SELF-ASSEMBLED NANOPARTICLES MADE OF DEXTRIN

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Abstract. *The amphiphilic molecule dextrin-VA-SC_{16} ($dexC_{16}$) was produced and studied in this work. $DexC_{16}$ has a hydrophilic dextrin backbone with grafted acrylate groups (VA), substituted with hydrophobic 1-hexadecanethiol (C_{16}). The dextrin degree of substitution with the hydrophobic chains (DS_{C16}, number of alkyl chains per 100 dextrin glucopyranoside residues) may be controlled. Materials with different DS_{C16} were prepared and characterized using ^1H NMR. $DexC_{16}$ self assembles in water through association of the hydrophobic alkyl chains, originating hydrogel nanoparticles. The properties of the hydrogel nanoparticle were studied by dynamic light scattering (DLS), fluorescence spectroscopy and atomic force microscopy (AFM).*

Keywords: *Nanoparticles, Dextrin, Self-assembling*

1. INTRODUCTION

Amphiphilic molecules, such as surfactants or lipids, spontaneously self-assemble in water, forming self-aggregates, such as micelles, bilayer membranes, tubes and vesicles. Amphiphilicity of biopolymers is one of the important factors for their self-organization in water (Akiyoshi and Sunamoto, 1996). Among the different types of amphiphilic polymers, water-soluble polymers with hydrophobic molecules grafted on side chains have received special attention. By self-assembling, the hydrophobic segments are segregated from the aqueous exterior, to form an inner core surrounded by hydrophilic chains. Polymer micelles or nanoparticles with hydrophobic core and hydrophilic shell are thus prepared. This kind of structure is suitable for trapping hydrophobic substances, such as fluorescent probes (Akiyoshi *et al.*, 1993, Nichifor *et al.*, 2004), proteins (Akiyoshi *et al.*, 1998), and hydrophobic pharmaceuticals (Liu *et al.*, 2005). Size, density and colloidal stability of nanoparticles can be controlled, by changing the degree of substitution of hydrophobes and its hydrophobicity (Akiyoshi and Sunamoto, 1996). The association mechanism is mainly governed by the alkyl chain concentration and length and is little influenced by the molecular weight of the polymer backbone (Petit-Agnely *et al.* 2000). However, with low-molecular weight polymers, the hydrophobic aggregates are not connected via the polymer backbone (Petit-Agnely and Iliopoulos, 1999). The study of nanogels (hydrogel nanoparticles) has intensified during the last decade due to enormous potential applications in the development and implementation of new environmentally responsive materials, biomimetics, biosensors,

artificial muscles and drug delivery systems (Akiyoshi and Lee, 2004). Solid nanoparticles made from biodegradable polymers have been widely investigated for long-term delivery of drugs (Hans *et al*., 2005). They can potentially provide benefits such as increased therapeutic effect, prolonged bioactivity, controlled release rate, and finally decreased administration frequency, thereby increasing patient compliance.
Nanostructures spontaneously form when the concentration of the polymer is higher than a concentration called critical micelle concentration (cmc). The self-assembly of hydrophobized dextrin in water ($dexC_{16}$) was investigated in this work. The structural change upon the dilution of the $dexC_{16}$ self-aggregates, in water, was investigated by fluorometry, in the presence of pyrene as the fluorescent probe (Magdassi and Nizri, 2005). Other relevant properties of the nanoparticles, such as the size, stability, and shape were also evaluated in this work.

2. EXPERIMENTAL SECTION

2.1. Materials

The dextrin-VA was synthesized as described in a previous paper (Carvalho *et al*., submitted). In this work, dextrin-VA with 20 acrylate groups per 100 dextrin glucopyranoside residues (DS_{VA} 20%) was used. Dimethylsulfoxide (DMSO), triethylamine (TEA) and deuterium oxide (D_2O) were from Aldrich. Regenerated cellulose tubular membranes, with 3500 MWCO, were obtained from Membrane Filtration Products. Pyrene (Py) was from Aldrich and was used after appropriate recrystallization. Distilled water was used for the preparation of aqueous solutions.

2.2. Synthesis of $dexC_{16}$

Dextrin-VA and 1-hexadecanethiol were dissolved in dimethylsulfoxide (conc. VA = 0.058 M). Different molar percentages (10, 20, 40, 60, 100% relatively to VA) of 1-hexadecanethiol were added to the reaction mixture such that different levels of grafting were obtained. Triethylamine (1 equivalent to VA) was added to the reaction. The medium was stirred for 24h, at 50ºC. The mixture was dialysed for 48h against water, with frequent water change. After freezing, the mixture was lyophilized and stored.

2.3. Sample Preparation

The lyophilized $dexC_{16}$ was dissolved in water under stirring at 50ºC, and then further sonicated for 20min. The degree of solubility depends on degree of substitution. Increasing the degree of substitution reduces the solubility.

2.4. Dynamic Light Scattering (DLS)

The size distribution was determined with a Malvern Zetasizer, MODEL NANO ZS (Malvern Instruments Limited, UK). A water dispersion (1 mL) of nanoparticles in ultra-pure water were analysed in a polystyrene cell, using a He-Ne laser - wavelength of 633 nm and a detector angle of 173º. The temperature used was 25ºC.

2.5. Fluorescence spectroscopy

Fluorescence measurements were performed on a JASCO FP-6200 spectrofluorometer in a quartz cell. The Pyrene spectra were obtained by exciting the solutions at 377 nm and recording the emission over the range 350-600 nm at the scan rate 250 nm/min. The slit width was set at 5 nm for the excitation and 5 nm for the emission. To increase the precision of the determination of the values of intensities of different vibronic peaks, the fluorescence intensities were measured at the maximum of each peak.

2.6. Atomic Force Microscopy (AFM)

Tapping mode imaging was carried out on a Multimode (Digital Instruments) scanning probe microscope. A silicon tip doped with phosphorus, with a radius curvature of less than 10 nm (RTESP, VEECO), was used. This tip has a typical resonance frequency of 288-328 kHz and a typical force constant of 20-80 N/m. A scan rate of 1 Hz was sufficient to maintain a good signal-to-noise ratio.

3. RESULTS AND DISCUSSION

3.1. Synthesis of the dextrin-VA- SC_{16}

The reaction between the thiol moiety and the acrylate group of dextrin-VA is a conjugate addition, with thiol acting as a nucleophile (Figure 1). Although the reaction was detected in the absence of TEA, the presence of the base substantially increases the reaction rate, as described ahead.

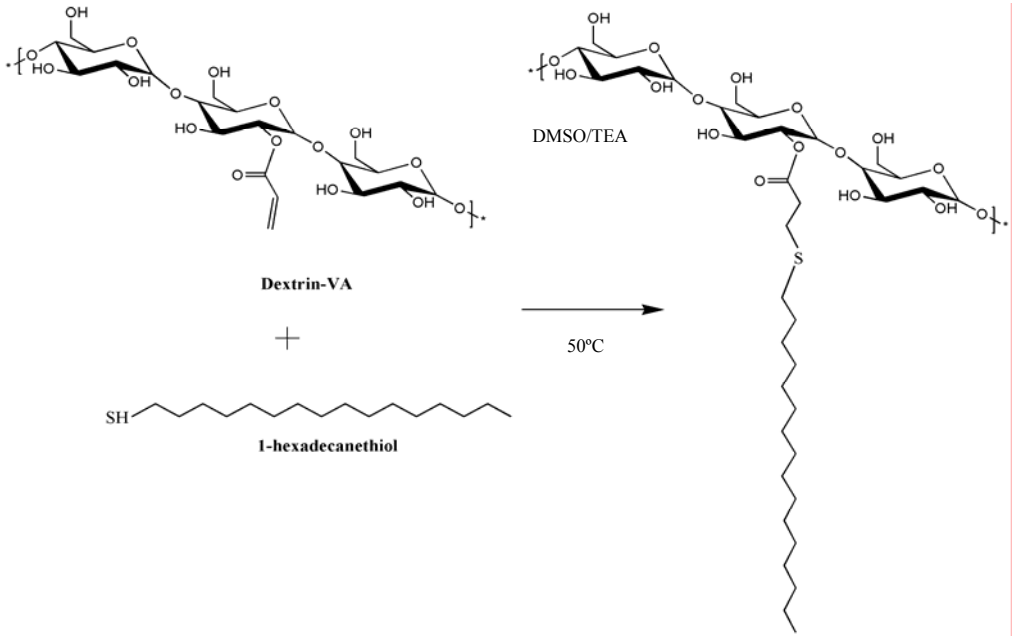

Figure 1. Synthesis of the $dexC_{16}$.

3.2. Formation of nanoparticles

The dissolution of $dexC_{16}$ in water is expected to five rise to micelle formation, owing to the amphiphilic nature of the molecules. The formation of hydrogel nanoparticles was accessed using both ^{1}H NMR and AFM.

Due to the limited mobility of the alkyl/hydrophobic chains inside of the nanoparticle, the shape of the proton peaks generated by the alkyl chain (2.0 - 0.6 ppm) is different depending on whether the 1H NMR spectra is obtained in deuterated water or DMSO. Peaks from methyl (0.8 ppm) and methylene (1.1 ppm) groups are visible in all spectra presented in Figure 2. They are sharp for DMSO solvent, and sharp yet progressively broadening at the base, for deuterated water solvent. As the concentration of water in the D_2O/DMSO mixtures increases, the larger is the broadening of the methylene protons. The shape of the alkyl chain peaks, in deuterated water, is characteristic of a superposition of peaks representing a collection of chemically identical species, yet possessing various degrees of mobility. This evidence suggests that alkyl chains have different environments, when dispersed in water.

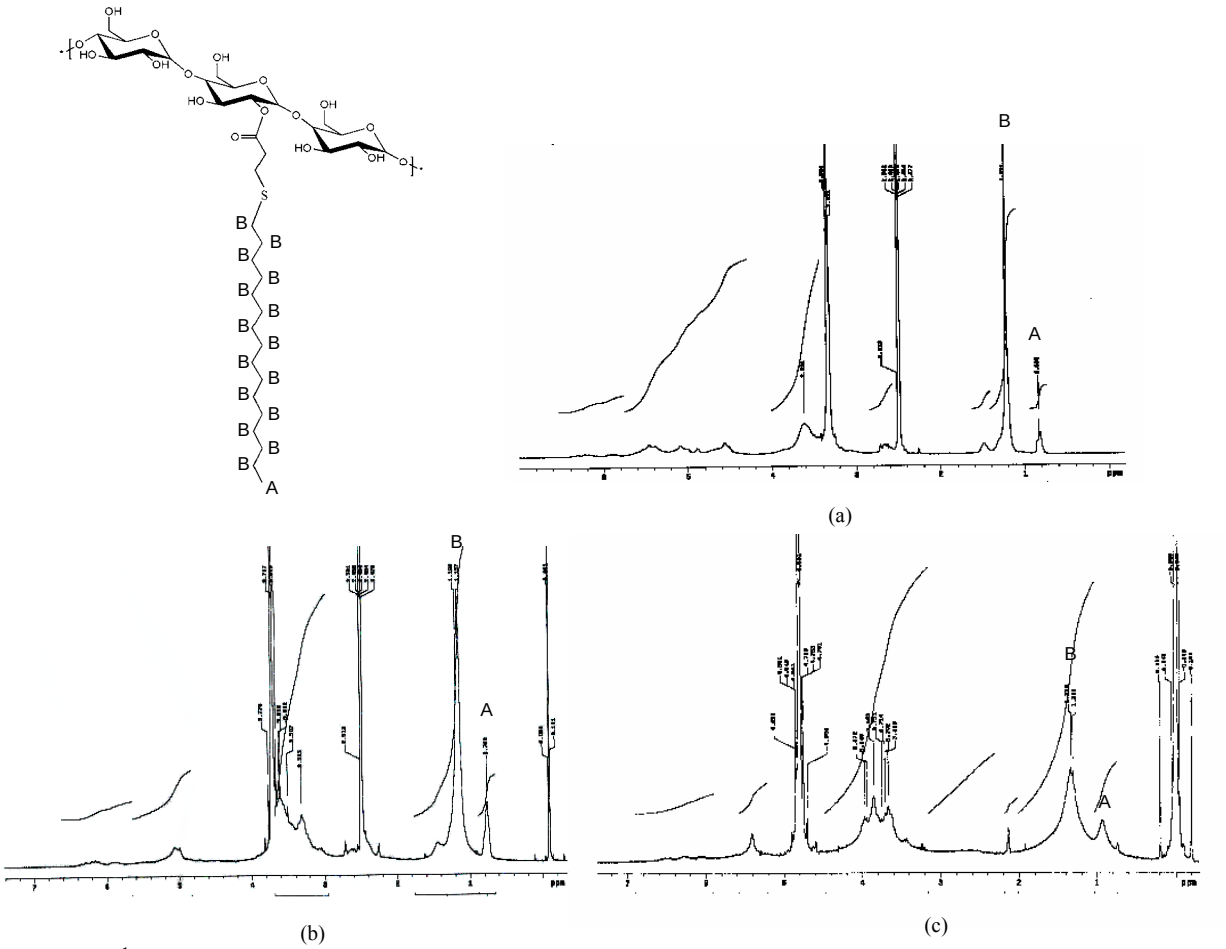

Figure 2. ^{1}H NMR spectra of dexC_{16} (10 mg/mL): (a) DMSO-d_6, (b) 10% D_2O in DMSO-d_6 and (c) D_2O.

In addition, the self-assembled particles were observed using AFM. The AFM images collected (Figure 3) allow direct visualization of the spherical nanoparticles, formed by self-assembling. The AFM results indicate that, for dexC_{16} with DS_{C16} 7%, the mean diameter of nanoparticles is roughly 20 nm. In general, the nanoparticles appear to have a narrow size distribution.

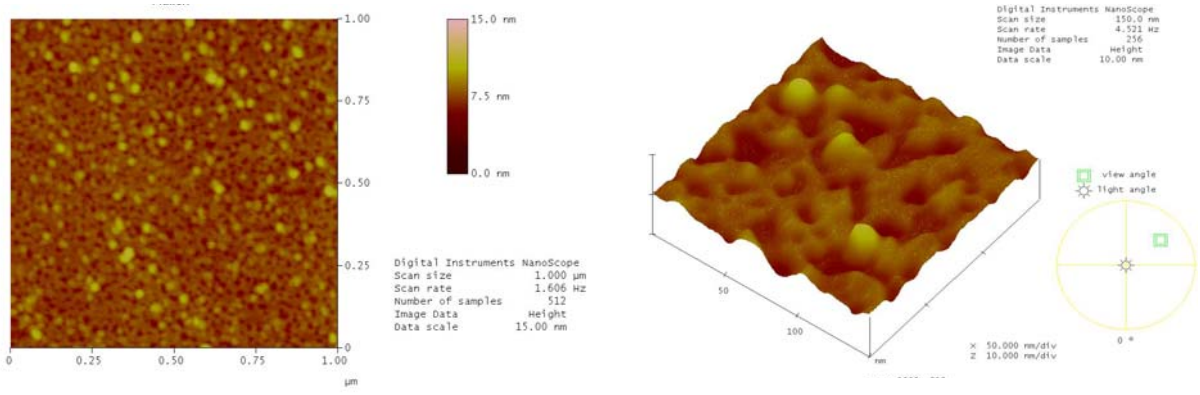

Figure 3. AFM images (a) and (b) three-dimensional of dexC_{16} (DS_{C16} 7%) at the concentration of 0.01g/dL.

3.3. Degree of substitution

The synthesis of dexC_{16} with different degrees of substitution with hydrophobic chains was successfully attempted, using different amounts of 1-hexadecanethiol in the reaction mixture. The ^{1}H NMR spectra of dexC_{16}, in deuterated water, was used to estimate the degree of substitution (DS_{C16}, amount of alkyl chains per 100 dextrin glucopyranoside residues). DS_{C16} was calculated as a peak area ratio in the NMR spectra, according to equation 1.

$$DS_{C16} = \frac{7 \times x}{37 \times y} \times 100 \qquad (1)$$

Where x is the average integral of the protons from alkyl moieties (2.0 - 0.6 ppm), and y is the integral of all dextrin protons (3.0 - 5.8 ppm). Table 1 presents the DS values obtained for dexC_{16} and the respective efficiency.

Table 1. Degree of substitution obtained relatively to acrylate groups and efficiency for dexC_{16}.

Theoretical DS [a] (%)	Obtained DS [b] (%)	Efficiency [c] (%)
10	2	20
20	4	20
40	14	35
60	23	38
100	59	59

(a) Calculated as molar ratio of 1-hexadecanethiol to acrylate groups ($\times 100$).
(b) Determined by ^{1}H NMR. The value shown is the calculated using equation 3, and multiplied by 5 to obtain the DS relatively to acrylate groups.
(c) Calculated as the ratio of the obtained to the theoretical DS ($\times 100$).

The results show that is possible to produce material with varying amounts of grafted alkyl chain, hence allowing the fine tunning of the properties of the obtained materials. Furthermore, different hydrophobic materials may be grafted, for instance thiocholesterol (data not shown).

3.4. Size and Size Distribution

The dynamic light scattering (DLS) studies were done using a Nano-ZS (Malvern) instrument. Samples of dexC_{16} with different degree of substitution and concentrations were analysed. The results of the DLS studies are given in Table 2. A representative size distribution by volume (Figure 4) is shown.

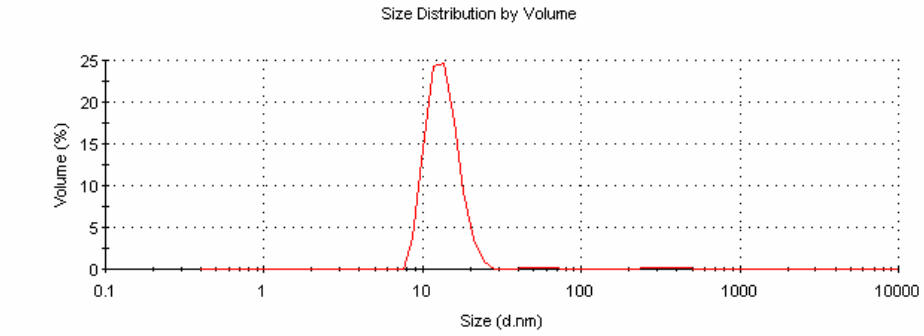

Figure 4. Size distribution by volume obtained by DLS for dexC_{16} with DS_{C16} 7%.

Table 2. Results of DLS studies with dexC_{16}.

		Size /nm (fraction of material/%)		
DS_{C16}	Conc.	Peak 1	Peak 2	Peak 3
7%	0.001	15.4 (98.7%)	187 (1.3%)	
7%	0.01	13.6 (98.5%)	57.3 (0.7%)	349 (0.8%)
7%	0.1	9 (97%)	61.6 (0.5%)	737 (2.5%)

Data in table 2 shows that the dilution process has no significant effect on the particle size, in the range of concentration used. The hydrodynamic diameter of the nanoparticles slightly increases with the decreasing of concentration. The size distribution generally presents three peaks, but the majority volume of the particles has around 10-20 nm in size.
The DLS results are in good agreement with AFM presented above. It was possible to observe the small nanoparticles, with a diameter around 20 nm, as the predominant population.

3.5. Critical Micelle Concentration of Hydrophobized Dextrin-VA

As reported by Akiyoshi [1996], pyrene may be used as a fluorescent probe to evaluate aggregate formation. The fluorescent properties of pyrene change when it is transferred from the aqueous environment to the hydrophobic microenvironment of the nanoparticles. To determine the *cmc*, the ratio of intensities (I_3/I_1) in the emission spectra was used.

The pyrene concentration is in all experiments very low (1×10^{-6}M), to prevent excimer formation (detectable at ≈ 470 nm). The I_3/I_1 values corresponding to samples with different polymer concentrations were calculated. The intensity ratio was plotted against polymer concentration, for dexC_{16} samples with different degrees of substitution. Figure 5a shows the variation of I_3/I_1 with concentration for dexC_{16} with DS_{C16} 7%.

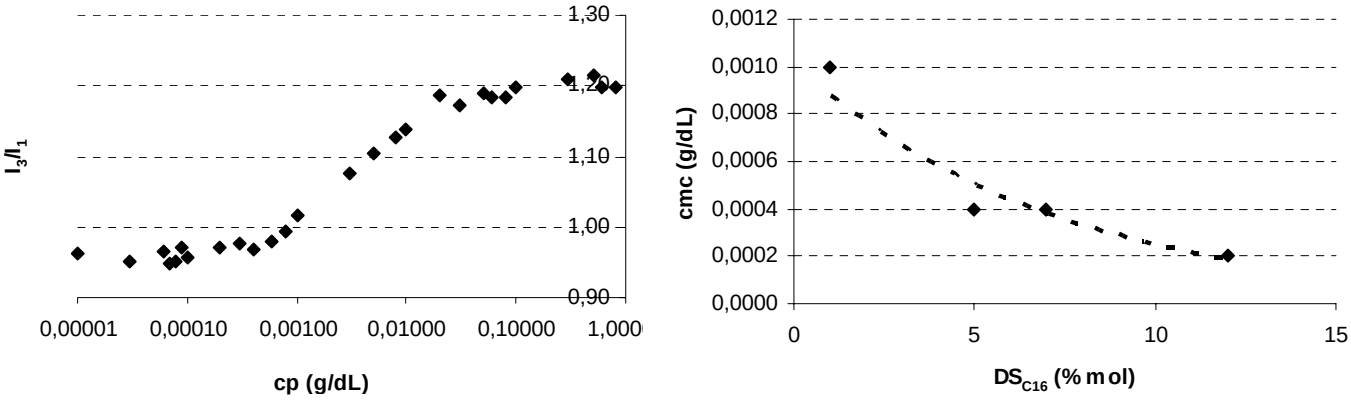

Figure 5. (a) Fluorescence intensity ratio I_3/I_1 as a function of the dexC_{16} concentration with DS_{C16} 7% and (b) variation of critical micelle concentration (*cmc*) of dexC_{16} with the degree of substitution.

At low polymer concentrations, I_3/I_1 is almost constant, i.e. pyrene experiences an aqueous environment. For higher polymer concentrations I_3/I_1 starts to increase, due to the solubilization of pyrene in the hydrophobic domains formed by self-assembly (for cp > 0.0004 g/dL), where it senses a less polar environment. The curve is sigmoidal, as observed for other amphiphilic polymers. The ratio I_3/I_1 reach a constant value, as there are enough nanoparticles for solubilising all pyrene molecules. The variation in pyrene fluorescence

properties is due the partition of the probe between water (where it is solubilized) and the hydrophobic microdomains, until equilibrium between the two regions (or probe saturation) occurs.

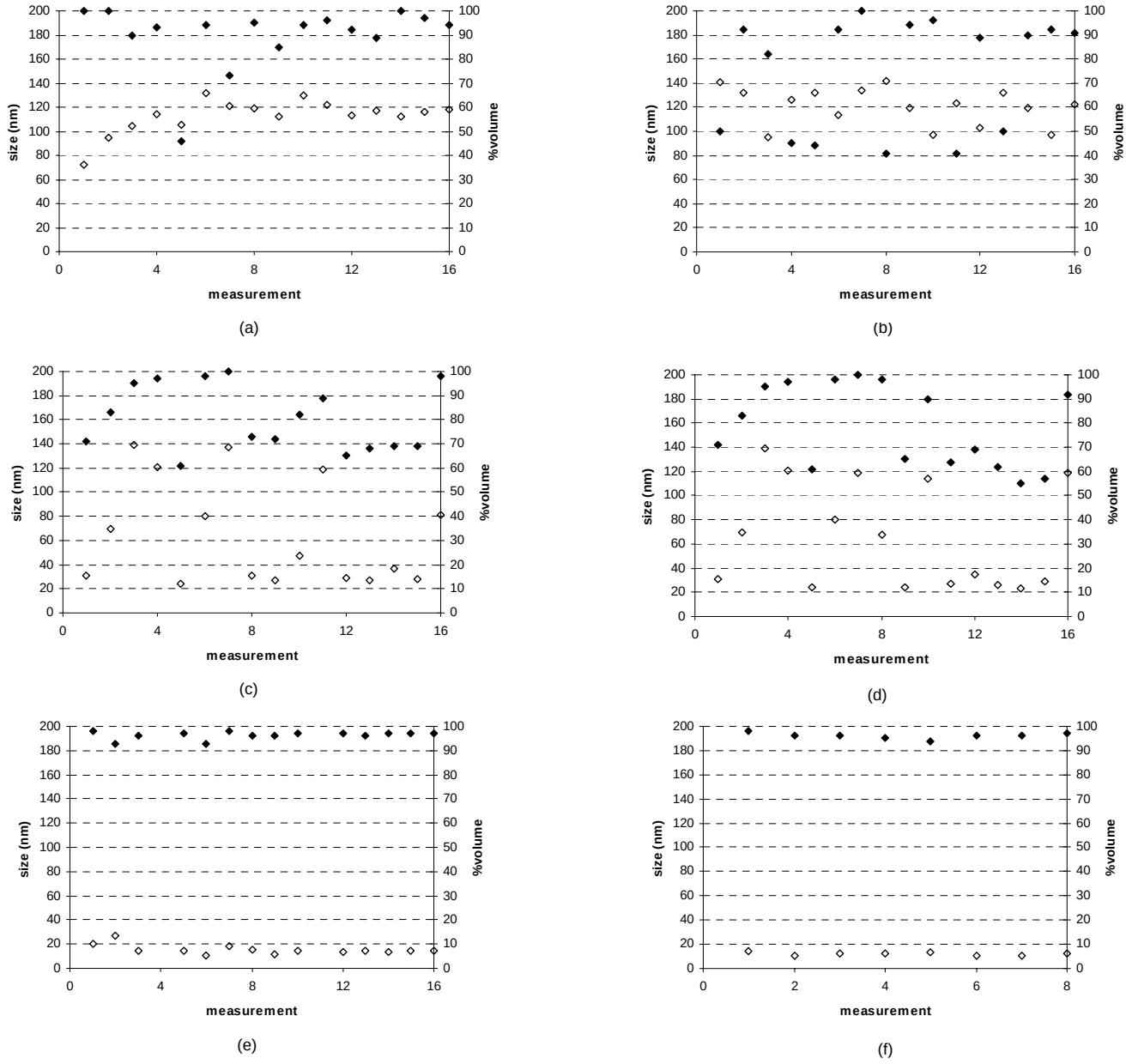

Figure 6. Size and the respective % volume for dexC_{16} with DS_{C16} 7% at (a) 0.00001 g/dL, (b) 0.0001 g/dL, (c) 0.0004 g/dL, 0.001 g/dL, (d) 0.01 g/dL and 0.1 g/dL.

The determination of *cmc* values from the fluorescence data is inaccurate, due to the large concentration over which the fluorophore response stretches. The onset and offset of the fluorophore response to polymer concentration, or the midpoint of this variation, were proposed to correspond to the *cmc* value (referências). In this study, the polymer concentration corresponding to the initial variation of pyrene fluorescence spectra which indicates that a significant event takes place at that particular concentration, is here considered the *cmc* value. This event can be an incipient association of side-chain hydrophobes (at least the very first association of two hydrophobes). As a consequence, the onset of the fluorophore response, as a function of polymer concentration, is considered the first critical micelle

concentration, corresponding to the very beginning of the hydrophobe association (by intra- or inter-molecular association).

Materials with different DS_{C16} were prepared, and the variation of the ratio I_3/I_1 with polymer concentration (for $dexC_{16}$ with different degree of substitution 1, 5, 7 e 12%) was analysed. The influence of the degree of substitution on *cmc* is shown in figure 5b. The cmc value decreases with increasing degree of substitution. For a higher DS_{C16}, the probability of finding two alkyl chains next to one another is higher, which in turn increases the association tendency.

The *cmc* can also be evaluated using the size distribution of the nanoparticles measured by DLS. Figure 6 shows the size and the respective % volume for $dexC_{16}$ with DS_{C16} 7% at different polymer concentrations, ranging between 0.00001 g/dL and 0.1 g/dL.

The results show that the *cmc* value for $dexC_{16}$ with DS_{C16} 7%, obtained through the fluorimetry experiments, was also confirmed by DLS. While at low and high polymer concentration, the measurement of particle size fine consistent results in consecutive analysis (± 120 nm and ± 18 nm for low and high concentration of polymer, respectively), for the cp values close to *cmc* the sample is apparently unstable, quite different values being determined in independent assays (figures 6c, d). Indeed the nanoparticles formation was detected at around 0.0004 g/dL

The values determined are lower than the reported for the most common low molecular weight surfactants. The low *cmc* may be due to the low solubility of dextrin.

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