Synergistic Stabilization of Perfluorocarbon-Pluronic F-68 Emulsion by Perfluoroalkylated Polyhydroxylated Surfactants

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One of the most serious limitations of the first generation of fluorocarbon emulsions destined to serve as injectable oxygen carriers (blood substitutes) is their insufficient stability. Considerable stabilization of such emulsions has now been achieved by using a combination of Pluronic F-68, a polyoxyethylene polyoxypropylene block polymer, and of a perfluoroalkylated polyhydroxylated surfactant (PPFS) comprising a C_6F_{13} or C_8F_{17} fluorophilic tail and a hydrophilic head derived from maltose or xylitol. These new surfactants are either soluble in water or dispersible in an aqueous Pluronic F-68 solution. The tensions at the surface of these solutions and dispersions and at their interface with a typical fluorocarbon, F-decalin, are discussed. A strong stabilizing effect on F-decalin emulsions was obtained when small amounts of PPFS were incorporated, the total amount of surfactant used remaining constant. For 20% w/v emulsions, the maximum stabilizing effect was reached when 20–30% of PPFS were present, resulting after one year at 25°C in an up to 10 times smaller increase in particle size than for a reference emulsion prepared with Pluronic F-68 alone. The effect is even stronger with more concentrated 50% w/v emulsions, reaching a stabilizing ratio of 17 after one year for a one-to-one PPFS/Pluronic F-68 combination. None of the PPFS, when taken alone, permitted the preparation of stable emulsions. The characteristic U-shape taken after aging, by the particle size vs PPFS/Pluronic F-68 ratio curves demonstrates a strong synergistic effect of the two surfactants. They also permitted the selection of optimal formulations for the emulsions. The evolution of the particle sizes against time for such formulations was followed for 20% and 75% w/v concentrated F-decalin emulsions at 4°C, 25°C and 50°C. The best emulsion in terms of stability was obtained with the xylitol derivative $C_8F_{17}CH=CHCH_2OCH_2(CHOH)_4H$; most significantly, temperature, from 4 to 50°C, had little or no effect on the aging of this emulsion over a two month period.

Fluorocarbon emulsions permit the administration of oxygen *in vivo* and therefore have a considerable potential in medicine (1-3). The surfactants used so far for preparing such injectable emulsions are Pluronic F-68, a synthetic polyoxyethylene polyoxypropylene block copolymer, and/or natural egg-yolk lecithins. It is a combination of these two surfactants which was used to prepare Fluosol-DA (Green Cross Corp., Japan), the first fluorocarbon emulsion to have undergone clinical trials, and more particularly now, in percutaneous transluminal coronary angioplasty (4).

Pluronic F-68 is one of the most biocompatible synthetic surfactants known, with an LD_{50} in the 12.5 g/kg body weight range i.v. in rats (5). It has found numerous applications in the biomedical field (6), for example, for preventing accumulation of fat emboli resulting from prolonged cardiopulmonary bypass, in the treatment of hemorrhagic shock, as a dispersant for drugs, as a wetting agent in preparations used to dissolve renal calculi, as an emulsifier for oral and intravenous therapeutic fat products, as well as for preparing oil-in-water emulsions of vitamins. On the other hand, Pluronic F-68 was found to be responsible for the transient anaphylactoid reaction observed with some of the patients who were given a test-dose of Fluosol-DA (7). However, this reaction lasts for only a few minutes and can be repressed by corticosteroids. Lecithins are used as emulsifiers, especially in fat emulsions for parenteral nutrition and for the preparation of liposomes for drug targeting (8); it has recently been used for preparing very highly concentrated emulsions of F-octyl bromide (9). However, neither Pluronic F-68 nor lecithins are particularly fluorophilic, and the stability of the emulsions they form with fluorocarbons is usually poor. Fluosol-DA, for example, has to be shipped and stored in the frozen state (10), which is inconvenient and strongly limits the scope of its uses.

In order to improve this situation we designed a range of new amphiphilic molecules susceptible of acting as co-surfactants with Pluronic F-68 in view of conferring an increased stability on fluorocarbon emulsions (11, 12).

From a structural standpoint the basic concepts which underline this approach are that these new molecules had to be i) more fluorophilic than Pluronic F-68, hence comprise a perfluoroalkylated chain, in order to improve the affinity of the surfactant for the fluorocarbon phase, and ii) able to bind themselves to the Pluronic F-68 molecules—this could involve only the latter's ether functions, which called for the creation of hydrogen bonds and hence for the use of hydroxyl-group-bearing polar heads. For biocompatibility reasons, these polyhydroxylated heads were chosen from among the natural, low-cost sugars and polyols. In this way we expected to build a synergism involving, on one hand, the largely steric-based particle stabilization effect of the polymer, and, on the other, the much stronger surface activity of the fluorinated surfactants.

Pluronic F-68 is actually a poor surfactant when only its ability to decrease the water/fluorocarbon interfacial tensions is considered. Its emulsion stabilizing capacity appears to be predominantly due to the ability of the polymer to spread at the particle's surface.

The present study shows that a strong stabilization of fluorocarbon emulsions can indeed be achieved by combining the properties of Pluronic F-68 with those of a polyhydroxylated perfluoroalkylated surfactant (PPFS), which indicates that the above-mentioned underlying concepts are valid. The surfactants chosen for this study were recently synthetized in our laboratory (13, 14). They consist in a derivative of xylitol:

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5-O-[3'-(F-octyl)-2'-propenyl]-xylitol 1 (Scheme 1) and two derivatives of maltose: 2'-(F-hexyl)-ethyl-4-O-(α-D-glucopyranosyl)-α/β-D-glucopyranoside 2 (Scheme 1) and 2'-(F-octyl)-ethyl-4-O-(α-D-glucopyranosyl)-α/β-D-glucopyranoside 3 (Scheme 1). In the case of 2 both the α and β anomers, 2α and 2β, were investigated.

EXPERIMENTAL

General methods. Surface tensions were measured at 20 ± 0.1°C on an automatic Lauda tensiometer based on the Lecomte du Noüy method, using a rigid platinum ring; samples were left to equilibrate for 24 hr before measurements were made. Critical micellar concentrations (CMC) were determined with a Philips (Pye Unicam PU 8650) spectrophotometer and Coomassic Brilliant Blue G (Aldrich) as a dye. Most dispersions and emulsions were prepared by pulsed mode sonication (Branson B-30 cell disruptor). Larger samples were prepared with a Manton Gaulin® high pressure homogenizer, model 15M-8TA, or a Microfluidics model M 110 Microfluidizer. The average particle size and the particle size distribution in the emulsions were evaluated by a sedimental centrifugation method coupled with optic density measurements on an automatic Horiba Capa 500 particle analyzer. Dilutions were made with a 50 ml Brand digital burette. The pH of the solutions was controlled with an Orion Research Microprocessor Ionalyzer/901.

Materials. The synthesis of the perfluoroalkylated surfactants was achieved in the laboratory, only batches displaying better than a 99% grade of purity (HPLC) were allowed to be tested. Pluronic F-68 and F-decalin (Flutec PP5) were purchased from Serva Feinbiochemica (Heidelberg, NY) and Imperial Smelting Chemicals Ltd. (Avonmouth, UK), respectively.

Dispersion of the perfluoralkylated surfactants in Pluronic F-68 solutions. The data collected in Table 1 were measured on clear solutions or dispersions. These were prepared by sonicating 0.1 g of the PPFS in a 0.1 g solution of Pluronic F-68 in 10 ml of water for 15 min. Water was then added to yield 100 ml of a dispersion containing 1 g of PPFS for 1 g of Pluronic F-68 per liter (1/1 g/l). Subsequent dilutions in a 1 g/l solution of Pluronic F-68 were achieved to obtain the 0.1/l and 0.01/l g/l dispersions.

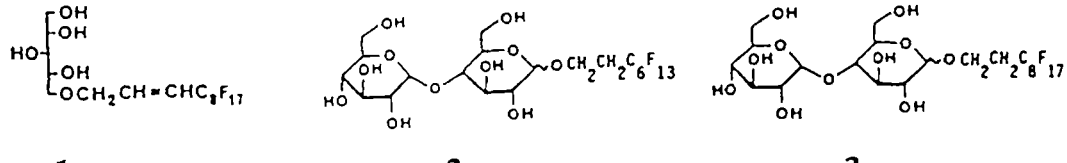
The dispersions in a 10 g/l solution of Pluronic F-68 were prepared by predispersing the desired amounts (0.5, 0.05, and 0.005 g) of the PPFS by sonication (5 min) in 100 ml of a 50 g/l solution of Pluronic F-68. These dispersions were then diluted with water to a 500 ml volume and run through the Manton Gaulin homogenizer (5 min, 350 bar), which yielded clear dispersions; these were further diluted in water to obtain the concentrations needed for establishing the curves in Fig. 1.

Preparation of emulsions by sonication. For the emulsion stabilization studies shown in Fig. 2–6, 10 ml samples of emulsions of F-decalin in water were prepared. All sonications were performed in the pulsed mode (0.5 s of sonication per s-cycle) at medium power with titanium probes of 3 mm of diameter. Great care was taken to apply the sonication procedure in as similar and reproducible a way as possible to all the samples within a given study: same size and shape of the vessel, location of the titanium probe, power and duration, the sample being kept under nitrogen; the probe was placed at the interface for 15 min, the sample being cooled in an ice bath. A 10 ml sample of emulsion was prepared for each surfactant/co-surfactant ratio and for each fluorocarbon concentration.

Preparation of emulsions by high pressure homogenization. The studies requiring larger amounts of emulsions (Fig. 7) were obtained by high pressure homogenization using a Manton Gaulin device on 400 ml samples. PPFS 1 (Scheme 1) was first dispersed by sonication in the aqueous solution of Pluronic F-68 using a 13 mm diameter probe in a 60 mm diameter glass container. The emulsification was then achieved at 300 bar; it required 7 min for the test emulsion and 25 min for the reference emulsion.

Preparation of emulsions by microfluidization. This method was used in the study presented in Fig. 8 and 9. 100 ml of concentrated emulsions containing 75% by weight of F-decalin and 5% of Pluronic F-68 (reference emulsion), or a mixture of Pluronic F-68 (2.5%) and of one of the PPFS (2.5%), were prepared. The solutions of surfactant mixtures were prepared as described above. The F-decalin/surfactant system was then pre-emulsified for 15 min by sonicating using the 13 mm probe, and an average particle size of 0.30–0.40 μm was obtained. These emulsions were then immediately run through the microfluidizer under the following conditions: 20 passages at 7800 psi (517 bar), the apparatus being maintained in a water bath at 45°C; the resulting emulsions had average droplet sizes in the 0.14–0.17 μm range.

Emulsion storage. Each batch of emulsion was divided in a sufficient number of 3–4 ml aliquots which were stored in 75 × 12 mm glass tubes placed in a refrigerator at 4 ± 1°C or in thermoregulated baths at 25 ± 1°C and 50 ± 1°C. When the samples were destined to be stored for longer periods of time, an antibiotic (Ampicillin 5 mg/l) and a fungicide (sodium azide 200 mg/l) were added.



1 2 3

SCHEME 1: Structure of compounds derived from xyletol (1) and maltose (2,3)

TABLE 1

Surface Active Properties of Perfluoroalkylated Surfactants Derived from Xylitol and Maltose

Compound mol. weight (g)	Solubility g/l	C.M.C.[a] g/l	Concentrations C (mM)	Concentrations C (g/l)	Solutions γ_s[b]	Solutions γ_i[b]	Dispersions[c] γ_s[b]	Dispersions[c] γ_i[b]
1	0.05		1.6	1			16.6	1.0
610			0.16	0.1			17.4	1.1
			0.016	0.01			19.5	2.8
2 ($\alpha + \beta$)	110	0.72	1.45	1	23.8	4.7	23.0	4.5
688			0.145	0.1	36.6	18.3	32.8	15.0
			0.014	0.01	51.2	33.1	41.3	23.9
2 α				1	25.3	5.6		
688				0.1	35.2	18.7		
				0.01	53.0	33.8		
2 β		0.70		1	23.3	4.7		
688				0.1	34.0	16.9		
				0.01	52.1	32.4		
3 ($\alpha + \beta$)	35	0.14	1.27	1	22.3	2.6	21.1	3.0
788	(gel)		0.127	0.1	23.0	3.6	20.7	3.3
			0.013	0.01	25.8	6.6	21.2	5.2
Pluronic F-68	>400			1	48	28		
9850				0.1	48.7	29.6		
				0.01	50.7	31.4		

[a]In water at 25 ± 1°C.
[b]In mN m^{-1} ± 0.3 at 20 ± 0.1°C. γ_i is measured with respect to F-decalin. $\gamma_s H_2O$ = 73 mNm^{-1}, $\gamma_s FDC$ = 22.9 mNm^{-1}, $\gamma_i H_2O/FDC$ = 55.0 mNm^{-1}.
[c]In 1 g/l Pluronic F-68 solution.

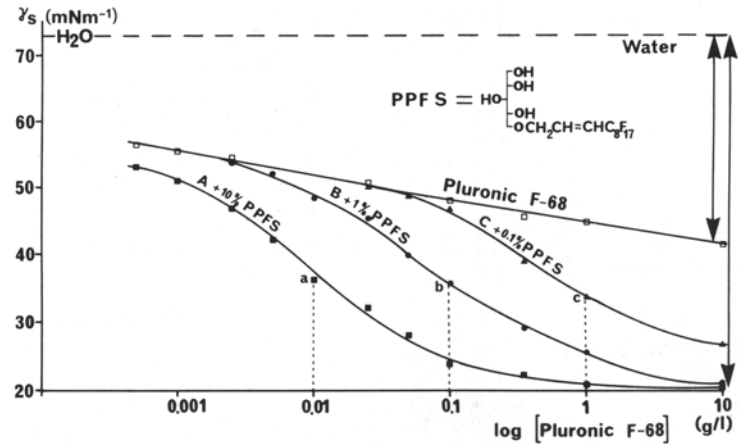


FIG. 1. Cosurfactive effect of surfactant 1 on an aqueous solution of Pluronic F-68. The ponderal percentages of 1 (10, 1, 0.1%) are given with respect to Pluronic F-68.

RESULTS AND DISCUSSION

The fluorinated surfactants 1-3 tested here have been selected from several newly synthesized families of sugar and polyol derived amphiphiles (13,14) on the following basis: ease of preparation and purification, solubility in water or dispersibility in an aqueous Pluronic F-68 solution, surface tension measurements. The three compounds chosen illustrate the range of situations that were encountered from the solubility standpoint, which drastically depends, as expected, on the length of the F-alkyl chain and on the size and number of hydroxyl groups of the head. Thus the solubility of the F-hexyl maltoside 2 in water is greater than 100 g/l at 25°C. Its F-octyl homolog 3 is already much less soluble, and starts forming gels for concentrations greater than 1 g/l, but it is highly dispersible in the Pluronic F-68 solution. For example, clear fluid dispersions of 45 g/l of 3 in a 5 g/l aqueous solution of Pluronic F-68 can easily be prepared. Finally, the solubility of the F-octyl xylitol ether 1 is very low (<0.1 g/l at 25°C) as a consequence of the lesser number of free hydroxyl groups, but it retains the capacity to be dispersed in Pluronic F-68, i.e., clear dispersions at concentrations as high as 50 g/l in a 15 g/l solution of Pluronic F-68 in water can be achieved.

The fact that 3, and to an even greater extent 1, which is almost insoluble in water, can be dispersed in high concentrations with the help of Pluronic F-68 (at least 10^3 times greater in the latter case than in the absence of the polymer) is a first strong indication that an association is taking place, most likely through

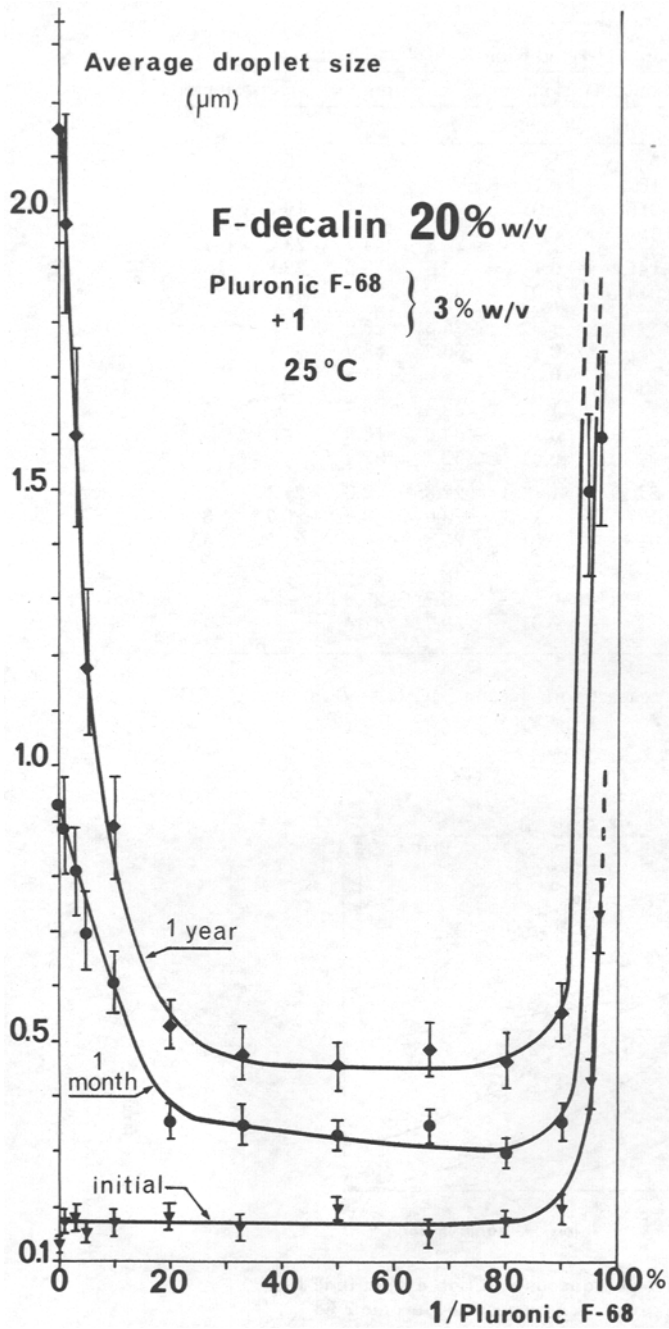


FIG. 2. Average droplet size (ads) (initial, after 1 month and 1 year) in an 20% w/v emulsion of F-decalin in water at 25°C as a function of the ratio of fluorinated surfactant 1 to Pluronic F-68 in the surfactant mixture; the total concentration of surfactant constantly being 30 g/l^{-1}.

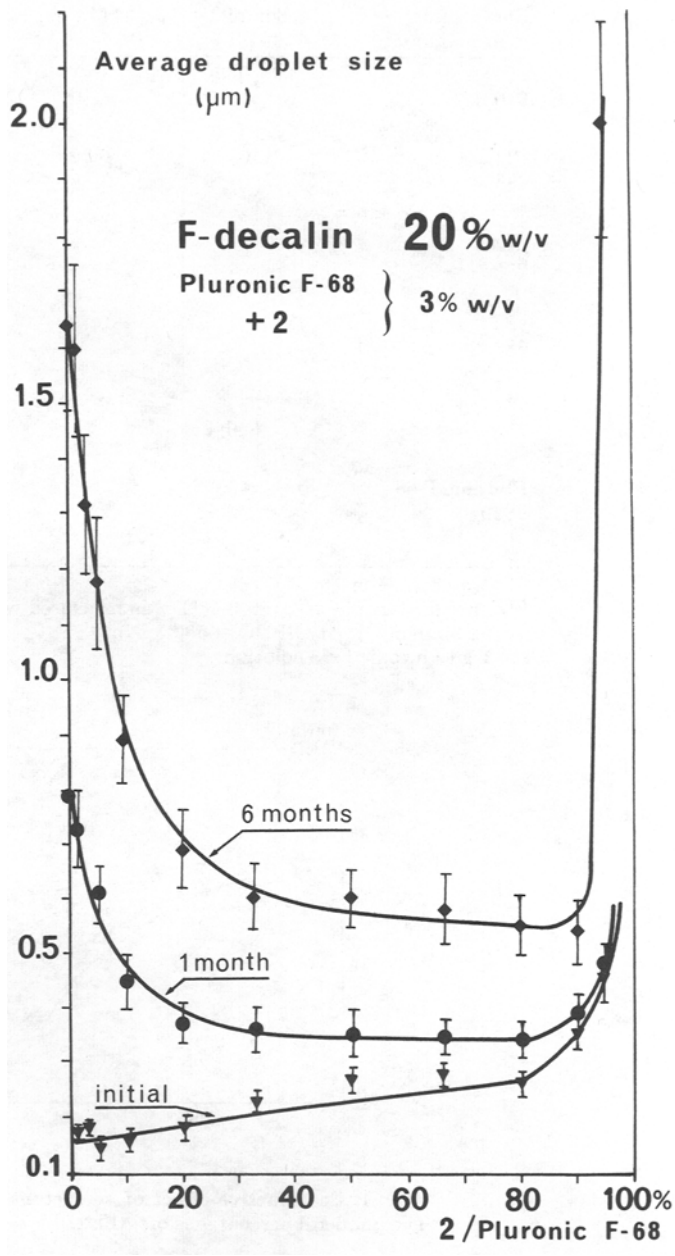


FIG. 3. Average droplet size (ads) (initial, after 1 month and 6 months) in an 20% w/v emulsion of F-decalin in water at 25°C as a function of the ratio of fluorinated surfactant 2 to Pluronic F-68 in the surfactant mixture; the total concentration of surfactant constantly being 30 g/l^{-1}.

hydrogen bonding, between the two surfactants. We first investigated the surface activity of the new surfactants, and their effect on the surface activity of Pluronic F-68 solutions.

The stabilizing effect of their addition to F-decalin/Pluronic F-68 emulsions was then evaluated at room temperature with a fixed total ponderal amount of the surfactant mixture, in order to define the best F-alkyl-surfactant/Pluronic F-68 ratio. Finally, the stability of emulsions based on this ratio was examined at various temperatures.

Several emulsification procedures have been used, sonication when the amount of sample required was smaller than 20 ml, high-pressure homogenization when amounts larger than 400 ml were needed, and microfluidization for intermediate sample sizes.

Effects of the polyhydroxylated perfluoroalkylated surfactants on the surface properties. The tension γ_s at the surface of aqueous solutions of the F-alkylated surfactants at various concentrations, and the tension γ_i at the interface between these solutions and F-decalin, are collected in Table 1 and compared with

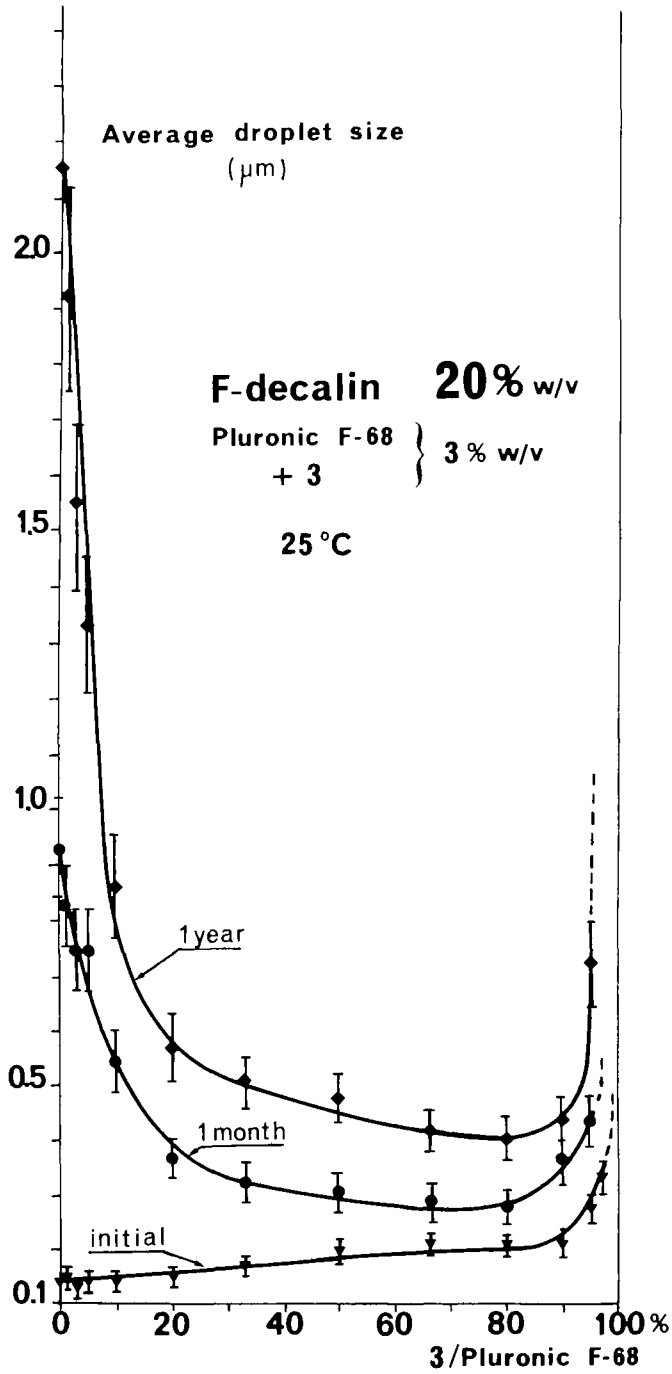


FIG. 4. Average droplet size (ads) (initial, after 1 month and 1 year) in an 20% w/v emulsion of F-decalin in water at 25°C as a function of the ratio of fluorinated surfactant 3 to Pluronic F-68 in the surfactant mixture; the total concentration of surfactant constantly being 30 g/l^{-1}.

those of water, F-decalin, and aqueous solutions of Pluronic F-68. The new compounds exhibit strong surfactant properties. Thus the γ_s and γ_i/F-decalin values can be lowered to values in the 22-25 and 1-4.5 mNm^{-1} ranges, respectively, with these compounds, compared to 48 and 28 mNm^{-1} with Pluronic F-68. The best results are obtained, as expected, for the molecules with the C_8F_{17} tail, which are still effective at very low concentrations, whether in water or in a 1 g/l Pluronic F-68 solution. A comparison of the data collected for 2 and 3, shows that the γ_s and γ_i are only slightly lower for the higher homologue, but that the increase in chain length markedly lowers the CMC.

In the two cases where measurements could be made on both solutions and dispersions, it was found that the lowest tensions that could be reached (i.e., when the concentration in PPFS was high) were not significantly different, but that there was a definite

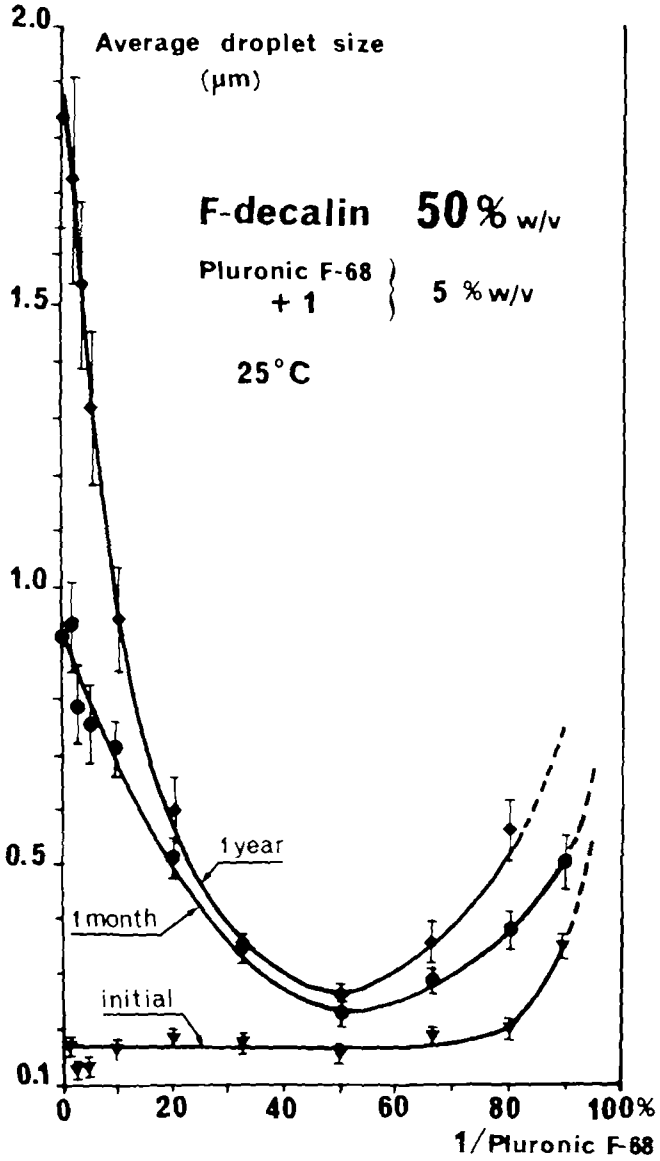


FIG. 5. Average droplet size (ads) (initial, after 1 month and 6 months) in an 50% w/v emulsion of F-decalin in water at 25°C as a function of the ratio of fluorinated surfactant 1 to Pluronic F-68 in the surfactant mixture; the total concentration of surfactant constantly being 50 g/l^{-1}.

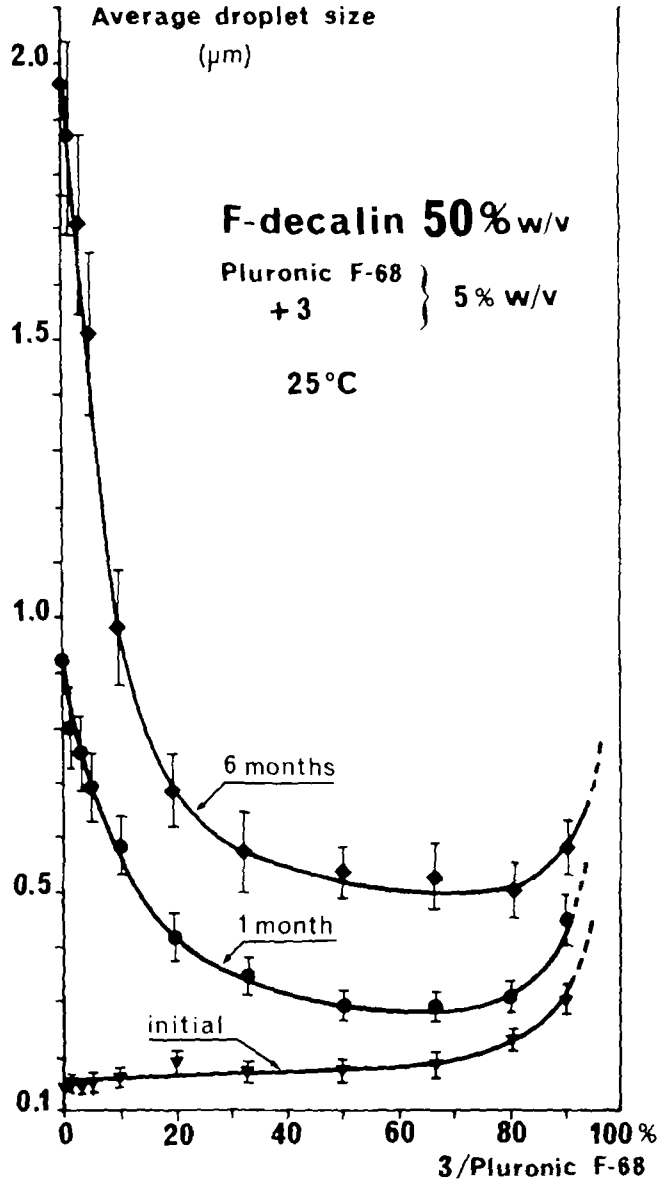


FIG. 6. Average droplet size (ads) (initial, after 1 month and 6 months) in an 50% w/v emulsion of F-decalin in water at 25°C as a function of the ratio of fluorinated surfactant 3 to Pluronic FK-68 in the surfactant mixture; the total concentration of surfactant constantly being 50 g/l^{-1}.

synergic effect for concentrations in PPFS lower than their CMC.

The F-alkylated maltosides 2 and 3 are produced as a mixture of the α and β anomers. An important question with respect to the product preparation issue was to know whether the surface properties of these anomers were significantly different or not. Therefore, we achieved a separation of the two anomers by HPLC for compound 2 and measured their γ_s and γ_i individually (Table 1). It was found that they had very similar performances for the relevant 1 g/l concentration, with only a marginal advantage for the β anomer. In view of the difficulty and cost involved in the isomer separation procedure it was decided to pursue the evaluation with the 3 (α/β) (30/70) anomeric mixture as it is obtained by synthesis (14).

An illustration of the effect of the F-alkylated surfactants on the surface tension of aqueous solutions of Pluronic F-68 is shown in Figure 1. Dispersions containing various percentages of 1 with respect to Pluronic F-68 were achieved, and allowed the curves A, B and C of Figure 1 to be drawn. The surface tension is considerably lowered by the presence of small amounts of 1, and can reach 20 mNm^{-1}.

The decrease in surface tension induced by 1 appears to be essentially independent of the concentration in Pluronic F-68 — thus points a, b, c on Figure 1 all correspond to the same concentration of 1, 1 mg/l,

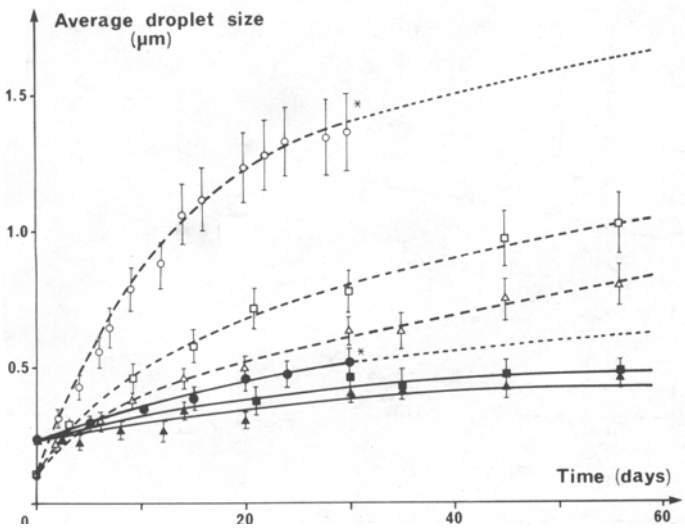


FIG. 7. Aging at 4, 25 and 50°C of diluted 20 w/v F-decalin emulsions prepared with 2% of Pluronic F-68 and 1% of surfactant 1 (full lines), and with 3% of Pluronic F-68 alone (dashed lines); triangles, 4°C; squares, 25°C; and circles, 50°C; * large droplets appear and progressive phase separation.

in solutions of widely different concentrations in Pluronic F-68 — in all three cases the surface tension is lowered to ca 35 mNm^{-1}.

Synergistic stabilization of fluorocarbon emulsions and determination of an optimal formulation. The stability of a fluorocarbon emulsion increases as the fluorocarbon's molecular weight increases and its vapor pressure decreases (15). Unfortunately the retention of the fluorocarbon in the organs also increases, and in an exponential way, as its molecular weight increases, which proscribes the use of compounds having a molecular weight higher than ca 520 in most medical applications. F-decalin has an organ retention halftime of ca 7 days, acceptable for i.v. use, but the attempts to produce emulsions of this compound stable enough to be practical, using Pluronic F-68 and/or egg-yolk lecithins as the surfactants, have failed so far (1,16). F-decalin was therefore an appropriate fluorocarbon for investigating whether our polyhydroxylated perfluoroalkylated surfactants and their combinations with Pluronic F-68 had a stabilizing effect on injectable fluorocarbon emulsions. For this we prepared fluorocarbon emulsions in which the PPFS/Pluronic F-68 ratios were stepwisely increased, the total amount of surfactant being held constant. Two concentrations were chosen for the fluorocarbon: 20% by weight with a total of 3% by weight of surfactant as in the so-called first-generation, Fluosol-type emulsions, and 50% by weight with a total of 5% of surfactants, which represents a more desirable goal in terms of therapeutic efficacy.

The emulsions were prepared by sonication, and their stability was appreciated by measuring the increase with time in their average particle size when stored at 25°C. The stabilization effect after D days, S_D, was expressed by the following ratio:

$$S_D = (d_D{}^R - d_O{}^R)/(d_D{}^T - d_O{}^T)$$

where $d_D{}^R$ and $d_O{}^R$ represent the average particle size in the reference emulsion prepared with Pluronic F-68 alone, at day D and day zero, and $d_D{}^T$ and $d_O{}^T$ the same parameters for the test emulsion.

When the F-alkylated surfactants **2** and **3**, which are soluble enough in water, were first used alone, we found that we were unable to obtain fine, stable emulsions of F-decalin; in spite of their strong surfactive properties, neither one, when taken alone, is effective in allowing the preparation of fluorocarbon emulsions.

On the contrary, when small amounts of **1**, **2** or **3** were incorporated in the formulation of Pluronic F-68-based emulsions, the emulsification process was facilitated, and the stability of the emulsion dramatically improved. The greater ease of emulsification is illustrated, for example, by the fact that when high-pressure homogenization was applied a particle size of 0.15 μm was reached in 7 min (17 passages) at 350 bar

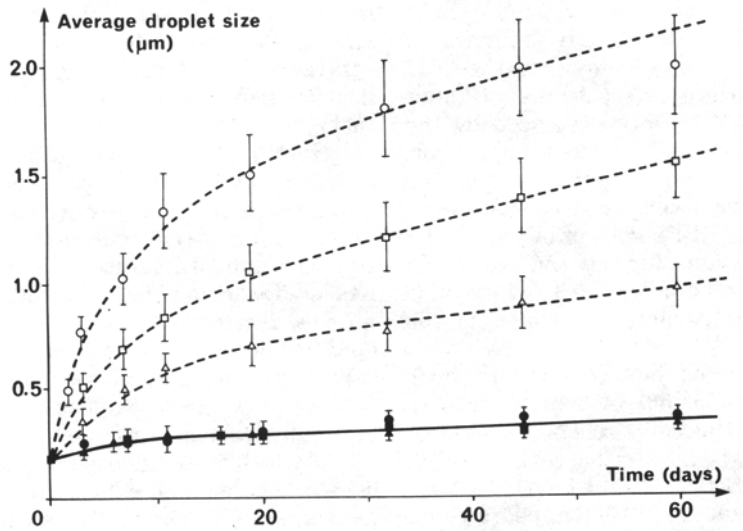


FIG. 8. Aging at 4, 25 and 50°C of concentrated 75% w/v F-decalin emulsions prepared with 2.5% of Pluronic F-68 and 2.5% of **1** (full lines), and with 5% of Pluronic F-68 alone (dashed lines). Triangles, 4°C; squares, 25°C; and circles, 50°C.

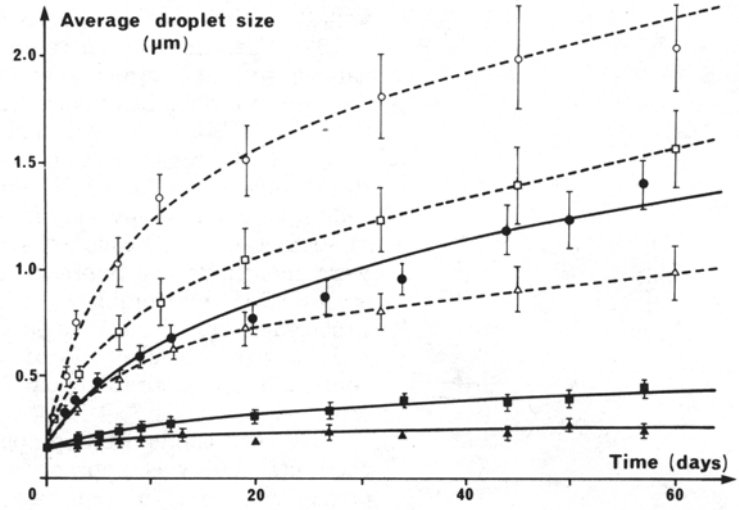


FIG. 9. Aging at 4, 25 and 50°C of concentrated 75% w/v F-decalin emulsions prepared with 2.5% of Pluronic F-68 and 2.5% of surfactant 2 (full lines), and with 5% of Pluronic F-68 alone (dashed lines). Triangles, 4°C; squares, 25°C; and circles, 50°C.

when one third of the Pluronic F-68 was replaced by the PPFS, compared to 25 min (60 passages) when Pluronic F-68 was used alone.

An interesting observation, most perceptible for the more concentrated emulsions, is that for essentially similar initial particle sizes, the viscosity decreases when the proportion of the polymer in the surfactant system diminishes.

The emulsion stabilizing effect of the added PPFS is clearly demonstrated in Figures 2-6. Figures 2-4 correspond to the emulsions formulated with 20% of F-decalin and a total of 3% of surfactants, Figures 5 and 6, to those with 50% of F-decalin and a total of 5% of surfactants.

The first noteworthy observation is that all these curves are similarly U shaped, independently of the surfactant used, whether it is strongly hydrophobic and insoluble in water like 1, or highly water-soluble like 2.

After one year, all these curves show a very sharp initial drop in particle size when small amounts of PPFS are incorporated in the emulsions. For the 20% w/v emulsions, this stabilizing effect levels off once 20-30% of the Pluronic F-68 has been replaced by the PPFS. The maximum stabilization ratio at one year, S_{365}, reaches values of 7 (Fig. 2) and 10 (Fig. 4), i.e., the increase in particle size after a year is in these cases 7 and 10 times smaller in the PPFS-containing emulsion than in the reference emulsion. Among the maltoside derivatives it is the higher homologue, 3, which also displays the strongest surface activity, which is the most efficient.

For the more concentrated emulsions, with 50% w/v of F-decalin and a total of 5% of the surfactants (Figs. 5 and 6), the S ratio already reaches values of 17 and 8 after one year and six months with 1 and 3, respectively (compared to $S_{180} = 9$ and 6 for the 20% emulsions), which means that the PPFS appears to be even more efficient in stabilizing concentrated emulsions. This is not a consequence of increased viscosity, as viscosity tends to decrease when the PPFS is added.

Once the proportion of PPFS becomes higher than 80-90% of the total amount of surfactants present, one observes in all cases a drastic decrease in stability of the emulsion, which can no longer be prepared above ca 95% of PPFS.

Thus it is found that neither Pluronic F-68 alone, and even less the PPFS alone, can help produce stable emulsions of F-decalin, while the combination of the two surfactants is effective in realizing this goal. The typical U shape of the curves of Fig. 2-6 is a clear demonstration of the synergy that develops between the two surfactants, and supports the concepts which led to the synthesis of these new perfluoroalkylated surfactants.

These results led to the defining of optimal formulations for the F-decalin emulsions: for the low-concentrated 20% w/v emulsion, a 1:2 ratio of PPFS to Pluronic F-68 was selected, as no significant increase in stabilization effect was noted above this ratio, while the cost of the PPFS is much higher than that of Pluronic F-68; for the higher fluorocarbon concentrations a 1:1 ratio was chosen, as maximum stabilization had not yet been reached with the above 1:2 ratio.

Stabilization of fluorocarbon emulsions by perfluoroalkylated polyhydroxylated Surfactants; influence of the temperature on the aging of the emulsions. The preservation of the emulsions' characteristics upon storage at room temperature is an essential goal, which has so far never been reached with F-decalin, even after a fluorocarbon with a higher molecular weight (and consequently longer retention time in the organs) (15), F-tripropylamine, had been added, as in Fluosol-DA, to slow down particle size increase by Ostwald's molecular diffusion process.

The effect of incorporating our new PPFS in F-decalin emulsions has been investigated at several temperatures: 4°C and 25°C, as they represent practical standard storage temperatures, and 50°C, which produces a significant acceleration of the aging process, and shortens the observation time needed for evaluat-

ing the emulsions' stability. Both diluted (20% w/v) and concentrated (75% w/v) emulsions were examined. Their formulations were established on the basis of the results discussed above. The concentrated emulsions are in line with the present trend of preparing more efficient emulsions with lower surfactant/fluorocarbon ratios (3). Both 2, the most hydrophilic of our surfactants, and 1, the least hydrophilic one, were tested. The emulsions were prepared either by high pressure homogenization (Fig. 7) or by microfluidization (Fig. 8 and 9). The increase in average particle size was measured against time, and compared with that of reference emulsions prepared with Pluronic F-68 as the sole surfactant (4% for the 20% w/v and 5% for the 75% w/v emulsions, respectively).

In all cases (Figures 7–9), and for any given temperature, the PPFS-containing test emulsion was significantly more stable than the reference emulsion. The highest stabilization effect was obtained with compound 1, and it is the most marked for the higher fluorocarbon concentration. This is particularly well demonstrated in Figure 8, where it can be seen that the increase in particle size over time is by far the slowest, even at 50°C, and, most strikingly, that the particle sizes remain the same, within experimental error, whether the emulsion is stored at 4, 25 or 50°C. The increase in viscosity from the 20% to the 75% emulsion may contribute in part to the stability of the more concentrated PPFS-containing emulsions, in addition to the fact that the higher-PPFS/Pluronic F-68 ratio leads to improved stability as found in the preceding section. But the increase in viscosity cannot account for any increase in stability with respect to the reference emulsions, as these, especially for the higher fluorocarbon concentration, were always significantly more viscous.

Comparison of Figures 8 and 9 shows that compound 1 is definitely more efficient in stabilizing fluorocarbon emulsions than compound 2, which may be related to the lower fluorophilicity of the latter compound.

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