-DBMA (dibutyl-methyl-amine) stabilized with home-made EO-PO-block copolymer emulsifier in 1986. The subsequent goal was the development of an improved emulsion with long term stability at room temperature with increased fluorocarbon content of 30 % w/v and reduced emulsifier of only 2.5 % [2].

Block copolymers have poor stabilizing properties but a reasonable toxicity, which was found to be dependent on hydrophile-lipophile balance number (HLB) [3]. On the contrary, fluorosurfactants exhibit superior emulsion stability, but they are not biocompatible [4]. Consequently, we passed over to phospholipids as biologically more acceptable materials. At that time little work had been done with lecithins, and there were no

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reports of stability parameters and the principal 'state of emulsion' [5]. Fung and co-workers, who have investigated interactions between PFC's and phosphatidyl choline vesicles did not get concentrated fluorocarbon filled liposomes, because of using the unsatisfactory REV-method [6].

EXPERIMENTAL

Egg-yolk as well as soy-bean lecithins were purified by aceton and subsequent ethanol extraction. The amounts of constituent phospholipids were determined by ^{31}P-nmr and were found to be comparable with a commercial grade lecithin (Fluka) consisting of 30 % phosphatidylcholine, 30 % phosphatidylethanolamine and phosphatidylinositol, used as well.

Phospholipids (PL) were presonicated to form aqueous vesicles. Likewise the method of depositing the PL from chloroform solution on a round bottom flask by rotary evaporation and dispersing the dried lipid film with water was practised. The obtained multilamellar aqueous vesicles (MLV) together with PFC's were dispersed by sonication with energy densities up to 300 W/cm^2 and by high pressure homogenization at 500 atm, respectively.

As gas carriers highly purified fluorocarbons e.g. perfluorodecalin (PFD), perfluorotributylamine (TBA), perfluorooctylbromide (PFOB) and newly developed N or/and O containing bicycles such as F-cyclohexylmethyl morpholine (CMM) and others were used. The encapsulated fluorocarbons were in the concentration range of 10 to 100 % w/v. Particle size and size distribution analyses were performed by means of the laser light PCS-spectrometer 'Coulter N-4MD' (Coultronics). Viscosities were measured with a capillary viscosimeter according to Ubbelohde.

All nmr measurements were carried out with a Varian XL-300 spectrometer at 282 MHz (^{19}F) and 121 MHz (^{31}P), respectively. About 0.01 % ethylmercury thiosalicyclic acid·Na-salt was added to the dispersion samples to prevent growth of microorganisms.

RESULTS AND DISCUSSION

Colloid and emulsion science have taught us to differentiate sharply between amphiphiles at interfaces (emulsion state), microemulsions (solubilized extended micelles) and hydrophobic bilayer lipid membrane structures. The latter self-organize to three dimensional aggregates to minimize their free and entropic energy. These vesicles (liposomes) are thermodynamically stable spheres able to carry liquids e.g. fluorocarbons in their inner cage. Unlike aqueous lecithin vesicles the fatty acid tails of fluorocarbon filled vesicles are oriented to the oil phase, because of reverse polarity. At any rate excess phospholipids build up bi- and/or multilamellar structures [7], which were confirmed by shape and line width of their ^{31}P-nmr signals [8].

Whereas emulsions are determined by interaction forces of emulsifier and fluorocarbon at the interface, vesicles are not (or almost not!) influenced this way in their stability.

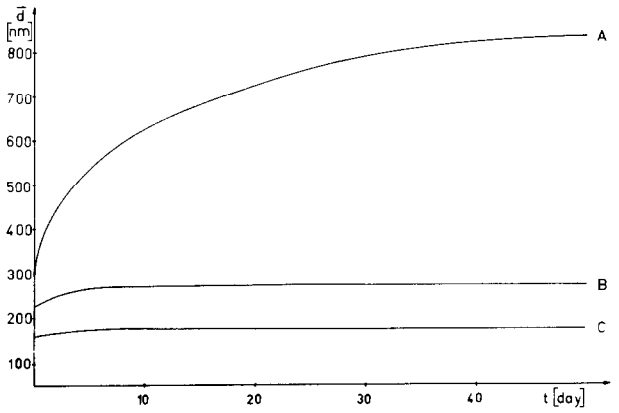

Fig. 1. Aging of fluorocarbon emulsions and filled vesicles stabilized with: A EO-PO-block copolymer, B lecithin, C F-amido-aminoxid-fluorosurfactant.

Figure 1 examplifies the discussion by representing three typical stabilizers for 50 % w/v aqueous PFD systems. Curve A shows the well known aging of a EO-PO-block copolymer stabilized fluorocarbon emulsion. Strong changes in particle diameter occur

immediately after preparation obviously due to coalescence as well as the Ostwald ripening process. Compositions represented by curves B and C do not suffer coalescence. The fluorosurfactant in C makes the most stable emulsion without aging at room temperature [4]. Curve B strikingly demonstrates that the particle size remains the same, within experimental error, in the case of PFD filled PL-vesicles.

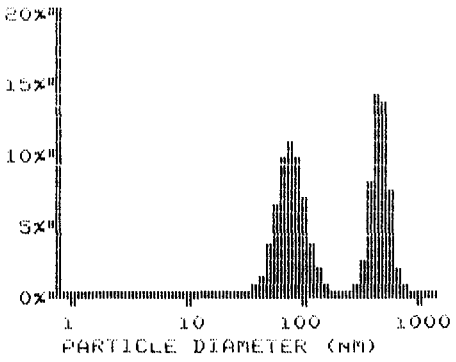

Fig. 2. Particle diameter of a 100 % PFD-lecithin dispersion obtained from a size distribution processing (SDP)-analyses.

The sample represented in Fig. 2 has a typical bimodal weight distribution with maxima at 76 nm (53 %) and 450 nm (47 %). The overall mean particle diameter results to 253 nm. The PFD-dispersion consists of small as well as large uni- and multilamellar vesicles, which can be concentrated or separated by high speed centrifugation.

According to our experiments, vesicle diameter was found to be almost independent of amount and type of fluorocarbon used. Assuming that the ratio of phospholipid/fluorocarbon is optimal concerning the surface area of the spheres, a relatively uniform distribution of vesicles will be obtained. Then, only the conditions of making them are decisive. Replying to Riess' view [9] judging the lecithins in terms of fluorophilicity, solubility and depression of interface tension, these terms are not relevant. However, in agreement with Rydhag and Wilton [7] the stabilizing properties of soy-bean lecithins are a result of the multilayer interfacial film, its swelling behaviour, i.e. incorporation of water, and the negatively charged surface. The latter was convincingly confirmed by zeta potential measurements in the case of PFD dispersions [10].

Another advantage of vesicle solutions consists in their viscosity behaviour. Whereas conventional PFC-emulsions above 60% become too viscous, vesicle solutions are of nearly constant viscosity in this concentration range. Therefore these dispersions do not negatively influence the extremely important rheology factor of human blood when they are used for injection experiments. Figure 3 presents viscosities and particle diameter of aqueous fluorocarbon systems using 5 % lecithin (Δ,•) and 5 % block copolymers (x, o), respectively, immediately after sonication.

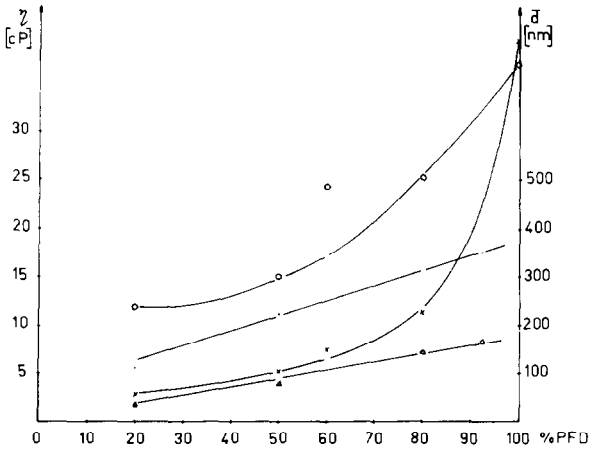

Fig. 3. Viscosities (Δ PL vesicles, x block polymer) and particle diameter (• PL vesicles, o block polymer) of PFD emulsions and lecithin dispersions.

A thermal stress of lecithin-stabilized aqueous fluorocarbon dispersions is without any drastic changes concerning particle size. The average diameter remains nearly constant, when aging the system in a temperature range up to 90 °C for several hours (Fig. 4). This stability enables a sterilization at 121 °C for twenty minutes.

A comparable system containing 5 % EO-PO-block copolymer emulsifier instead of lecithin is much more affected in stability with increasing temperature (Fig. 5). Expectedly, higher temperatures enhance both droplet coalescence as well as iso-

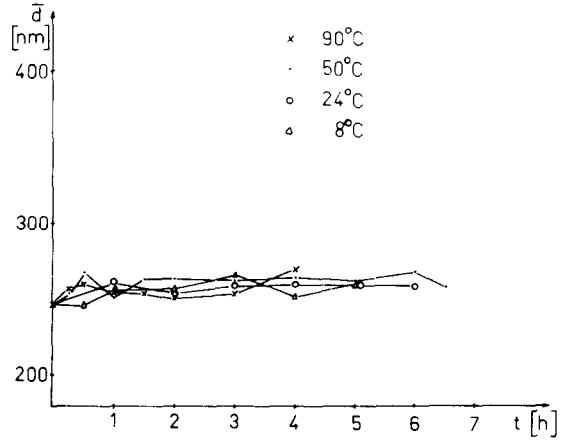

Fig. 4 . Temperature aging of a 50 % w/v PFD-lecithin dispersion.

thermal distillation (Ostwald ripening), which contribute to the overall emulsion destabilization.

^{19}F-Nmr spectra obtained from PL-vesicles filled with PFD, PFOB, TBA and F-CMM are of high resolution at room temperature as well as at 45 °C, that means above the gel to liquid cristal phase transition temperature of membrane lipids. The observed chemical shifts correspond to that of bulk fluorocarbons. Nmr tomographic measurements of such systems could be useful as medical probes in living organisms, especially when all fluorine intensities are combined in only one nmr signal.

^{31}P-Nmr investigations allow the study of head group conformations of phospholipids and their interactions with membrane soluble compounds.

Generally, phospholipids in bilayer membranes are characterized by broad lines [11]. Sonication leads to an asymmetric distribution of phospholipids in vesicle membranes and the typical inside-outside signals disappear. In our samples the peaks for sonicated PL-vesicles become sharper and the line width of the major signal at -0.08 ppm (with respect to phosphoric acid as standard) is 18.7 Hz. Whereas in the case of 50 % w/v TBA vesicles the signal remains unchanged, highly concentrated F-CMM vesicles increase the line width to 30.1 Hz. When going on to PFOB and PFD filled vesicles a steady increase takes place both at room temperature and at 45 °C, too.

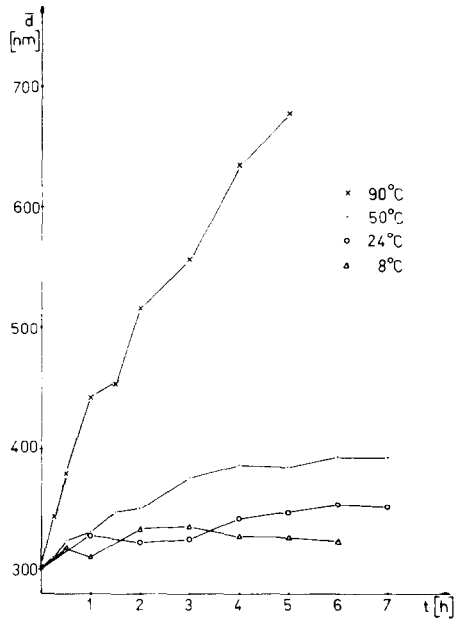

Fig. 5. Time depending particle growth of 50 % w/v PFD-emulsions stabilized by block polymers at fixed temperatures.

TABLE 1

^{31}P-Nmr line width of soy lecithin in PFC filled vesicle membranes

Sample	Line width 25 °C (Hz)	Line width 45 °C (Hz)	CST (°C)
soy lecithin (sonicated)	18,7		
TBA	19,4		61
F-CMM	30,1		38,5
PF0B		169	-24,5 ±2*
PFD	201,6	230	22

*PF0B separates from n-hexane solution at -12 °C as a solid. The value was extrapolated from measurements of PF0B mixtures with other PFC's (to be published).

The changes in line shape and width of the ^{31}P-nmr signal obviously correlate with PFC solubilities in lipid membranes (Table 1). In agreement with the low critical solution temperature in n-hexane

(CST), PFD and PFOB are the most lipophilic fluorocarbons, contrary to the nearly insoluble TBA. The well known similarity between bilayer vesicle membranes and biological membranes allows one to transfer these consequences to cell membrane structures. Therefore, PFD is very likely to have a high solubility in biological membranes, fulfilling a precondition for possible interactions in liver microsomes and mitochondrial membranes, which could provoke biochemical alterations [12].

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