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# Novel nanoparticles made from amphiphilic perfluoroalkyl α-cyclodextrin derivatives: Preparation, characterization and application to the transport of acyclovir

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ABSTRACT

The preparation of aqueous suspensions of nanoparticles of the fluorinated amphiphilic α-cyclodextrins hexakis[6-deoxy-6-(3-perfluoroalkylpropanethio)-2,3-di-*O*-methyl]-α-cyclodextrin and their hydrocarbon analogues was studied. The complexation of acyclovir by modified α-cyclodextrin, the encapsulation efficiency and release profile were measured as an assessment of the properties of such nanoparticles regarding drug delivery applications.

Stable aqueous suspensions of nanoparticles were prepared using nanoprecipitation method without using surface-active agent. The organic solvent (ethanol) and cyclodextrin concentration (0.4 mM) were carefully selected. The nanoparticles prepared from these new amphiphilic α-cyclodextrin derivatives according to optimized conditions have an average diameter of 100 nm for fluorinated derivatives and 150 nm for hydrocarbon analogues. Suspensions were stable over at least 9 months. Acyclovir forms inclusion complexes of 1:1 stoichiometry and high stability constants (from 700 mol $L^{-1}$ to 4000 mol $L^{-1}$ in ethanol) as assessed from UV/vis spectroscopy and Electrospray Ionization Mass Spectroscopy. Satisfactory loading of acyclovir inside the nanoparticles was achieved according to the "highly loaded" preparation method (encapsulation efficiency ≈ 40%). Nanoparticles based on the fluorinated compounds delayed the drug release up to 3 h with little initial burst release.

Fluorinated amphiphilic α-cyclodextrins self-assemble in the form of nanospheres that encapsulate acyclovir and allow sustained release, showing their potential for applications to drug delivery.



## 1. Introduction

The solubilization and transport of drugs of medium polarity is a difficult issue in pharmaceutical formulation because such molecules are neither soluble enough in water, nor in the apolar oils that are commonly used for the preparation of emulsions. Organic solvents of medium polarity such as alcohols, DMSO and acetone are not suitable in pharmaceutical applications. Solubilization in surfactants assemblies such as micelles or liposomes is open to major drawbacks. Micelles leave their content upon dilution as they pass the critical micellar concentration (cmc); most water-soluble surfactants cause either irritancy or hemolysis. The poor colloidal stability of liposomes severely limits their utilization. Solubilization as an inclusion complex in the hydrophobic cavity of cyclodextrins (CD) is an alternative. The internal cavity of CDs is indeed hydrophobic but also a medium polarity. Not only highly hydrophobic molecules can enter the cavity as an inclusion complex, molecules of moderate polarity can do the same if their size fits the cavity. Even water-soluble molecules may form a favourable inclusion complex, which may appear a beneficial effect when encapsulation inside CD aims at the stabilization of fragile molecules. Amphiphilic cyclodextrin derivatives are of considerable interest for pharmaceutical applications because of their capacity for self-organization in water (Uekama et al., 1998). Supramolecular assemblies of amphiphilic CDs retain the complexation properties of the parent CD and bring about supplementary benefits such as improved stabilization of the drug, better contact with biological membranes and better delivery of the drug. Synthesis methods have recently been reviewed by Salas and Darcy (2008) and showed that most of the previous investigations of amphiphilic CDs dealt with alkyl grafted β-CDs (Terry et al., 2001;

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Dubes et al., 2001, 2003) because β-cyclodextrin is readily available from biotechnological production process. β-cyclodextrin is weakly hydrophilic compared to α- and γ-CD and the grafted alkyl chains coming from fatty acids are generally of moderate length (6–12 carbon atoms). A definite improvement is expected using the more hydrophilic α-cyclodextrin on the one hand; and more hydrophobic fluorinated chains on the other hand. Use of α-cyclodextrin derivatives may favour both interactions with water and also organization in molecular assemblies (Tchoreloff et al., 1995). More hydrophobic chains should enhance intermolecular interactions inside the supramolecular assemblies. In recent years, fluorinated surfactants are widely studied due to their potential activity in biomedical research (Krafft et al., 2001). Vesicles and nanocapsules made from fluorinated surfactants are usually more stable and less permeable than those made from non-fluorinated surfactants (Guittard and Geribaldi, 2001). However, nanospheres based on short fluorocarbon chain β-cyclodextrin show poor stability (Granger et al., 2000), longer fluorinated chains attached to the native β-cyclodextrin lead to enhanced stability of the nanospheres (Peroche and Parrot-Lopez, 2003; Peroche et al., 2005). The objective of the present investigation on amphiphilic fluorinated α-cyclodextrins is to develop an efficient synthesis scheme, investigate the preparation method and the stability of aqueous suspensions of nanoparticles, and lastly evaluate the encapsulation and release of a model drug. We thus synthesized α-cyclodextrins substituted at the C-6 position by perfluoroalkylpropanethiol chains and their O-2-, O-3-methylated analogues (Bertino Ghera et al., 2007), the methyl ether derivatives showing good solubilities in organic solvent and being suitable candidates for the preparation of nanoparticles.

9-[(2-Hydroxyethoxy)-methyl]-guanine (Acyclovir, Fig. 1) was chosen as the model drug. This antiviral drug is an acyclic synthetic analogue of purine nucleosides, which is active against *Herpes simplex virus* (HSV1 and HSV2), *Varicella zoster virus* (VZV), *Epstein-Barr virus* (EBV) and *cytomegalovirus* (CMV) (Laskin et al., 1982). Acyclovir (ACV) inhibits the viral DNA polymerase after phosphorylation by viral thymidinekinase. Acyclovir formulations do not allow suitable drug levels at target sites following oral, local or parenteral administration, due to the low water solubility and low lipid bilayer solubility of the drug (Fresta et al., 1999).

This paper reports the optimized preparation of colloidal systems of nanoparticle made from amphiphilic α-cyclodextrins substituted by perfluoroalkylpropanethiol chains at the primary hydroxyl face and permethylated at the secondary hydroxyl face. Such aqueous suspensions of nanoparticles are characterized in terms of their particle size distribution and stability on comparison with hydrocarbon analogues. The potential utility of such nanoparticles for drug delivery is assessed through an investigation of the complexation and encapsulation of acyclovir as a model drug. A detailed investigation of the complexation of acyclovir by such substituted cyclodextrins in ethanol is presented. Encapsulation efficiency and release profile of acyclovir are reported and show the beneficial effect of the fluorinated chains.

## 2. Materials and methods

### 2.1. Materials

All chemicals were purchased from Acros Organics or Sigma–Aldrich and used without further purification. Other solvents were of chemical grade and were used as received. α-cyclodextrin was purchased from Roquette Frères (Lestrem, France). Amphiphilic fluorinated α-cyclodextrins and their hydrocarbon analogues (Fig. 1) were synthesized as previously described (Bertino Ghera et al., 2007). Briefly, after the selective protection of the primary hydroxyl groups with *tert*-butyldimethylsilyl groups, all the secondary hydroxyl groups were methyl-substituted using sodium hydride and methyl iodide. Removal of the *tert*-butyldimethylsilyl groups was performed with tetrabutylammonium fluoride in THF and introduction of the methanesulfonyl groups with methanesulfonyl chloride. Finally, the hydrophobic chains (fluorinated or hydrocarbon) were introduced by nucleophilic substitution of the leaving groups by the thiolate derivative, generated *in situ* by the basic hydrolysis of the 3-perfluoroalkylpropane (or alkyl) isothiouronium salts with cesium carbonate. The structure and the purity of these amphiphilic derivatives were verified by $^{1}H$ and $^{13}C$ NMR and MALDI mass spectroscopy. In order to simplify the name of each compound, shorter name are chosen. For example, αCDF13OMe refers to hexakis[6-deoxy-6-(3-perfluorohexylpropanethio)-2,3-di-*O*-methyl]-α-cyclodextrin.

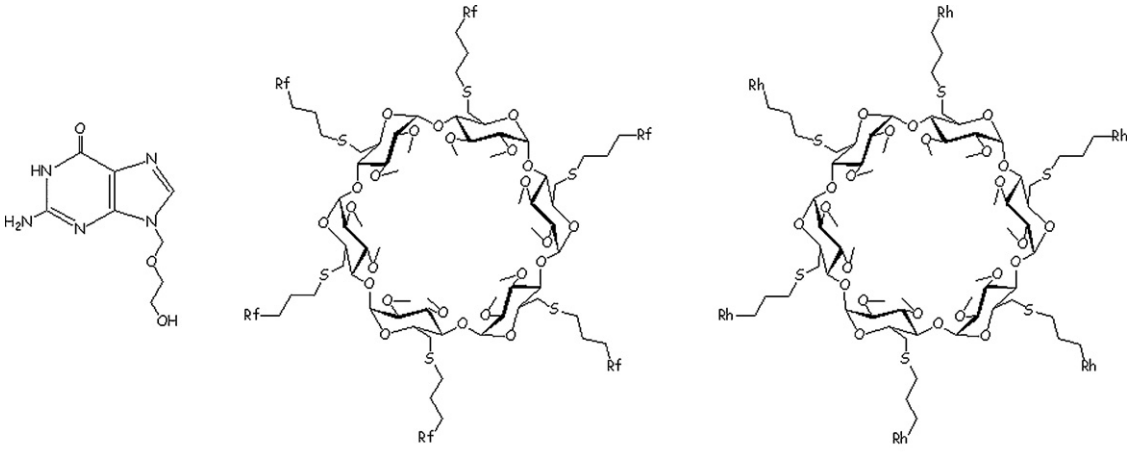


**Fig. 1.** Schematic molecular structures of acyclovir, fluorinated and hydrocarbon amphiphilic α-cyclodextrins.

### 2.2. Methods

#### 2.2.1. Preparation of nanoparticles

A challenging issue is designing the preparation process. The "nanoprecipitation" method is the most popular (Fessi et al., 1988). Accordingly, a solution of amphiphilic cyclodextrin in a polar organic solvent (e.g. acetone, ethanol, DMSO, THF) is mixed with water; diffusion of the polar organic solvent in water leaves the amphiphilic cyclodextrin as a supersaturated solution in water. The amphiphilic cyclodextrins precipitate as nanoparticles in suspension in water. A too large concentration of amphiphilic cyclodextrin in water leads to emulsification failure because the precipitation occurs from a too much supersaturated solution (Ganachaud and Katz, 2005; Vitale and Katz, 2003). Designing the nanoprecipitation process consists in choosing the polar organic solvent and the concentration of amphiphilic cyclodextrin that allow the formation of a stable aqueous suspension of nanoparticles. This is a difficult issue in the case of fluorinated amphiphiles because fluorinated molecules generally have low solubilities in water and organic solvents. The nanoprecipitation process is a suitable technique as it avoids the use of additional surfactants and gives more homogeneous dispersions for amphiphilic cyclodextrins (Duchêne et al., 1999). However, the drug might remain mostly adsorbed on the nanoparticle surface rather than in the CD cavity or inside the hydrophobic core of the particle, resulting in fast release of the drug from the nanoparticles (Wouessidjewe et al., 1996). The inclusion complex is formed either in the organic solvent before nanoprecipitation, or during the nanoprecipitation process. The choice of the organic solvent type and of the concentrations matters with this respect.

The nanoparticles based on 2,3-di-*O*-methyl amphiphilic α-cyclodextrins were prepared by the nanoprecipitation technique. The relevant amphiphilic cyclodextrin was dissolved in acetone, THF or ethanol (12.5 mL) and the solution was poured within 1 min into deionized water (25 mL) and stirred at 400 rpm. A slightly turbid emulsion of nanospheres formed spontaneously. Solvent and a part of water were evaporated under reduced pressure and the total volume adjusted to 20 mL.

#### 2.2.2. Particle size measurements

The mean particle diameter (nm) and the polydispersity index (PI) of nanospheres were measured by photon correlation spectroscopy (PCS) by using a Malvern Nanosizer (Malvern Instruments, UK). The measurements were carried out at 25 °C. Each value is the average of three measurements.

#### 2.2.3. Transmission electronic microscopy (TEM)

The morphology of nanoparticles was characterized by TEM using a Philips CM210 microscope (Philips, Netherlands) at an acceleration voltage of 80 kV. After dilution of nanoparticles suspensions down to lower than 1%, a drop of solution was placed on a metal probe (copper/formvar/carbon). Excess liquid was absorbed with a paper sheet and then evaporated overnight at atmospheric pressure.

#### 2.2.4. Stability of the suspensions

Nanoparticles suspensions were stored at +4 °C. Particle size and PI measurements were performed on aliquots every month.

#### 2.2.5. Characterization of inclusion complexes between amphiphilic cyclodextrins and ACV

*2.2.5.1. UV/vis spectroscopy.* The continuous variation method was used to determine the stoichiometry of the fluorinated and hydrocarbon amphiphilic cyclodextrin/acyclovir complexes. Two solutions of equal concentration ($10^{-4}$ M) of amphiphilic cyclodextrins and acyclovir were prepared in ethanol. These solutions were mixed in different portions (molar ratio from 0 to 1) without variation of the final volume, and stirred during 5 days. The absorbance ($A_{read}$) of each solution was measured at 256 nm wavelength ($\lambda_{maxACV}$). The absorbance change ($\Delta A = A_{read} - A_{ACV}$) was plotted in Job plots ($\Delta A = f\{[ACV]/([CD]+[ACV])\}$) that show a maximum or a minimum at a specific molar ratio indicating the stoichiometry of the complexes. For the determination of the equilibrium constant ($K_{11}$) and the molar extinction coefficient ($\varepsilon_{11}$) of the 1:1 inclusion complexes, the double-reciprocal (Benesi/Hildebrand) plot was used: $l/\Delta A = 1/\Delta\varepsilon_{11}K_{11}[CD][ACV]_t + 1/\Delta\varepsilon_{11}[ACV]_t$ where $l$ is the path length, $\Delta A$ the absorbance change, $[ACV]_t$ the total acyclovir concentration, $K_{11}$ the stability constant, $\Delta\varepsilon_{11}$ the difference of molar extinction coefficient between the complexed and free ACV and [CD] the cyclodextrin concentration. Absorbance of ACV at constant concentration was measured in ethanol at room temperature at 256 nm as function of added amphiphilic cyclodextrins.

*2.2.5.2. Electrospray ionization mass spectrometry (ESI–MS).* Electrospray ionization mass spectrometry provides confirmation of the interaction of acyclovir with the amphiphilic cyclodextrins. According to such a soft ionization technique, ions existing in solution are transferred into gas phase without breaking non-covalent interaction, which are predominant forces in the host/guest interaction. Fixed quantities of ACV and amphiphilic cyclodextrins (molar ratio 5:1 for ACV:CD derivatives) were dissolved in ethanol and left to equilibrate for 5 days.

#### 2.2.6. ACV loading of nanospheres based on amphiphilic α-cyclodextrins

Nanospheres based on amphiphilic α-cyclodextrins were loaded with ACV, according to the following methods (Memisoğlu et al., 2003):

*2.2.6.1. Conventionally loaded nanospheres.* Nanospheres were prepared by mixing water and a solution of amphiphilic α-cyclodextrins (0.4 mM) and ACV (0.6 mM) in organic solvent (ethanol) using the nanoprecipitation technique described above.

*2.2.6.2. Preloaded nanospheres.* The 1:1 ACV:CD complex was prepared by freeze-drying prior to nanoprecipitation. Nanospheres were prepared directly from a 0.4 mM solution of preformed 1:1 ACV:CD complexes in ethanol according to the above described nanoprecipitation technique.

*2.2.6.3. Highly loaded nanospheres.* The nanoprecipitation method was performed using a 0.4 mM solution of preformed 1:1 ACV:CD complexes overloaded with an additional amount of ACV in the organic phase. The total concentration of ACV was 1.2 mM (ACV/CD = 3).

The loading efficiency was assessed as follows. After the formation of nanospheres suspensions, unbound antiviral drug and the loaded nanospheres were separated by centrifugation at 50,000 rpm for 1 h at +4 °C using an Optima™ MAX-E ultracentrifuge from Beckman–Coulter. The sediment was dried overnight and the resulting powder containing the loaded nanospheres was dissolved in ethanol. The clear solution was analyzed for ACV by UV–vis spectrophotometry using a Shimadzu UV-2401PC spectrophotometer at a wavelength of 256 nm. Loading capacity expressed as the associated drug percentage was calculated according to the following equation:

$$\text{Associated drug\%} = 100 \times \left[\frac{\text{Determined drug quantity}(\mu\text{mol})}{\text{Initial drug quantity}(\mu\text{mol})}\right]$$

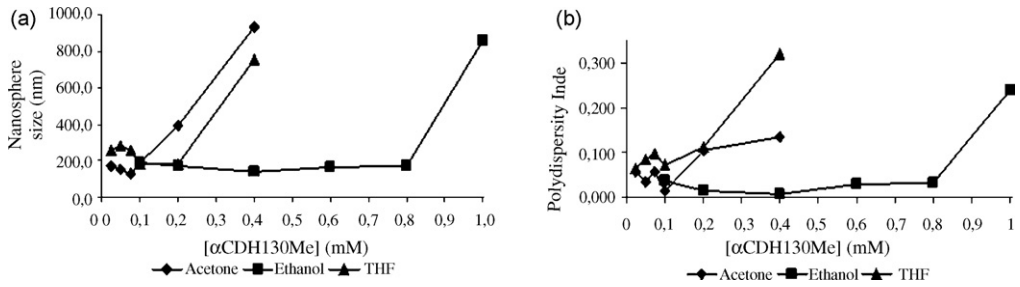


**Fig. 2.** (a) Mean diameter (nm) and polydispersity index (b) of nanospheres prepared by the nanoprecipitation method from organic solutions as a function the αCDH13OMe concentration (mM) in the organic solution in acetone, THF and ethanol.

*2.2.7. Controlled release studies*

The suspension of nanoparticles loaded with ACV (1 mL) was introduced into a dialysis tube (cut-off 5000 Da) and placed in a 20 mL bath of phosphate buffer solution (pH 7.4). Aliquots of 1 mL were collected at time intervals (1, 2, 5, 10, 15, 30, 60, 120, 180, 210, 240 min) so as to measure the fractions of released and encapsulated molecules from the UV absorbance at 256 nm.

## 3. Results and discussion

### *3.1. Characteristics of amphiphilic α-cyclodextrins nanospheres*

In order to determine optimized conditions for the nanospheres preparation, the influence of two parameters was tested using αCDH13OMe derivative as model: the amphiphilic α-cyclodextrin concentration (from 0.025 mM to 1.0 mM) and the nature of the organic solvent (acetone, THF, ethanol). The results are given in Fig. 2. In the case of nanospheres prepared from a THF solution, homogeneous milky suspensions of nanoparticles of average diameter around 220 nm and narrow particle size distribution were obtained for concentration of amphiphilic α-cyclodextrin in THF below than 0.2 mM. Above this concentration, the diameter was larger and the particle size distribution was wider; the average diameter increased with respect to the concentration of cyclodextrin. In the case of acetone, homogeneous suspensions were obtained below 0.4 mM cyclodextrin concentration; the nanosphere mean diameter continuously increased with respect to the concentration of αCDH13OMe. Therefore THF and acetone were not suitable for the preparation of nanospheres. In contrast, ethanol gave satisfactory results. The diameter of nanospheres was constant (170 nm) and the polydispersity index was lower than 0.040 for concentrations ranging from 0.1 mM to 0.8 mM. In view of the above observations, a set of optimized conditions was established to prepare nanospheres from amphiphilic α-cyclodextrins: 0.4 mM concentration of amphiphilic cyclodextrin derivative in ethanol. The results obtained for all the amphiphilic α-cyclodextrins are presented in Table 2. Nanospheres have a similar size, ranging from 96 nm for those based on αCDF13OMe to 177 nm for those generated with αCDH9OMe. Introduction of fluorinated chains to the amphiphilic cyclodextrins did not affect dramatically the nanosphere sizes. The differences between hydrocarbon and fluorocarbon chained-cyclodextrins were significant however. For a same length of the hydrophobic chain, the mean diameter of fluorinated nanospheres was less than for the hydrocarbon nanosphere analogues. A slight larger nanospheres size was also observed for amphiphilic cyclodextrins having the longest chains. For example, the size of nanospheres of αCDH17OMe was 177 nm against 140 nm for nanospheres based on αCDH9OMe and αCDH13OMe. All the particle size distributions were monomodal and narrow; lower polydispersity indexes were measured for hydrocarbon derivatives. Shape and size of the nanoparticles were analyzed by transmission electron microscopy. As example, Fig. 3 shows TEM images of nanospheres based on αCDF13OMe. All particles were spherical, the mean diameter measured from the pictures were around 100 nm and the particle size distribution was narrow, in agreement with mean diameters obtained by PCS (95.9 ± 0.2 nm). Some nanospheres appeared deformed or spread, possibly because of the drying step required for sampling the suspensions before visualization by TEM. The full results show the capacity of the new fluorinated amphiphilic α-cyclodextrins to form small and spherical nanospheres in water using the nanoprecipitation method, without addition of surface-active agent.

### *3.2. Stability the suspensions of amphiphilic α-cyclodextrin nanospheres*

Fig. 4 displays stability assessment of fluorinated and hydrocarbon amphiphilic α-cyclodextrin nanospheres. The suspensions of nanospheres prepared from all of the amphiphilic cyclodextrins were stable. No significant variation of size and polydispersity indexes could be detected during the storage period of 9 months. Both fluorinated and hydrocarbon amphiphilic cyclodextrins formed stable emulsions of nanospheres in aqueous media.

### *3.3. Characterization of ACV: amphiphilic cyclodextrins inclusion complexes*

The inclusion complexes between fluorinated or hydrocarbon amphiphilic α-cyclodextrin derivatives and ACV in ethanol were firstly characterized by UV/vis spectroscopy, allowing the determination of the stoichiometry, the stability constant ($K_{11}$) and the molar extinction coefficient ($\varepsilon_{11}$) of each inclusion complex.

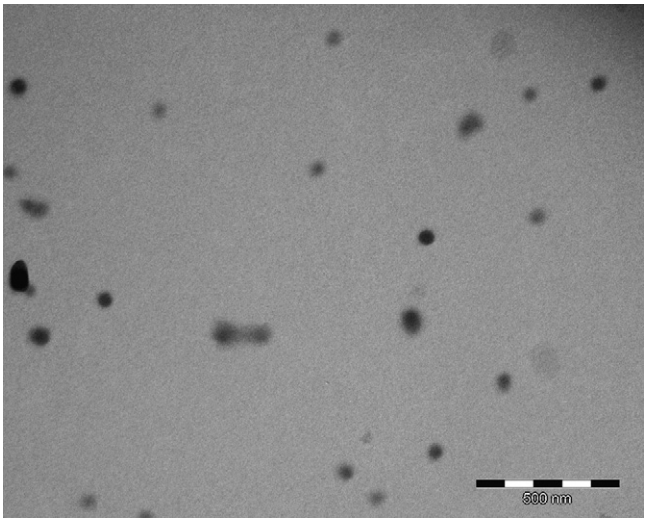


**Fig. 3.** TEM images of nanospheres based on αCDF13OMe.

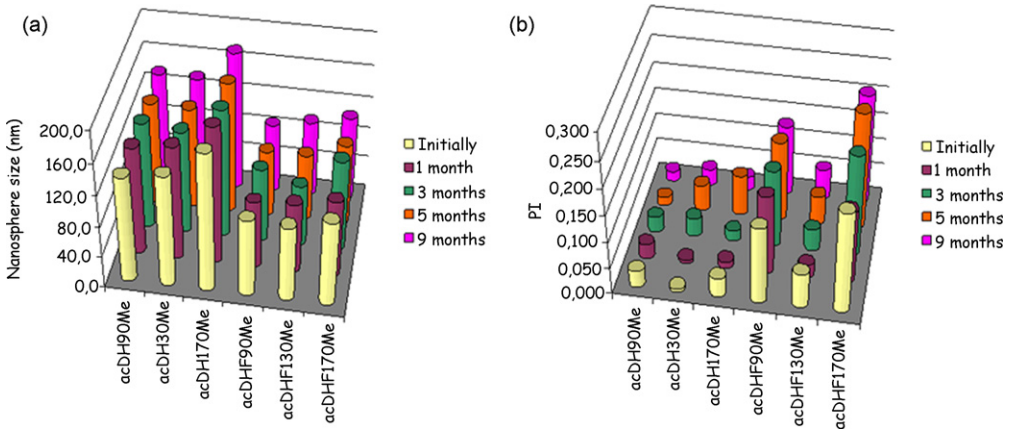


**Fig. 4.** Stability of nanospheres based on amphiphilic α-cyclodextrins: (a) mean diameter (nm) and (b) polydispersity index (PI).

**Table 1**
Mean diameter (nm) and polydispersity index (PI) of nanospheres based on fluorinated and hydrocarbon amphiphilic α-cyclodextrins.

| Derivatives | Nanosphere size (nm) | PI |
|---|---|---|
| αCDH9OMe | 135,6 ± 3,1 | 0,031 ± 0,025 |
| αCDH13OMe | 142,8 ± 2,1 | 0,007 ± 0,007 |
| αCDH17OMe | 176,7 ± 5,0 | 0,037 ± 0,032 |
| αCDF9OMe | 99,2 ± 0,2 | 0,143 ± 0,011 |
| αCDF13OMe | 95,9 ± 0,2 | 0,065 ± 0,022 |
| αCDF17OMe | 109,0 ± 1,7 | 0,190 ± 0,007 |

**Table 2**
Stoichiometry, stability constants ($K_{11}$) and molar extinction coefficients ($\varepsilon_{11}$) of complexes in ethanol.

| Complex | Stoichiometry | $K_{11}$ (mol L$^{-1}$) | $\varepsilon_{11}$ (L mol$^{-1}$ cm$^{-1}$) |
|---|---|---|---|
| αCDF9OMe/ACV | 1:1 | 1427 | 19,438 |
| αCDF13OMe/ACV | 1:1 | 2560 | 18,378 |
| αCDF17OMe/ACV | 1:1 | 3725 | 19,213 |
| αCDH9OMe/ACV | 1:1 | 687 | 8,290 |
| αCDH13OMe/ACV | 1:1 | 746 | 6,631 |
| αCDH17OMe/ACV | 1:1 | 4225 | 6,489 |

The results are summarized in Table 1. For all of the amphiphilic cyclodextrins either fluorinated or the hydrocarbon analogues, the Job plots (Fig. 5a) showed a maximum at a molar ratio of 0.5 indicating a 1:1 stoichiometry of the complexes. The stability constant and the molar extinction coefficient of the complexes were calculated from Benesi–Hildebrand plots (Fig. 5b). The plots were linear (correlation coefficient from 0.987 to 0.997) and the $K_{11}$ values are given in Table 2. The $K_{11}$ values for the fluorinated amphiphilic α-cyclodextrin derivatives increased with the chain length; they were ranging from 1427 mol L$^{-1}$ for αCDF9OMe to 3725 mol L$^{-1}$ for αCDF17OMe. The same behaviour was observed for the hydrocarbon analogues with $K_{11}$ values ranging from 687 mol L$^{-1}$ to 4225 mol L$^{-1}$. The stability constants were higher for the fluorinated derivatives than for the hydrocarbon analogues for a same length of hydrophobic chain; an exception was the case of the longest chain derivatives having a similar $K_{11}$ value. This variation of the stability constants with the chain length suggested a contribution of ACV complexation by insertion in between the hydrophobic chains. In contrast with the stability constant values, the molar extinction coefficient of the complex were independent of the lengths of the hydrophobic chains, but depended of the nature of the chains. $\varepsilon_{11}$ values were around 19,000 L mol$^{-1}$ cm$^{-1}$ for the fluorinated derivatives and around 7000 L mol$^{-1}$ cm$^{-1}$ for the hydrocarbon analogues, indicating that ACV was located in different chemical environments for the fluorinated and the hydrocarbon derivatives. The inclusion inside cyclodextrin cavity could be ruled out however. At least partial inclusion of ACV inside the cavity may take place in addition to insertion in between the hydrophobic chains. The contribution of the possible partial inclusion in the cavity looked weak however. Indeed, the complexation of ACV with the native α-cyclodextrin did not take place in the same experimental conditions. The α-cyclodextrin cavity is not suitable for the ACV inclusion. Inclusion complex with β-cyclodextrin only is described in the literature (Von Plessing Rossel et al., 2000). All of these results demonstrated that ACV was located in between the hydrophobic chains of the amphiphilic cyclodextrins inside the nanoparticles.

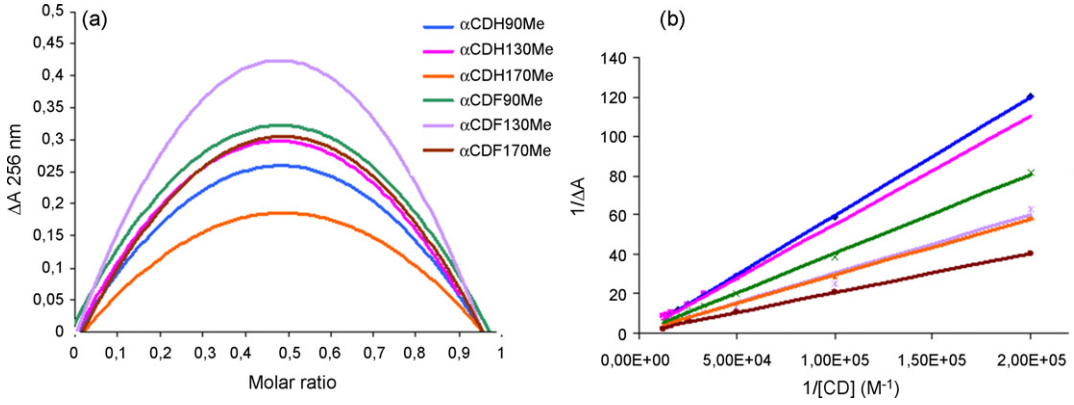


**Fig. 5.** (a) Job plot and (b) Benesi–Hildebrand plot for amphiphilic α-cyclodextrins. ACV at constant concentration ($8 \times 10^{-5}$ mol L$^{-1}$) in the presence of increasing concentrations of amphiphilic α-cyclodextrin derivatives (from $5 \times 10^{-6}$ mol L$^{-1}$ to $8 \times 10^{-5}$ mol L$^{-1}$) measured at 256 nm.

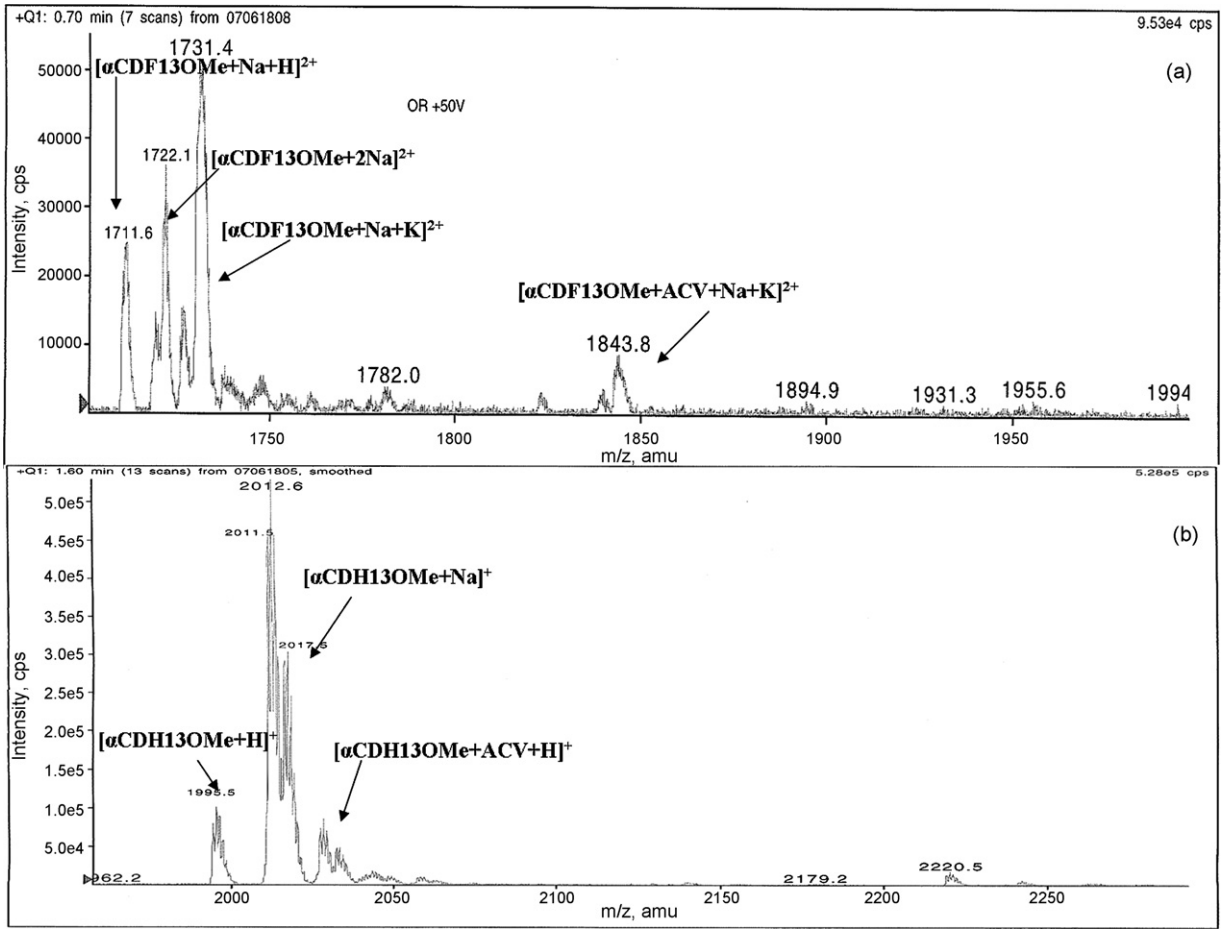


**Fig. 6.** Electrospray mass spectra for (a) αCDF13OMe/ACV complex and (b) αCDH13OMe/ACV complex in ethanol.

It is worth noticing that similar observations were made regarding the complexation of ACV with sulfated amphiphilic α- and β-cyclodextrins (Dubes et al., 2003) where the antiviral agent interacted only with the lipophilic environment generated by the acyl chain grafted onto the secondary hydroxyl face of the cyclodextrin.

Electrospray ionization mass spectrometry provided confirmation of the interaction of acyclovir with the amphiphilic cyclodextrins. This experiment allows the determination of the quantity and the nature of ions present in the gas phase after nebulisation and ionization of the liquid solution. The spectra give the percentage of ion as a function of their mass on charge ($m/z$). Electrospray mass spectra for αCDF13OMe/ACV and αCDH13OMe/ACV are given in Fig. 6. For fluorinated amphiphilic cyclodextrin αCDH13OMe (Fig. 6a), the molecular ions of αCDH13OMe were observed at $m/z$ = 1711.6, 1722.1 and 1731.4 corresponding respectively to the $[\alpha CDF13OMe + Na + H]^{2+}$, $[\alpha CDF13OMe + 2Na]^{2+}$ $[\alpha CDF13OMe + Na + K]^{2+}$ cations. Only the 1:1 stoichiometry complex with ACV was present on the spectrum at $m/z$ = 1843.8 corresponding to $[\alpha CDF13OMe + ACV + Na + K]^{2+}$ cation. For complex of hydrocarbon amphiphilic cyclodextrin αCDH13OMe (Fig. 6b), only the molecular ions of αCDH13OMe and the complex with ACV of 1:1 stoichiometry were observed at $m/z$ = 1995.5, 2012.6, 2017.5 and 2220.5 corresponding to $[\alpha CDH13OMe + H]^{+}$, $[\alpha CDH13OMe + NH_4]^{+}$, $[\alpha CDH13OMe + Na]^{+}$ and $[\alpha CDH13OMe + ACV + H]^{+}$ cations respectively. These results obtained from electrospray mass spectra confirmed the 1:1 stoichiometry of the complex between the amphiphilic cyclodextrins and ACV.

### 3.4. ACV loading of nanospheres based on amphiphilic α-cyclodextrins

The encapsulation process of ACV into αCDF13OMe and αCDH13OMe nanospheres was optimized with respect to the loading method referred to as: conventionally loaded, preloaded or highly loaded). The results are given in Table 3. The particle sizes were slightly different for loaded and blank particles. For loaded nanospheres made from αCDH13OMe, the nanospheres sizes were ranging from 100 nm to 115 nm; they were slightly smaller than those formed without ACV (142.8 ± 2.1 nm). On the other hand, loaded nanospheres based on αCDF13OMe had a size around 120 nm, slightly larger than those formed without ACV (95.9 ± 0.2 nm). All of the particle size distributions remained narrow (PI values lower than 0.20). The drug loading method had a significant influence on the loading efficiency (percentage of associated drug) (Table 4). The preparation of the complex with ACV before the nanoprecipitation process improved the loading in comparison to the conventional loading techniques. For example, αCDF13OMe nanospheres loaded using the conventional loading method gave 14% of associated drug. This value increased up to 24% when the complex was preformed. The highly loaded method that combined the first two methods allowed further improving these results up to 32% of associated drug. Slow loading was observed after the emulsion has been prepared by the nanoprecipitation method. Indeed, the measured loading efficiency increased if the separation of the nanoparticles and aqueous phase by centrifugation was delayed. Therefore, part of the free ACV present in solution in the aqueous phase could slowly enter the nanoparticles. In all

**Table 3**
Characteristics of loaded nanospheres based on αCDF13OMe and αCDF13OMe (PI: polydispersity index).

| Methods | Nanosphere size (nm) | PI | Time (days) | Associated drug (%) |
|---|---|---|---|---|
| αCDH13OMe | | | | |
| Conventionally loaded | 114.0 ± 2.4 | 0.118 ± 0.007 | 0 | 4 |
| | 108.0 ± 1.9 | 0.107 ± 0.007 | 1 | 7 |
| Preloaded | 101.2 ± 2.2 | 0.111 ± 0.036 | 0 | 7 |
| | 102.5 ± 1.3 | 0.092 ± 0.022 | 1 | 16 |
| Highly loaded | 114.0 ± 2.1 | 0.089 ± 0.009 | 0 | 23 |
| | 114.0 ± 1.8 | 0.094 ± 0.030 | 1 | 25 |
| αCDF13OMe | | | | |
| Conventionally loaded | 117.0 ± 2.8 | 0.115 ± 0.036 | 0 | 14 |
| | 121.3 ± 4.4 | 0.124 ± 0.023 | 1 | 25 |
| Preloaded | 126.5 ± 0.8 | 0.127 ± 0.006 | 0 | 24 |
| | 125.3 ± 1.5 | 0.129 ± 0.016 | 1 | 29 |
| Highly loaded | 140.4 ± 2.7 | 0.172 ± 0.011 | 0 | 32 |
| | 147.7 ± 3.0 | 0.141 ± 0.027 | 1 | 39 |

**Table 4**
Characteristics of loaded nanospheres made from amphiphilic α-cyclodextrins.

| Derivatives | Nanosphere size (nm) | PI | Associated drug (%) |
|---|---|---|---|
| αCDH9OMe | 115.5 ± 1.8 | 0.071 ± 0.027 | 41 |
| αCDH13OMe | 114.0 ± 1.8 | 0.094 ± 0.030 | 25 |
| αCDH17OMe | 128.6 ± 0.8 | 0.088 ± 0.007 | 41 |
| αCDF9OMe | 123.1 ± 0.4 | 0.154 ± 0.018 | 45 |
| αCDF13OMe | 147.7 ± 3.0 | 0.141 ± 0.027 | 39 |
| αCDF17OMe | | Precipitation | |

cases, an increase of loading was observed upon storage during one day. The loading did not increase further after this storage period. 39% loading inside αCDF13OMe nanoparticles could be reached according to the highly loaded method and after one-day delay. Loading efficiency of the same order of magnitude (40%) were obtained with fluorinated cyclodextrins of various chain lengths (Table 4). Nanospheres made from fluorinated cyclodextrins gave better encapsulation of ACV than those based on the hydrocarbon analogues for a same protocol. αCDF17OMe nanoparticles could not be loaded by ACV because the nanospheres flocculated when the organic solvent was evaporated. This result was surprising because αCDF17OMe alone formed stable nanospheres and complexation of acyclovir by αCDF17OMe was possible.

### 3.5. Controlled release studies

The release profiles of ACV from highly loaded nanospheres based on three derivatives (αCDF9OMe, αCDF13OMe and αCDH17OMe) in water are shown in Fig. 7. In the absence of nanospheres, concentration equilibrium between the compartments inside and outside the dialysis bag was reached within 30 min. The release from loaded nanoparticles was significantly delayed. The release profile showed little fast initial release (burst release), of the order of 10% of the full load. Burst release is caused by the presence of drug molecules adsorbed at the surface of the nanoparticle instead of encapsulated inside. The absence of burst effect showed that the loaded drug was actually encapsulated inside the nanoparticles as complexes with the amphiphilic cyclodextrins. Large burst release from nanoparticles of amphiphilic cyclodextrins were observed in some instances (Wouessidjewe et al., 1996). Drug release from highly loaded nanospheres reached completion within two to 3 h. These results clearly indicate the positive effect of the nanoparticles on the controlled release. The length of the fluorinated chains looked a critical factor for the drug release. Nanospheres from αCDF9OMe released the full drug in 2 h, whereas 3 h were necessary for particles made with αCDF13OMe. After 1 h, only 30% of the encapsulated drugs were released for the fluorinated nanospheres versus 40% for the hydrocarbon analogues. With regard to sustained release, the fluorinated NPs showed better characteristics than the hydrocarbon-chained systems.

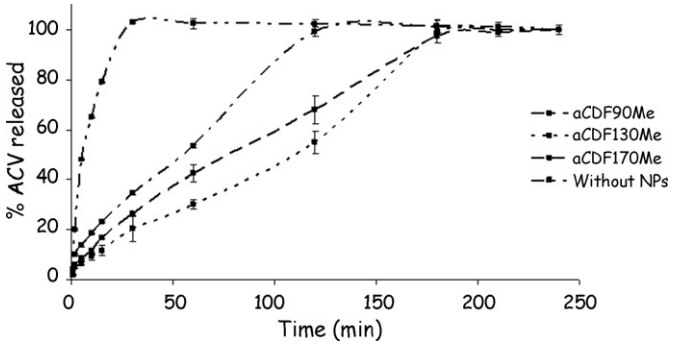


**Fig. 7.** Release profiles of ACV from highly loaded nanospheres based on αCDF9OMe, αCDF13OMe and αCDH17OMe in water.

## 4. Conclusion

New amphiphilic cyclodextrins substituted by perfluoroalkyl chains on the O-6 positions and permethylated on the O-2 and O-3 positions are able to self-organize in water in the form of nanospheres. Optimized preparation conditions have been established: 0.4 mM of amphiphilic cyclodextrins in ethanol. Characterization of nanoparticles by means of PCS and TEM methods allowed us to confirm nanospheres formation. Nanoparticles were spherical, small in size (100 nm), and the particle size distributions were monomodal and narrow. Suspensions of nanospheres were also stable over nine months of storage at +4 °C. The amphiphilic derivatives formed a 1:1 inclusion complex with acyclovir; the drug being probably located between the hydrophobic chains. Acyclovir could also be encapsulated in these carriers at satisfactory loading (∼40%) using the highly loaded method. Significant sustained release was observed (complete release in 3 h). These preliminary results show the benefits of the fluorinated chains with respect to hydrocarbon analogues and the potential regarding the application to the delivery of bioactive molecules (hydrophilic or lipophilic) coming from the self-organization of amphiphilic perfluoroalkylpropanethio-α-cyclodextrin derivatives as nanoparticles.

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