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Research paper

Synthesis and characterisation of novel nanospheres made from amphiphilic perfluoroalkylthio-β-cyclodextrins

Sandrine Péroche[a], Ghania Degobert[b], Jean-Luc Putaux[c], Marie-Geneviève Blanchin[d], Hatem Fessi[b], Hélène Parrot-Lopez[a,*]

[a]Université Claude Bernard Lyon 1, Laboratoire de Méthodologie de Synthèse et Molécules Bioactives, Villeurbanne Cedex, France
[b]Université Claude Bernard Lyon 1, Laboratoire d'Automatisation et du Génie des Procédés, Villeurbanne Cedex, France
[c]CERMAV-CNRS, Grenoble Cedex 9, France
[d]Université Claude Bernard Lyon 1, Laboratoire de Physique de la Matière Condensée et Nanostructures, Villeurbanne Cedex, France

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Abstract

This work describes the synthesis of new amphiphilic perfluorohexyl- and perfluorooctyl-propanethio-β-cyclodextrins and the comparison of the ability of these molecules and alkyl analogue, nonanethio-β-cyclodextrin to form nanospheres. Nanospheres were prepared using nanoprecipitation method (perfluoroalkylthio-β-cyclodextrin in THF [0.11×10^{-3}M], stirring rate 700 rpm, addition of aqueous phase at 64 °C into organic phase at 50 °C). They were characterised by Photon Correlation Spectroscopy (PCS) and by electron microscopy (SEM and cryo-TEM). The nanospheres prepared from these new β-cyclodextrin derivatives have an average size of 260 nm, and appear to be spherical in cryo-TEM images. Whereas alkyl analogue forms polydisperse aggregates with sizes in the range 60–350 nm.

Keywords: Amphiphilic cyclodextrins; Fluoroalkylthio-β-cyclodextrins; Alkylthio-β-cyclodextrins; Nanospheres; PCS; SEM; Cryo-TEM

1. Introduction

β-Cyclodextrins (β-CDs) are cyclic oligosaccharides composed of seven α-D-glucopyranose residues. Amphiphilic β-CD derivatives are of considerable interest for pharmaceutical applications in view of their capacity for self-organization [1]. With the aim of providing versatile carriers and delivery systems for hydrophilic and lipophilic drugs, modified cyclodextrins have been synthesised. Amphiphilic cyclodextrins can be obtained by the introduction of lipophilic groups at primary face and/or secondary face [2,3]. The self-organisation depends on the number and the length of hydrophobic chains at the different faces. For examples, nanospheres [4] and solid lipid nanoparticles [5] were prepared from amphiphilic cyclodextrins substituted at the secondary face by hexanoyl chains. Substitution of β-cyclodextrin primary face by alkylthio chains leads to organisation as liquid crystals [2] and substitution of both faces with alkylthio chains at the primary face and oligoethylene glycol chains at the secondary face facilitates vesicles formation [6].

In recent years, highly fluorinated surfactants have been increasingly studied in view of their unique hydrophobic and colloidal self-assembly properties [7]. Vesicles and nanocapsules made from fluorinated surfactants are usually more stable and less permeable than those made from non-fluorinated surfactants [8]. The combination of the self-assembly properties of amphiphilic cyclodextrins and those of fluorocarbon chains should produce carriers exhibiting new physical properties, higher hydrophobicity and possibly greater stability compared to their alkylated analogues.

Previously [9], we described the synthesis of new amphiphilic perfluoroalkylthio- cyclodextrins in which the primary face was mono-, di- and hepta-substituted with

* Corresponding author. Université Claude Bernard Lyon 1, Laboratoire de Méthodologie de Synthèse et Molécules Bioactives, UMR CNRS 5181, 43 Boulevard du 11 Novembre 1918, 69622 Villeurbanne Cedex, France. Tel.: +33 4 72 43 15 32; fax: +33 4 72 44 84 38.
E-mail address: h.parrot@cdlyon.univ-lyon1.fr (H. Parrot-Lopez).

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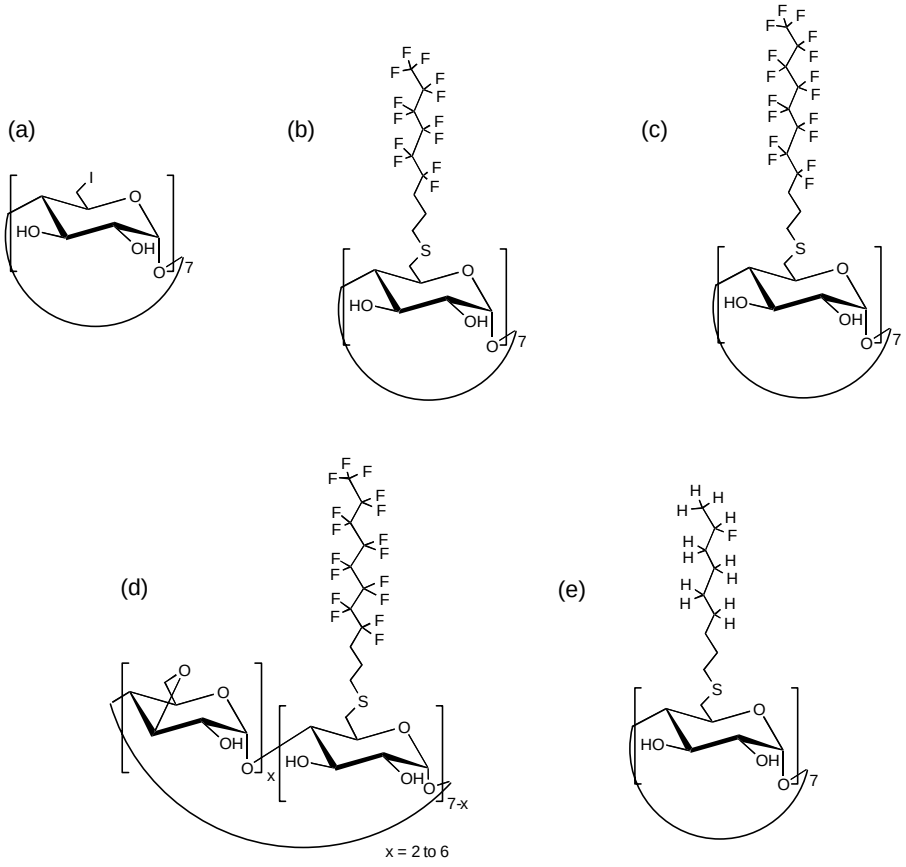

Fig. 1. Formulae of (a) β-CD(I)$_7$, (b) β-CD-(C$_6$F$_{13}$)$_7$, (c) β-CD(C$_8$F$_{17}$)$_7$, (d) β-CD(C$_8$F$_{17}$)$_4$ and (e) β-CD(C$_6$H$_{13}$)$_7$.

perfluorohexylpropanethiol chains. These new derivatives were obtained by thio-alkylation of different β-CDs having tosyl or iodo groups at C-6 position.

In this paper, we describe the synthesis of novel perfluorooctylpropanethio-β-cyclodextrins derivatives with either seven or an average of four (DS) perfluorooctylpropanethiol chains (Fig. 1(c) and (d), respectively) at the primary face and the capacity of these new molecules and heptakis-perfluorohexylpropanethio-β-CD (Fig. 1(b)) to form nanospheres in water. We also synthesised alkylated analogous β-CD, heptakis-nonanethio-β-CD (Fig. 1(e)) and compared chain fluorination effect on their capacity to self-assemble as nanospheres.

2. Materials and methods

2.1. Materials

Tetrahydrofuran (99.5%) and dimethylformamide (99.8%) were purchased from SDS (France). Benzophenone (99%) and Calcium Hydride (99%) were purchased from Aldrich (France). Tetrahydrofuran was purified by distillation under nitrogen atmosphere over sodium and benzophenone. Dimethylformamide was purified by distillation under nitrogen atmosphere over Calcium Hydride. 6-deoxy-6-iodo-β-cyclodextrin (β-CD(I)$_7$) was synthesized according to the method of Gadelle and Defaye [10]. 3-Perfluorooctylpropaneguanidinium salt and 3-perfluorooctylpropanethiol were obtained according to our previously published method [9]. The structure and purity of the products were verified by ^{1}H, ^{19}F and ^{13}C NMR spectroscopy at 300 K using a Brucker 500 MHz spectrometer, pyridine d$_5$ as the solvent and TMS and CFCl$_3$ as the references. Substitution degree (DS) was determined by MALDI mass spectrometry.

2.2. Synthesis

The heptakis-[6-deoxy-6-(3-perfluorohexylpropanethio)]-β-cyclodextrin, referred to as β-CD(C$_6$F$_{13}$)$_7$ (Fig. 1(b)) was synthesised by nucleophilic displacement of the iodide group at O-6 position (Fig. 1(a)) by a solution of 3-perfluorohexylpropanethiolate salt in dry DMF under nitrogen atmosphere according to our method [9]. The solution of 3-perfluorohexylpropanethiolate salt was prepared by deprotonation of the corresponding thiol (0.73 g) by 1.3 ml NaOMe 1 M in 5 ml MeOH [11]. The mixture was stirred during 2 h at room temperature. The solvent was removed and 5 ml anhydrous DMF was added to the residue under a nitrogen atmosphere. To this solution was added a solution of 0.25 g 6-deoxy-6-iodo-β-CD in 7 ml anhydrous DMF. The solution was stirred at 70 °C for

24 h. The substituted cyclodextrin was precipitated by addition of acetone. The residue was washed with acetone, water and then acetone to give β-CD(C_6F_{13})$_7$ as a powder (0.49 g, 99% yield); mp 210 °C; I.R. ν (cm^{-1}) 3306 (OH free); 2926 (C–H stretch); 1237–1153 (C–F stretch); ^{1}H NMR (Pyridine d_5, 500 MHz) δ (ppm): 2.14–2.21 (m, 14H, C*H*$_2$–CH$_2$–S), 2.38–2.60 (m, 14H, C*H*$_2$–S–), 2.70 (m, 14H, C*H*$_2$–CF$_2$), 3.27 (dd, 7H, H-6, J=8 and 14.0 Hz), 3.70 (m, 7H, H-6′), 4.06 (t, 7H, H-5, J=8.0 Hz), 4.21 (m, 7H, H-4), 4.49–4.71 (m, 14H, H-2, H-3), 5.56 (d, 7H. H-1, J=3.2 Hz), 7.60 (m, 7H, OH-3), 8.15 (m, 7H, OH-2); ^{13}C NMR (Pyridine d_5) δ (ppm): 105.7 (C_1), 88.4 (C_4), 76.7, 76.3, 75.8 (C_2, C_3,C_5), 36.0 (C_6), 34.7 (*C*H$_2$–S–), 31.9 (*C*H$_2$–CF$_2$), 22.8 (*C*H$_2$–CH$_2$–S); ^{19}F NMR (Pyridine d5, $CFCl_3$) δ (ppm): −80.8 (m, 3F, CF_3), −113.5 (m, 2F, C*F*$_2$–CH$_2$), −121.5 (m, 2F, CF_2), −122.5 (m, 2F, CF_2), −122.8 (m, 2F, CF_2), −125.9 (m, 2F, C*F*$_2$–CF_3); MALDI MS *m/z:* [M+Na]$^+$ 3789.

The heptakis-[6-deoxy-6-(3-perfluorooctylpropanethio)]-β-cyclodextrin, referred to as β-CD(C_8F_{17})$_7$ (Fig. 1(c)) was synthesised using 0.35 g of 6-deoxy-6-iodo-β-cyclodextrin, 1.62 g of 3-perfluorooctylpropaneguanidinium salt in 10 ml anhydrous DMF at 60 °C for 72 h in presence of 1.25 g Cs_2CO_3. The substituted cyclodextrin was precipitated by addition of acetone. The residue was washed with acetone, water ad then acetone to give β-CD(C_8F_{17})$_7$ as a white powder (0.57 g, 99% yield); mp 215 °C; I.R. ν (cm^{-1}) 3325 (OH free); 2919 (C–H stretch); 1232–1146 (C–F strech); ^{1}H NMR (Pyridine d_5, 500 MHz)δ (ppm): 2.14–2.21 (m, 14H, C*H*$_2$–CH$_2$–S), 2.38–2.60 (m, 14H, C*H*$_2$–S–), 2.70 (m, 14H, C*H*$_2$–CF$_2$), 3.32 (m, 7H, H-6), 3.72 (m, 7H, H-6′), 4.12 (t, 7H, H-5, J=8.0 Hz), 4.18 (m, 7H, H-4), 4.49–4.71 (m, 14H, H-2, H-3), 5.64 (d, 7H. H-1, J=3.2 Hz), 7.60 (m, 7H, OH-3), 8.15 (m, 7H, OH-2); ^{19}F NMR (Pyridine d_5, $CFCl_3$) δ (ppm): −80.6 (m, 3F, CF_3), −112.9 (m, 2F, C*F*$_2$–CH$_2$), −121.0 (m, 6F, CF_2), −122.4 (m, 2F, CF_2), −122.6 (m, 2F, CF_2), −125.5 (m, 2F, C*F*$_2$–CF_3); MALDI MS *m/z:* [M+Na]$^+$ 4489.4.

A mixture of different substituted cyclodextrins was obtained with a average degree of substitution (DS) of 4 is referred to as β-CD(C_8F_{17})$_4$ in basic conditions (Fig. 1(d)). The solution of 1.23 g perfluorooctylpropanethiol, 1.3 ml NaOMe 1 M in 5 ml anhydrous THF and 0.34 g of 6-deoxy-6-iodo-β-cyclodextrin was stirred at 70 °C for 72 h. The crude product was precipitated by addition of acetone. The residue was washed with acetone, water and then acetone and precipitated with cold EtOH. The cyclodextrin β-CD(C_8F_{17})$_4$ is obtained in 76% yield; ^{1}H NMR (Pyridine d_5, 500 MHz)δ (ppm): 2.17 (m, 8H, C*H*$_2$–CH$_2$–S), 2.49 (m,8H,C*H*$_2$–S–), 3.03 (m, 8H, C*H*$_2$–CF$_2$), 3.28 (m, 7H, H-6), 3.71 (m, 7H, H-6′), 4.04–4.22 (m, 14H, H-4, H-5), 4.61 (m, 14H, H-2, H-3), 5.57 (m, 7H. H-1); 7.65 (m, 7H, OH-3), 8.22 (m, 7H, OH-2); ^{19}F NMR (Pyridine d_5, $CFCl_3$) δ (ppm): −80.8 (m, 3F, CF_3), −113.5 (m, 2F, C*F*$_2$–CH$_2$), −121.7 (m, 6F, CF_2), −122.9 (m, 4F, CF_2), −126.1(m, 2F, C*F*$_2$–CF_3); MALDI MS *m/z*: [M−H+Na]$^+$ 3008.9.

The heptakis-[6-deoxy-6-(nonanethio)]-β-cyclodextrin is referred to as β-CD(C_6H_{13})7 (Fig. 1(e)) was synthesised using the procedure previously described [2]. The spectra and chemical shifts of β-CD(C_6H_{13})$_7$ were in agreement with the literature; mp 250 °C; I.R. ν (cm^{-1}) 3341 (OH free); 2955–2852 (C–H stretch); ^{1}H NMR (Pyridine d_5, 500 MHz) δ (ppm) 0.92 (s, 21H, CH3), 1.12–1.71 (m, 84H, CH2), 1.83 (m, 14H, CH2), 2.95 (m, 14H, CH2–S), 3.39 (m, 7H, H-6), 3.64 (m, 7H, H-6′), 4.14 (m, 14H, H-2, H-4), 4.62 (m, 14H, H-3, H-5), 5.56 (d, 7H, H-1, J=3.2 Hz), 7.57 (m, 7H, OH-3), 8.09 (m, 7H, OH-2); ES *m/z* [M+Na]+2153.1.

2.3. Preparation of nanospheres

Nanospheres were prepared using the nanoprecipitation technique [12]. The amphiphilic cyclodextrins were dissolved in THF. One phase (aqueous or organic) was slowly added to the other, under magnetic stirring, via a syringe equipped with a fitted capillary. Organic solvent was removed under vacuum and the dispersion was concentrated to yield a total volume of 10 ml. Cyclodextrin amount, stirring rate and addition order of one phase of the other were varied.

2.4. Characterisation of the nanospheres

2.4.1. Photon correlation spectroscopy

The particle size and the polydispersity index (PI) were measured by Photon Correlation Spectroscopy (PCS) on a Malvern Zetasizer 3000 HS_A spectrophotometer. All values were measured at an angle of 90 in 10 mm diameter cell. The system was thermostated at 25 °C. Particle analysis was carried out using the Malvern software package and multiple mode analysis. All samples were prepared in triplicate and all measurements were repeated four times for each sample. The variance of the measurements was less than 5%.

2.4.2. Scanning electron microscopy

Scanning electron microscopy (SEM) was performed on a FEG Hitachi S 800 microscope operated at an accelerating voltage of 15 kV.

2.4.3. Cryo-transmission electron microscopy

Using methods described elsewhere [13], specimens for cryo-transmission electron microscopy (cryo-TEM) were prepared by fast-freezing thin liquid films of nanospheres suspensions formed on ‘lacey’ carbon films. The vitrified samples were observed at low temperature (−180 °C) in a Gatan 626 specimen holder, under low dose conditions, using a Philips CM200 ‘Cryo’ microscope operated at 80 kV. Micrographs were recorded on Kodak SO163 films.

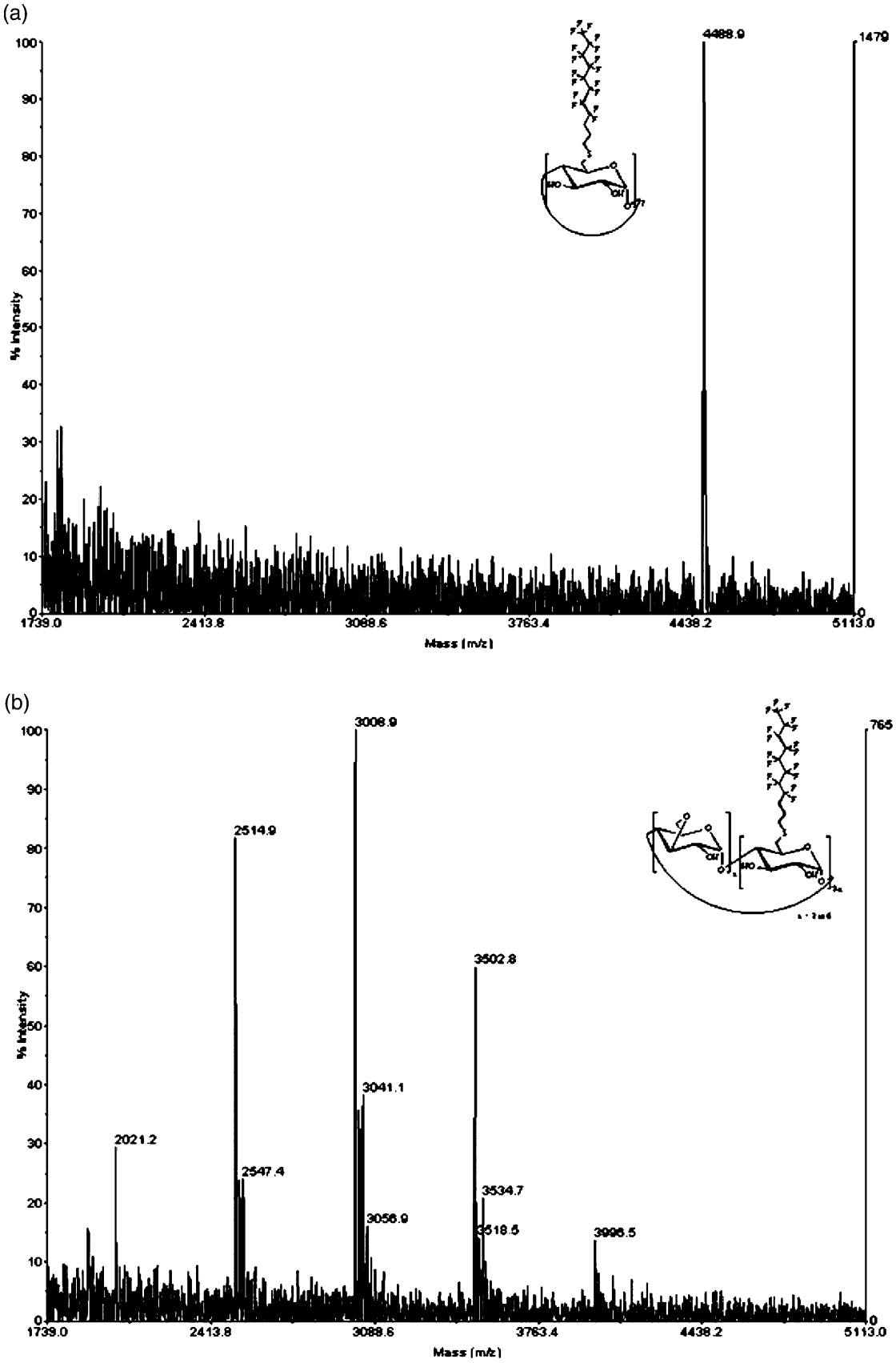

Fig. 2. MALDI spectra of (a) β-CD(C_8F_{17})$_7$ and (b) β-CD(C_8F_{17})$_4$.

2.5. Stability

Samples were kept at room temperature as aqueous suspensions and particle size measurements were repeated every month.

3. Results and discussion

3.1. Synthesis

In our previous studies we synthesised heptakis-(6-deoxy-6-trifluoromethylthio)-β-cyclodextrin and studied their ability to form nanospheres [14]. Unfortunately no stable dispersions were obtained. We assumed that inability to yield stable nanospheres was due to a too short chain length. In order to overcome this problem we decided to increase the chain length and to synthesize by substitution of an iodine group, β-CDs having perfluoroalkylthiol chains of six and eight carbon atoms. The ^{1}H NMR spectra confirm total substitution of the primary face, in particular by the presence of a sharp doublet ($J = 3.2$ Hz) corresponding to anomeric protons, H-1 at 5.64 ppm. The ^{19}F NMR confirmed the presence of perfluoroalkylated chain. *Per*-substitution was confirmed by MALDI mass spectrometry (m/z 4489.4 [β-CD$(C_8F_{17})_7$+Na]$^+$) and no under-substituted derivatives were detected (Fig. 2(a)). The ^{1}H NMR spectrum of β-CD$(C_8F_{17})_4$ was typical of a mixture. Anomeric proton were not present as a clear doublet but as a multiplet (not shown). MALDI mass spectrometry (Fig. 2(b)) clearly demonstrated that β-CD$(C_8F_{17})_4$ was a mixture of derivatives resulting from coupling of 2 to 6 perfluorooctylpropanethiol chains with an average degree (DS) of 4: 2021.2 [β-CD$(C_8F_{17})_2$−H+Na]$^+$; 2514.9 [β-CD$(C_8F_{17})_3$ −H+Na]$^+$; 3008.9 [β-CD$(C_8F_{17})_4$ −H+Na]$^+$; 3502.8 [β-CD$(C_8F_{17})_5$−H + Na]$^+$; 3996.5 [β-CD$(C_8F_{17})_6$−H+Na]$^+$). In basic conditions, the iodine atoms were eliminated by intramolecular nucleophilic substitution by hydroxyl groups on C-3 position leading to anhydro-glucopyranose. This phenomenom has already been observed for cyclodextrins possessing leaving groups in the primary face [15].

3.2. Nanospheres formation and PCS measurements

Organic solvents such as methanol, ethanol or acetone are commonly used for preparation of nanospheres by the nanoprecipitation method. Their elimination by evaporation under vacuum is easy. The nanoprecipitation method can also be used with THF as the organic solvent, given that our perfluoro derivatives are only soluble in THF, it was decided to use this solvent for both the fluorinated and alkylated systems. Nanospheres were prepared using 10 ml distilled THF as organic solvent and 20 ml distilled water as aqueous phase. No surfactant was added to determine the fluoroalkylated chains effect on nanopheres stability. Different parameters were studied: addition of organic phase to aqueous phase or vice versa, stirring rate (300 or 700 rpm), amount of β-CD (12.5 or 25 mg), as well as phases temperature.

Table 1
Effect of the addition of aqueous phase into organic phase or vice versa, amount of CD and stirring rate on nanosphere size, made from β-CD$(C_6H_{13})_7$, at room temperature

	Amount of CD (mg)	Stirring rate (rpm)	Diameter (nm)	Polydispersity index
Aqueous phase into organic phase	12.5	300	317±7	0.08±0.06
		700	315±10	0.10±0.04
	25.0	300	292±6	0.13±0.01
		700	249±1	0.16±0.02
Organic phase into aqueous phase	12.5	300	150±2	0.11±0.01
		700	118±1	0.13±0.02
	25.0	300	168±1	0.14±0.01
		700	145±1	0.13±0.01

Table 1 summarises the results obtained for amphiphilic β-CD$(C_6H_{13})_7$ nanospheres obtained under different preparation conditions. Whatever, the amount of alkylated β-CD used and the stirring rate, the addition of the aqueous phase to the organic phase leads to the formation of nanospheres with a large size (249–317 nm). Samples were polydisperse and satisfactory reproducibility was difficult to achieve. The obtained diameter was inversely proportional to the stirring speed: at 300 rpm nanospheres of 317 nm diameter are observed whereas at a faster stirring rate 700 rpm lower particle values size 249 nm were observed. When aqueous phase was added to organic phase, particle size increased with increasing amount of β-CD, however when organic phase was added to aqueous phase, effect was inversed.

In view of the above observations, a set of conditions was established to obtain small nanospheres (118 nm) from β-CD$(C_6H_{13})_7$. These conditions were: room temperature, 12.5 mg (5.86×10^{-3} mmol, 0.19×10^{-3} M) of β-CD in THF/H_2O (1:2), high stirring rate (700 rpm) and addition of organic phase into aqueous phase (Table 1).

In contrast to the results obtained for β-CD$(C_6H_{13})_7$ for all the perfluoroalkylthio-β-cyclodextrin derivatives [β-CD$(C_6F_{13})_7$, β-CD$(C_8F_{17})_7$ and β-CD$(C_8F_{17})_4$] addition of the organic phase into the aqueous phase led only to clear non-birefringant solutions. After evaporation of the organic solvent, precipitation of the cyclodextrin derivatives was observed.

For β-CD$(C_6F_{13})_7$ addition of aqueous phase to organic phase led to formation of nanospheres of diameter 512 nm but with relatively low polydispersity index. However in the case of β-CD$(C_8F_{17})_7$, precipitation occurred after solvent evaporation (Table 2). β-CD$(C_8F_{17})_4$ gave nanospheres with a diameter of 312 nm but with a high polydispersity index.

Heating solutions of amphiphilic cyclodextrins and particularly aqueous phase led lower nanosphere sizes than those obtained at room temperature. For β-CD$(C_6F_{13})_7$,

Table 2
Effect of the addition of aqueous phase into organic phase or vice versa, organic and aqueous phase temperature on nanospheres size made from perfluoroalkylthio-β-cyclodextrins (0.11×10^{-3}M of CD and stirring rate of 700 rpm)

	CD	Temp. aqueous phase (°C)	Temp. organic phase (°C)	Diameter (nm)	Polydispersity index
Organic phase into aqueous phase	β-CD(C_6F_{13})$_7$	rt	rt	No particles	
	β-CD(C_8F_{17})$_7$	50	50		
	β-CD(C_8F_{17})$_4$	64	50		
Aqueous phase into organic phase	β-CD(C_6F_{13})$_7$	rt	rt	512±23	0.10±0.02
		50	50	362±5	0.08±0.03
		64	50	264±4	0.04±0.02
	β-CD(C_8F_{17})$_7$	rt	rt	No particles	
		50	50		
		64	50		
	β-CD(C_8F_{17})$_4$	rt	rt	312±122	0.37±0.06
		64	50	263±4	0.08±0.01

mean diameter of nanospheres prepared at room temperature were measured by PCS to be 512 nm, this decreased to a mean diameter of 264 nm when the temperature of aqueous phase was 64 °C and that of organic phase 50 °C. A similar decrease in size was also observed for nanospheres derived from β-CD(C_8F_{17})$_4$. This decrease may be due to a better dispersion of molecules in organic solvent. For all temperatures above room temperature, polydispersity index was lower than 0.1 indicating a homogeneous dispersion.

Thus, for fluoroalkylated systems, a different set of standard preparation conditions can be established; 12.5 mg (3×10^{-3} mmol, 0.11×10^{-3} M) of fluoroalkylated-β-cyclodextrin in THF/H_2O (1:2), stirring rate 700 rpm, addition of aqueous phase at 64 °C into organic phase at 50 °C.

Under these conditions nanospheres based on perfluoroalkylthio-β-CDs, having good polydispersity indices and low mean diameter were easily reproduced.

3.3. Temporal stability

Tables 3 and 4 display nanospheres temporal stability made from alkylthio- and perfluoroalkylthio-β-cyclodextrins, respectively.

Nanospheres prepared from alkylthio-cyclodextrins were in general stable (Table 3). For preparations made using a

Table 3
Nanospheres temporal stability made from β-CD(C_6H_{13})$_7$ (stirring rate of 700 rpm and addition of organic phase into aqueous phase at room temperature)

Amount of CD (mg)	Stirring rate (rpm)	Diameter (nm) PI	Diameter (nm) PI after 2 months	Diameter (nm) PI after 5 months
12.5	300	150±2 0.11±0.01	155±2 0.12±0.02	149±2 0.09±0.04
	700	118±1 0.13±0.02	121±2 0.14±0.04	Precipitation
25.0	300	168±1 0.14±0.01	171±1 0.11±0.01	170±3 0.10±0.05
	700	145±1 0.13±0.01	150±2 0.12±0.03	153±4 0.11±0.02

Table 4
Nanospheres size temporal stability made from perfluoroalkylthio-β-cyclodextrins

CD	Temp. aqueous phase (°C)	Temp. organic phase (°C)	Diameter (nm) PI	Diameter (nm) PI after 2 months	Diameter (nm) PI after 5 months
β-CD(C_6F_{13})$_7$	rt	rt	512±23 0.10±0.02	308±19 0.11±0.01	321±27 0.15±0.05
	50	50	362±5 0.08±0.03	308±5 0.09±0.02	324±10 0.11±0.09
	64	50	264±4 0.04±0.02	244±4 0.07±0.03	249±7 0.07±0.04
β-CD(C_8F_{17})$_4$	64	50	263±4 0.08±0.01	253±3 0.08±0.02	254±5 0.09±0.05

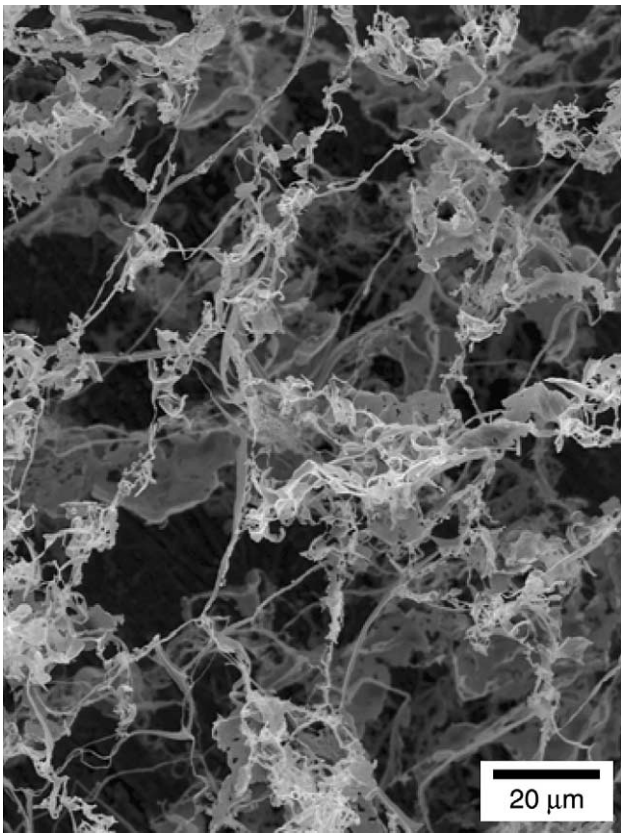

Fig. 3. SEM image of freeze-dried nanoparticles made from β-CD(C_6H_{13})$_7$.

slow stirring rate during addition, no increase in particle size is observed and indeed the polydispersity index decreases after storage for 5 months at room temperature. Samples prepared using a faster stirring rate (700 rpm) with 12.5 mg of β-CD(C_6H_{13})$_7$ are stable up to 2 months.

Preparations made from perfluoroalkylthio-β-CDs under previously determined conditions were stable over a minimum of 5 months. No increase in particle size was observed and polydispersity index stayed below 0.07 for β-CD(C_6F_{13})$_7$ and 0.09 for β-CD(C_8F_{17})$_4$. These results suggest that dispersions prepared from perfluoroalkylthiocyclodextrins were more stable than those made from alkylthio ones. Once again, fluoroalkylated chains showed their ability to form stable nanospheres in aqueous media.

3.4. Electron microscopy

Shape, size and surface morphology of the samples were analysed by electron microscopy. Fig. 3 shows SEM images of freeze-dried samples made from β-CD(C_6H_{13})$_7$. No nanospheres were observed: the sample mostly appeared as a filamentous network. This phenomena may be due to the lyophilization method used for sample preparation leading to desagregation. For freeze-dried nanospheres made from β-CD(C_6F_{13})$_7$, an overview of the sample showed fused nanospheres as clusters (Fig. 4(a)). Only a few individualized nanospheres were observed (Fig. 4(b)). Their mean diameter (about 250 nm) was consistent with that measured by PCS (260 nm).

In order to avoid the detrimental effect of lyophilization, cryo-TEM was used to observe the nanospheres embedded in vitreous ice. Those obtained from β-CD(C_6H_{13})$_7$ appeared as non spherical objects. Contrary to the amphiphilic β-CD nanospheres observed by Gèze et al. [16] using the same technique, a variety of objects can be seen in our images (Fig. 5). The low contrast with respect to surrounding ice leads us to consider that they are flat objects. Their mean diameter measured from the micrographs varies from 60 to 350 nm.

As observed by SEM, nanospheres made from fluoroalkylthio-β-CDs seem to be spherical, in particular with β-CD(C_8F_{17})$_4$ (Fig. 6(a) and (b)). In Fig. 6(a) clear area viewed in some particles suggests that they contain a cavity near their surface. This behaviour is similar to liposome

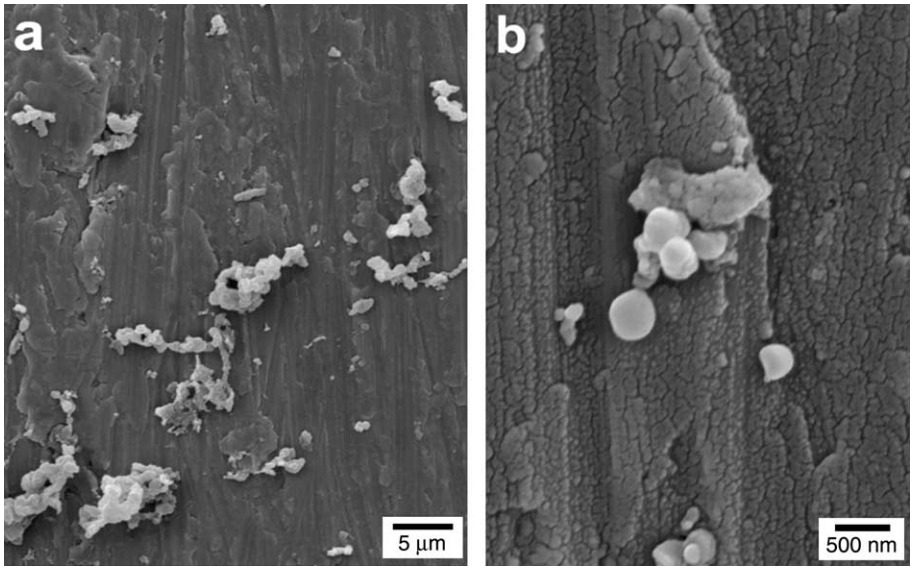

Fig. 4. SEM images of freeze-dried nanoparticles made from β-CD(C_6F_{13})$_7$.

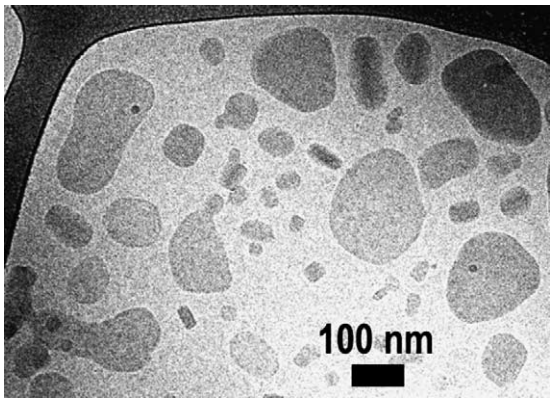

Fig. 5. Cryo-TEM image of nanoparticles made from β-CD$(C_6H_{13})_7$, embedded in vitreous ice.

formation but no bilayer is observed. Mean diameter measured from these images (250 ± 20 nm) was consistent with data obtained by PCS (263 nm). Concerning β-CD$(C_6F_{13})_7$ samples, two kinds of objects were visualised in Fig. 6(b): spherical nanospheres as in β-CD$(C_6F_{13})_7$ samples with a 250 nm mean diameter were in agreement with PCS measurements, other objects exhibiting a lower contrast were comparable to those observed in β-CD$(C_6H_{13})_7$ specimens and may correspond to softer particles deformed in the liquid film.

From the present study, it was clear that fluoroalkylated chains introduced onto β-cyclodextrin allow nanospheres preparation. We can note that PCS measurements used in conjunction with cryo-TEM observations allow useful characterisation of the particles in terms of morphology and size.

4. Conclusion

We synthesized new amphiphilic cyclodextrins substituted at primary face by seven perfluorohexylpropanethiol, by seven perfluorooctylpropanethiol chains or by an average of four perfluorooctylpropanethiol chains. These molecules were able to self-organize into nanospheres in aqueous media contrary to alkylated analogue which forms

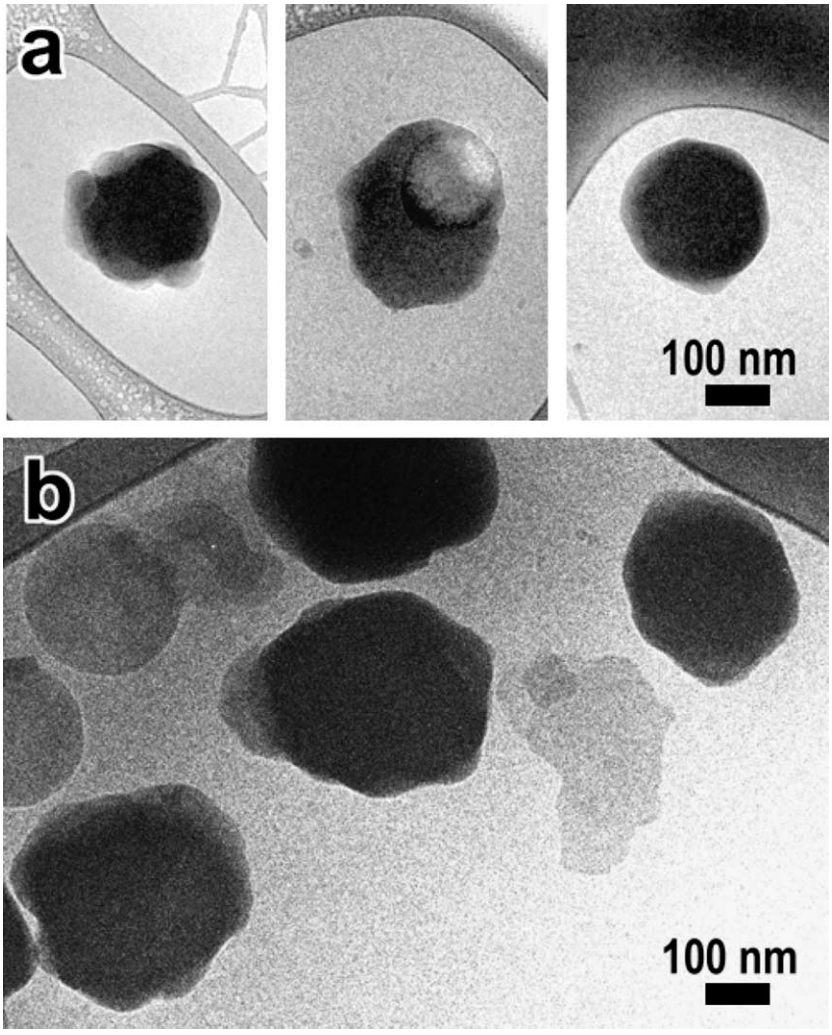

Fig. 6. Cryo-TEM images of nanospheres made from (a) β-CD$(C_8F_{17})_4$, (b) β-CD$(C_6F_{13})_7$, embedded in vitreous ice.

aggregates. Standard preparation conditions have been established; 0.11×10^{-3} M of perfluoroalkylthio-β-CDs in THF/H_2O (1:2), stirring rate 700 rpm and addition of aqueous phase at 64 °C into organic phase at 50 °C. Dispersions made from these perfluoroalkylthio-β-CDs were stable over 5 months of conservation at room temperature contrary to those made from alkylthio-β-CDs. Using different microscopy methods (PCS, SEM, cryo-TEM) allowed us to confirm nanospheres formation. As we expectred, incorporation of fluorine chains allowed nanospheres stabilisation and cohesion without using co-surfactants. In order to determine the interest of these new nanospheres in pharmaceutical research, investigations on drug encapsulation are currently being undertaken.

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