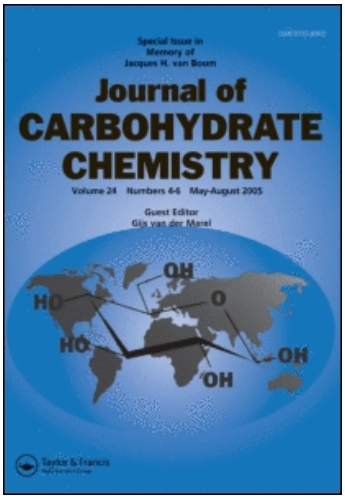

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Hydrophobic Derivatives of Dextran Polysaccharide: Characterization and Properties

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Hydrophobic Derivatives of Dextran Polysaccharide: Characterization and Properties

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This study reports the synthesis and characterization of hydrophobic derivatives of dextran in which long alkyl chains substituted a proportion of the hydroxyl groups. These derivatives were characterized by ^{13}C and ^{1}H NMR and infrared spectroscopy. Information about hydrophobic associations in aqueous solutions was obtained by fluorescence spectroscopy in the presence of pyrene and nabumetone probes. These results, in addition to the swelling-index data of derivatives, showed that there are perspectives of using them as a starting point for models of drug delivery.

Keywords Dextran, Esterification, Drug-delivery systems, NMR and infrared spectroscopy, Pyrene and nabumetone

INTRODUCTION

In the past few years, studies about hydrophobically modified derivatives of polysaccharides have increased owing to the possible applications.[1–4] Such derivatives present a set of properties useful in many fields of basic research, tendency to self-aggregation in aqueous medium due to intra- and/or intermolecular interactions of the hydrophobic groups being one of these properties. These polysaccharides have been applied in medical therapies with considerable success as drug-delivery systems.[5,6]

Dextran is a water-soluble polysaccharide that consists mainly of α-1, 6-linked D-glucopyranose residues with a low percentage of α-1,2; α-1,3; and α-1,4 linkages. It is essentially non toxic and commonly used as a blood

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expander to maintain or replace blood, and also in investigations as a drug delivery system.[2,7]

Different processes to obtain dextran derivatives have been developed based on distinct reaction methods. Dextran partially modified with cyanoethyl groups was prepared with acrylonitrile in alkaline medium.[8] Acyldextran has been synthesized by reaction with benzoyl and valeryl chlorides.[9,10] Carboxymethyl and benzoylamide groups have been used to produce carboxymethyldextrans-benzylamide derivatives.[11] Generally speaking, polysaccharides have been modified to obtain derivatives capable of forming micelle-like structures in aqueous solution and thus to dissolve organic molecules [1,12] such as drugs and probes.[2,3,13]

In this study, the idea of generating hydrophobic dextran derivatives by incorporating long alkyl chains was investigated. Dextran was modified by reactions with octanoyl and lauroyl chlorides and its products were characterized. Polymer characteristics such as degree of substitution (DS), swelling-index, and aggregation behavior in aqueous solution were studied. The results may be used in future specific contexts such as applications of these derivatives as models for drug delivery.

In view of the success in utilizing fluorescent probes to characterize the conformational structure of a number of microenvironments[2,3,14–18] pyrene and nabumetone were chosen to evaluate the structure of dextran derivatives in aqueous solutions. These luminescent molecules have been well characterized with respect to their photophysical properties in different environments; however, only a few reports are described on the associative behavior of hydrophobized polysaccharides in aqueous solution and its dependence on the DS.

EXPERIMENTAL

Chemicals

Dextran was purchased from Pharmacia Uppsala and acyl chlorides (octanoyl, 99% and lauroyl, 98%) were purchased from Acros Organics. Triethylamine (TEA, 99%) and KBr (99 + %FTIR) were purchased from Acros Organics and Aldrich Chemical, respectively. The pyrene and nabumetone photophysical probes were purchased from Fluka and Sigma, respectively. Other chemicals were reagent grade and used without previous purification.

Synthesis of Dextran Derivatives

Dextran 20% (w/v) was dissolved in aqueous solution under stirring and maintained under constant temperature (18 ± 0.01°C). TEA and acyl chloride were added to this solution and the mixtures were stirred for 1 hr. The purification

was conducted according to reported produces.[9] The DS was controlled by the amount of acyl chloride or TEA. The ratios of dextran (DX), octanoyl or lauroyl chloride (Ch), and TEA are shown in Table 1.

Characterization of Dextran Derivatives

NMR Spectroscopy

^{13}C and ^{1}H NMR measurements were performed on a AC200 Bruker NMR spectrometer at 200 MHz, using DMSO as internal reference. ^{1}H NMR measurements were also used to determine the degree of acyl chloride substitution on dextran (DS).

FT-Infrared (IR) Measurement

Infrared spectra were obtained by Fourier Transform Infrared Spectroscopy (FT-IR, Spectrum 2000 Perkin Elmer). The samples were prepared in thickness KBr pellets (5 mg in 200 mg of KBr) and stabilized under controlled relative humidity before acquiring the spectrum. For each sample, 30 scans were recorded from 4000 to 500 cm^{-1} with a resolution of 4 cm^{-1}.

Swelling Test

The swelling index of dextran derivatives was calculated by the following equation:

$$\text{Swelling index, } \% = \frac{W_s - W_o}{W_o} \times 100 \quad (1)$$

where *Wo* is the weight of a dried dextran derivative and W_s is the weight of a swollen derivative after the immersion in water at time *t*.

Table 1: Molar ratios for the derivatives of dextran with dextran (DX), acyl chloride (Ch), and TEA amounts.

Octanoyl derivatives	Molar ratio DX : Ch : TEA	Lauroyl derivatives
TDX-O1	1.0 : 1.0 : 5.0	**TDX-L1**
DX-O1	1.0 : 1.0 : 6.7	**DX-L1**
DX-O2	1.0 : 0.70 : 6.7	**DX-L2**
DX-O3	1.0 : 0.33 : 6.7	**DX-L3**
DX-O4	1.0 : 0.15 : 6.7	**DX-L4**
DX-O5	1.0 : 0.10 : 6.7	**DX-L5**

Fluorescence Spectroscopy

Fluorescence experiments were carried out at rt using a Hitachi F-4500 spectrofluorimeter. Pyrene ($1 \times 10^{-6}\,\text{mol}\cdot\text{L}^{-1}$) and nabumetone ($5 \times 10^{-5}\,\text{mol}\cdot\text{L}^{-1}$) were excited at 334 nm and 317 nm, respectively. The absorption spectra of nabumetone were measured with a Hitachi U-2000 spectrophotometer.

The vibrational structure of the fluorescence bands of pyrene is known to be sensitive to the local polarity of the microenvironment. Band I of the fluorescence spectrum (~372 nm) shows significant intensity enhancement in polar solvents. Thus, the ratio between the fluorescence intensities of peaks I and III (~384 nm) of the emission spectrum of pyrene, I_1/I_3, was used to evaluate the polarity of the local environment and to determine the critical aggregation concentration (CAC).[13]

Nabumetone is a drug with absorption and emission spectra sensitive to changes in the molecular microvicinity.[17] Therefore, it was employed to monitor the solution properties of derivatives.

RESULTS AND DISCUSSION

Characterization of Dextran Derivatives

The chemical shifts of the ^{13}C NMR signals of dextran are given in Figure 1 and they are in agreement with literature.[19,20] The intense signal at ~40 ppm

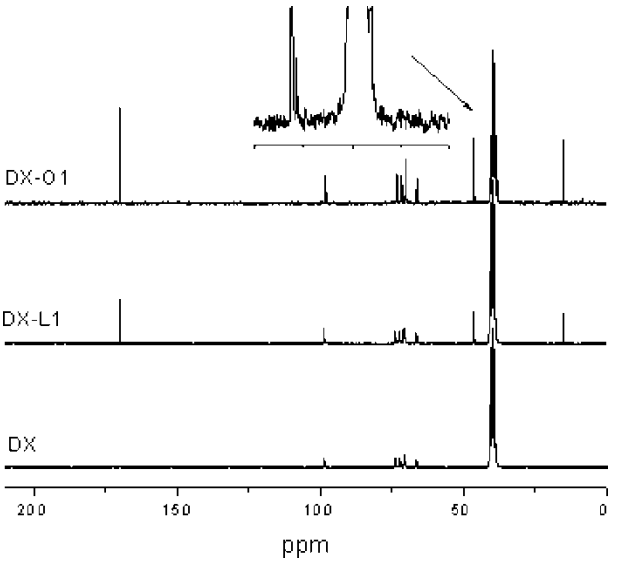

Figure 1: ^{13}C NMR spectra of dextran (DX), lauroyl-derivative (DX-L1), and octanoyl-derivative (DX-O1).

corresponds to the DMSO-d_6.[21] The ^{13}C NMR spectra of all derivatives confirm the chemical modifications, as exemplified by DX-L1 and DX-O1 derivatives (Fig. 1). This figure shows the new signals at 170 ppm attributed to the carbonyl carbon,[20,22] and 15.00 and 45.96 ppm attributed to the $-CH_3-$ and $-CH_2-$ groups, respectively.[21–23] Spectra of the $-CH_2$ group (long alkyl chains) must present peaks in a range of shifts between 15 and 55 ppm.[21] In the insert of Figure 1, when the spectrum of derivative (DX-O1) was extended, other peaks had been observed. The intense signal at ~40 ppm (DMSO-d_6) is hidden by other peaks. The spectra of the other derivatives have shown the same behavior.

The substitution was also confirmed by FTIR measurements by the appearance of a carbonyl band at ~1750 cm^{-1} that corresponds to the ester group of the substituent,[2,19,22] which was absent in the spectrum from the dextran (results not shown).

Figure 2 (A–C) shows the chemical shifts of the ^1H NMR signals for DX-O3 and DX-O1 together with the dextran. The chemical shifts of the ^1H NMR signals of dextran are in agreement with reported values.[19,20,22] The peak at 2.50 ppm was due to solvent, DMSO-d_6. After esterification, new peaks appeared and modifications occured in the spectrum as a consequence of a different electronic environment, as described.[24] A new peak at 1.20 ppm can be attributed to protons of the alkyl chain of the hydrophobic moiety.[22,25] This has different intensities depending on the DS. Derivatives show two anomeric proton peaks, one at 4.50 ppm corresponding to dextran and the other at 4.70 ppm. The latter was assigned to anomeric protons (C-1) in which the hydroxyl on the neighboring C-2 carbon reacted to form an

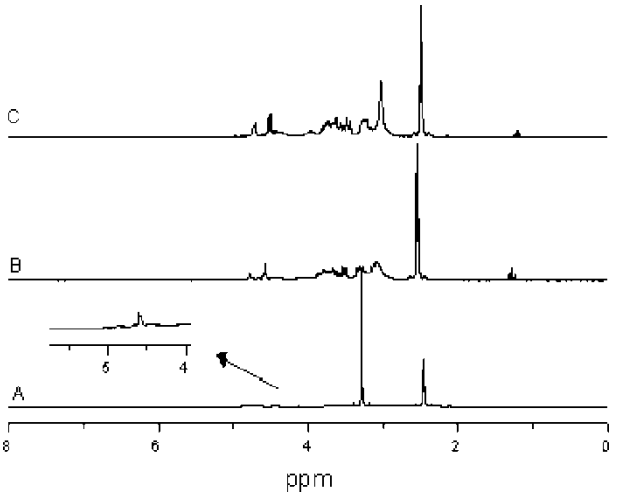

Figure 2: ^1H NMR spectra of (A) dextran and octanoyl-derivative, (B) DX-O3, and (C) DX-O1.

Table 2: DS and CAC for both octanoyl (DX-O) and lauroyl (DX-L) dextran derivatives.

Derivatives	DS	CAC $\times 10^{-2}$ ($g \cdot L^{-1}$)	Derivatives	DS	CAC $\times 10^{-2}$ ($g \cdot L^{-1}$)
TDX-L1	0.063	12.6	TDX-O1	0.063	11.8
DX-L1	0.11	4.93	DX-O1	0.12	4.60
DX-L2	0.097	10.5	DX-O2	0.11	9.00
DX-L3	0.090	11.6	DX-O3	0.098	10.8
DX-L4	0.053	15.1	DX-O4	0.058	14.0
DX-L5	0.048	16.4	DX-O5	0.052	17.5

ester.[9] The new peaks confirm the chemical modification, as exemplified to DX-O3 and DX-O1 derivatives in Figure 2 (B and C).

The DS was estimated by ^{1}H NMR measurements (Table 2). The values were obtained from the integral intensities of protons from the substituent (1.20 ppm) by relating them to the integral intensity due to the anomeric proton (4.50 ppm). Reported analyses for both dextran[9,10] and chitosan[26] alkyl derivatives substituted suggest a more favorable reaction with a high concentration of acyl choride, and a bigger value of DS the smaller the length of the substituent chain. The values reported here show a discrete indication in the same direction.

Swelling Test

The swelling kinetics for dextran derivatives were studied, as exemplified by DX-L1 and DX-L4 (Fig. 3). The curve for the DX-L4 presented a significant

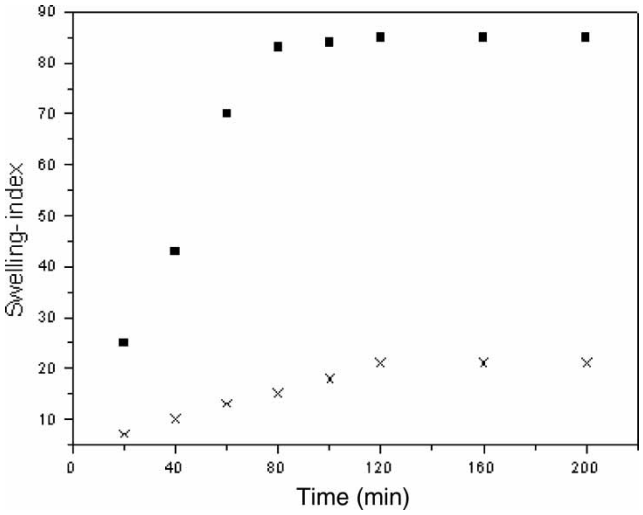

Figure 3: Swelling kinetics of the dextran derivatives: (x) DX-L1 and (■) DX-L4.

change when compared with DX-L1, suggesting that the swelling process is faster and a stable state is reached much more rapidly. This may be due to the lesser hydrophobic content.

The behavior of swelling was related to the DS for two groups of derivatives. Figure 4 shows that the swelling index of dextran derivatives decreases with the increase of the DS, in agreement with the literature.[27] Smaller values of swelling index (derivative with bigger degree of substitution) can be attributed to a tighter and more compact structure that limits the water uptake. Moreover, the number of carbon atoms of the alkyl chain also seems to influence the swelling, in accordance with literature.[26,28]

CAC and Self-Aggregation Studies for Dextran Derivatives

The ratio of intensities I_1/I_3 of the pyrene fluorescence spectrum was used to investigate the formation and properties of aggregated dextran derivatives. The I_1/I_3 values range from 1.87 in water to 0.6 in aliphatic hydrocarbon solvents. Values of 1.1 to 1.2 are typical of aqueous micelles, indicating that pyrene is located in the surface region of the hydrocarbon core.[13] When the pyrene is incorporated into the polymeric aggregates, the ratio of intensities I_1/I_3 becomes smaller compared with that in aqueous media. Figure 5 shows a plot of the monitored parameter I_1/I_3 as a function of DX-L3 concentration. All derivatives presented the same behavior and CAC values were determined from the threshold concentration, where the intensity ratio of I_1/I_3 started to decrease markedly. In Table 2 it is possible to verify that CAC decreases with the increase of DS.

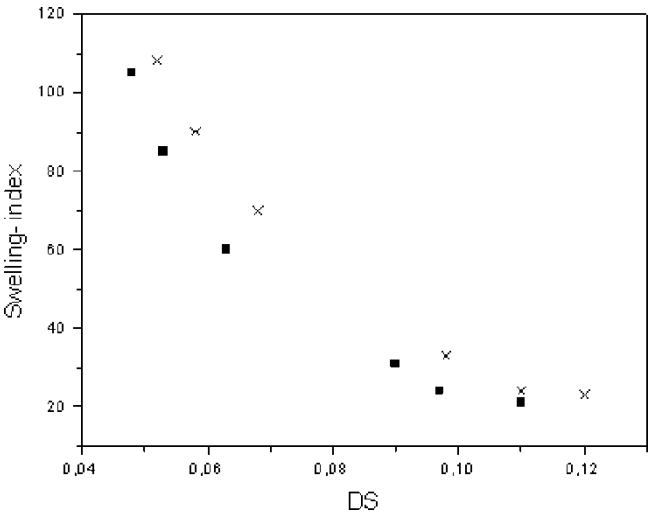

Figure 4: Swelling index versus degree of substitution (DS) of (×) octanoyl and (■) lauroyl dextran derivatives.

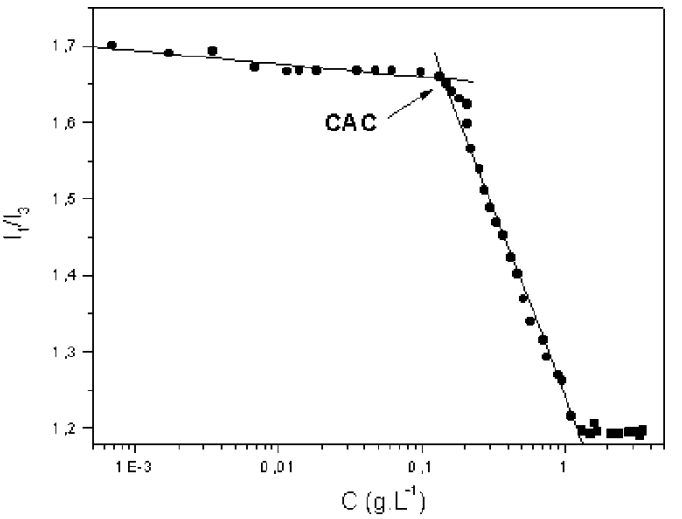

Figure 5: Plot of the fluorescence intensity ratio I_1/I_3 as a function of derivative dextran (DX-L3) concentration (C, $g \cdot L^{-1}$).

The variation of the excimer emission of pyrene ($\sim$470 nm)[13] was used to verify the region where it was solubilized. Figure 6 shows that (a) the excimer emission appears when free pyrene molecules are transferred to preaggregates of the hydrophobized dextran and (b) the excimer emission is more pronounced at low concentrations of DX-L3 and disappears when the derivative's

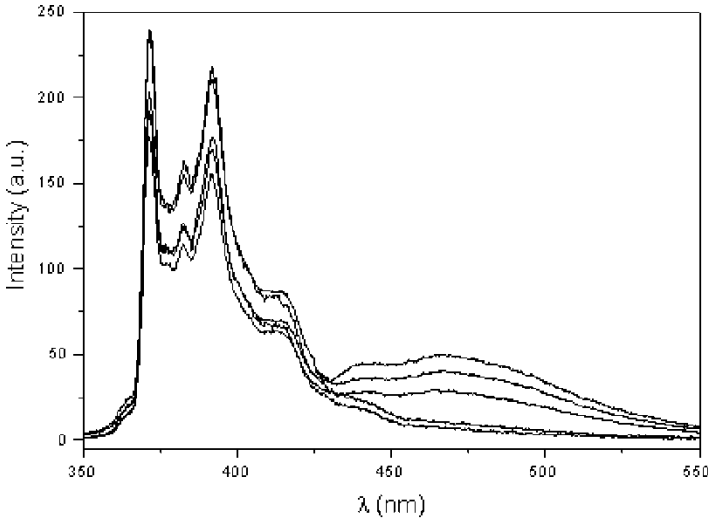

Figure 6: Fluorescence spectra of pyrene (Py) as a function of DX-L3 concentration ($10^{-2} g \cdot L^{-1}$): water; 10.0; 8.50; 3.50; 1.30 (from the base to the top).

concentration is bigger than the CAC. This is caused by the redistribution of pyrene molecules among the polymer aggregates.

Intra- and/or intermolecular interactions have been reported as responsible for the formation of aggregates in native polysaccharides such as dextran,[2] sodium hyaluronate,[3] and pullulan,[29] as well as in synthetic polymers.[13–15] The increase of the hydrophobic content in the flexible chain of dextran, caused by the increase of substitution, favors the aggregation. These interactions were studied by photophysical data of pyrene and strengthened by the photophysical behavior of nabumetone. This probe has a weak fluorescence in water and different photophysical behavior in other solutions.[16,17]

Absorption and emission spectra of nabumetone solubilized in different dextran derivative concentrations are presented in Figures 7 and 8, respectively. Figure 7 shows a typical absorption spectrum of a naphthalene 2-substituted compound.[30] As expected, the longer wavelength band is the most sensitive to changes in the solvent polarity[30] and the maximum absorption can be related to the relative permeability of the drug environment.[17] The spectra are shifted to longer wavelengths when the dextran derivative concentration increases, indicating that nabumetone molecules are transferred from water to a less polar environment inside the aggregates of dextran derivative. This is a known trend[16,31] and was observed in all derivatives.

When nabumetone is dissolved in the dextran derivative solution, its emission spectrum shows a significant modification with the concentration increase (Fig. 8). When the derivative's concentration increases, the intensity

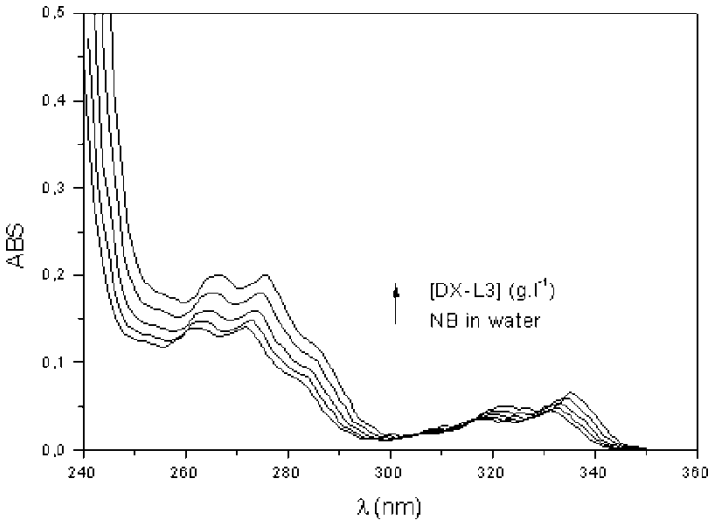

Figure 7: Absorption spectra of nabumetone as a function of DX-L3 concentration ($10^{-2}\,g \cdot L^{-1}$): water; 1.30; 3.50; 8.50; 9.50; 10.0 (from the base to the top).

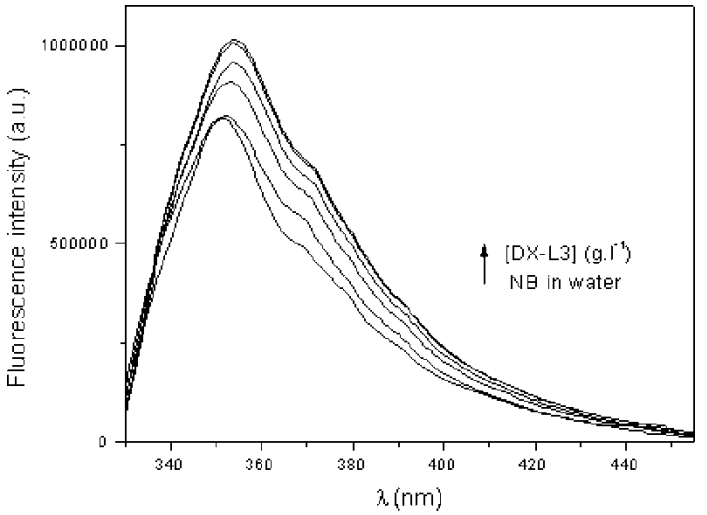

Figure 8: Fluorescence spectra of nabumetone as a function of DX-L3 concentration (10^{-2} g · L^{-1}): water; 1.30; 3.50; 8.50; 9.50; 10.0 (from the base to the top).

of the emission spectrum (band centered around ~350 nm) also increases, indicating that the probe has been transferred to a more hydrophobic microenvironment where it is protected from water quenching. When the derivative concentration achieves a value near that of CAC (obtained values with pyrene), the emission spectra remain practically constant, showing that the nabumetone probe was incorporated into the aggregates.

CONCLUSION

The analysis of lauroyl and octanoyl derivatives of dextran in aqueous solution showed that the increase of DS favors the self-aggregation process. This fact is due to the interactions among the alkyl chains of the hydrophobic moiety, which lead to the formation of a more hydrophobic microenvironment where the probe is solubilized. In addition, photophysical studies using pyrene and nabumetone were used to determine the CAC values (concentration where hydrophobic molecules such as drugs can be better solubilized).

The swelling values showed that the derivatives are expected to be good models of drug-delivery systems. The ability to swell and retain large volumes of water in a swollen structure without dissolution is required for taking the drug to the specific place. A limited number of available free hydroxyl groups generate a tighter and more compact structure for limiting water uptake.

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