# Perfluoroalkylated Fatty Acid Monoesters of Trehalose and Sucrose for Biomedical Applications: Remarkable Emulsifying Properties of 6-*O*-[3′-(Perfluorooctyl) propanoyl]-Trehalose

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**A new series of perfluoroalkylated fatty acid monoesters of α,α-trehalose and sucrose has been evaluated with respect to their physicochemical and biological properties for possible biomedical use. These water-soluble compounds strongly reduce the water surface tension and fluorocarbon/water interfacial tension. As co-surfactants in perfluorodecalin/Pluronic F-68 type emulsions they significantly increase the stability of these emulsions. Remarkably stable concentrated perfluorodecalin-in-water (50% w/v) emulsions were obtained when the $C_8F_{17}CH_2CH_2C(O)$-α,α-trehalose monoester was used as the sole surfactant, while no emulsion could be obtained with its maltoside analogue. No significant effect on the growth and viability of Namalva cell cultures and no hemolytic activity on human erythrocytes at concentrations up to 50 g/L were detected for these amphiphiles in spite of their high surface activity. The $LD_{50}$ was found to be in the range of 250–375 mg/kg of body weight *i.v.* in mice.**

KEY WORDS: Fluorocarbon emulsion, perfluorodecalin emulsion, perfluoroalkylated fatty acid monoester of sucrose, perfluoroalkylated fatty acid monoester of trehalose, perfluoroalkylated surfactant, 6-*O*-[3′-(perfluorooctyl) propanoyl]-trehalose.

The recognition that the acquired immunodeficiency syndrome (AIDS) could be transmitted by blood has focused attention on problems connected with blood transfusions, such as immunodepressive effects, risk of infection and the always increasing cost for safe blood (1). This has given new impetus to research and development of a "blood substitute" capable of delivering oxygen *in vivo*. One major approach uses perfluorocarbon compounds such as oxygen carriers (2). These synthetic, chemically and biologically inert compounds are, however, insoluble in water and must be emulsified to become injectable intravenously. Fluorocarbon emulsions are under investigation not only as substitutes for red blood cells but also for treating other situations where oxygen is required, such as cardio- and cerebrovascular diseases, and for use in surgery, organ preservation, radio- and chemiotherapy of cancer, and diagnosis (3).

Recently, the first fluorocarbon emulsion Fluosol® (4) was approved by the Food and Drug Administration for providing the myocardium with oxygen during percutaneous transluminal coronary angioplasty (5). The usefulness and efficacy of Fluosol is, however, limited by its low stability, which requires it to be stored and shipped in the frozen state, and by its low fluorocarbon content, only 20% by weight, *i.e.*, 11% by volume. In recent years, significant advances have been made in developing more effective emulsions containing up to 100% w/v (*i.e.*, 52% by volume) of fluorocarbon (6), which have significantly higher oxygen carrying capacity. They are based on perfluorooctyl bromide (PFOB) as the $O_2$-carrier and egg-yolk lecithins as the surfactant. They are stable at room temperature for over a year. Further improvement of our mastery over fluorocarbon emulsions is desirable in order to allow the optimal adaptation of the emulsion's characteristics for each specific therapeutic application and to extend their field of applications. This objective prompted us to conceive and synthesize new perfluoroalkylated surfactants derived from sugars and sugar derivatives (7–11).

When used as co-surfactants, perfluoroalkylated surfactants derived from xylitol or maltose have been shown to exercise a strong synergistic stabilizing effect in fluorocarbon emulsions in which Pluronic F-68® (a polyoxyethylene polyoxypropylene block polymer) was the main surfactant (7). This synergistic effect was expected to occur as a result of the presence of a perfluorinated segment that interacts with the fluorocarbon phase and of a polyhydroxylated head capable of forming hydrogen bonds with the ether functions of Pluronic F-68. But the use of Pluronic F-68 is controversial because it (or some impurities it may contain) is considered responsible for some transitory anaphylactoid reactions observed in certain patients (12). On the other hand, it proved impossible to obtain stable emulsions with any of the sugar or polyol-derived perfluoroalkylated surfactants reported previously (7–11) when taken as the sole surfactant. Our next objective was therefore to avoid the use of Pluronic F-68 by developing new, superior, and safe emulsifying agents capable of giving stable fluorocarbon emulsions when they are used alone. Analysis of the previous results led us to look for esters of disaccharides, which had properties that were a compromise between those of the very water-soluble maltosides and of other, less soluble compounds such as galactosides and glucosides, or the insoluble and difficultly dispersible galactose and glucose monoesters.

Fatty acid esters of sucrose, especially the mono- and diesters, are well known for their surfactant properties. They are used as biodegradable emulsifying agents in the food and cosmetic industries (13–14). They also play a role in biochemical research as detergents for the isolation and purification of membrane proteins (15–16). α,α-Trehalose esters, especially the diesters, also possess important biological properties and have been investigated as immunostimulants (17). We therefore undertook a study of the emulsifying properties and biocompatibility of series of newly synthesized perfluoroalkylated monoesters of α,α-trehalose and sucrose (18) (Fig. 1). A variable-size hydrophobic tail was devised, which consists of variable-length fluorinated chains and hydrocarbon spacers. This combination allows the stepwise variation of the solubility and the adjustment of the hydrophilic-lipophilic-fluorophilic character of such amphiphilic molecules.

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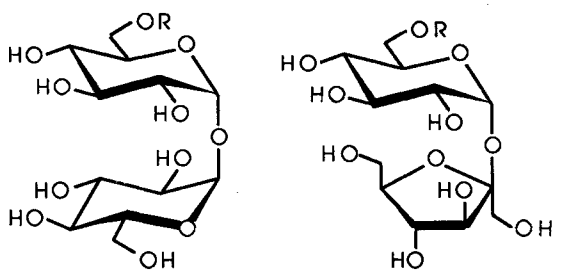

1 2

$R = R_F(CH_2)_nC(O)$

| Compound | a | b | c | d | e |
|---|---|---|---|---|---|
| n | 2 | 4 | 4 | 10 | 10 |
| $R_F$ | $C_8F_{17}$ | $C_6F_{13}$ | $C_8F_{17}$ | $C_4F_9$ | $C_6F_{13}$ |

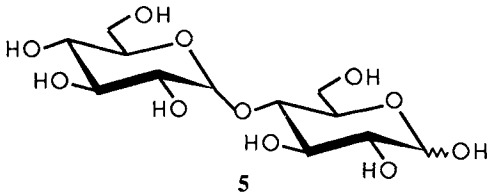

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**FIG. 1. Structures of trehalose (1) and sucrose (2), perfluoroalkylated fatty acid monoesters, and of maltose (5).**

## EXPERIMENTAL PROCEDURES

*Materials.* The perfluoroalkylated surfactants (FS), $\alpha,\alpha$-trehalose and sucrose fatty acid monoesters, and their hydrocarbon analogues used for this study were prepared by means of the Mitsunobu reaction (19). A solution of a linear perfluoroalkylated acid $R_F(CH_2)_nCO_2H$ ($R_F$ = $C_4F_9$, $C_6F_{13}$, $C_8F_{17}$; n = 2, 4, 10), palmitic or undecanoic acid (1 equiv.) and diisopropylazodicarboxylate (2 equiv.) in *N,N*-dimethylformamide (15 mL) was added at 0°C in a one-step procedure to a solution of $\alpha,\alpha$-trehalose or sucrose (3 equiv.) and triphenylphosphine (2 equiv.) in anhydrous *N,N*-dimethylformamide (15 mL). After three days with stirring at room temperature, the reaction mixture (after treatment and purification by chromatography) afforded the 6-esters of $\alpha,\alpha$-trehalose, 1, and sucrose, 2 (Fig. 1) in *ca.* 40% and 25% yields, respectively (18). The batches tested displayed a better than 99% grade of purity high-performance liquid chromatography (HPLC). Pluronic F-68 and perfluorodecalin (FDC, Flutec® PP5) were purchased from Serva Feinbiochemica (Heidelberg, NY) and Imperial Smelting Chemicals Ltd. (Avonsmouth, U.K.), respectively. Perfluorooctylbromide (PFOB) was a gift from Atochem (Pierre-Benite, France).

*General methods.* Surface tension and interfacial tension measurements on aqueous solutions, at 20 ± 0.2°C, were carried out on an automatic Lauda tensiometer based on the Lecomte du Noüy concept, with a rigid platinum ring. Samples were given 12 hr to reach equilibrium before measurements were made. Critical micellar concentrations (CMC) were determined by plotting surface tensions as a function of the logarithm of the surfactant concentration. Dispersions and emulsions were prepared by pulsed-mode sonication (Model B-30, 350 W, 20 kHz, power dial 6, 13 mm-diameter titanum probe, Branson Ultrasonic Co., Danbury, CT) in an ice-cooled rosette cell. The average particle size and size distribution of the emulsions were assessed with Horiba CAPA 500 or 700 particle analyzers (Horiba Instruments, Tokyo, Japan) based on light absorption measurements during centrifugal sedimentation. For the study of emulsion aging, 15-mL samples of emulsions were prepared. Each batch was divided into 4- to 5-mL aliquots, which were stored in 75 × 12 mm glass tubes at 4, 25 and 50°C ± 1°C. The perfluoroalkylated fatty acid esters and some of their hydrocarbon analogues were tested *in vitro* (toxicity on cell cultures and hemolysis) and *in vivo* (intravenous injections in mice of solutions or dispersions in 0.9% NaCl water) as described by Zarif *et al.* (11).

## RESULTS AND DISCUSSION

*Physicochemical properties: Solubility in water.* The solubility and behavior of the fluorinated surfactants (FS) 1 and 2 in water depend on three parameters: i) the structure of the hydrophilic head; ii) the number of carbons in the hydrocarbon spacer; and iii) the number of carbons in the perfluorinated terminal group. It was found that:

i) For a given hydrophobic tail, the FS derived from sucrose are more soluble than their homologues derived from $\alpha,\alpha$-trehalose, thus, for example, for the $C_6F_{13}(CH_2)_{10}$ chain, the solubility of the monoester of $\alpha,\alpha$-trehalose, **1e**, is about 1 g/L, while that of the sucrose ester, **2e**, is larger than 10 g/L. This reflects the large difference of solubility that exists between the two hydrophilic heads, trehalose and sucrose.

ii) The solubility of the FS decreases sharply when the length of the hydrocarbon spacer increases. Thus, for the same hydrophilic head ($\alpha,\alpha$-trehalose), **1b**, with a $C_6F_{13}(CH_2)_4$ chain, is soluble at 50 g/L, while the solubility of **1e** with a $C_6F_{13}(CH_2)_{10}$ chain is about 1 g/L.

iii) Within a given series of FS and for a given total number of the carbons in the acyl chain, the behavior depends on the length of the perfluorinated fragment. For example, while at 10 g/L, **1a**, [with a $C_8F_{17}(CH_2)_2$ chain], gives a gel at 50 g/L, **1b** [with a $C_6F_{13}(CH_2)_4$ chain], and the hydrocarbon analogue (6-*O*-undecanoyl-$\alpha,\alpha$-trehalose) **3**, give syrupy and flowing solutions, respectively. These macroscopic observations (20) indicate that changes in molecular aggregation in aqueous solution depend on the presence and length of the fluorinated tail.

*Dispersibility.* The aqueous solutions of certain FS cannot be injected intravenously in mice because their viscosity is too high. We found that this viscosity can be lowered by dispersing them by sonication in solutions of Pluronic F-68. Significant amounts of Pluronic F-68 may be used in view of its high $LD_{50}$ of about 7.7 g/kg (21).

*Thermal stability.* One requirement for the FS to be useful for biomedical applications is that their emulsions and solutions can be sterilized. The stability of their solutions was therefore checked under FDA sterilization standards. HPLC analysis has shown no detectable change in

**TABLE 1**

**Surface Activity of the Trehalose and Sucrose Perfluoroalkylated Fatty Acid Monoesters**

| Compound [MW (g)] | Surface activity | Concentrations (g/L) 1 | 0.5 | 0.1 | 0.01 | 0.001 | 0.0001 | CMC (mM) |
|---|---|---|---|---|---|---|---|---|
| **1a** | $\gamma_s$ | 21.7 | — | 21.6 | 21.6 | 40.4 | 51.8 | 0.007 |
| (816.4) | $\gamma_i$/FDC | 2.7 | — | 4.2 | 5.6 | 30.8 | 50.5 | |
| | $\gamma_i$/PFOB | 5.9 | — | 6.2 | 9.4 | 29.3 | 42.2 | |
| **1b** | $\gamma_s$ | — | 21.5 | 21.6 | 37.6 | 53.1 | 62.9 | 0.10 |
| (744.5) | $\gamma_i$/FDC | — | — | 6.3 | 21.4 | 39.5 | 49.1 | |
| | $\gamma_i$/PFOB | — | — | 5.4 | 21.3 | 36.2 | 48.7 | |
| **1c** | $\gamma_s$ | — | 20.9 | 21.4 | 22.6 | 33.8 | 61.1 | 0.004 |
| (844.5) | $\gamma_i$/FDC | — | — | 3.8 | 7.1 | 40.6 | 53.5 | |
| | $\gamma_i$/PFOB | — | — | 3.5 | 6.4 | 28.3 | 41.5 | |
| **1d** | $\gamma_s$ | 25.3 | 25.3 | 25.3 | 24.4 | 40.4 | 61.0 | 0.0075 |
| (728.6) | $\gamma_i$/FDC | 3.9 | — | 5.1 | 21.4 | 49.7 | 50.7 | |
| | $\gamma_i$/PFOB | 2.9 | — | 3.9 | 18.6 | 43.2 | 45.5 | |
| **1e** | $\gamma_s$ | — | 24.4 | 24.5 | 25.1 | 36.3 | 60.8 | 0.007 |
| (828.6) | $\gamma_i$/FDC | 3.0 | — | 3.6 | 4.3 | 16.2 | 46.5 | |
| | $\gamma_i$/PFOB | 2.9 | — | 3.5 | 3.7 | 13.4 | 40.5 | |
| **2a** | $\gamma_s$ | 23.5 | 23.5 | 23.4 | 23.4 | 45.4 | 64.6 | 0.010 |
| (816.4) | $\gamma_i$/FDC | 4.0 | — | 4.7 | 6.6 | 38.3 | 54.6 | |
| | $\gamma_i$/PFOB | 3.2 | — | 4.2 | 5.6 | 36.9 | 50.7 | |
| **2d** | $\gamma_s$ | 24.3 | 24.3 | 24.2 | 23.8 | 41.4 | 56.8 | 0.008 |
| (728.6) | $\gamma_i$/FDC | 3.9 | — | 4.8 | 13.1 | 42.2 | 45.5 | |
| | $\gamma_i$/PFOB | 2.9 | — | 3.7 | 6.0 | 40.9 | 44.7 | |
| **2e** | $\gamma_s$ | 24.6 | 24.5 | 24.6 | 24.9 | 32.9 | 50.5 | 0.008 |
| (828.6) | $\gamma_i$/FDC | 2.9 | — | 6.5 | 11.4 | 40.9 | 43.9 | |
| | $\gamma_i$/PFOB | 2.6 | — | 2.7 | 3.7 | 33.8 | 42.2 | |
| **3**[a] | $\gamma_s$ | 38.3 | 38.4 | 43.3 | 59.2 | 56.4 | — | 0.39 |
| (510.6) | $\gamma_i$/FDC | 15.8 | — | 27.2 | 50.5 | 50.1 | — | |
| | $\gamma_i$/PFOB | 13.6 | — | 26.5 | 43.4 | 45.3 | — | |
| **4**[b] | $\gamma_s$ | — | 34.9 | 35.4 | 35.9 | 43.9 | 63.8 | |
| (580.7) | $\gamma_i$/FDC | — | 15.3 | 15.7 | 16.9 | 34.4 | 53.2 | |
| | $\gamma_i$/PFOB | — | 5.1 | 5.6 | 6.1 | 17.4 | 30.9 | |

$\gamma_s$, $\gamma_i$/FDC, $\gamma_i$/PFOB measured in $mNm^{-1}$ ($\pm 0.3$) at $20 \pm 0.1°C$.
$\gamma_s(H_2O) = 73$ $mNm^{-1}$, $\gamma_i(H_2O/FDC) = 56$ $mNm^{-1}$, $\gamma_i(H_2O/PFOB) = 51$ $mNm^{-1}$.
[a]6-*O*-Undecanoyl-$\alpha,\alpha$-trehalose, a hydrocarbon analogue of **1a** and **1b**.
[b]6-*O*-Palmitoylsucrose.

aqueous solutions (1 g/L) of these surfactants after heating them at 121°C for six hours.

*Surface activity and CMC.* Surface tension measurements at various concentrations on aqueous solutions of **1a–e** and **2a,d,e** are summarized in Table 1, and compared to those measured for their hydrocarbon analogues **3** and 6-*O*-palmitoyl-sucrose, **4**. A considerable decrease of the surface tension ($\gamma_s$), of water, down to 21–25 $mNm^{-1}$, and of the interfacial tension ($\gamma_i$), between water and fluorocarbons (FDC and PFOB), down to 2.5–6 $mNm^{-1}$, has been observed.

The performance of a surfactant is appropriately defined by its efficiency and effectiveness (22). The curves obtained by plotting surface tension against the logarithm of concentration for homologous $\alpha,\alpha$-trehalose fatty acid monoesters (Fig. 2) show that efficiency (concentration of surfactant required to produce a given surface effect) increases when an increasing portion of the hydrocarbon tail is replaced by a fluorinated chain. Thus, for example, the molar concentration required to attain the same surface tension of 38 $mNm^{-1}$ is almost 400 times smaller for **1a** [with a $C_8F_{17}(CH_2)_2$ chain], than for **3** (which is not fluorinated). For a given acyl tail length, the larger the number of fluorine atoms, the more efficient is the FS. Compound **1a** is 10 times more efficient than **1b** [with a $C_6F_{13}(CH_2)_4$ chain]. Likewise, Figure 2 shows clearly that the FS **1a** and **1b** display very strong effectiveness (the maximum surface effect that the surfactant can produce, regardless of the amount used), compared to their hydrocarbon analogue **3**.

Table 1 shows that the critical micellar concentration (CMC) of homologous series of FS decreases as the hydrophobic chainlength increases. It also shows that the CMC of $\alpha,\alpha$-trehalose monoesters with comparable acyl chainlength decreases when the number of hydrogen atoms substituted by fluorine atoms increases. Thus, **1a**, **1b** and **3** possess CMCs values of 0.007, 0.1 and 0.39 mM, respectively.

*Emulsification of fluorocarbons.* It is desirable that intravenously injectable perfluorocarbon emulsions used as oxygen carriers be prepared from pure and rapidly excreted fluorocarbons and surfactants. To meet practical requirements, Fluosol 20% consists of a mixture of two different fluorocarbons, perfluorodecalin (FDC) and perfluorotripropylamine (FTPA), in 70:30 ratio. This formulation allows for a compromise between the retention

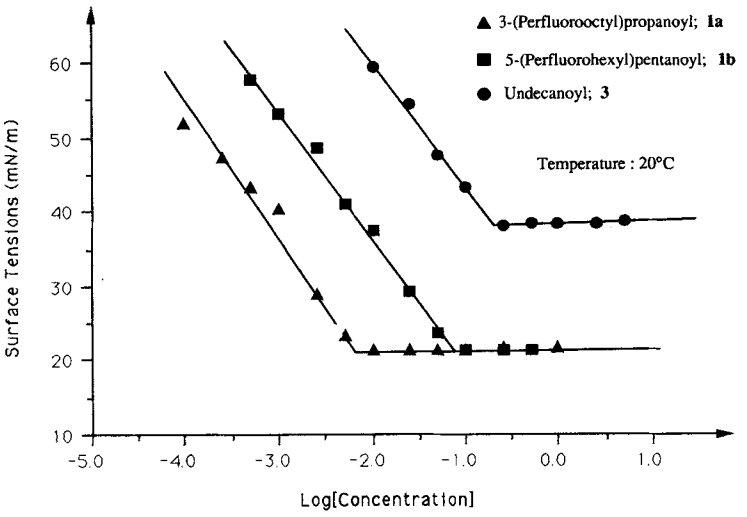


**FIG. 2. Surface tensions *vs.* logarithm of concentration, in g/L, for aqueous solutions of the trehalose fatty acid monoesters.**

of the fluorocarbons in the organs and the stability of their emulsion (2). FTPA gives relatively stable emulsions, but its retention half-time in the organs ($T_{1/2}$) is very long (~65 days). On the other hand, FDC, which has a $T_{1/2}$ of about seven days, gives rather unstable emulsions. One of our goals was to circumvent the disadvantages of such formulations by achieving stable emulsions with only the most biologically acceptable perfluorocarbon. Another goal was to prepare more concentrated emulsions (50% w/v and more) which consequently have higher $O_2$-delivering capacity. We therefore investigated the emulsifying and stabilizing properties of our FS both as sole surfactants and as co-surfactants in association with Pluronic F-68 for the preparation of 50% w/v FDC emulsions.

*Emulsifying properties.* Our efforts to dispense totally with Pluronic F-68 in the formulation previously had been unsuccessful with perfluoroalkylated surfactants derived from galactose, glucose, maltose and related polyols (7–11,23).

The new FS (**1a–e, 2e**) were tested as sole surfactants in a 50% w/v FDC, 5% w/v surfactant formulation. 6-*O*-[3′-(perfluorooctyl) propanoyl]-$\alpha,\alpha$-trehalose, **1a**, showed excellent emulsifying and stabilizing properties while none of the others allowed the formation of any emulsion. This major difference is particularly remarkable in view of the close structural relationships that exist among the series of surfactants tested. The amount of surfactant **1a** in the formulation was then reduced to 3% w/v, which was sufficient to stabilize a 50% w/v FDC emulsion significantly with respect to a reference emulsion prepared with the same amount of Pluronic F-68. Figure 3 illustrates this stabilizing effect. It shows the evolution of the particle sizes as a function of time at 4, 25 and 50°C in these emulsions. It is outstanding that after two months of storage no significant change in the average particle size was observed for the fluorinated surfactant-containing emulsion, whether at 4, at 25 or at 50°C, in comparison with the reference emulsion.

It should be reemphasized that the closely related maltose-derived surfactant 5 with the same hydrophobic-fluorophilic tail [$C_8F_{17}(CH_2)_2$ in position 1], when taken alone, did not permit the preparation of stable emulsions (7,23). This may be related to this surfactant's high CMC (0.18 mM) compared to that of the ester **1a** (0.007 mM). Moreover, the structure of the hydrophilic head of **1a** could favor the organization of this surfactant around the fluorocarbon droplets; a relative lack of flexibility of the $\alpha,\alpha$-trehalose molecule in aqueous solution has recently been reported (24). However, in spite of its low CMC (0.004 mM), the trehalose ester **1c** [with the $C_8F_{17}(CH_2)_4C(O)$ acyl group] did not give stable emulsions.

*Co-surfactant effect with Pluronic F-68.* We have previously shown that, with respect to a 50% w/v FDC, 5% w/v Pluronic F-68 reference emulsion, maximum stabilization could be gained when 30 to 50% of the Pluronic F-68 was replaced by a perfluoroalkylated polyhydroxylated surfactant derived from maltose of xylitol (7). It has also been established that the volume, v, of the droplets in the fluorocarbon/Pluronic F-68 emulsions increases linearly with time (25). The linearity of $v = f(t)$ is lost and a deviation towards more stable emulsions was observed when Pluronic F-68 was progressively replaced by a perfluoroalkylated polyhydroxylated surfactant (23). The stabilizing effect on the latter fluorocarbon emulsions was expressed as the ratio (R) between the slopes of straight lines drawn for the reference emulsion and that obtained by joining the $d_0^3$ and $d_D^3$ for the stabilized test emulsion.

$$R = (d_D^{Ref})^3 - (d_0^{Ref})^3/(d_D^{Test})^3 - (d_0^{Test})^3$$

where $d_0^{Ref}$ and $d_D^{Ref}$ are the average diameters of the particles in the reference emulsion at 0 and D days, respectively, and $d_0^{Test}$ and $d_D^{Test}$ are the same parameters for the test emulsion.

This formula was applied to the emulsions prepared with the FS *vs.* the reference emulsion after 60 days; the

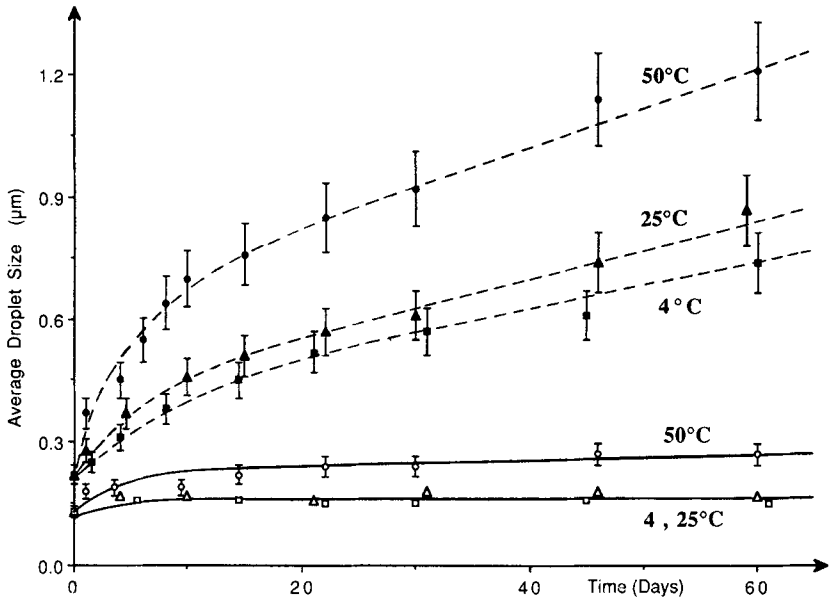


**FIG. 3. Stabilization at 4, 25 and 50°C of 50% w/v perfluorodecalin emulsions prepared with 3% w/v of 6-*O*-[3'-(perfluorooctyl) propanoyl]-α,α-trehalose 1a (full lines), and with 3% (w/v) of Pluronic F-68 (dashed lines).**

**TABLE 2**

**Stabilizing Effect of the Trehalose and Sucrose Perfluoroalkylated Fatty Acid Monoesters on a 50% w/v Perfluorodecalin/5% w/v Pluronic F-68 Emulsion**

| | Average droplet size (± 10%, μm) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 4°C | | 25°C | | 50°C | | $S_{60}$[b] | | |
| Compound | $D_0$[a] | $D_{30}$ | $D_{60}$ | $D_{30}$ | $D_{60}$ | $D_{30}$ | $D_{60}$ | 4°C | 25°C | 50°C |
| **1a** | 0.14 | 0.16 | 0.18 | 0.26 | 0.30 | 0.52 | 0.70 | 267 | 92 | 15 |
| **1b** | 0.17 | 0.21 | 0.20 | 0.36 | 0.47 | 0.81 | 1.09 | 267 | 23 | 4 |
| **1c** | 0.18 | 0.19 | 0.19 | 0.24 | 0.24 | 0.47 | 0.61 | 804 | 280 | 24 |
| **1d** | 0.15 | 0.19 | 0.21 | 0.32 | 0.41 | 0.90 | 1.13 | 140 | 34 | 4 |
| **1e** | 0.19 | 0.20 | 0.21 | 0.21 | 0.26 | 0.43 | 0.79 | 344 | 209 | 11 |
| **2e** | 0.20 | 0.24 | 0.25 | 0.33 | 0.36 | 0.63 | 0.84 | 108 | 58 | 9 |
| **3**[a] | 0.16 | 0.29 | 0.34 | 0.52 | 0.62 | 1.30 | 1.60 | 23 | 10 | 1 |
| Pluronic F-68 | 0.16 | 0.70 | 0.94 | 0.91 | 1.31 | 1.32 | 1.74 | 1 | 1 | 1 |

[a]$D_x$, average droplet size after x days.
[b]Stabilizing effect after 60 days.
[c]6-*O*-Undecanoyl-α,α-trehalose, a hydrocarbon analogue of **1a** and **1b**.

results are summarized in Table 2. They show that for a given hydrophilic head and a given length of the hydrophobic chain, the stability R of the emulsion increases when the length of the fluorinated tail increases in the acyl chain. At 4 and 25°C, the FS derived from α,α-trehalose, **1a** and **1b**, lead to more stable emulsions (Fig. 4a and 4b) as compared to their analogue **3** (6-*O*-undecanoyl-α,α-trehalose). The R parameter for the emulsions prepared with **1a** or **1b** are 10 times higher than that for the emulsion prepared with **3**. The difference is more marked at 50°C (Fig. 4c); in this case the stability of the emulsion prepared with the hydrocarbon co-surfactant **3** is comparable to that of the reference. This clearly illustrates the advantage of having a fluorinated chain on the hydrophobic tail of the surfactant for stabilizing fluorocarbon emulsions. Moreover, for the trehalose fatty acid monoester family the stability ratio (R) increases when the length of the hydrocarbon spacer increases and when the CMC decreases. Thus, at 4 and 25°C, the $C_8F_{17}(CH_2)_4$ ester (**1c**, CMC = 0.004 mM) stabilizes the emulsion three times more than the $C_8F_{17}(CH_2)_2$ ester (**1a**, CMC = 0.007 mM). One can also observe that for the $C_6F_{13}(CH_2)_{10}$ acyl chain, the trehalose ester is a more efficient co-surfactant than the sucrose ester.

*Preliminary biological studies.* The fluorinated surfactants investigated here are intended for use in the formulation of intravenously injectable emulsions. This requires that they be biologically acceptable. To determine biological compatibility, several preliminary biological tests have been undertaken on these compounds.

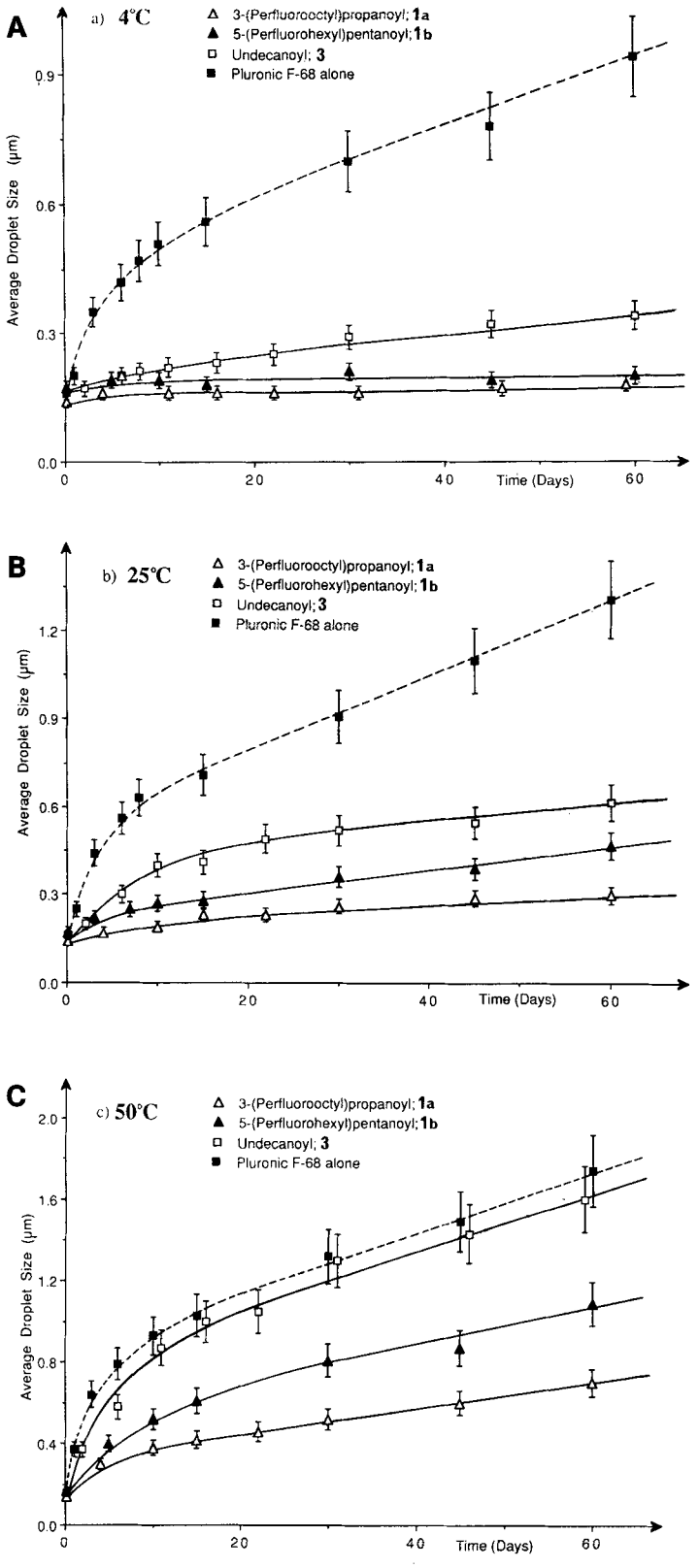


**FIG. 4. a: Influence of the perfluoroalkylated chainlength of homologous series of trehalose monoesters on the stabilization of a 50% w/v FDC emulsion. Aging at 4°C of emulsions prepared with 2.5% w/v of Pluronic F-68 and 2.5% w/v of the trehalose esters (full lines); and with 5% w/v of Pluronic F-68 alone (dashed lines). b: Aging at 25°C. c: Aging at 50°C.**

**TABLE 3**

**Toxicity of the Trehalose and Sucrose Perfluoroalkylated Fatty Acid Monoesters Toward Namalva Lymphoblastoid Cell Cultures**

| Compound | Concentration (g/L) | Results (% *vs.* control) growth/viability |
|---|---|---|
| **1a** | 1 | 33/41 |
| | 0.1 | 99/97 |
| **1b** | 1 | 93/103 |
| | 0.1 | 108/101 |
| **1c** | 1 | 36/44 |
| | 0.1 | 108/105 |
| **1d** | 1 | *b* |
| | 0.1 | 100/103 |
| **1e** | 1 | 70/87 |
| | 0.1 | 95/106 |
| **2e** | 1 | *b* |
| | 0.1 | 101/101 |
| **3**[a] | 1 | *b* |
| | 0.1 | 78/94 |

[a]6-*O*-Undecanoyl-α,α-trehalose, a hydrocarbon analogue of **1a** and **1b**.
[b]Cells died.

In vitro *tests*. The *in vitro* cell culture test used is particularly sensitive. The Namalva lymphoblastoid cells were chosen because of the stability of their strain and of the reproducibility of their growth parameters (26). In this test, the growth and viability of the cells in the presence of the compound tested were compared to those of control cells grown under the same conditions. The FS have been tested in physiological water solution (0.9% NaCl) at 1 and 0.1 g/L concentrations. The results summarized in Table 3 show that none of the FS caused any significant inhibition of cell growth and viability at 0.1 g/L compared to controls, in spite of their considerable surface activity. No significant toxicity has been found either with compounds **1b** and **1e** at 1 g/L concentrations.

No hemolytic activity on a suspension of human red blood cells in an isotonic 0.9% NaCl solution was detected for solutions of **1b**, even in the high 10–50 g/L concentration range, while its hydrocarbon analogue is already strongly hemolytic at 1 g/L. Dispersions of **1a** and **1c** in an 8-g/L solution of Pluronic F-68 showed no hemolytic activity at 30- and 20-g/L concentrations, respectively. It should be noted that the most surface-active compounds are the least hemolytic. Analysis of Table 4 allows several observations concerning the relationship between hemolytic activity and the structure of the FS: i) Hemolytic activity decreases sharply when the length of the perfluoroalkylated tail increases; ii) Hemolytic activity increases with the length of the hydrocarbon spacer. Thus **1b**, with a $(CH_2)_4$ spacer, is non-hemolytic at 50 g/L while **1e**, with a $(CH_2)_{10}$ spacer, is highly hemolytic at 0.1 g/L. These two observations are in line with previous observations made with other perfluoroalkylated surfactants (27) and iii) In addition, when the more hemolytic compounds with the longer hydrocarbon spacers are examined, 0.1-g/L solutions of the FS derived from sucrose, **2d** and **2e**, are less hemolytic than those derived from trehalose, **1d** and **1e**.

TABLE 4

**Hemolytic Activity of the Trehalose and Sucrose Perfluoroalkylated Fatty Acid Monoesters**

| Compound | Concentration | | Hemolysis | |
|---|---|---|---|---|
| | g/L | mM | Visual | %[a] *vs.* Control |
| **1a** | 30/8[b] | 36.7 | 0 | 0 |
| **1b** | 50 | 67.1 | 0 | 0 |
| **1c** | 20/8[b] | 23.7 | 0 | 0 |
| **1d** | 0.1 | 0.14 | ++++ | 128 |
| | 0.01 | 0.014 | + | 11 |
| | 0.005 | 0.0068 | 0 | 0 |
| **1e** | 0.1 | 0.12 | ++++ | 128 |
| | 0.01 | 0.012 | + | 10 |
| | 0.005 | 0.006 | 0 | 0 |
| **2d** | 1 | 1.37 | ++++ | 117 |
| | 0.1 | 0.14 | 0 | 0 |
| **2e** | 1 | 1.2 | ++++ | 117 |
| | 0.5 | 0.6 | 0 | 0 |
| | 0.1 | 0.12 | 0 | 0 |
| **3**[c] | 1 | 1.96 | ++++ | 146 |
| | 0.1 | 0.19 | 0 | 0 |
| NaCl 0.9% | control(−) | | 0 | 0 |
| $H_2O$ | control(+) | | ++++ | 100 |

[a]Hemolysis % = $100(OD_{test}-OD_{NaCl})/(OD_{H_2O}-OD_{NaCl})$. Optical densities were measured at 540 nm.
[b]A/B: Dispersion of A g/L of the compound in B g/L of Pluronic F-68 in water.
[c]6-*O*-undecanoyl-α,α-trehalose hydrocarbon analogue of **1a** and **1b**.

TABLE 5

**Toxicity Tests *i.v.* in Mice of the Trehalose and Sucrose Perfluoroalkylated Fatty Acid Monoesters**

| Compound | Concentration (g/L) | Injected dose (mg/kg) | Survival ratio | $LD_{50}$ (mg/kg) |
|---|---|---|---|---|
| **1a** | 10/4[a] | 250 | 10/10 | 250–375 |
| | 15/6[a] | 375 | 2/10 | |
| **1b** | 5 | 125 | 10/10 | 250–375 |
| | 10 | 250 | 9/10 | |
| | 15 | 375 | 0/5 | |
| **1c**[b] | 5 | 125 | 10/10 | >250 |
| | 10/4[a] | 250 | 10/10 | |
| **1d** | 10/4[a] | 250 | 10/10 | ~375 |
| | 15/6[a] | 375 | 6/10 | |
| **1e** | 1 | 25 | 10/10 | >125 |
| | 5/2[a] | 125 | 10/10 | |
| **2e** | 1 | 25 | 10/10 | 250–375 |
| | 10/4[a] | 250 | 10/10 | |
| | 15/6[a] | 375 | 2/10 | |
| | 20/8[a] | 500 | 2/10 | |
| **3**[b] | 5 | 125 | 6/10 | ~125 |

[a]A/B: Dispersion of A g/L of the compound in B g/L of Pluronic F-68 in water.
[b]6-*O*-undecanoyl-α,α-trehalose, a hydrocarbon analogue of **1a** and **1b**.

In vivo *tests*. The intravenous injection (tail vein) of 0.5 mL of solutions or dispersions of the FS in saline were performed in mice. Death and any other symptoms were recorded; growth of the animals was compared to that of control animals who received only saline. The results, collected in Table 5, show that none of the FS tested caused death or perturbed the normal growth of the mice at doses of 250 mg/kg of body weight. An $LD_{50}$ of 250–375 mg/kg was found for **1a** and **1b**, while compound **3**, their nonfluorinated analogue, was more toxic with an $LD_{50}$ around 125 mg/kg.

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