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The aggregative behavior of hydrophobically modified chitosans with high substitution degree in aqueous solution

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Abstract

Nowadays chitosan, CHIT, is becoming one of the most interesting raw materials because of the actual and potential applications in several technological fields due to its chemical versatility, natural abundance and ecological compatibility. By applying a very versatile and quantitative amination reaction, linear aliphatic chains of length variable from five to 12 carbon atoms have been grafted on the chitosan backbone at 10% in mole of the glycosidic units.

Newtonian viscosity and fluorescence probe emission have been used to analyze the intra- and inter-aggregation properties of these modified chitosans, C*n*-CHITs, and to compare them with those of chitosan itself. Viscosity in the dilute concentration range showed that for CHIT and C5-CHIT the behavior is quite similar highlighting that the insertion of this short aliphatic chain does not modify the rigidity and/or the interaction with the solvent. For C6, C8, and C10-CHIT, the increasing length of the pendant promotes a progressively more efficient intra-aggregation of the polymer as shown by the reduction of its hydrodynamic radius.

Converging evidences are obtained by the analysis of the fluorescence spectra of pyrene, used as probe, in the aqueous solutions of the grafted polymers, which show how different is the environment in which pyrene is located, if short or long chained polymers are analyzed. In fact, the spectra in CHIT, C5-CHIT, and C6-CHIT are quite similar among them and indicate a scant hydrophobic quality of the aggregates for these short grafted polymers even in concentrated solution. In contrast, the presence of C8-CHIT, C10-CHIT, and C12-CHIT concentrated solutions gives rise to a progressive and evident modification of the pyrene spectra, which resemble those of this fluorescent probe in a hydrocarbon solvent, thus indicating the formation of well-defined hydrophobic pools. However, in the limit of very concentrated solutions, the specificity of the grafted chains of different length is lost.

Keywords: Alkylated chitosan; Hydrophobic aggregation; Hydrophobic modulation

1. Introduction

Chitin is the most abundant organic raw material on earth after cellulose. It is the main constituent of the carapace of Crustacea and it is constituted by several (1→4)-2 acetamido-2-deoxy-β-glucopyranose units, playing in the animal kingdom the role that cellulose plays in the vegetable one. Chitin is essentially water-insoluble. The partial de-acetylation (Bough, Wu, Campbell, Holmes, & Perkins, 1978; Wu & Bough, 1977) of this molecule gives rise to chitosan that, having several free aminic functions, becomes soluble if pH is properly lowered ($\text{pH} < 5$) (Varum, Ottoy, & Smidsrod, 1994). The abundantly available, biocompatible, atoxic, inexpensive chitosan has been used in many different fields (Prashanth & Tharanathan, 2007) such as waste water treatment (Chi & Cheng, 2006; Chung, 2006; Qin, Shi, & Liu, 2006), drinking water treatment (Nowak, Kaminski, Albinska, & Jaros, 2005; Zhang & Schiewer, 2006), food processing (Bornet & Teissedre, 2005; No & Kim, 2006), agriculture (Bautista-Banos et al., 2006), biotechnology (Kirkkopru, Alpaslan, Omay, & Guvenilir, 2006; Kumar, Jena, Behera, Lockey, & Mohapatra, 2005; Qi et al., 2006; Issa, Koeping-Hoegg-aard, & Artursson, 2005; Wu et al., 2003; Acosta et al.,

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2003), cosmetics (Hasebe & Ohno, 2006), medical application (Chen & Dai, 2006; Ding, Guan, Ouyang, Wang, & Yu, 2005; Iwu, 2006; Ohkawa, Minato, Kumagai, Hayashi, & Yamamoto, 2006; Whang, Kirsch, Zhu, Yang, & Hudson, 2005; Lin et al., 2005), so that the interest on chitosan is not limited to the academic science, as demonstrated by the thousands of patents related to its applications. The largest part of them is related to the ability of this polymer to bind a lot of substances for the contemporary presence on its backbone of hydrophobic units, the acetamide groups, and hydrophilic units, the amino groups, and also to the capacity of chitosan to self-aggregate giving rise to gel systems.

The physico-chemical properties of chitosan can be varied by chemical modification generating a lot of materials with further wide technological applications. These modifications are easy to perform by a versatile reductive amination procedure suggested by Yalpani (Yalpani & Hall, 1984), being the presence of an aldehydic or keto function on the chemical modifier the only condition requested. For example, the introduction on chitosan of a lot of linear or cyclic carbohydrates makes it water-soluble, while its tunable partial alkylation produces polymers that can be still soluble in water at acidic pH, but that also efficiently self-aggregate in water giving rise to hydrophobic pools. This is particularly important in the drug release field (Liu, Sun, Zhang, & Yao, 2003) for the possibility to dissolve both hydrophilic and hydrophobic drugs into the chitosan matrix. Several papers dealing with the physico-chemical properties of alkylated chitosans are present in the literature and several experimental approaches, such as rheology (Kjøniksen et al., 1997; Desbrieres, 2002; Esquenet & Buhler, 2001; Esquenet, Terech, Boué, & Buhler, 2004; Rinaudo, Auzely, Vallin, & Mullagaliev, 2005; Philippova et al., 2001), interfacial tension (Rinaudo et al., 2005), scattering techniques (Esquenet & Buhler, 2001; Esquenet et al., 2004; Kjøniksen, Iversen, Nyström, Nakken, & Palmgren, 1998) and fluorescence (Philippova et al., 2001), have been applied. However, only the physico-chemical behavior of alkylated chitosans of low alkylation degree, AD, generally 2–4% in mole of the cyclic units, has been taken into account. The increase of the AD value could be relevant in order to obtain hydrophobic pools well suited to solubilize hydrophobic guests. This paper is focused on the study of the physico-chemical behavior of a series of substituted chitosans with aliphatic chains of different length, at 10% AD with the aim to give a quantitative insight about the self-aggregation behavior of these grafted polymers in solution.

2. Experimental

2.1. Materials and methods

Chitosan (CHIT), classified by the seller as medium molecular weight, batch 08028CD, was purchased from Aldrich. Its acetylation degree, 5%, was determined by NMR (Desbrieres, Martinez, & Rinaudo, 1996) with a Varian 300 spectrometer. It is in very good agreement with that reported by the seller.

Pentanal (declared purity ∼97%), hexanal (∼98%), octanal (∼99%), decanal (∼95%), dodecanal (∼92%), sodium cyanoborohydride $NaCNBH_3$ (>95%), glacial acetic acid (∼99.8%), all from Aldrich, and pyrene (⩾99%) from Fluka, were used as received.

To synthesize the alkylated chitosans (C*n*-CHITs) in mild condition, we followed a reductive amination procedure widely discussed in the literature. This method was suggested earlier by Yalpani (Yalpani & Hall, 1984) and modified by Rinaudo (Rinaudo et al., 2005; Desbrieres et al., 1996). It gives rise to a quantitative grafting of the aliphatic chains on the chitosan backbone. In summary, the procedure involves the equilibrium reaction of an aldehyde with an aminic function of chitosan in acidic solution to form the chitosan imminium ion followed by its quantitative reduction by sodium cyanoborohydride. The alkylation degree of C*n*-CHIT samples was checked by NMR (Rinaudo, Desbrieres, Le Dung, Thuy Binh, & Dong, 2001) confirming the almost complete yield of the synthesis.

The samples of CHIT and C*n*-CHITs were checked for humidity by TGA (Thermal Gravimetric Analysis) finding values that depend on the considered sample, but that are generally around 5% by weight; hydration water was taken into account in computing solutions' concentration.

2.2. Viscosity

Polymer stock solutions were prepared by weighing CHIT or C*n*-CHITs and the solvent: an aqueous solution of acetic acid 0.3 M, sodium acetate 0.2 M. The solutions were stirred for at least 24 h at room temperature and then filtered on Millipore Millex HA Filters 0.45 μm and diluted directly with the described solvent inside the Ubbhelode viscosimeter used for the measurements. To convert the concentration of the stocks from mass to volume units, the density of the concentrated solutions was measured by an Anton Paar ultrasonic density-meter.

2.3. Fluorescence spectroscopy

Pyrene was used as fluorescent probe to put in evidence the presence of hydrophobic domains inside CHIT and C*n*-CHIT solutions as suggested by Amiji (1995), in that the ratio between the intensities of the peaks at 370 and 381 nm, is sensitive to the hydrophobicity of the environment in which pyrene is located. A 0.6 μM pyrene solution acidified by HCl at pH 4 was used as solvent for all the solutions prepared. A titrating procedure was followed by adding small aliquots of a ∼10 mg g_{sol}^{-1} CHIT or C*n*-CHIT solution at pH 4 to a weighed amount of the solvent. Only in the case of C12-CHIT, the solution containing the polymer had an initial lower concentration, ∼4 mg g_{sol}^{-1} for solubility reasons. The spectrofluorimeter was a

Fluorolog3-11 instrument from Jobin Yvon. The sample cell was kept at 25.0 °C by an external water circulation. The excitation wavelength was 338 nm, while spectra were recorded in the interval 350–500 nm at an integration time of 1.0 s, with excitation and emission slit openings of 3.0 and 0.5 nm, respectively.

3. Results and discussion

3.1. Viscosity

Viscosity was adopted both to determine the mean viscosimetric molecular weight of chitosan and to study the influence of the grafted aliphatic chains on the intra-molecular interactions of the chemically modified polymers in dilute solution.

The CHIT mean viscosimetric molecular weight was calculated through the Mark–Houwink equation

$$[\eta] = kM^{\alpha} \tag{1}$$

In Eq. (1) $[\eta]$ is the intrinsic viscosity, defined as: $[\eta] = \lim_{C\to 0} \frac{\eta_{\text{spec}}}{C}$, where k and α are constants depending on the quality of interaction between the solute and the solvent. Low α values (0.5–0.8) are compatible with flexible random coil, high α values (1.0–1.7) are measured in the case of highly extended chains (Flory, 1953). M is the molecular weight. In the intrinsic viscosity definition, C is the polymer concentration in g $\text{cm}_{\text{sol}}^{-3}$. The specific viscosity is defined as: $\eta_{\text{spec}} = \frac{t}{t_0}\frac{d_0}{d} - 1$ with t and t_0, and d, d_0 being the flowing time and the density of the solution at each composition and of the solvent, respectively. In the case of dilute solutions as those of interest, the ratio d_0/d can be taken unitary.

For the viscosity measurements we chose the solvent 0.3 M AcH/0.2 M AcNa because it demonstrated to be a very good solvent for chitosan for which Rinaudo (Rinaudo, Milas, & Le Dung, 1993) very accurately determined the k and α values, finding 0.08 and 0.76, respectively. For CHIT, $[\eta] = 973 \pm 3\ \text{cm}^{-3}\ g_{\text{sol}}^{-1}$ so that the mean viscosimetric molecular weight results to be 237 ± 2 kDa.

In relation to the investigation of the influence of the aliphatic chains on the intra-molecular interactions, the values of η_{spec}/C of CHIT and Cn-CHITs at 10% alkylation degree are reported in Fig. 1. The data of C12-CHIT are not shown for its very limited solubility that does not allow to measure viscosity values significantly different from that of the solvent. All the samples in the investigated concentration range behave as Newtonian fluids, allowing the determination of the Huggins constant, k_H, defined in the following equation and whose values are shown in Table 1

$$\eta_{\text{spec}} = \frac{\eta - \eta_0}{\eta_0} = [\eta]C + k_H(C[\eta])^2 \tag{2}$$

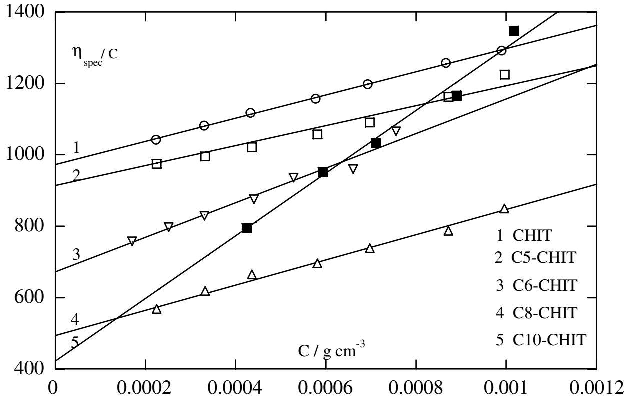

Fig. 1. Viscosimetric behavior of CHIT (1); C5-CHIT (2); C6-CHIT (3); C8-CHIT (4); C10-CHIT (5) in 0.3 M acetic acid/0.2 M sodium acetate at 25 °C.

Table 1
Influence of the chain length on the viscosimetric behavior of alkylated chitosan

	$[\eta]/\text{cm}^{-3}\ \text{g}_{\text{sol}}^{-1}$	k_H	M_w/kDa	r_h/nm
Alkylation degree 10%				
CHIT	973 ± 3	0.34 ± 0.01	237	33.2 ± 0.1
C5-CHIT	920 ± 3	0.27 ± 0.01	249	33.1 ± 0.1
C6-CHIT	673 ± 3	1.06 ± 0.02	251	29.9 ± 0.1
C8-CHIT	492 ± 2	1.49 ± 0.01	255	27.1 ± 0.1
C10-CHIT	422 ± 2	4.94 ± 0.60	259	25.9 ± 0.1
C12-CHIT			263	

From a general viewpoint, the intrinsic viscosity, $[\eta]$, shows a descending trend with increasing the length of the aliphatic chain, thus suggesting a shrinking effect of the polymer chain driven by the hydrophobic interactions. In Eq. (2) the constant k_H describes the interaction resulting only from differences in the chemical structure of the polymer and/or the nature of the solvent. A low interaction among the dissolved macromolecules, expressed by a low value of k_H, reflects a high solvent power for the specific polymer (Desbrieres, 2002).

The increasing value of k_H, together with the decreasing values of $[\eta]$ summarized in Table 1, are converging indications that the polymer becomes more squeezed by increasing the length of the aliphatic chain, and in turn the greater shrinkage can be probably imputed to the greater efficiency of the hydrophobic interactions among the longer aliphatic chains. $[\eta]$ and k_H parameters are indirect means to evaluate the polymer–solvent interactions, in particular the intrinsic viscosity is an expression of the hydrodynamic interference between the polymer and the solvent. Particularly, if $[\eta]$ and the molecular weight, M, are known, and assuming that the polymer coils are hard spheres, the hydrodynamic radius, r_h, can be calculated (Kwaambwa, Goodwin, Hughes, & Reynolds, 2007) from Eq. (3)

$$r_h/m = \left(\frac{1}{2.5N_A}\,\frac{3[\eta]M}{4\pi 10^6}\right)^{1/3} \tag{3}$$

The r_h data are collected in Table 1 together with the molecular weights of Cn-CHIT determined by using the mean viscosity molecular weight of CHIT and taking into account the acylation and alkylation degrees of the chemically modified samples. From the inspection of the table it appears evident that while CHIT and C5-CHIT have almost the same hydrodynamic radius, by further increasing the length of the aliphatic chain, it is progressively reduced because of the more efficient intra-molecular hydrophobic interactions.

3.2. Fluorescence

In Fig. 2 the fluorescence spectra of pyrene in chitosan aqueous solutions acidified by HCl at pH 4, are reported. As it can be observed in the figure, the spectra shape changes strongly with increasing chitosan concentration as shown by the comparison of spectrum (a) at $C_{\mathrm{CHIT}} = 0$ with spectrum (b) at $C_{\mathrm{CHIT}} = 0.00613\ \mathrm{g\ g_{sol}^{-1}}$. Spectrum (a) is typical of a very hydrophilic pyrene environment while spectrum (b) is a clear indication that pyrene experiences a more hydrophobic localization, see for comparison the pyrene spectrum in cyclohexane in the inset of Fig. 2. The ratio of the intensities at 370 and 381 nm is a quantitative measurement of this experimental evidence (Kalyanasundaram & Thomas, 1977; Turro, Baretz, & Kuo, 1984); in fact, by increasing the polymer concentration, the ratio I_{370}/I_{381}, goes down from 2.25 to 0.83 showing that chitosan itself is able to form hydrophobic domains in which pyrene can be located, see Fig. 3.

The presence of aliphatic chains on the chitosan backbone can promote the formation of more structured hydrophobic pools. In this contest, it is interesting to compare the pyrene fluorescence spectra of CHIT and C*n*-CHITs at the highest concentration used: $C \sim 10\ \mathrm{mg\ g_{sol}^{-1}}$ for all the samples, except for C12-CHIT because of its lower solubility. By inspection of Fig. 4 it appears that even in comparable concentration conditions, pyrene in the presence of modified chitosans is located in qualitatively very different hydrophobic pools. In fact, the spectra in CHIT, C5-CHIT, and C6-CHIT concentrated solutions are quite similar among them while going from C8-CHIT to C12-CHIT the intensity of the third peak becomes more evident so that the shape of the spectrum resembles that of pyrene in an apolar solvent, see the inset of Fig. 2. This evidence confirms the lower ability of the short aliphatic substituents, even in concentrated solution, to tune hydrophobic aggregation; in contrast, C8-CHIT, C10-CHIT, and C12-CHIT are able to form structured hydrophobic pools.

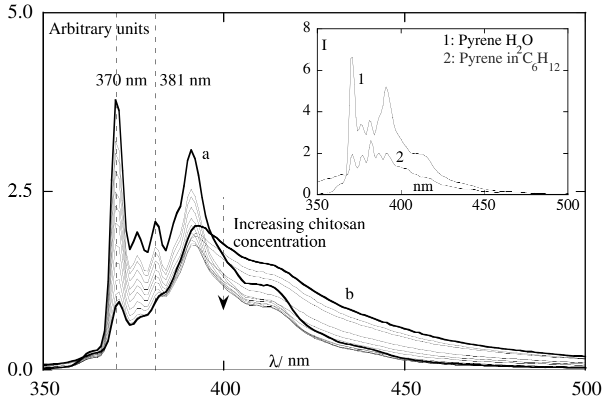

Fig. 2. Fluorescence spectra of pyrene in the presence of chitosan aqueous solution of different concentration at pH 4 and 25 °C. Spectrum (a) $C_{\mathrm{CHIT}} = 0$; spectrum (a) $C_{\mathrm{CHIT}} = 0.00613\ \mathrm{g_{sol}^{-1}}$.

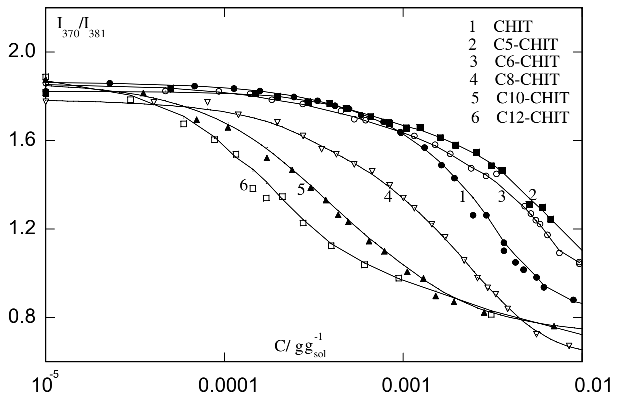

Fig. 3. Polarity parameter I_{370}/I_{381} of pyrene vs CHIT (1); C5-CHIT (2); C6-CHIT (3); C8-CHIT (4); C10-CHIT (5); C12-CHIT (6) concentrations at pH 4 and 25 °C.

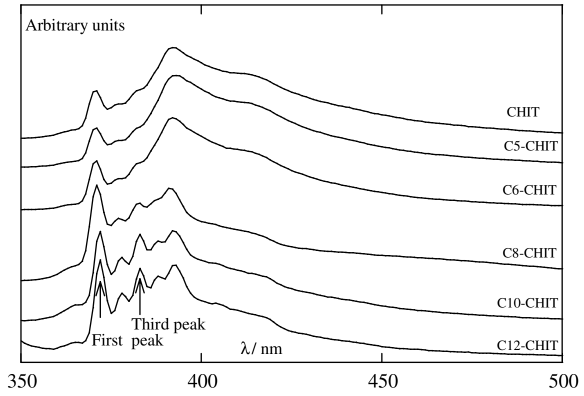

Fig. 4. Fluorescence emission spectra of pyrene in hydrophobically modified chitosans aqueous solutions for CHIT, C5-CHIT, C6-CHIT, C8-CHIT, C10-CHIT, C12-CHIT near the solubility limit of the polymer, namely at: 0.009, 0.100, 0.0097, 0.0085, 0.0070, 0.003 g $\mathrm{g_{sol}^{-1}}$, respectively.

In Fig. 3 the ratio I_{370}/I_{381} is reported as a function of the concentration of the hydrophobically modified polymers in semi-log scale. From the inspection of the figure it appears that the curves related to C5-CHIT and C6-CHIT pass over that of the unmodified chitosan, while all the other curves relative to the longer C*n*-CHITs pass under it. This is probably an indication that the introduction of short aliphatic chains on the chitosan backbone is not yet able to promote more efficient hydrophobic interactions among the chains, but in contrast, making more rigid the polymer structure thus opposing its coiling, promotes a more efficient interaction between C*n*-CHIT and the solvent. This interpretation is supported by the values of the Huggins constant, k_{H}, in Table 1 showing that for C5-CHIT the interaction with the solvent is stronger than that of the chitosan itself.

In the case of C6-CHIT both fluorescence and viscosity show that the system is stiff enough even if both techniques indicate that the hydrophobic interactions among the aliphatic chains begin to play a role.

To achieve a quantitative evaluation of the tendency of these polymers to aggregate via hydrophobic interaction, it is necessary to define a parameter able to quantify this ability. In the case of surfactants, the concentration at which they begin to aggregate cooperatively, named critical micellar concentration, cmc, is generally chosen. Unfortunately for CHIT and C*n*-CHITs the aggregation process is a-cooperative, as shown for CHIT, used as example in Fig. 5. In fact the ratio I_{370}/I_{381} lowers even when the polymer is extremely diluted, not evidencing any critical aggregation concentration. However, from Fig. 5 it appears clear the higher tendency, even at very low concentration, of C12-CHIT to aggregate, as shown by the higher negative slope of I_{370}/I_{381} data at the axis origin: $\lim_{C\to 0}\left(\frac{\partial(I_{370}/I_{381})}{\partial C}\right)$. In the expression above defined, C is the concentration expressed in g g_{sol}^{-1}. In contrast, the extrapolation of I_{370}/I_{381} at very high polymer concentration, that is: $\lim_{1/C\to 0}(I_{370}/I_{381})$ is almost the same, namely ~0.7 for CHIT and for all C*n*-CHITs, not far from the value of 0.56 expected for a hydrocarbon solvent (Kalyanasundaram & Thomas, 1977).

This evidence is quite surprising, indicating that in this extreme condition the pyrene environment should be that proper to a liquid hydrocarbon no matter of the presence of aliphatic chains on the polymer structure. The reason probably depends on the presence of acetyl residues that, at very high concentration, play the same hydrophobic role of the alkyl chains. From the evidences exposed above, we define as C*n*-CHIT' "critical aggregation concentration" (cac) the concentration at which the straight lines

$$\frac{I_{370}}{I_{381}} = \left(\frac{I_{370}}{I_{381}}\right)_{c=0} + \left(\frac{\partial\left(\frac{I_{370}}{I_{381}}\right)}{\partial C}\right)_{c=0} \times C \quad \text{and}$$

$$\frac{I_{370}}{I_{381}} = \lim_{1/C\to 0}\left(\frac{I_{370}}{I_{381}}\right) \tag{4}$$

crosses, so that

$$\text{cac} = \frac{\left(\frac{I_{370}}{I_{381}}\right)_{1/C=0} - \left(\frac{I_{370}}{I_{381}}\right)_{C=0}}{\left(\frac{\partial\left(\frac{I_{370}}{I_{381}}\right)}{\partial C}\right)_{C=0}} \tag{5}$$

These cac values are presented in Table 2 and shown in Fig. 6.

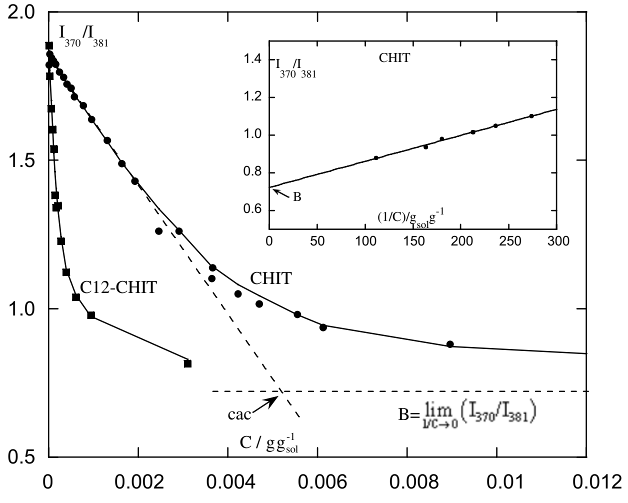

Fig. 5. Comparison between the aggregation profile of chitosan, CHIT and C12-CHIT grafted polymer through pyrene fluorescence measurements. The lines are guides for the eye. In the inset: example of the method to achieve I_{370}/I_{381} values for high chitosan concentration.

From inspection of Fig. 6 the borderline role of C5-CHIT very clearly appears. In fact CHIT and C5-CHIT present comparable cac values, and more than that, the C5-CHIT cac is slightly higher, confirming that chain stiffness due the presence of the grafted short aliphatic chains does not favour the polymer aggregation. Starting from C6-CHIT and going in the sense of longer hydrophobic chains, this effect begins to be balanced by the entropic gain due to the more and more efficient hydrophobic interactions.

4. Conclusions

This paper has been focused on the study of the aggregation properties in solution of a series of hydrophobically modified chitosans by the insertion of different aliphatic chains on the amine residues of the glucose units. Viscosity and fluorescence probe emission measurements give rise to very converging results, stating that in dilute solution the grafting of a short chain of five carbon atoms is not yet able to hydrophobically promote intra-aggregation of the polymer molecules but in contrast, making more rigid the chitosan structure, improves the polymer–solvent interaction, as shown by its bigger hydrodynamic radius and higher cac value. By increasing the length of the aliphatic chain this rigidity effect begins to be partially compensated if a six carbon atoms chain is present, while the entropic gain

Table 2
Influence of the chain length on the aggregative properties of alkylated chitosan

	A[a]	B[b]	cac/g g_{sol}^{-1}
Alkylation degree 10%			
CHIT	−223	0.72	0.0052
C5-CHIT	−179	0.75	0.0062
C6-CHIT	−290	0.79	0.0037
C8-CHIT	−615	0.63	0.0020
C10-CHIT	−2410	0.67	0.00049
C12-CHIT	−3599	0.74	0.00031

[a] $A = \lim_{C\to 0}\left(\frac{\partial(I_{370}/I_{381})}{\partial C}\right)$.
[b] $B = \lim_{1/C\to 0}(I_{370}/I_{381})$.

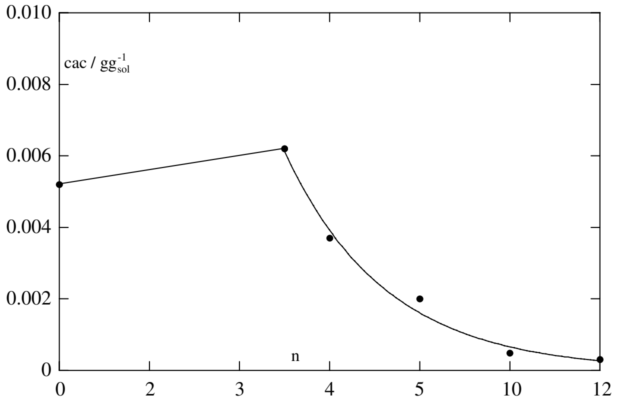

Fig. 6. Critical aggregation concentration, cac, as defined in Eq. (4) vs the length of the grafted chain, *n*. The line is a guide for the eye.

due to intra- and inter-hydrophobic interaction becomes preeminent for longer chains.

Fluorescence spectra in concentrated solution of all the examined polymers highlight that the aliphatic chains and the acetylic units of chitosan promote the formation of local environments whose hydrophobicity is comparable to that of liquid hydrocarbon, thus showing that in concentrated solution the specificity of the grafted chains of different length is lost.

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