# Liposomes and liposome-like vesicles in perfluorocarbon emulsions

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**Abstract**

Transmission electron microscopic examinations of perfluorocarbon emulsions have been made to investigate the influence of liposomes and liposome-like vesicles on the stability of these emulsions. Lecithin and the fluorinated quaternary ammonium salt $F_{13}C_6CH(OH)CH_2NHC_2H_4N(CH_3)_2C_{12}H_{25}Br$ were used as emulsifiers. In contrast to what had been expected, no indications of perfluorocarbon inclusions in liposome-like vesicles were found.

## Introduction

Gases are soluble to a great extent in many organic liquids. Of these liquids, perfluorocarbons (PFCs) play an important role as a result of their chemical inertness, a quality of great interest for biological and medicinal applications. However, for many such functions, especially when used as oxygen-carrying media in biological systems – better known as 'blood substitutes' – perfluorocarbons must be emulsified [1–5].

In addition to ethylene oxide/propylene oxide block polymers such as 'Pluronic F68', lecithins are the most suitable surfactants for perfluorocarbon emulsions, as they are biocompatible and contribute towards increasing the stability of the emulsions [6].

For the development of new and more stable perfluorocarbon emulsions, commonly used surfactants should be replaced by fluorosurfactants due to the increased interfacial activities of the fluorophilic part of the fluorosurfactant relative to lecithins [7, 8]. First results indicate that not only the application of fluorosurfactants but also their synergistic use as cosurfactants together with lecithin or Pluronic results in an increase in stability [9]. Many fluorosurfactants are not suitable as emulsifying agents, because they are only interfacially active and have no emulsifying properties [10].

Lecithins are characterized by their ability to build liposomes [11]. Only a few groups of surfactants are capable of building liposome-like vesicles, e.g. quaternary ammonium salts. When used as fluorosurfactants, fluorinated quaternary ammonium salts generate perfluorocarbon emulsions having a shelf-life time comparable with that of emulsions stabilized with lecithin [12, 13]. This is explained by the fact that the perfluorocarbon liquids are ingested completely or partly by vesicles. The fluorinated quaternary ammonium salt $F_{13}C_6CH(OH)CH_2NHC_2H_4N(CH_3)_2C_{12}H_{25}Br$ (**1**) has been synthesized as a liposome-building fluorosurfactant [14] for our investigations.

In contrast to emulsified particles – where the participating perfluorocarbon is completely surrounded by a layer of surfactant which in turn may be coated with a further layer or a greater number of layers of surfactant molecules – vesicles are formed in a more complicated manner. Thus, emulsified perfluorocarbon particles may be present as inclusions in the centre of vesicles surrounded by a bilayer, and also as free particles outside the vesicles. In this way, a separating bilayer reduces the growth of the emulsified particles inside the vesicles [15].

A further possible way in which perfluorocarbons may be ingested is by the inclusion of perfluorocarbon liquid in the bilayer, resulting in an increased thickness of the bilayer. On the basis of the highly fluorinated space within the bilayer, we assume that vesicles originating from fluorosurfactant **1** are included in this way [16, 17]. To investigate how perfluorocarbons are included in vesicles, we have emulsified perfluorodecalin in conjunction with either soyabean lecithin or fluorosurfactant **1** as well as studying the surfactants on their own. Transmission electron microscopic exami-

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nations of the emulsions and vesicles were undertaken in order to study the behaviour of these systems.

## Results

The following mixtures were prepared for electron microscopic examination:

(1) An emulsion analogous to Fluosol DA 20 (Green Cross Corp.) with ethylene oxide/propylene oxide block polymer Synperonic F68 (Serva) as surfactant and 20% (w/v) perfluorodecalin.

(2) Soyabean lecithin liposomes in water.

(3) Liposome-like vesicles derived from **1** in water.

(4) Soyabean lecithin and perfluorodecalin emulsions (1:1) in water.

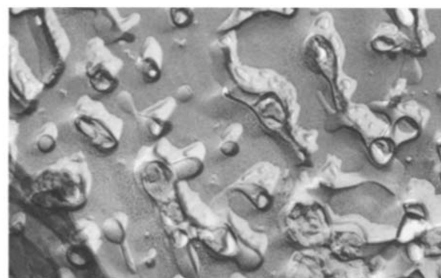
(5) (a) Liposome-like vesicles derived from **1** and perfluorodecalin (1:1) in water.

(b) Liposome-like vesicles derived from **1** and perfluorodecalin (1:1.5) in water.

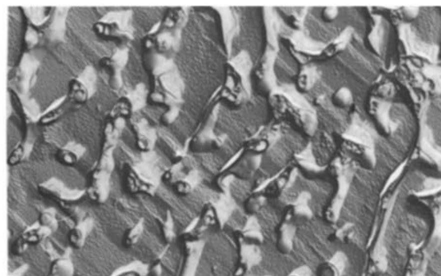
(c) Liposome-like vesicles derived from **1** and perfluorodecalin (1:2) in water.

**Case 1:** Electron microscopic examination of a carbon–platinum mask which had been generated from a perfluorodecalin emulsion by the etch-freezing method showed the existence of spherical particles having diameters of 50–220 nm, as shown in Fig. 1(a) and (b). These diameter ranges were confirmed by photocorrelation spectroscopy.

**Case 2:** To generate soyabean lecithin liposomes, 2.5 g soyabean lecithin was dissolved in 100 ml chloroform. The solution was then slowly transformed to a thin coating on the wall of a rotating glass flask in a rotary evaporator. Subsequently, 100 ml distilled water were added and the flask shaken continuously for 24 h. This procedure resulted in the formation of a milky liquid containing liposomes with diameters of 100–1500 nm. In contrast to the results shown in Fig. 1, a number of broken vesicles or typical liposomes can be seen in the micrographs depicted in Fig. 2(a) and (b). Within some liposomes, a second or even a third bilayer may be recognized [Fig. 2(c)]. These inner liposomes are not positioned close to the outer bilayer and appear to have been included randomly. This is confirmed by

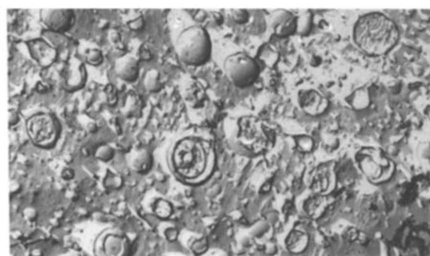


(a) —— 500 nm

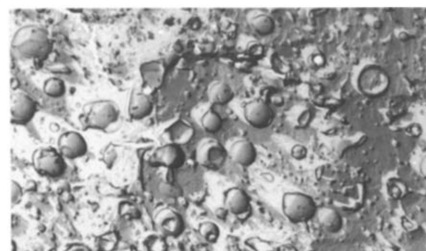


(b) —— 1000 nm

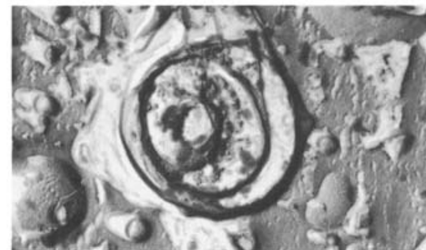
Fig. 1. PFC emulsion.



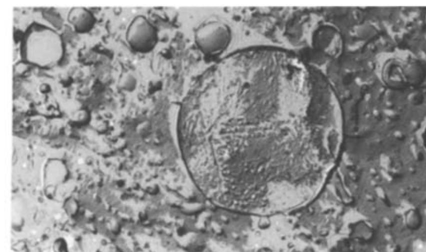
(a) —— 1000 nm



(b) —— 1000 nm



(c) —— 500 nm



(d) —— 1000 nm

Fig. 2. Lecithin liposomes.

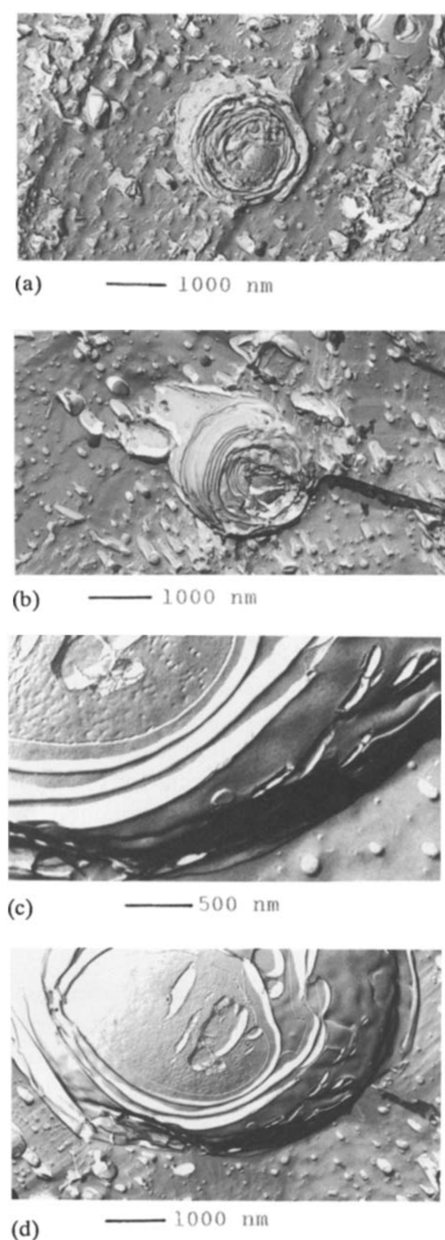



Fig. 3. Vesicles from $F_{13}C_6CH(OH)CH_2NHC_2H_4N(CH_3)_2C_{12}H_{25}Br$.

the fact that no inner structures have been found in larger liposomes [Fig. 2(d)].

These initial results show that emulsified particles (bubbles) and liposomes can be distinguished by means of electron microscopy. Differences in appearance originate from the method of sample preparation. The samples were shock-frozen, broken and subsequently coated with carbon–platinum vapour. In their frozen state, liposomes form spherical hollow bodies in a matrix of frozen water. These spherical hollow bodies appear as broken samples following freeze-etching. In the frozen emulsion, the perfluorocarbon bubbles exist as compact particles which are not destroyed when the sample is broken.

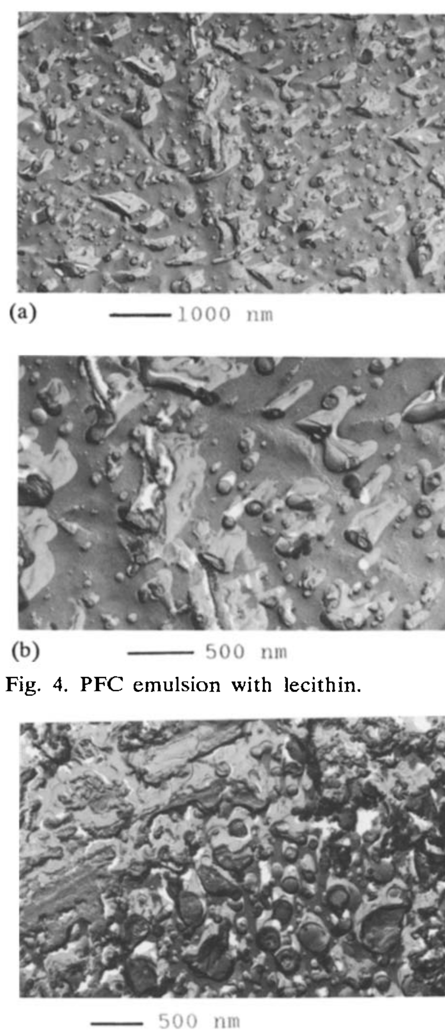



Fig. 4. PFC emulsion with lecithin.

Fig. 5. PFOB emulsion (80% w/v) with lecithin.

**Case 3:** The compound $F_{13}C_6CH(OH)CH_2NHC_2H_4$-$N(CH_3)_2C_{12}H_{25}Br$ was dissolved in water at a temperature of 70 °C using ultrasound. Electron microscopy (TEM) revealed the simultaneous existence of small spherical particles together with large multilaminary vesicles [Fig. 3(a)–(c)]. Individual bilayers can be recognized in this case. The narrow space between the bilayers prevented the formation of an aqueous layer between these two layers. It was not possible to estimate the dimensions of the aqueous interiors by electron microscopy [Fig. 3(c) and (d)]. The small spherical particles are unilaminar vesicles (SUV) with particle diameters greater than 120 nm.

**Case 4:** For the reasons outlined above, a perfluorodecalin emulsion with soyabean lecithin as the emulsifying agent (1:1) was prepared. When observed through an electron microscope, only small spherical particles (bubbles) which are typical of emulsions could be observed [Fig. 4(a) and (b)]. No liposomes were found. Comparative electron microscopic examinations of per-

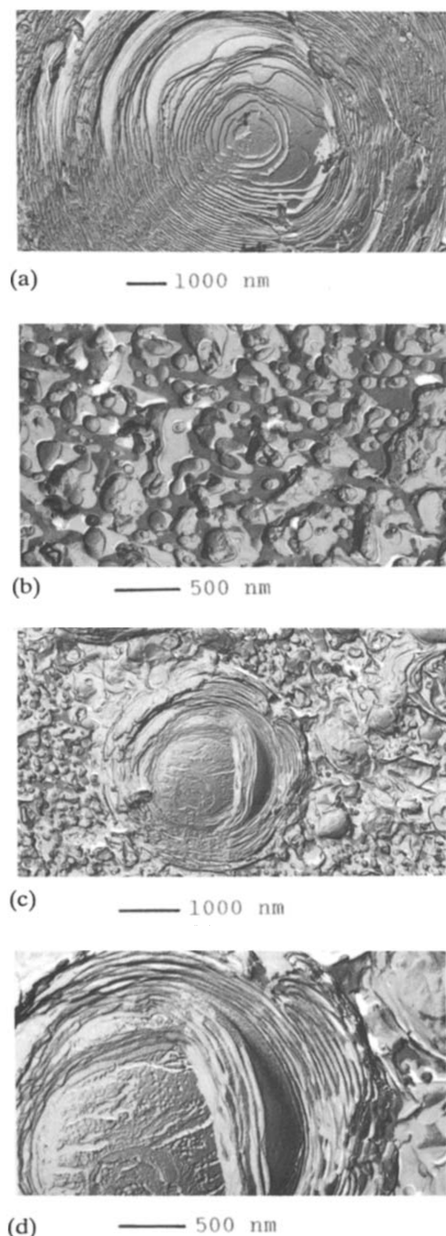



Fig. 6. PFC emulsion with $F_{13}C_6CH(OH)CH_2NHC_2H_4N(CH_3)_2C_{12}H_{25}Br$.

fluorocarbon (PFC)/lecithin emulsions and an 80% (w/v) perfluorooctylbromide (PFOB)/lecithin emulsion (Fig. 5) were also made. These supported the view that perfluorocarbon/lecithin emulsions do not contain liposomes but only typical bubbles, i.e. emulsified particles.

The absence of liposomes is explained by the high energy level applied in the preparation, since the emulsification of perfluorocarbons in the presence of liposomes requires ultrasound or high pressure homogenization. If lecithin is replaced by emulsifier **1**, a homogeneous mixture is generated by the application of the same moderate methods as necessary for the formation of surfactant vesicles.

**Case 5:** Mixtures of perfluorodecalin and the surfactant $F_{13}C_6CH(OH)CH_2NHC_2H_4N(CH_3)_2C_{12}H_{25}Br$ in water were exposed to ultrasound at a temperature of 70 °C and examined by electron microscopy. All samples exhibited the presence of both perfluorocarbon bubbles and vesicles [Fig. 6(a) and (b)]. The thickness of the bilayer of the liposomes was the same as that found in mixtures prepared without perfluorodecalin. Other irregularities indicating perfluorodecalin inclusions in the bilayer were also observed [Fig. 6(c) and (d)].

## Discussion

We have compared, via electron microscopy, perfluorocarbon emulsions containing vesicle-forming surfactants (lecithin or compound **1**) and solutions of the same surfactants without perfluorocarbon additives. The results obtained indicate the formation of emulsified particles containing the perfluorocarbon in all cases. No perfluorocarbon inclusion was found in vesicles. Hence, the greater stability of perfluorocarbon emulsions with vesicle-building surfactants relative to emulsions with other surfactants cannot be explained by perfluorocarbon inclusion in bilayers.

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## References

1 E. Dellaherie, P. Labrude, C.L. Vigneron and J.G. Riess, *CRC Crit. Rev. Therap. Drug Carrier Systems, 3* (1987) 41.
2 R.P. Geyer, *Biomater., Art. Cells, Art. Org., 16* (1988) 31.
3 T. Fujita, T. Sumaya and K. Yokoyama, *Eur. Surg. Res., 3* (1971) 436.
4 K.C. Lowe, *Comp. Biochem. Physiol., 87A* (1987) 825.
5 H. Meinert, U. Gross, M. Kupfer, S. Rüdiger and L. Kolditz, *Period. Biol., 87* (1985) 141.
6 U. Gross, H. Reichelt and E. Döring, *Tenside Surfact. Deterg., 25* (1988) 284.
7 A. Cambon, J.-J. Delpuech, L. Matos, G. Serratrice and F. Szönyi, *Bull. Soc. Chim. Fr.,* (1986) 965.
8 J.-J. Delpuech, G. Mathis, J.C. Ravey, C. Selve, G. Serratrice and M.J. Stebe, *Bull. Soc. Chim. Fr.,* (1985) 578.
9 L. Zarif, A. Manfredi, C. Varescon, M. Le Blanc and J.G. Riess, *J. Am. Oil Chem. Soc., 66* (1989) 1515.
10 D. Myers, *Surfactant Science and Technology,* VCH Publishers, New York, 1988, pp. 209–253.

11 J.M.A. Kemps and D.J.A. Crommelin, *Pharm. Weekbl., 123* (1988) 457.
12 H. Ringsdorf, B. Schlarb and J. Venzmer, *Angew. Chem., 100* (1988) 117.
13 U. Gross, L. Kolditz, G. Papke and S. Rüdiger, *J. Fluorine Chem., 53* (1991) 163.
14 S. Szönyi, *Thèse de doctorat,* Université de Nice, 1991.
15 D. Lasic, *Am. Sci., 80* (1992) 20.
16 J.-H. Gu, S.-M. Luo, H.-K. Kong and Y.Z. Hui, *Acta Chim. Sinica,* (Chinese edition), *46* (1988) 913.
17 C.F. Kong, B.M. Fung and E.A. O'Rear, *J. Phys. Chem., 89* (1985) 4386.