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Perfluoroalkylated telomers derived from tris(hydroxymethyl)acrylamidomethane as surfactants and co-surfactants in fluorocarbon emulsions

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Abstract

The ability of perfluoroalkylated telomers derived from tris(hydroxymethyl)acrylamidomethane (TAC) to stabilize fluorocarbon emulsions has been investigated. For this purpose, 50% w/v emulsions of perfluorodecalin (FDC) and perfluoro-octyl bromide (PFOB) were prepared with a total 3% w/v of surfactant and were compared with emulsions prepared with Pluronic F-68® or egg yolk phospholipids (EYP). When used as the sole surfactant, telomers **1** (TAC with a C_6F_{13} chain and a number average degree of polymerization $n \sim 6$) and **2** (C_8F_{17}TAC, $n \sim 6$) produced FDC emulsions that were more stable than with Pluronic F-68 alone; when compared to EYP no improvement was found. When associated to other, less hydrophilic perfluoroalkylated surfactants, such as the monoadduct **3** (C_6F_{13}TAC, $n=1$) or [1-*O*-(perfluoro-octyl)-2′-propenyl]xylitol (**4**), for certain formulations these telomers resulted in somewhat enhanced stabilization of both FDC and PFOB emulsions. In some cases, the emulsions were as stable as those prepared with EYP alone. When telomer **1** was used as a co-emulsifier with EYP, no noticeable stabilization was observed; with Pluronic F-68 emulsion stability was reduced.

Keywords: Perfluoroalkylated telomers; Tris(hydroxymethyl)acrylamidomethane; Surfactants; Co-surfactants; Fluorocarbon emulsions; Stability

1. Introduction

The range of potential therapeutic applications of fluorocarbons and other highly fluorinated materials is expanding from temporary blood substitutes to drug delivery systems and contrast agents for diagnosis [1,2]. The problem of the shelf-life stability of fluorocarbon emulsions has been extensively investigated [3,4]. One way of stabilizing such emulsions consists in adding to the fluorocarbon phase a small quantity of a higher boiling lipophilic fluorocarbon [5], or a so-called 'dowel' molecule, i.e. a mixed fluorocarbon/hydrocarbon compound such as $C_nF_{2n+1}C_mH_{2m+1}$ or $C_nF_{2n+1}CH{=}CHC_mH_{2m+1}$ [4,6,7]. However, improved mastery of other characteristics and properties of fluorocarbon emulsions, such as particle size, size distribution, surface charge, in vivo recognition, intravascular persistence, response to phagocytosis and biodistribution, remains an important challenge [2]. The nature and characteristics of the surfactant film are crucial in this respect. The surfactant also determines the characteristics of other colloidal systems such as liposomes destined to drug targetting and delivery [2]. As yet very few commercial surfactants are deemed acceptable for large-dose injectable preparations. They include essentially natural egg yolk phospholipids (EYP) and Pluronic F-68 (a synthetic poloxamer), both of which are present in the first injectable fluorocarbon emulsion, Fluosol® (Green Cross Corp., Osaka, Japan), to have been approved by the United States Food and Drug Administration for human use [1]. EYP is also a component of Oxygent™ (Alliance Pharm. Corp., San Diego, USA), a recently developed emulsion with higher shelf stability and improved O_2-delivery efficacy [1,8]. Nevertheless, these surfactants have their drawbacks and limitations [1,9].

This situation led us to design and synthesize a range of new surfactants fitted with a perfluoroalkylated tail in order to increase their fluorophilicity and facilitate the dispersion of fluorocarbons in water [10–12]. Their

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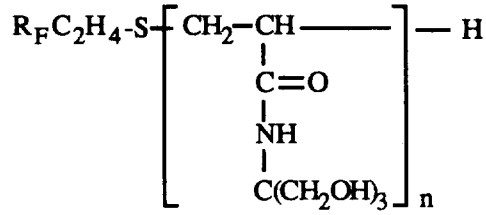

1: $R_F = C_6F_{13}$, n≃6

2: $R_F = C_8F_{17}$, n≃6

3: $R_F = C_6F_{13}$, n=1

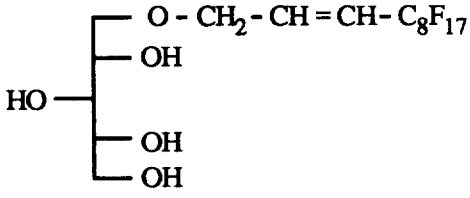

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Fig. 1. Structure of perfluoroalkylated surfactants derived from tris(hydroxymethyl)acrylamidomethane (**1**, **2**, **3**) and xylitol (**4**).

hydrophilic head was generally derived from natural products such as sugars, polyols, aminoacids, phosphocholines and analogues of phospholipids. A series of telomeric perfluoroalkylated amphiphiles was also developed [13] whose polar heads are composed of repeated tris(hydroxymethyl)acrylamidomethane moieties (compounds **1** and **2**, Fig. 1). These compounds showed promising preliminary biocompatibility data including absence of hemolytic activity and intravenous LD_{50} values in mice larger than 4 g kg^{-1} body weight [14].

These amphiphiles are now tested as emulsifiers of perfluorodecalin (FDC) and perfluoro-octyl bromide (PFOB, perflubron). Their use as co-emulsifiers with Pluronic F-68 or EYP or other perfluoroalkylated surfactants has also been investigated since it is known that mixtures of surfactants often lead to more stable and finer emulsions than those obtained with a single surfactant [15].

2. Experimental details

Perfluorodecalin and perfluoro-octyl bromide were supplied by Imperial Smelting Chemicals Ltd. (Avonmouth, UK), and Hoechst (Corpus Christi, USA) respectively; EYP came from Lipoïd KG (Frankfurt, Germany), Pluronic F-68 was supplied by Serva Feinbiochemica (Heidelberg, Germany) and the Dubelcco phosphate buffer solution (PBS) by Gibco BRL (Cergy Pontoise, France).

Dispersions and emulsions were prepared by sonication (50% pulse mode: 0.5 s per s-cycle) with a Branson B-30 cell disruptor, at a frequency of 20 kHz, at a medium power of 5 (on a scale of 10 corresponding to 350 W) with a titanium probe (13 mm) placed at the water/fluorocarbon interface. Average droplet sizes and droplet size distributions were measured with a Horiba Capa-700 automatic particle size analyzer.

Concentrated emulsions formulated with 50% by weight of fluorocarbon and 3% of surfactant in aqueous PBS (2.16 g l^{-1} of Na_2HPO_4) were prepared by sonication for 7 min (dial 5). For the water-insoluble surfactants, pre-dispersion of the surfactant in the aqueous phase was carried out by sonication (1 min for EYP, power at dial 7; 2 min, dial 5 for **3** and **4**) prior to the addition of the fluorocarbon. Great care was taken to apply the sonication procedure in as similar and reproducible a way as possible (same rosette cell, volumes of water and fluorocarbon, location of the probe, power and duration).

Samples of the emulsions (15 ml) were prepared, then sterilized by heating for 15 min at 121 °C (or 102 °C for preparations containing Pluronic F-68) and stored at 40 °C in an incubator.

3. Results and discussion

3.1. Methodology

Sonication was used as a means of determining the emulsification efficiency of the new surfactants because it requires only small quantities of ingredients. The stability of the emulsions was followed by monitoring the evolution of the droplet's sizes for four months at 40 °C. This temperature was chosen in order to accelerate the aging process and hence shorten the time needed for evaluating the stability of the emulsions. The stability of the emulsions investigated was compared with that of reference emulsions prepared with Pluronic F-68 or EYP.

To facilitate the study, two parameters were introduced: I, the ratio of the emulsion's mean particle size (MPS) increase at day D to the initial MPS measured after sterilization, and R, the ratio of the test emulsion's MPS increase to the MPS increase of the reference emulsion. Both were expressed as percentages [16]:

$$I = 100[(d_D - d_0)/d_0]$$

$$R = 100[(d_D - d_0)/(d'_D - d'_0) - 1]$$

where d_D and d_0 represent the mean diameters of the emulsions tested at day D and day zero (after sterilization) respectively, and d'_D and d'_0 the mean diameters of the reference emulsion at day D and day zero (after sterilization).

The telomeric compounds **1** and **2** (Fig. 1) with perfluorohexyl and perfluoro-octyl tails, respectively, and similar degrees of polymerization ($DP_n \sim 6$), were selected for this study. Compound **1** was also investigated as a co-emulsifier with Pluronic F-68 and with EYP. These admixtures were designed as a means of introducing the fluorophilic character which is missing in hydrocarbon surfactants. Compound **1** and **2** were also investigated in association with two other, less hydrophilic perfluoroalkylated surfactants, **3** and **4** (Fig. 1). Compound **3**, a perfluorohexyl-tailed monoadduct compounds ($n = 1$) belongs to the same family as **1** and **2**. Compound **4**, (1-*O*-[3-(perfluoro-octyl)-2-propenyl]xylitol), is xylitol-derived surfactant previously developed in our laboratory [16,17]. Surfactants **3** and **4**, which are water-insoluble, were chosen since a mixture of highly water-soluble and low water-soluble surfactants has often proved to be effective [15–17]. When mixtures of surfactants were used, two surfactant compositions (2.5/0.5 and 2/1% w/v) were tested for each combination.

FDC was chosen because it does not give stable emulsions easily and PFOB because it stands out as one of the most promising fluorocarbons for medical application [1,2]. Average emulsion particle sizes are collected in Table 1, and curves representative of the evolution of the mean diameter vs. time are displayed in Fig. 2.

3.2. Stabilization effect of telomers when used as sole surfactant

The ability of telomers **1** and **2** to emulsify FDC and PFOB was compared with that of the commercial surfactants EYP and Pluronic F-68. Both telomers allowed the preparation of fluorocarbon emulsions. For a given telomer the emulsions prepared with PFOB had smaller average particle sizes than those prepared with FDC, but the rate of enlargement of the particles was similar (Table 1). Increasing the fluorophilic character of the telomers did not improve the emulsion stability significantly. Both telomers gave more stable FDC emulsions than Pluronic F-68, but with PFOB the emulsions prepared with **1** or **2** or Pluronic had comparable stabilities (see *I* and *R*, Table 1).

When compared with EYP, no improvement in stabilization or particle size reduction was observed with FDC or PFOB. In both cases, EYP was the most efficient emulsifier (Table 1).

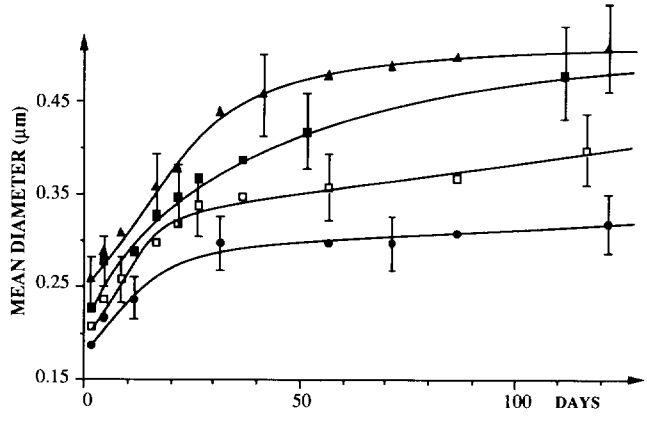

Fig. 2. Evolution with time, at 40 °C, of the mean droplet diameter of 50% w/v FDC emulsions prepared with 2% w/v of **2** and 1% w/v of **4** (●); 2.5% of **2** and 0.5% of **4** (■); and reference emulsions with 3% of **2** (▲); and 3% of EYP (□). For clarity, the error bars are not shown.

3.3. Co-surfactant effect between telomers and Pluronic F-68

Pluronic F-68 is not very effective in decreasing the water/fluorocarbon interfacial tension. Its capacity to stabilize fluorocarbon emulsions appears to result predominantly from its ability to spread on the particle's surface (steric stabilization). It has been demonstrated previously that non-ionic, monomeric polyhydroxylated perfluoroalkylated surfactants display significant synergic stabilization effects when associated with Pluronic F-68 [16,17]. We examined whether a similar synergic effect could be obtained when the telomeric polyhydroxylated perfluoroalkylated surfactant **1** is associated with Pluronic F-68.

However, such association led to emulsion destabilization irrespective of the composition of the Pluronic/**1** mixture used (2/1% w/v or 2.5/0.5% w/v) in the case of the PFOB emulsions (Table 1) ($R = +140\%$ or $+200\%$ depending on formulation). No significant effect was found for the FDC emulsions ($R = +20\%$ or $+40\%$). This destabilization phenomenon may be due to steric exclusion between the two types of polymers.

3.4. Co-surfactant effect between telomers and EYP

We investigated the effect of **1** as a co-emulsifier with EYP by replacing 0.5% or 1% of EYP by **1** in the formulations. Both compositions led to coarser FDC and PFOB emulsions than with EYP alone. One of the formulations (50/2.5/1 PFOB/EYP/**1**) led to an unstable emulsion ($R = +135\%$). However, except for this preparation, the stabilities of the emulsions were comparable with that of the EYP-based reference emulsions.

3.5. Co-surfactant effect between telomers 1 or 2 and the fluorinated monoadduct 3 or 4

The effect of association between the perfluoroalkylated telomers **1** or **2** and the perfluoroalkylated

Table 1
Evolution with time, at 40 °C, of the mean droplet diameter in FDC and PFOB emulsions formulated with EYP, Pluronic F-68 and the fluorinated surfactants **1**, **2**, **3** and **4**

Formulation	Compcsition (% w/v)	Mean diameter (μm) ±10%		*I* (%)	*R* [a] (%)
		Initial	After 4 months		
FDC/EYP	50/3	0.20	0.39	95	–
PFOB/EYP	50/3	0.20	0.34	70	–
FDC/Pluronic F-68	50/3	0.21	1.07	410	+355
PFOB/Pluronic F-68	50/3	0.14	0.47 **	235	+135
FDC/**1**	50/3	0.22	0.58	165	+90
PFOB/**1**	50/3	0.19	0.49	160	+115
FDC/**2**	50/3	0.25	0.50	100	+30
PFOB/**2**	50/3	0.19	0.42	120	+65
FDC/Pluronic F-68/**1**	50/2.5/0.5	0.22	1.41 *	540	+49 [b]
FDC/Pluronic F-68/**1**	50/2/1	0.22	1.25 *	470	+20 [b]
PFOB/Pluronic F-68/**1**	50/2.5/0.5	0.18	0.98 *	445	+140 [b]
PFOB/Pluronic F-68/**1**	50/2/1	0.16	1.16 *	625	+200 [b]
FDC/EYP/**1**	50/2.5/0.5	0.28	0.49	75	+10
FDC/EYP/**1**	50/2/1	0.28	0.54	95	+35
PFOB/EYP/**1**	50/2.5/0.5	0.24	0.37	55	−5
PFOB/EYP/**1**	50/2/1	0.20	0.53	165	+135
FDC/**1**/**3**	50/2.5/0.5	0.23	0.49	95	+35
FDC/**1**/**3**	50/2/1	0.20	0.37	85	−10
PFOB/**1**/**3**	50/2.5/0.5	0.24	0.42 **	75	+30
PFOB/**1**/**3**	50/2/1	0.23	0.38 **	80	+5
FDC/**2**/**4**	50/2.5/0.5	0.22	0.47	115	+30
FDC/**2**/**4**	50/2/1	0.18	0.31	70	−30
PFOB/**2**/**4**	50/2.5/0.5	0.22	0.37 **	70	+5
PFOB/**2**/**4**	50/2/1	0.17	0.55 **	225	+170

[a] With EYP as a reference unless stated otherwise.
[b] With Pluronic F-68 as a reference.
* After 100 d.
** After 105 d.

monomeric surfactants **3** or **4** on the stabilization of fluorocarbon emulsions was investigated.

Irrespective of the preparation tested, the particle size increase rates were similar (Table 1) with one exception, i.e. the (50/2/1% w/v) PFOB/**2**/**4** emulsion, which was significantly less stable.

Both the FDC and PFOB emulsions prepared with a mixture of **1** and **3** were somewhat finer and more stable than those prepared with **1** alone (Table 1). Compared with the EYP references, emulsions composed of 2% of **1** and 1% of **3** were as stable as those prepared with EYP. The best results were obtained with the FDC emulsion emulsified with 2% of **1** and 1% of **3**, but this emulsion was only as stable as the reference EYP emulsion and had similar particle sizes.

A similar trend was found for emulsions prepared with a mixture of **2** and **4**, the best result being obtained with the FDC emulsion with 2% of **2** and 1% of **4**. This emulsion was slightly finer than the EYP-based reference emulsion and had a similar stability (Table 1).

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