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Amphiphilic polysaccharides. Evidence for a competition between intra and intermolecular associations in dilute system

S. Simon, J.Y. Dugast, D. Le Cerf, L. Picton*, G. Muller

Laboratoire Polymères, Biopolymères, Membranes, U.M.R. 6522, CNRS-Université de Rouen, 76821 Mont-Saint-Aignan, France

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Abstract

Amphiphilic carboxymethylpullulans differing in their degree of octyl-bearing groups were investigated in dilute solution. Viscometry, flow field-flow fractionation with on-line coupling multi-angle laser light scattering (F4/MALLS) were used to examine conformations and aggregation states in dilute solutions. Polymer/polymer interactions tendency increases with grafting degree. At lower grafting degree, the main species in solution are composed of isolated chains, the conformation of which is close to the precursor. For greater grafting degree, strongly compact structure has been evidenced due to strong intramolecular hydrophobic associations. The transition between dilute and semi-dilute cannot be described by a classical overlapping of chains. The critical concentration (C_{cr}) appears to be largely function of the content of C_8 groups and presents a minimum for about 20% of octyl groups: at this critical content aggregation trend is found maximum.
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Keywords: Amphiphilic polysaccharide; F4/MALLS; Light scattering

1. Introduction

Water-soluble polysaccharides have broad importance in many applications where the environmental considerations, the biocompatible and/or biodegradable aspect take a growing importance. Their importance depends on the properties they impart to solutions and gels. For specific applications, optimizing the final properties by a suitable control of the chemical structure and/or physical properties is necessary. In this context grafting of alkyl groups varying in length provide amphiphilic polysaccharides with adaptive properties.

Amphiphilic polymers carrying long alkyl chains demonstrated interesting behavior useful for control of aqueous phases rheological properties [1]. Polymers bearing shorter alkyl groups may also present some interesting properties notably at the interfaces [2]. The field of interfacial applications may concern the dilute polymer regime and it is of great interest to have good knowledge and understanding of the specific conformations adopted by amphiphilic polymers in aqueous solutions. Many reported data [3] evidenced that intramolecular and/or intermolecular (aggregates) specific 'hydrophobic' interactions could exist in dilute solutions. Nevertheless there are very few investigation according to the competition between intramolecular and intermolecular interactions notably as a function of concentration.

The aim of the present work was to examine the conformation and the aggregation of a set of hydrophobically modified carboxymethylpullulans (CMP) containing various grafting degree (0 to 45%) of octyl (C_8) alkyl chains. Our attention was focused on conformational properties in dilute medium and during the dilute-semi-dilute transition.

In this study, we have used mainly the flow field-flow fractionation (FFFF or F4) technique coupled on-line with multi-angle laser light scattering (MALLS). This technique was earlier reported suited for the characterization of amphiphilic polymers [4–7]. F4 is an universal separation method which can separate macromolecules as a function of their hydrodynamic diameters [8,9] and is useful in situation where the classical SEC fails because of specific interactions between amphiphilic polymer and the stationary phase. Therefore F4/MALLS allows the determination of the molar masses and gyration radii distributions of the

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* Corresponding author. Tel.: +33-2-35-14-65-43; fax: +33-2-35-14-65-43.

E-mail address: luc.picton@univ-rouen.fr (L. Picton).

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sample without any ambiguity. Dilute and semi dilute characterization have also been studied by viscometry.

2. Experimental section

2.1. Products

Pullulan (a flexible, neutral and linear polysaccharide) was purchased from Hayashibara Biochemical Laboratory (Okayama, Japan). The different products used hereafter were commercially available and used without further purification. Water was from a Milli-Q water reagent system. Carboxymethylpullulan (CMP) was prepared according to the method described in a previous work [10]. The degree of substitution DS (number of carboxylic groups per anhydroglucose unit) was determined by conductimetry and it has been found about 0.85. The hydrophobically modified carboxymethylpullulans (HMCMP) were prepared by covalent bonding of n-bromooctan (C_8) onto carboxylic acid groups of the obtained CMP [11]. The degree of modification (τ), i.e. the number of alkyl chains per anhydroglucose units was determined using gas chromatography after saponification of all ester groups [11,12]. It should be noted that the charge density (DS) decreases as the degree of hydrophobic modification, i.e. alkyl group increases. The HMCMP samples are code-named as following: a carboxymethylpullulan which was substituted by τ octyl chains, per 100 anhydroglucose units, is coded as CMP-τ-C_8.

2.2. Solution properties

2.2.1. Preparation of solutions

For F4 measurements, the polymer was directly dissolved in the carrier of flow field-flow fractionation ($LiNO_3$ 0.1 M + NaN_3 0.02%, 0.1 μm filtered) and gently stirred during 48 h then filtered through 0.45 or 8 μm type membrane (*Millipore*). Afterwards, solutions are used at once. For viscosity measurements, the polymer was dissolved in Milli-Q water and stirred for 2 days, then the required amount of NaCl was added and the new solution was again stirred during 24 h. All samples where analyzed under their fully ionized form.

Let us note that $LiNO_3$ and NaCl give the same ionic strength and are quite similar as concerned the screening effect onto the charged polymers. We have used $LiNO_3$ in F4/MALLS analysis to avoid any corrosive consequences which have been observed using NaCl.

2.3. Physico-chemical analysis

2.3.1. Viscometry

Viscosity measurements were performed in the Newtonian domain at low shear rate ($\approx 5\ s^{-1}$) using a Contraves LS30 viscometer (controlled temperature: 25 °C).

2.3.2. SEC and F4/MALLS

Fractionation of polymers is currently realized using size exclusion chromatography (SEC). For amphiphilic polymers SEC analysis is difficult for many reasons. Due to interactions with the stationary phase a low recovery of sample is detected at the outflow of the column and the retention time is not only due to size exclusion effects [10]. By using flow field-flow fractionation (F4) we have shown that such effects can be avoided [7]. The principle of the method is described elsewhere [8,9,13].

The following procedure was used here. The F4 is an universal fractionator model F-1000, from PostNova (*Munchen*, *Germany*). The channel dimensions are the following: Length = 27.7 cm, breadth = 2 cm and thickness = 254 μm. The accumulation wall is coated with a cellulose based membrane (M_w cut off = 10,000 g mol^{-1}). The linear channel flow rate (F_L) is regulated with an intelligent pump HPLC flom 301, while the crossflow (F_C) is generated by a P-500 dual piston syringe pump (*Pharmacia Biotech*) piloted by the fitted software 'Flow 160'. The sample-injected volume consists of a 100 μl full loop. When the sample reaches the head of the channel, the linear flow is diverted to keep particles under the cross flow during the time needed for equilibrium. This relaxation time, determined by the 'Flow 160' software is three times the sweep time of the cross flow and therefore is proportional to the applied F_C. Carrier is $LiNO_3$ 0.1 M + NaN_3 0.02%, 0.1 μm filtered and degassed (ERC-413). For all measurements, we have respected the following conditions: $F_L = 0.5$ ml min^{-1}, $F_C = 1$ ml × min^{-1} for 5 min, a decay of 10 min (exponential factor -1) until 0.3 ml min^{-1}, a plateau at 0.3 ml min^{-1} for 20 min then a last plateau at 0.05 ml min^{-1} until the end of analysis. Particles with diameters lower than about 1 μm are eluted in the so-called normal mode, the smaller particles being eluted before the larger ones [14].

The SEC line consisted of an OHPAK SB-G guard column as protection and two OHPAK SB 804 and 806 HQ columns (Shodex) in series. The column packing is a polyhydroxymethylmetacrylate gel. $LiNO_3$ 0.1 M used as carrier, was filtered through 0.1 μm filter unit (Millipore), carefully degassed (ERC-413), eluted at 0.6 ml min^{-1} flow rate (Flom HPLC pump 301), and clarified through a 0.45 μm filter upstream columns. The sample was injected through a 100 μl full loop.

Both F4 and SEC are coupled on line with MALLS. The MALLS photometer, a DAWN-F (DSP) from Wyatt Technology inc. (Santa Barbara, USA) is equipped with a K5 cell and a He–Ne laser ($\lambda = 633$ nm). The collected data were analyzed using the Astra V-4.50 software package. The SEC-MALLS technique has been described elsewhere [13,15]. The concentration of each eluted fraction has been determined with the differential refractive index (DRI) (ERC 7515A) from the measured value of dn/dC (0.140 ml g^{-1} for all the studied samples).

3. Results and discussion

3.1. Molecular conformation in dilute solution

The change in the intrinsic viscosities [η] and Huggins' coefficient k' as a function of the grafting degree (τ) for HMCMP are reported in Fig. 1. The value of k', found about 0.2 for CMP is in agreement with the usual value for a polymer in good solvent [10]. On the other hand the Huggins' coefficient of the HMCMP samples increases with increasing τ and reaches abnormally high values (31 for CMP-45-C_8). This reflects an increase in the polymer–polymer interactions and a decrease in the polymer-solvent interactions with the hydrophobic content (aggregation trend). This result may indicate 'theta' solvent conditions which have already been evidenced on hydrophobically modified hydroxy ethyl cellulose (HMHEC) bearing 0.6% of C_{16} alkyl group for which a negative value of the second Virial coefficient have been found by light scattering (LS) measurements [17]. In fact metastable equilibrium of solvatation should be expected even in dilute solution. It is the reason why all solutions have been studied in the same state (i.e. 72 h after total solubilization), since during this time any instability has been observed. Concerning the values of the intrinsic viscosities and according to the experimental uncertainties, [η] is found nearly constant for modification degrees varying from 0 to 11%. As molar mass is constant in this range (see the following F4/MALLS results) and as the product $[\eta] \times M$ is proportional to hydrodynamic radius (Rh) (Fox Flory relation), this suggests that the average hydrodynamic radius of the isolated species in solution is roughly constant. For larger grafting degree, intrinsic viscosities decrease with the alkyl chains content and reach very low values. This indicates that the species in solution, for the samples CMP-23, 33 and 45-C_8, have a lower average hydrodynamic radius than CMP, and shows that the species in solution are composed either of compact individual species due to intramolecular interactions or of very compact aggregates due to intra and intermolecular interactions. A similar behavior (increase of k' and lower value of [η] for a hydrophobically modified polymer) has been reported for many different products [3,16,17]. From viscosity measurements, it is difficult to have precise information about the different species existing in solution for a particular sample and it is necessary to use a separation technique (F4) suited to analyze amphiphilic samples.

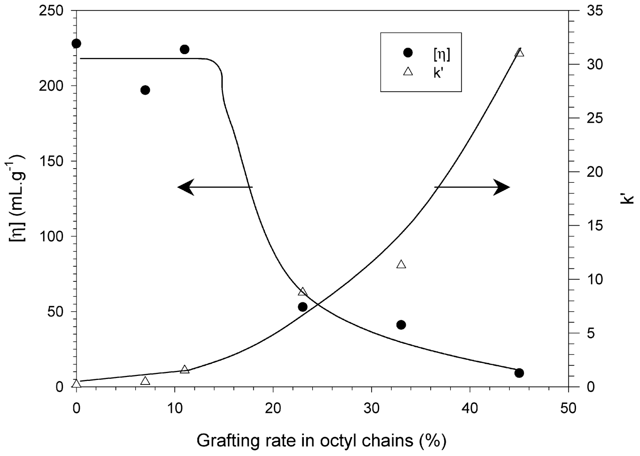

Fig. 1. Intrinsic viscosities and Huggin's constants for HMCMP as a function of the grafting degree in NaCl 0.1 M at 25 °C.

In a first step, we have checked the efficiency of F4 method compared to the well-known SEC. The hydrophilic CMP precursor was analyzed using both SEC and F4 methods. The results are reported in the first part of Table 1 and shown that both methods lead to number and weight average molar masses ($\overline{M_n}$ and $\overline{M_w}$, respectively) in good agreement. Therefore F4 can be used confidently for analyzing amphiphilic CMP.

Fig. 2 illustrated the fractionation profiles (both DRI and (LS) at 90° responses) together with the molar masses distributions of CMP and CMP-7-C_8 in $LiNO_3$ 0.1 M. Table 1 summarizes the analysis of these fractograms (i.e. the main DRI responses). It has been verified by DRI that no loss of product occurs for both samples during the filtration step and in the channel of F4 since the recovery of products determined by refractometric detector after separation is close to 100%. Concerning the CMP-7-C_8 sample, the MALLS detector, indicates the presence of two populations whereas only one population exists for CMP.

For both CMP and CMP-7-C_8, only one population (1) is observed according to the DRI response (directly proportional to the concentration) whereas two populations are evidenced when looking at LS response for the modified polymer CMP-7-C_8. The second population of CMP-7-C_8, eluted at high elution volumes (between 18 and 30 ml) shows a very intensive LS signal without any DRI response. This evidences the presence of very large aggregates in size but in very low proportion (quite nothing). The first population, the characteristics of which ($\overline{M_n}$, $\overline{M_w}$, I_p and the elution volume for the DRI peak (V_{peak})) are reported in Table 1, represents almost all the detected mass (DRI response). From 2 to 8 ml (more than 85% of the modified sample), the molar masses distribution of CMP-7-C_8 is superimposed on the precursor (CMP) evidencing that the

Table 1
Characteristics of CMP (from both SEC/MALLS and F4/MALLS) and of HMCMP (from F4/MALLS) according to the DRI response

Sample	$\overline{M_n}$ (g mol^{-1})	$\overline{M_w}$ (g mol^{-1})	I_p	$\overline{Rg_w}$ (nm)	V_{peak} (ml) from DRI
CMP_{SEC}	170,000	320,000	1.9	35	–
CMP_{F4}	160,000	300,000	2.0	30	5.2
CMP-7-C8	160,000	400,000	2.5	30	5.1
CMP-11-C8	180,000	760,000	4.2	43	5.1
CMP-23-C8	160,000	600,000	3.7	30	4.8
CMP-33-C8	160,000	570,000	3.5	20	3.7
CMP-45-C8	150,000	330,000	2.2	<20	3.3

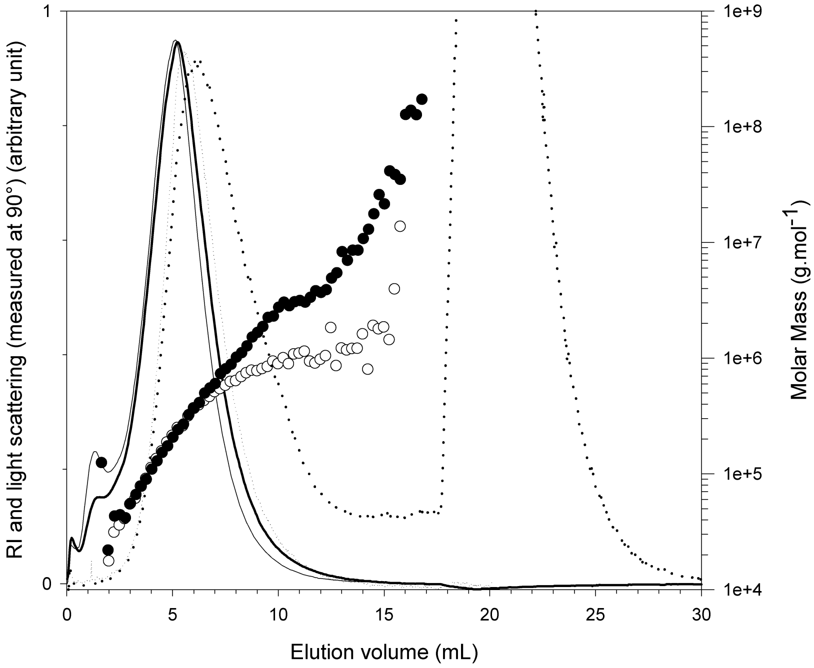

Fig. 2. Elution profiles from refractive index (full lines) and light scattering (dotted lines) of CMP (hairline) and CMP-7-C_8 (bold line) together with molar mass distributions of CMP (O) and CMP-7-C_8 (●) determined by F4/MALLS in $LiNO_3$ 0.1 M, Cp = 3.5 g l^{-1}.

main part of the modified sample is very similar to the CMP. This is reinforced by the value of $\overline{M_n}$ (significant of most species) and V_{peak} from DRI signal that are the same for both modified and precursor polymers. However, noticeable differences between precursor and CMP-7-C_8 can be observed for $\overline{M_w}$ together with a clear deviation of the modified polymer distribution in molar masses for higher eluted volumes. The reported data indicate the presence of few modified CMP aggregates (about 15%) which mainly influence the weight average molar mass $\overline{M_w}$.

In Fig. 3 are reported the fractionation profiles and molar masses distributions of CMP and CMP-45-C_8 whereas the molecular characteristics are indicated in Table 1 (the data concern the sample eluted between 1.5 and 17 ml according to the DRI response). As previously, the quasi totality of the sample was recovered in such a way that it is considered that most of the population was detected and analyzed. The examination of the values of V_{peak} for each sample indicates that modified CMP is eluted at lower volume than its precursor CMP. This evidences that the overall dimension of CMP-45-C_8 is lower than that of CMP. Since both CMP-45-C_8 and its precursor show quite similar value of $\overline{M_n}$, it can be argued that no degradation occurs during the modification step. As a consequence one can explain this result by the existence of strong intramolecular associations that induce a compactness of the macromolecule. This result clearly evidences the presence of intramolecular hydrophobic interactions due to the large amount of alkyl groups. Therefore, the presence of a second LS response according to the high elution volume together with any DRI signal which has been obtained for modified polymer as compared to its parent shows that very few aggregate structures coexist in the sample with a major part of isolated and collapsed chains.

The plot of elution volume at the maximum of the RI profile (V_{peak}) vs. the degree of modification (Fig. 4) largely agrees with that has been obtained with the variation of the intrinsic viscosity. The CMP-33-C_8 and CMP-45-C_8 are the most compact samples. By taking into account this fact and their large content in hydrophobic chains, it can be assumed that the conformation of such polymer in aqueous solution involve the presence of alkyl groups mainly inside the coil in order to limit contacts between octyl chains and water [4]. From the viscosity and F4/MALLS data a plausible explanation could be proposed concerning the conformation of the C_8 modified CMP series. This is illustrated in Fig. 5.

It is interesting to notice that upon the modification of few parameters (e.g. molar mass) it is possible to obtain noticeable change in conformation. In a previous work [7], which presents similar study on the same hydrophobically modified CMP only varying by a lower molar mass ($\overline{M_n}$ of precursor close to 20,000 g mol^{-1}) compared to the samples of present work. It has been reported that samples with lower molar masses and larger amount of C_8 display aggregation due to the existence of hydrophobic intermolecular interactions. This behavior can be explained as follow: with lower masses, there is less probability to obtain intramolecular interactions than with a sample with longer isolated chains, simply due to the decrease of both the number of alkyl chains per macromolecule and also the intrinsic flexibility.

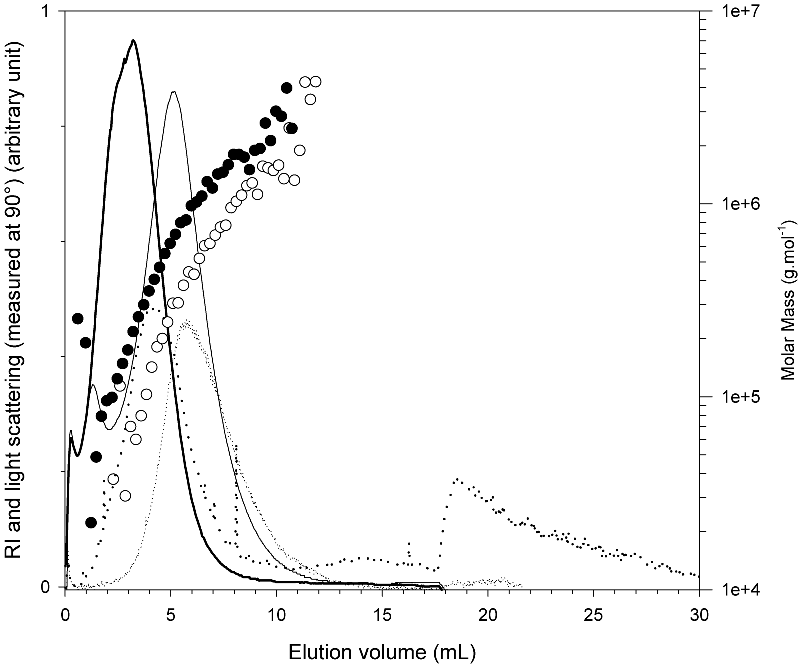

Fig. 3. Elution profiles from refractive index (full lines) and light scattering (dotted lines) of CMP (hairline) and CMP-45-C_8 (bold line) together with molar mass distributions of CMP (O) and CMP-45-C_8 (●) determined by F4/MALLS in $LiNO_3$ 0.1 M, Cp = 1 g l^{-1}.

3.2. Dilute/semi-dilute transition

After characterization of isolated chains and sometime aggregates of modified polymers and precursor, it seemed of interest to study the transition between dilute and semi-dilute solution. This has been done by using viscometry. When polymer concentration is increased, the typical behavior shows that the chains begin to overlap each other above a critical concentration called C^*. The Utracki-Simha representation [18] that is the bi-logarithmic plot of the zero-shear specific viscosity ($\eta_{spe} = (\eta - \eta_o)/\eta_o$ with η_o the solvent viscosity) as a function of polymer concentration, permits the determination of the C^* which corresponds to the observed break in the plot. A example of a such representation is given in Fig. 6 according to the CMP-33-C_8 sample. The C^* can be considered as a measurement of the degree of occupancy space of the polysaccharide [18]. The break in this plot (C^*) corresponds to the transition from dilute to semi-dilute solution therefore to the beginning of chain entanglement. According to the overlapping theory, the product $[\eta] \times C^*$ (coil overlap parameter) represents the filling of space by polymer coils and should be constant [19]. Fig. 7 shows the variation of both the critical concentration and the coil overlap parameter as a function of the grafting degree. In our case, this product decreases when the grafting degree increases. This observed behavior does not indicate a classical entanglement of the polymer chain and should be explained by the presence of interconnection between alkyl chains, creating intermolecular interactions. In fact, it seems better to design this phenomenon by a critical concentration C_{cr} and not by the inappropriate use of the term of C^*. Fig. 7

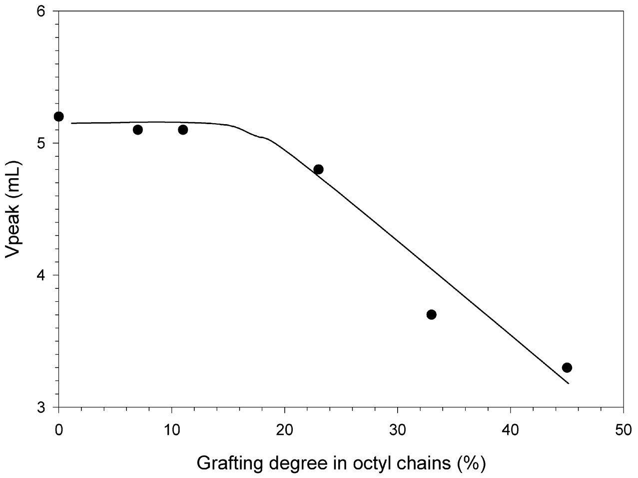

Fig. 4. Variation of V_{peak} (from DRI response) determined by F4/MALLS as a function of the grafting degree in $LiNO_3$ 0.1 M.

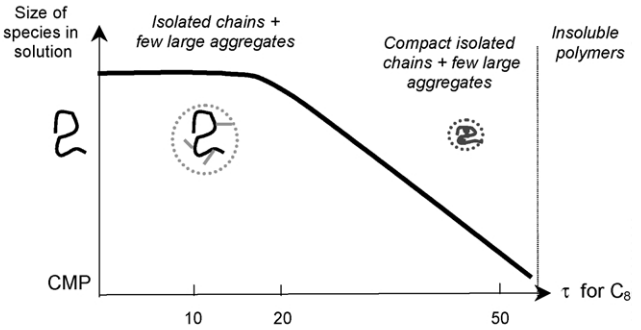

Fig. 5. Schematic representation of size evolution of HMCMP in solution as a function of the grafting degree (NaCl 0.1 M).

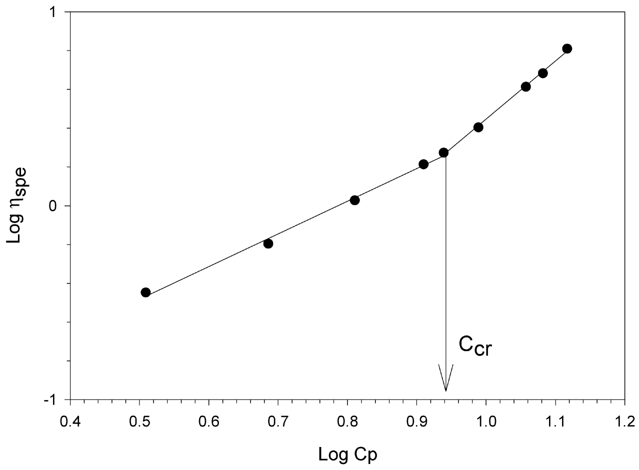

Fig. 6. Bi logarithmic plot of η_{sp} vs. Cp used for the determination of C_{cr}. Example shown for the CMP-33-C_8 in NaCl 0.1 M at 25 °C.

clearly shows a minimum value of C_{cr} as the amount of grafted C_8 increases. Up to 30% of grafted hydrophobic moieties, the decrease of C_{cr} should be explained by an increase of intermolecular interactions. Interconnected chains allow to an easier packing of the space. Then this class of amphiphilic polymers should be eventually used for their thickening properties. On the contrary, when the degree of grafting is above 30%, C_{cr} increases together with a very low [η]. We have shown that for large amount of C_8, intramolecular associations predominate and the conformation of polymer exhibits a very compact structure inducing very small hydrodynamic volume. Therefore higher concentrations seem necessary to occupy the same space. Nevertheless, the low value of the product [η]C_{cr} obtained for high amount of C_8 indicates that the dilute/semi-dilute transition is not only due to space packing but also to specific associations between coil and/or aggregates. As for the most grafted CMP (i.e. the 45% of C_8), the viscosity has been observed to become superior to the precursor's one (result not shown here) for sufficiently high concentration (i.e. above 50 g l^{-1}). It should be proposed that intramolecular associations can be progressively changed into intermolecular associations by 'opening' the compact coils with increasing concentration. To verify this, the aggregation tendency of hydrophobically modified CMP derivatives was investigated as a function of their concentrations in dilute solution.

Fig. 8 represents the normalized DRI profiles at several concentrations, 7-C_8, 11-C_8, 23-C_8 and 33-C_8 respectively, obtained by F4/MALLS analysis. We are aware of F4 technique is not really appropriate to describe the influence of the concentration since this it is an unknown way in F4 channel by the processes of focusing and elution. Nevertheless, the following results have to be taken as a confirmation of the previous observations mainly viscometric ones. Results from Fig. 8 will be discussed in terms of qualitative trend, in addition to the value of average weight molar masses ($\overline{M_w}$) showed on Table 1.

One can discern the following features: at low (CMP-7-C_8) and high (CMP-33-C_8) degree of grafted alkyl chains the profiles do not show any dependence on the concentration; at intermediate degree of modification (CMP-11 and 23-C_8) the main peak is widened toward higher elution volume in agreement with the presence of species of large size. As discussed about Fig. 2, CMP-7-C_8 shows a DRI profile quite similar to that observed for the CMP precursor but more interesting here (Fig. 8(a)) one can see any influence of the concentration on the profiles which indicates that no aggregation occurs in this dilute range of concentration. This result may evidence that the aggregation tendency is very low for the CMP-7-C_8, as previously showed by both the value of [η] and C_{cr} similar to the CMP precursor values (Figs. 1 and 7, respectively). For intermediate grafting degrees (11-C_8: Fig. 8(b) and 23-C_8: Fig. 8(c)) a peak train appears in the DRI profile which becomes more pronounced as polymer concentration

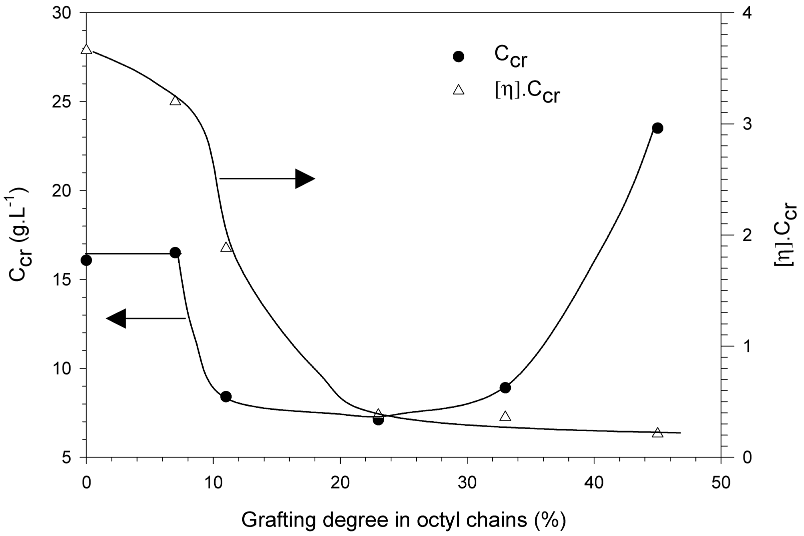

Fig. 7. C_{cr} and (Ccr × [η]) as a function of the grafting degree for HMCMP derivatives in NaCl 0.1 M at 25 °C.

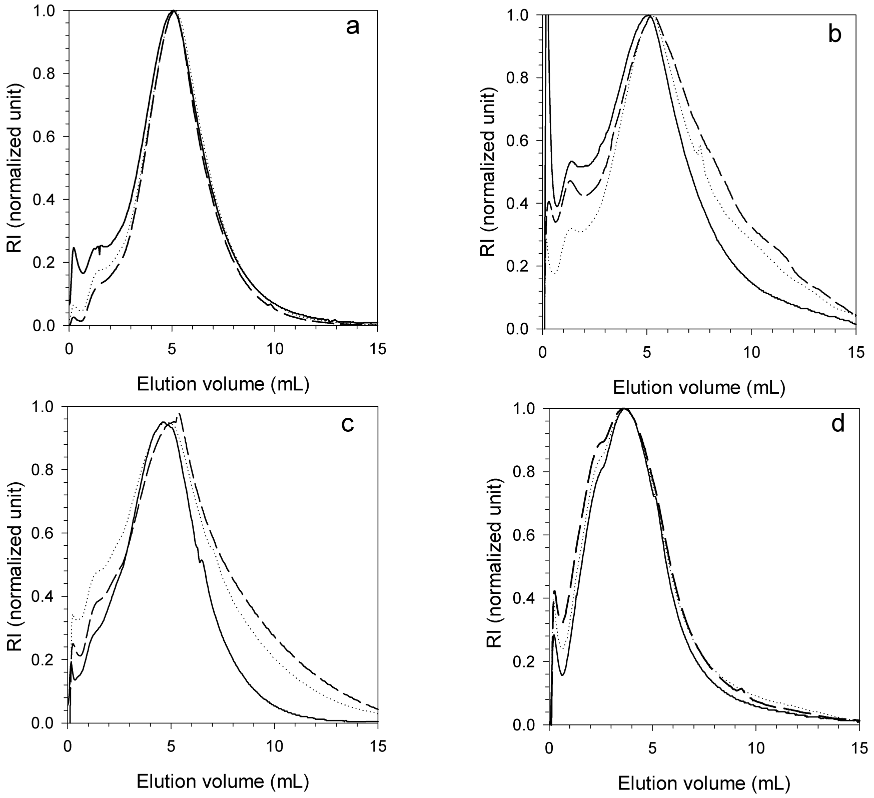

Fig. 8. Normalized RI profiles of CMP-7-C_8 (a), CMP-11-C_8 (b), CMP-23-C_8 (c) and CMP-33-C_8 (d) for various concentrations determined by F4/MALLS in $LiNO_3$ 0.1 M (samples filtered through 8 μm filters). 1 g l^{-1} (solid line), 3.5 g l^{-1} (dotted line), 6 g l^{-1} (dash line).

increases, in accordance with an increasing number of species with large hydrodynamic radius. For CMP-33-C_8 (Fig. 8(d)) the fractogram profiles are unchanged in the studied concentration range in agreement with a very compact main population. The same result has been observed for the sample CMP-45-C_8 (results not shown here). For the higher amount of C_8, and in the explored dilute regime, it can be argued that intramolecular interactions prevail over intermolecular interactions. The maximum obtained in the aggregation trend seems to be correlated in term of grafted amount, with the minimum obtained for critical concentration (C_{cr}). To have a seeing of that remark we have plotted on Fig. 9, both the $\overline{M_w}$ from Table 1 (that is a clear aggregation evidence) and the critical concentration (C_{cr}) as a function of C_8 grafting degree. We can easily observe the correlation between the maximum of $\overline{M_w}$ and the minimum of the C_{cr}.

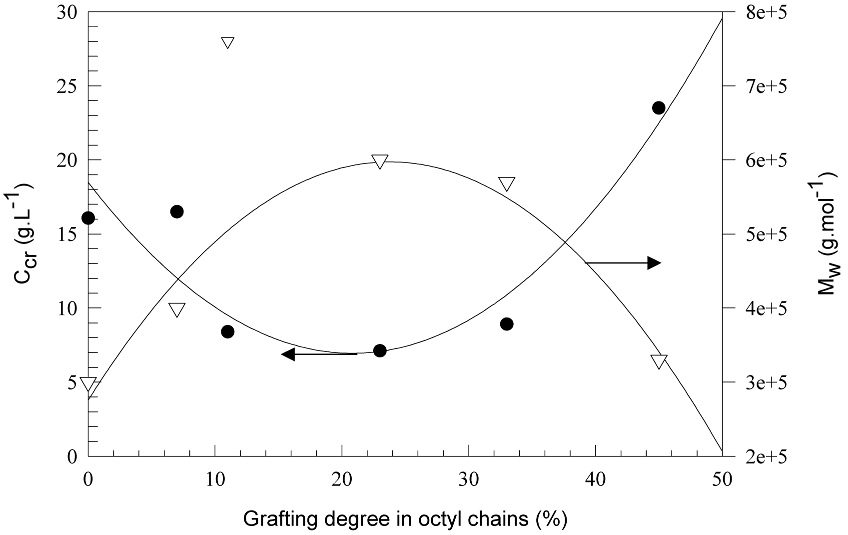

Fig. 9. Critical concentration (●) and $\overline{M_w}$ (▽) vs. grafting degree of C_8.

4. Conclusion

In summary, very different conformations and behaviors are exhibited according to varied amount of. C_8 moieties. For a lower grafting degree, samples have a conformation close to the precursor with very few aggregates. By contrast higher grafting degree leads to very compact species in solution. In all the cases, there is competition between intramolecular and intermolecular interactions depending mainly on hydrophobic degree and concentration. The transition between dilute and semi-dilute regime is not a classical overlapping phenomenon and we have shown that hydrophobic intermolecular associations permit the connection of amphiphilic chains for lower critical concentration (C_{cr}) than without hydrophobic 'stickers'. Moreover, a correlation has been evidenced between the aggregation tendency and the decrease of C_{cr}. In the same time, for the higher amount of C_8, the transition between intramolecular association and intermolecular associations seems difficult to obtain in low concentration range. It should be proposed that competitive forces take place between intramolecular interactions and internal stress according to intrinsic flexibility of polymer backbone on one hand and intermolecular interactions and meeting probability of chains on the other hand.

We plan to correlate the bulk properties and the adsorption properties onto polystyrene latex for this series of amphiphilic polymer in a future work.

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