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Sulfated and non-sulfated amphiphilic-β-cyclodextrins: Impact of their structural properties on the physicochemical properties of nanoparticles

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Abstract

Propose: The aim of this work was to study the preparation of nanospheres from amphiphilic β-cyclodextrins formed (a) by different acylation degrees (DA) at the secondary hydroxyl face (DA = 14 and 21) followed by varying (b) the sulfatation degrees (DS) at the primary hydroxyl face (DS = 0, 4 and 7).
Methods: The physicochemical properties of the synthesized compounds such as molecular weights, the theoretical HLB values and the critical micellar concentration values and their surface area were presented. The nanoparticles prepared from amphiphilic β-cyclodextrins were characterized by mean size, zeta potential and their morphology.
Results: The compounds presented hydrophile–lipophile balance values ranging from 5.6 to 10. For sulfated amphiphilic β-cyclodextrins having HLB values higher than 8, were able to self-organize in water to form nanoparticles. However, for the amphiphilic β-cyclodextrins that HLB values lower than 6.6 are insoluble in water but soluble in organic solvents rendering possible the preparation of nanoparticles by nanoprecipitation technique.
Conclusion: An interesting correlation between the amphiphilic-β-cyclodextrin structures and their ability to form nanospheres has been established. The association of sulfated amphiphilic-β-CDs to the peracylated amphiphilic-β-CDs was interesting, it led to improve the stability of nanospheres size and probably confer them a biological activity.

Keywords: Sulfated amphiphilic β-cyclodextrins; Nanoparticles; Chemical structure; Stability

1. Introduction

During the last few years, a new concept in drug delivery system was considered. It was based on using amphiphilic cyclodextrin molecules in preparing nanoparticles (Duchene et al., 1999a,b; Duchêne and Wouessidjewe, 1996) or nanocapsules (Memisoglu et al., 2002). Cyclodextrins (CDs) are a well-known cyclic oligosaccharide able to form an inclusion complex inside their hydrophobic cavity with a wide range of lipophilic molecules. Cyclodextrins can improve: (i) the apparent solubility of poorly water-soluble drugs, (ii) the stability of labile drugs against hydrolysis, oxidation. . . and (iii) drug absorption and so their bioavailability (Szejtli, 1982; Uekama et al., 1998).

The hydrophilic outer surface of these molecules results in a weak interaction with biologic membranes. To circumvent this problem, many authors have proposed to graft hydrocarbon chains to both the primary and the secondary faces of cyclodextrin. Different series of amphiphilic cyclodextrins were prepared including lollipops which resulted from grafting only one aliphatic acid chain on a 6-amino-β cyclodextrin, Medusa-like cyclodextrins were obtained by grafting alkyl chains with length from C_{10} to C_{16} to all the primary hydroxyl groups of β-cyclodextrin, skirt-shaped cyclodextrin which corresponded

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to the esterification of all the secondary hydroxyl groups and finally bouquet-shaped cyclodextrins resulting from the grafting of 14 polymethylene chains to 3-monomethylated β cyclodextrin (Duchene et al., 1999a,b; Duchêne and Wouessidjewe, 1996). It was found that amphiphilic cyclodextrins were of considerable interest for pharmaceutical applications in view of their capacity for to self-assemble in water at physiological pH, to form micelles (Auzely-Velty et al., 2000), nanospheres (Dubes et al., 2003a; Lemos-Senna, 1998; Peroche et al., 2005), nanocapsules (Memisoglu et al., 2002) and liposomes (Donohue et al., 2002).

The introduction of sulfate groups onto the hydroxyl groups of cyclodextrins gave rise to a new class of modified cyclodextrin. The sulfate groups confer to cyclodextrins an interesting biological activity, such as anti-inflammatory and anti-lipemic activities, similar and sometimes superior to those of heparin on such derivatives (Uekama et al., 1998). It is also mentioned that the biological activity of the cited sulfate compounds depend on the number of sulfate groups introduced. Presently, two products containing sulfobutyether-β-cyclodextrin as excipients are marketed in Europe and in the United State with the trade mark Vfend® (Pfiezer) and Zeldox® (Geodon) containing as active ingredients variconazol and Ziprasidone mesylate, respectively. The first product is intended for intravenous administration and the second one for intramuscular administration.

In this context, it appeared very interesting to associate to cyclodextrins on the one side a biological activity by grafting sulfate groups on their primary hydroxyl face. And on the other side, to render these compounds amphiphilic, hydrophobic chains were grafted on their secondary hydroxyl face. The synthesis of the original compounds were described by the authors (Dubes et al., 2001).

In this paper, we studied the feasibility of producing colloidal systems from amphiphilic-β-cyclodextrins (a) having different degrees of acylation (DA) at the secondary hydroxyl face (DA = 14 and 21) followed by varying the degrees of sulfatation (DS) at the primary hydroxyl face (b) (DS = 0, 4 and 7). The characterization of the colloidal systems was carried out by measuring particle size (photon correlation spectroscopie) and zeta potential (Zetasizer instrument). Also, an imaging by scanning electronic microscope of different nanoparticles was achieved.

2. Materials and Methods

2.1. Materials

All chemicals were purchased from Acros Organics and used without further purification. β-Cyclodextrin was purchased from Wacker (France), and amphiphilic sulfated β-cyclodextrins were synthesized (as previously described by (Dubes et al. (2001)) by protecting all primary hydroxyl groups with tert-butyldimethylsilyl chloride; then acylation was performed with either hexanoic anhydride or hexanoyl chloride in the presence of 2,4-dimethylaminopyridine. Removal of tert-butyldimethylsilyl groups was performed with boron trifluoride etherate and sulfatation with sulfur trioxide pyridine complex. βCD14C$_6$ (Fig. 1a) is a pure non-sulfated derivative with seven hexanoyl chains at the O-2 position and seven hexanoyl chains

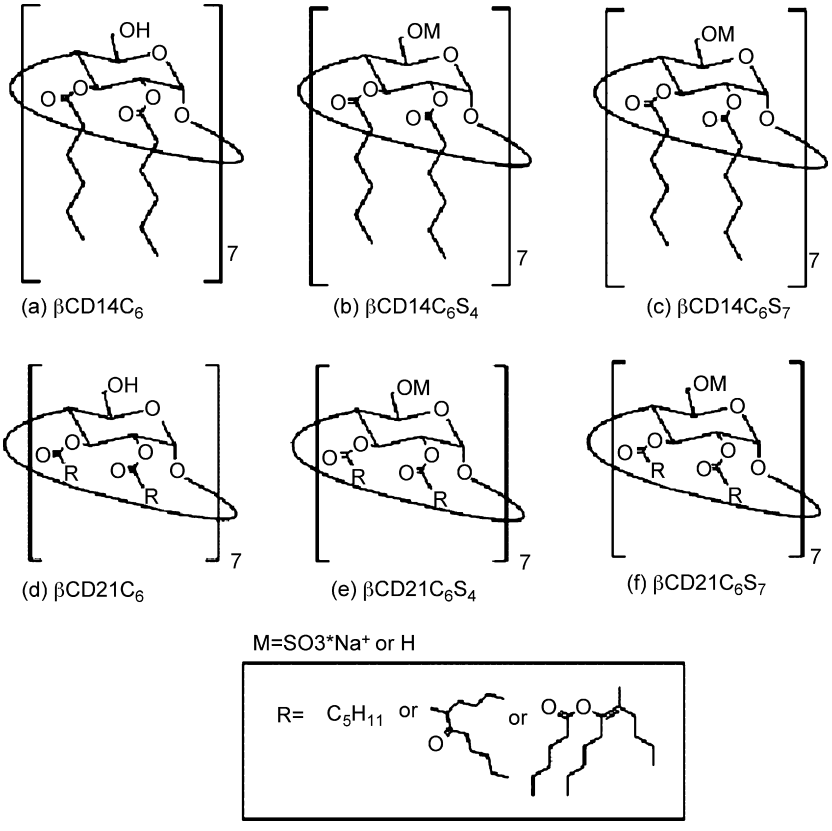

Fig. 1. Molecular structures of sulfated and non-sulfated amphiphilic-β-cyclodextrins.

at O-3 position; $\beta CD14C_6S_7$ is a pure sulfated product with seven hexanoyl chains at the O-2 position, seven hexanoyl chains at O-3 position, and seven sulfate groups at the O-6 position. $\beta CD14C_6S_4$ (Fig. 1b), $\beta CD21C_6$ (Fig. 1d), $\beta CD21C_6S_4$ (Fig. 1e), and $\beta CD21C_6S_7$ (Fig. 1f) are derivatives with an average degree of substitution of 21 hexanoyl chains, 14 hexanoyl chains and 4 sulfates, 21 hexanoyl chains and 4 sulfates, and 21 hexanoyl chains and 7 sulfates, respectively.

2.2. Methods

2.2.1. Preparation of nanoparticles

The amphiphilic-β-cyclodextrins nanoparticles were prepared by the nanoprecipitation technique. The relevant amphiphilic cyclodextrin ($\beta CD21C_6$, $\beta CD21C_6S_7$ and $\beta CD21C_6S_4$, 35 mg) was dissolved in ethanol (12.5 ml) and the solution was added for 1 min to water (25 ml) stirred at 400 rpm. The ethanol and a part of water were removed under reduced pressure and the total volume adjusted to 20 ml.

2.2.2. Particle size measurements

The mean particle size and the polydispersity index (PI) of amphiphilic nanospheres were measured by photon correlation spectroscopy (PCS) by using Malvern spectrometer 7032 (Malvern instruments, UK) which analyses the fluctuations in scattered light intensity generated by diffusion of the light in diluted suspension of nanoparticles. The measurements were carried out at 25 °C. Each value is the average of three measurements.

2.2.3. Zeta potential measurements

The surface charge of nanoparticles was determined by measurement of zeta potential of the particles extracted from their electrophoretic mobility. Nanospheres were suspended in 10^{-3} M KCl and measurements were realized by using Zetasizer 3 (Malvern Instruments, France). The measurements were made in triplicate at 25 °C.

2.2.4. Freeze drying of nanospheres

The lyophilization of nanospheres prepared with $\beta CD21C_6$ and stabilised with sodium dodecyl sulfate was performed by using a pilot freeze-dryer Usifroid SMH45 (Usifroid, France). The conditions applied during our study were the following: freezing for 2 h at −45 °C with a cooling profile of 1 °C/min, sublimation at a shelf-temperature −15 °C and a pressure of 100 μbar for 15 h and finally, secondary drying at 25 °C and 50 μbar for 6 h. 0.5 ml of nanosphere suspension without any protectant agent was filled into 5 ml freeze-drying vials (Fisher Bioblock scientific, France).

2.2.5. Scanning electronic microscopy (SEM)

The morphology of nanoparticles was characterized by SEM using a Hitachi S-800 microscope (Hitachi, Germany) at an accelerating voltage of 15 KV. A drop of the nanoparticle suspension was placed on a metallic probe, immersed in liquid nitrogen for 10 min and then evaporated under vacuum. For the freeze-dried samples, they were mounted on aluminium pins using double-sided adhesive tape. Prior to microscopic examination, the samples were coated with a gold/palladium layer under vacuum with a cathodic pulverizer technics Hummer II (6 V–10 mA).

3. Results and discussion

3.1. Physicochemical characterization of amphiphilic β-cyclodextrins

3.1.1. Molecular configuration

Six different sulfated and non-sulfated amphiphilic β-cyclodextrins have been synthesized and the characterization of the molecules has been realized in our previous paper (Dubes et al., 2001). Their structures are presented in Fig. 1, and their physicochemical properties are shown in Table 1. Molecular weight of the compounds ranged from 2507 to 3907 g/mol. Three of them were derivatives with a average degree of substitution of 21 hexanoyl chains abbreviated β-$CD21C_6$ and three others with average degree of substitution of 14 hexanoyl chains abbreviated β-$CD14C_6$. In each series, there was a molecule without sulfatation, a molecule with average degree of sulfatation of 4 (β-$CD14C_6S_4$, β-$CD21C_6S_4$) and a molecule with a degree of sulfatation of 7, namely for this later the whole hydroxyls at the primary face were sulfated ($\beta CD14C_6S_7$, $\beta CD21C_6S_7$). All the

Table 1
Physicochemical properties of non-sulfated and sulfated amphiphilic cyclodextrins and their influence on the properties of produced nanospheres

Amphiphilic β-CD	Characteristics of amphiphilic cyclodextrins					Characteristics of nanospheres		
	Acylation degree	Sulfatation degree	Molecular weight (g/mol)	HLB[a] values	CMC (μM)	Diameter (nm)	P.I.[b]	Zeta potential (mV)
$\beta CD21C_6$	21	0	3193	5.6	–	137.2 ± 3.4[c]	0.04 ± 0.038	−20 ± 1.2
$\beta CD21C_6S_4$		4	3601	7.2	–	132 ± 1.5	0.06 ± 0.03	−50.6 ± 0.7
$\beta CD21C_6S_7$		7	3907	8.2	10	41–92[d]	–	–
$\beta CD14C_6$	14	0	2507	7.1	–	159 ± 5.5	0.09 ± 0.074	−15 ± 1.7
$\beta CD14C_6S_4$		4	2915	9	120	29–86[d]	–	–
$\beta CD14C_6S_7$		7	3221	10	110	33–84[d]	–	–

[a] Hydrophile Lipophile Balance.
[b] Polydispersity index.
[c] Unstable preparation and formation of aggregates after keeping it in an aqueous suspension for 1 h.
[d] We have a bimodal distribution of the size.

molecules presented above have surfactant-like structures with distinguish parts: a polar head group (cyclodextrin ring bearing or not sulfates) and 14 or more of hydrophobic acyloyl tails. The structure should bring amphiphilicity and led the self-assemble into a well-defined microstructure in order to minimize solvent interactions.

3.1.2. Solubility

The following derivatives β-CD14C_6S_4, β-CD14C_6S_7 and β-CD21C_6S_7 are slightly soluble in water due to the introduction of sulfated groups. Whereas, β-CD14C_6, β-CD21C_6S_4 and β-CD21C_6 are water-insoluble, but soluble in organic solvents such as acetone and ethanol. Due to the high acylation degree of β-CD21C_6S_7, the introduction of 7 sulfate groups has been necessary to render it soluble in water, whereas for β-CD14C_6S_4 four sulfate groups have been sufficient.

3.1.3. Determination of Hydrophilic–Lipophilic balance (HLB) of amphiphilic β-CDs

In order to correlate the structural characteristics of amphiphilic β-CDs with their ability to self-assemble, it seemed interesting to calculate the ratio between the hydrophilic and the hydrophobic character by determining the theoretical HLB values of the different compounds Fig. 2.

The calculated HLB is in analogy to the Griffin's equation proposed for non-ionic surfactants, and already applied on the amphiphilic γ-cyclodextrins by Lemos-Senna (1998).

$$\mathrm{HLB} = \frac{W_{\mathrm{hydro}}/W_{\mathrm{CD}}}{5} \times 100$$

where: W_{hydro}: molecular weight corresponding to the hydrophilic moiety (cyclodextrin ring including sulfate groups when existing). W_{CD}: molecular weight of the corresponding amphiphilic β-CD.

The calculated HLB for the different compounds ranges from 5.6 to 10 (Table 1). The HLB values of β-CD21C_6, β-CD21C_6S_4 and β-CD14C_6 are respectively 5.6, 7.2 and 7.1. These HLB values were lower than those calculated for β-CD21C_6S_7, β-CD14C_6S_4 and β-CD14C_6S_7, which were 8.2, 9 and 10, respectively. When comparing β-CD14C_6, β-CD14C_6S_4 and β-CD14C_6S_7, we see that the addition of sulfated head groups led to an increase in the HLB values; this increase was of 1.28 folds for β-CD14C_6S_4 versus β-CD14C_6 and of 1.5 folds for β-CD14C_6S_7 versus β-CD14C_6.

In order to characterize the amphiphilic behaviour, surface tension was measured for the water-soluble compounds in solution of increasing concentrations in pure water at 25 °C. The critical micellar concentration (CMC) values for β-CD14C_6S_4, β-CD14C_6S_7 and β-CD21C_6S_7 are respectively of 120, 110 and 10 μM. These results showed that the sulfatation degree did not influence the CMC whereas an increase of acylation degree provoked its decrease with a ratio of 10 due to a larger hydrophobic tail of β-CD21C_6S_7 compared to β-CD14C_6S_7. The same behaviour was observed by Cavalli et al. (2007) for three alkylcarbonates of γ-cyclodextrin, i.e. hexyl, octyl and dodecylcarbonate. The authors observed a decrease of the CMC values as the alkyl chain length increased.

3.2. Characteristics of water-soluble amphiphilic β-cyclodextrin nanospheres

It is mentioned above that the following derivatives β-CD14C_6S_4, β-CD14C_6S_7 and β-CD21C_6S_7 having HLB values greater than 8 are water-soluble. These molecules were able to self-assemble in nanoparticulate systems beyond their critical micellar concentration when dispersed in water at low concentrations. The measured particle size has a bimodal distribution, the first majority population has a diameter which varied between 30 and 40 nm, and the second population was located around 85 nm. These results let suppose that we were in presence of aggregates and not of micelles. For high concentrations of the water-soluble amphiphilic β-cyclodextrins, lamellar structures were observed in water.

3.3. Characteristics of water-insoluble amphiphilic β-cyclodextrin nanospheres

With water-insoluble amphiphilic β-cyclodextrins (βCD14C_6, βCD21C_6 and βCD21C_6S_4) having HLB values lower than 7.3, the preparation of nanospheres was realized without surfactant using nanoprecipitation technique. Each of the water-insoluble amphiphilic β-cyclodextrin was dissolved in ethanol. The spontaneous formation of amphiphilic β-cyclodextrin nanospheres occurred when the organic phase was injected in water phase. Table 1 presents the influence of sulfatation and acylation degree on the values of the size, polydispersity index and zeta potential of the formulated nanospheres.

The surface of non-sulfated βCD14C_6 and βCD21C_6 nanoparticles were negatively charged as shown by the zeta potential values (Table 1). These values varied from −15 to −20 mV as previously reported (Memisoglu-Bilensoy et al., 2005). The negative charge indicated the presence of the hydroxyl groups of β-CD oriented toward the water, with surface hydrophilicity, and with the alkyl chains in the inner part of the nanoparticles. As expected, an increase in the zeta potential value from −20 to −50 mV was observed for β-CD21C_6 nanospheres compared to β-CD21C_6S_4 bearing 4 sulfates, respectively. It was probably due to the negative charge of sulfate groups localised at the surface of nanoparticles.

Particle size of β-CD21C_6 and β-CD21C_6S_4 nanoparticles are very near about 137 and 132 nm, respectively with a very high degree of monodispersity about 0.03. β-CD21C_6 nanoparticles were observed in SEM just after the preparation, they were spherical in shape. For β-CD21C_6S_4 nanoparticles, spherical to elongated shape was observed, but more stable than β-CD21C_6 nanoparticles. Indeed, after keeping the preparation in aqueous suspension for 1 h, we observed instability of β-CD21C_6 nanospheres conducting to the formation of aggregates with particle size of 360 nm. These results were correlated with those obtained by different authors (Geze et al., 2002; Lemos-Senna et al., 1998; Ringard-lefebvre et al., 2002; Skiba et al., 1996). Ringard-lefebvre et al. (2002) stated on that for concentrations higher than 0.6 mM of amphiphilic β-CDC$_6$, the instability of nanospheres was inevitable. Furthermore, they proved that molecular surface area of β-CDC$_6$ plays an important role. In

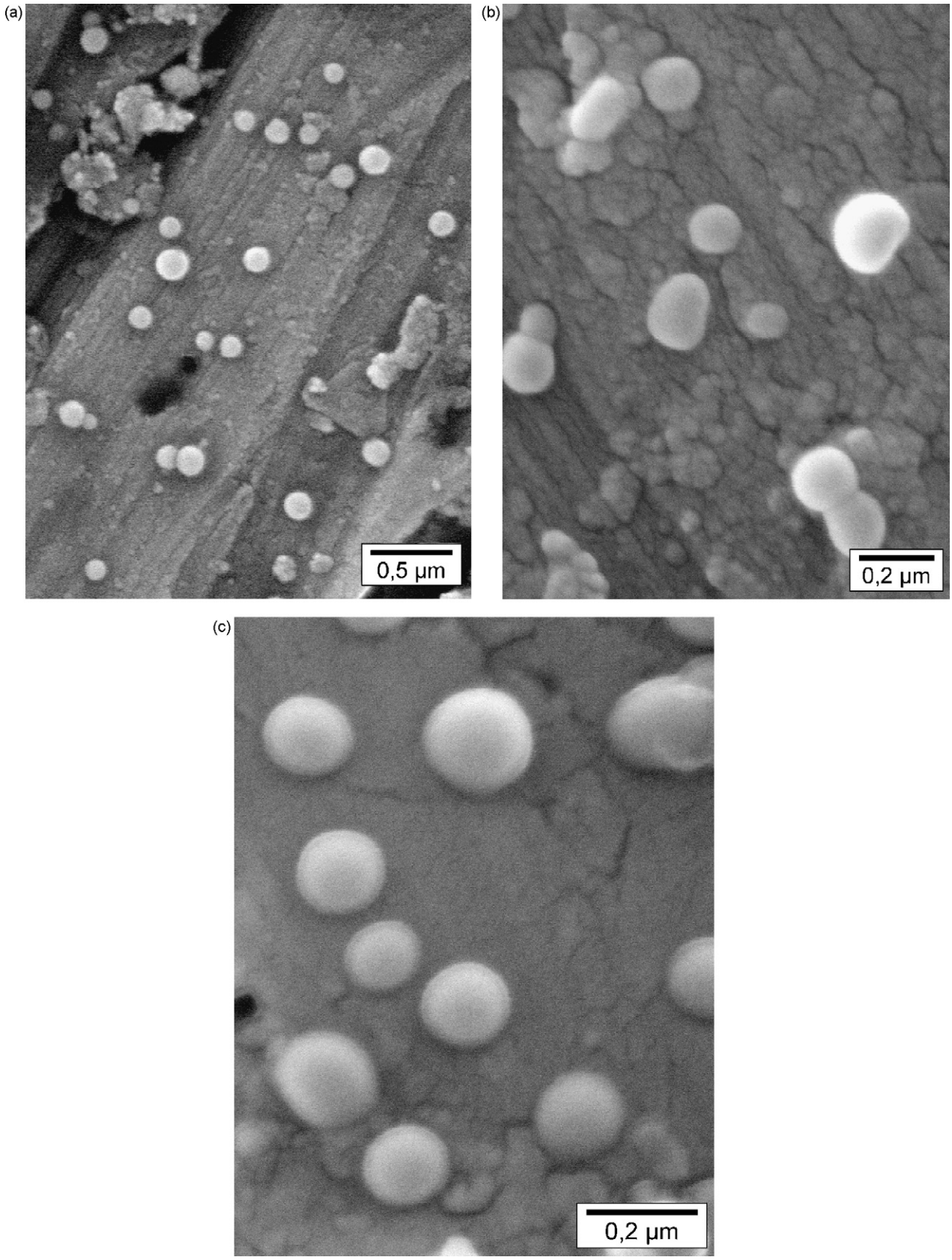

Fig. 2. Scanning electron microscopy photographs of amphiphilic β-cyclodextrins: (a) nanospheres of β-CD21C_6, (b) nanospheres of β-CD14C_6 and (c) nanospheres of mixed β-CD21C_6 with β-CD21C_6S_7.

Table 2
Influence of mixing sulphated (β-CD21 C_6 S_7) and non-sulphated (β-CD 21 C_6) amphiphilic β-cyclodextrin on nanoparticle properties

β-CD 21 C_6% (w/w)	β-CD21 C_6 S_7% (w/w)	Mean size (nm)	Polydispersity Index	Zeta Potential (mV)
85	15	84.4	0.21	−31.5
70	30	71	0.188	−33.2
50	50	69.4	0.338	−35.8
30	70	76.6	0.481	−33.6
15	85	80.4	0.391	−30.15

fact, the authors used 6-*N*-CDC_6 molecule with smaller molecular surface area (218 Å) than β-CDC_6 (370 Å), more stable nanospheres were obtained. In order to check this hypothesis, an extended study of the interfaciale behaviour of amphiphilic β-CDs presented in this work has been realized earlier, confirmed this hypothesis (Dubes et al., 2003b). The molecular area of β-$CD21C_6$ shows an apparent molecular area of 353 $Å^2$ higher than the apparent molecular area of β-$CD21C_6S_4$ which is of 302 $Å^2$.

3.4. Influence of mixing non-sulfated and sulfated amphiphilic β-CDs on the stability of produced nanospheres

As mentioned above the preparation of stable nanospheres from peracylated cyclodextrins is not possible. This instability is observed either for peracylated β-CDs (Skiba et al., 1996) or peracylated γ-CDs (Lemos-Senna et al., 1998) in the two cases, the compounds bearing 14 hydrocarbon chains. Skiba et al. (1996) reported that even in the presence of surfactant, the instability of the nanosphere suspensions occurred. In this study, an investigation was carried out for exploring the effect of mixing sulfated and non-sulfated amphiphilic β-CDs on the stability of nanoparticles prepared. β-$CD21C_6$ and β-$CD21C_6S_7$ were chosen for realizing this approach, on the one hand because β-$CD21C_6$ produces instable nanoparticle suspensions, on the other hand because β-$CD21C_6S_7$ also possess 21 hydrocarbon chains that can increase the interaction between these two compounds during the nanoprecipitation step. Table 2 presents the results obtained from mixing those molecules with different percentages using the same formulation described previously. It was found from particles size observation that the introduction of 15% (w/w) of β-$CD21C_6S_7$ in the formulation resulted in an important decrease of particles size from 137.2 nm for non-sulfated nanospheres to 84.4 nm for a mixture. Beyond this percentage the size of the nanospheres decreased until 50% to increase till 80 nm for 85% (w/w) of β-$CD21C_6S_7$. On the other hand, the continual increase in polydispersity index is observed with increasing the percentage of β-$CD21C_6S_7$ ranging from 0.21 to 0.48 for percentage ranging from 15 to 85%. For understanding this phenomenon, an hypothesis was considered. It was appeared, that sulfated β-$CD21C_6S_7$ molecule could solubilize the amphiphilic β-$CD21C_6$ compounds. Such effect had been studied (Lemos-Senna et al., 1998). These authors investigated the solubilizing effect of surfactant on nanospheres prepared from amphiphilic 2,3-di *O*-hexanoyl cyclomaltooctaose (γCDC6) using pluronic F68 as a model surfactant. However, the nanoparticles prepared from mixing two cyclodextrins molecules became very polydisperse. This polydispersity might be a result from the formation of aggregates of mixed micelles composed from the two amphiphilic molecules. It was obvious from zeta potential values that sulfate groups of β-$CD21C_6S_7$ were localized at the surface of nanoparticles.

4. Conclusion

β-CDs used in this study have been formed by an acylation at the secondary hydroxyl face and the sulfatation at the primary face of cyclodextrins. The results obtained showed an interesting correlation between the structure of the amphiphilic-β-CDs and their ability to form nanospheres has been established. Indeed, for the compounds that HLB values were greater than eight are water soluble, able to self organize in water to form nanospheres. Whereas, for the compounds with HLB values lower than 7.4 are soluble in organic solvent rendering the preparation of nanoparticles by nanoprecipitation technique possible.

In this study, we demonstrated that the association of sulfated amphiphilic-β-CDs to the peracylated amphiphilic-β-CDs was interesting because it led to improve the stability of nanospheres size and probably to confer them a biological activity.

Current study is under way for including acyclovir, an antiviral drug. The combination of antiviral activity of sulfated amphiphilic β-cyclodextrin nanoparticles with that of acyclovir may present a potential treatment for the herpetic encephalitis. Further investigations are recommended for evaluating this synergism and for assuring the absence of haemolytic effects of these nanoparticles.

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