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# New amphiphilic modified polysaccharides with original solution behaviour in salt media

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ABSTRACT

New water soluble and amphiphilic PCL-grafted-alginates with two different molar masses of PCL (530 and 1250 g mol$^{-1}$) and molar hydrophobe contents from 3.5 to 15% have been prepared by new aqueous micellar grafting technique. Physico-chemical behaviour in aqueous solution (pure water and NaCl 0.1 M) has been studied by means of viscosity measurements and on-line Flow field flow fractionation/Multi-angle laser light scattering/Quasi-elastic light scattering/Differential refractive index analyses. Expected associative behaviour has been evidenced in pure water whatever the length of the PCL chains. In salt media, the associative behaviour strongly depends on the length of PCL chains. For PCL chains of 530 g mol$^{-1}$, intramolecular hydrophobic interactions are predominant, even in semi-dilute regime. This non-classical behaviour for an associative polyelectrolyte opens the way to the conception of amphiphilic matrices with hydrophobic clusters for controlled release applications.



## 1. Introduction

The conception of new amphiphilic matrices used in aqueous media with well defined hydrophobic clusters knows a significant interest mainly for the development of controlled release systems particularly designed for biomedical or pharmaceutical applications (Inoue, Chen, Nakamae, & Hoffman, 1997). Due to their good biocompatibility and their ability to form hydrophobic clusters, amphiphilic associative polysaccharides are suitable candidates (Akiyoshi & Sunamoto, 1996; Henni-Silhadi et al., 2007; Leonard, Rastello de Boisseson, Hubert, Dalençon, & Dellacherie, 2004; Mocanu, Mihai, Le Cerf, Picton, & Muller, 2002; Mocanu, Mihai, Le Cerf, Picton, & Muller, 2004). Water soluble behaviours of associative polymers are now well defined by important literature (Blaz Vieira, Moscardini, Oliveira Tiera, & Tiera, 2003; Charpentier et al., 1997; Glinel, Huguet, & Muller, 1999; Ouchi, Nishizawa, & Ohya, 1998; Pelletier, Hubert, Lapicque, Payan, & Dellacherie, 2000; Simon, Dugast, Le Cerf, Picton, & Muller, 2003; Sinquin, Hubert, & Dellacherie, 1993; Volpert, Selb, & Candau, 1998). Such systems lead to either intra and/or intermolecular interactions, according to the polymer concentration, the intrinsic characteristics of the main polymer chain (structure and flexibility of the polymeric chain, neutral or polyelectrolyte character), the grafting rates and intrinsic characteristics of the hydrophobe moieties (size and/or structure) and/or external conditions such as pH, salinity, temperature or co-solutes. The presence of hydrophobic groups bound to the polysaccharide chain can lead to the formation of large aggregates (Duval-Terrié, Huguet, & Muller, 2003), which can change solution properties such as viscosity (Akiyama et al., 2005), surface tension (Henni et al., 2005) and solubility (Simon, Mocanu, Picton, Le Cerf, & Muller, 2004). Charged associative polysaccharides permit to conjugate high amount of grafted hydrophobic moieties together with good water solubility. Their solution behaviour is governed, especially in the dilute regime, by the occurrence of both long range electrostatic repulsions and hydrophobic attractions. According to the salt concentration in the aqueous polymer solution, the resulting conformations may be fully expanded or on the contrary, shrunken and tight. In the most cases, associative polyelectrolytes present a classical behaviour in salt media; i.e. hydrophobic interactions are predominantly intramolecular at low concentrations and become predominantly intermolecular at higher concentrations (Simon et al., 2003).

Thus it appears difficult to obtain hydrophobic clusters (intramolecular associations) at high polymer concentration. However, semi-dilute range of concentration is always needed to elaborate hydrogels by chemical or physical crosslinking.

The aim of this work is to obtain associative charged polysaccharides able to form intramolecular hydrophobic associations (clusters) in salt media along a wide range of concentration, suitable for physically cross-linked matrices used in controlled release systems.

The chosen polysaccharide is sodium alginate, a polyanionic polysaccharide originally extracted from brown seaweed algae. It is a linear binary copolymer consisting of (1→4)-linked β-D-mannuronic acid (*M*) and α-L-glucuronic acid (*G*) residues (Fig. 1). Via

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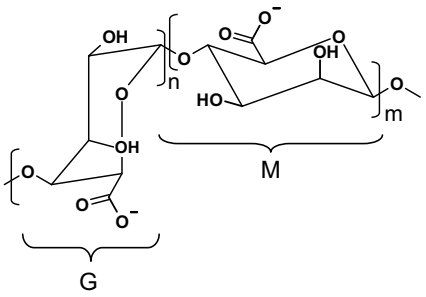


**Fig. 1.** Chemical structure of alginate: *G* is a Guluronate group, *M* is a Mannuronate group.

*G* residue, alginate is able to give gels with divalent cations such as $Ca^{2+}$, suitable for controlled release (Rousseau, Le Cerf, Picton, Argillier, & Muller, 2004). This polysaccharide is often used in the biotechnological industry as a thickening or gelling agent, and in pharmaceutical applications (Pepperman, Kuan, & McCombs, 1991).

Alkyl moieties usually grafted onto polysaccharides generally lead to intermolecular association in semi-dilute regime and seems not suitable in this case. We have chosen for this study, poly(ε-caprolactone) (PCL) (Fig. 2a) because such a pendant hydrophobic group is longer than alkyl one. PCL is a biodegradable aliphatic polyester readily synthesized chemically by the polymerization of ε-caprolactone (ε-CL) by cationic, anionic and coordination polymerization as well as by free radical initiation (Sinha, Bansal, Kaushik, Kumria, & Trehan, 2004). Due to its biocompatibility, non-toxicity and excellent mechanical strength, it has been intensively investigated as a biomedical material (Dai, Williamson, Khammo, Adams, & Coombes, 2004). It is also suitable for controlled release matrix for active compounds (drugs, pesticides...) due to its high permeability to many small drug molecules (Sinha et al., 2004).

One way to obtain PCL-grafted-polysaccharides is a catalytic ring-opening polymerization of ε-CL in the presence of polysaccharides (Dubois, Krishnan, & Narayan, 1999; Shi & Burt, 2004) or protected polysaccharides by silylation, of hydroxyl groups for example, to reduce the number of active sites (Chen et al., 2005; Liu, Li, Fang, & Chen, 2005; Nouvel, Dubois, Dellacherie, & Six, 2004; Rutot, Duquesne, Ydens, Degée, & Dubois, 2001). The disadvantage of this method is the polydispersity of the length of PCL chains thus obtained.

Another way is the connection of monodisperse PCL macromonomers on the hydroxyl groups of polysaccharide. However in this case, it is necessary to functionalize the end group polyester and/or the polysaccharide (Gref, Rodrigues, & Couvreur, 2002). Moreover, these syntheses are realized in organic solvent at high temperature that is generally favourable to high substitution rates for non water soluble materials. We propose in this paper a new route of synthesis by emulsion way in aqueous media to obtain water soluble polysaccharides, based on alginate, grafted by PCL (PCL-*g*-alginate). The coupling reaction between alginate and PCL is conducted between alcohol functions of the commercial PCL used (Fig. 2b) and carboxylic functions of protonated alginate thanks to a carbodiimide activator in heterogeneous phase, stabilized by sodium dodecyl sulfate (SDS) surfactant.

Solution behaviour in aqueous media, and notably hydrophobic associations, were studied by viscometry measurements, and by coupling a fractionation technique with both static and dynamic light scattering (F4/MALLS/QELS). These techniques allowed us to examine the conformation and the aggregation state of a set of hydrophobically modified alginate, with various grafting rate and length of PCL chains, in dilute media and during the dilute/semi-dilute transition. Our attention was especially focused on associative properties and conformation of these samples according to the length of PCL chains and the used media (pure water and NaCl 0.1M).

## 2. Experimental section

### 2.1. Materials

Sodium alginate of number average molar mass ($\overline{Mn}$) 194,000 g $mol^{-1}$, polymolecular index (Ip) 1.8 (determined from SEC/MALLS measurements, Rousseau et al., 2004) and with a ratio $M/G = 0.5$ was purchased from Degussa company. 1-ethyl-3-[3-(dimethylamino)-propyl] carbodiimide hydrochloride (EDCI), sodium dodecyl sulfate (SDS), dichloromethane ($CH_2Cl_2$) and hydrochloric acid (HCl) were purchased from Sigma-Aldrich. PCL-diol used was also purchased from Sigma-Aldrich and two different molar masses were studied (530 and 1250 g $mol^{-1}$) (Fig. 1b). This poly(ε-caprolactone) was bi-functionnal with an hydroxyl group on both extremities. All compounds and solvents were used without further purification. Water was purified with the Milli-Q reagent system (Millipore).

### 2.2. Synthesis

Due to the difference of solubility of both components, the reaction takes place in heterogeneous medium (water/dichloromethane) between hydroxyl groups of PCL (dichloromethane soluble) and carboxylate groups of alginate (water soluble). Hydroxyl groups of PCL cannot react easily on carboxylate groups of alginate and consequently these last were "activated" by EDCI. Moreover, as the activator reacts exclusively with carboxylic functions, alginate was first partially transformed into its acidic form thanks to the addition of hydrochloric acid. However, it is well-known that alginate has the ability to form gels at acidic pH (Draget, Skjåk-Bræk, Christensen, Gåserød, & Smidsrød, 1996), and precipitate below pH 3.0 (Ibáñez and Umetsu, 2002). Below pH 3.6 the viscosity of alginate solution (2 g/$L^{-1}$) dramatically increases (data not shown). In order to avoid a too high difference of viscosity between the two phases (organic and aqueous), leading to a decrease of the reactivity between "activated" acid and alcohol groups, the pH has been downed to this value. The pKa of alginate was found to be around 3.4–4.5 depending on the characteristics of the sample (ratio *M*/*G*, molar mass...) (Sreeram, Shrivastava, & Unni Nair, 2004) and therefore, it can be assumed that enough carboxylic functions were

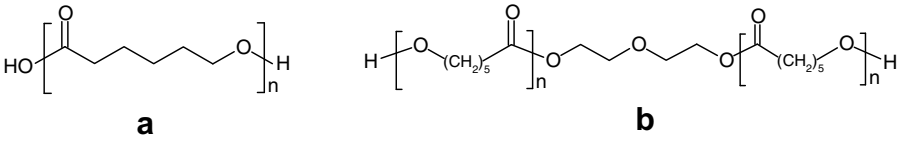


**Fig. 2.** Chemical structure of (a) Poly(ε-caprolactone) and (b) commercial PCL-diol used.

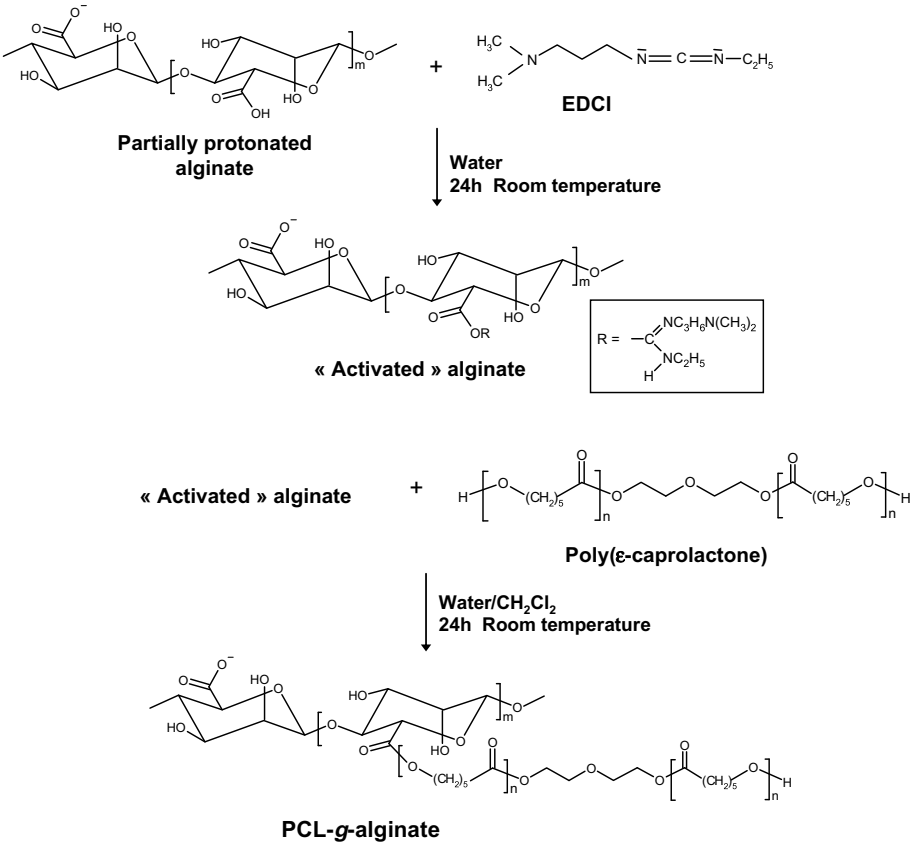


**Fig. 3.** Synthesis of PCL-*g*-alginate.

present at pH 3.6. According to the scheme in Fig. 3, polyester chains are thus linked to the polysaccharide backbone via ester functions.

Aqueous solution of alginate (0.2% w/v) was prepared by solubilizing 2 g of sodium alginate in 1L of water and the solution was stirred during 24h at room temperature at pH 3.6. Then a milli-Q water solution of EDCI freshly prepared at a desired concentration (see Table 1) was added to the solution under vigorous stirring. The reaction between EDCI and –COOH functions of partially protonated alginate was carried on 24 h at room temperature. The surfactant was then added to the aqueous phase at 0.35% (w/v) (at a higher concentration than the critical micellar concentration of SDS, equal to 0.23% (w/v)). An adequate mass of PCL was solubilized in 100 mL of dichloromethane and this organic phase was finally added drop by drop in the aqueous phase. The reaction was carried on 24 h at room temperature. After evaporation of dichloromethane, the aqueous solution containing hydrophobically modified sodium alginate was purified by successive dialysis against water to remove unreacted EDCI and the surfactant. Bu, Kjøniksen, Elgsaeter, and Nyström (2006) have shown existence of interactions between hydrophobically modified alginate (by grafting of *n*-octylamine groups (C8)) and SDS at very low surfactant concentration and therefore, it was necessary to carry on the dialysis during several weeks to remove all the SDS. The aqueous phase was then evaporated and the product was precipitated in an excess amount of acetone to remove unreacted PCL. The final reaction product obtained was extracted one more time with acetone in a Soxhlet apparatus and PCL-*g*-alginate derivative was finally dried 24 h at 40 °C.

**Table 1**
Graft copolymerization conditions and results of efficiency grafting rates

| Samples | $M_{pcl}$ (g mol$^{-1}$) | $n_{EDCI}/n_{COOH}$ (%) | $n_{PCL}/n_{COOH}$ (%) ($n_{EDCI}/n_{PCL}$) | Theoretical grafting rate (%) | Efficiency grafting rate (%) |
|---|---|---|---|---|---|
| 1 | 530 | 5 | 20 (1:4) | 5 | 4 |
| 2 | 530 | 10 | 30 (1:3) | 10 | 8 |
| 3 | 530 | 20 | 40 (1:2) | 20 | 15 |
| 4 | 1250 | 5 | 20 (1:4) | 5 | 3.5 |
| 5 | 1250 | 10 | 30 (1:3) | 10 | 7.5 |
| 6 | 1250 | 20 | 40 (1:2) | 20 | 13.5 |

The spectra FTIR of SDS was superimposed to the FTIR spectra of several samples of PCL-*g*-alginate (not shown) to verify the eventual presence of residual surfactant. It was found that almost all SDS has been removed from PCL-*g*-alginate.

Modified alginates are coded as following: Molar mass of the grafted PCL – real incorporating rate ($x$) (example: 530–15 for an alginate grafted with a 530 g mol$^{-1}$ PCL and a substitution degree of 0.15 or 15% in respect with uronic acid units).

It is important to notice the possibility of alginate-PCL-alginate coupling cross-linked reaction. Nevertheless, the probability of such reaction is expected poor, due to the low concentration of alginate and consequently the poor probability of this reaction. No gel has been observed and the obtained molar masses of grafted alginate (see below) argue that no such reaction occurred.

### 2.3. Solutions preparation

Polymer solutions were prepared under vigorous stirring for 48 h at 40 °C. The pH was then adjusted at pH 7.0 with NaOH 0.1 M and samples were analyzed under their fully ionized form.

When necessary, a solution of salt at adequate concentration has been added to the previously prepared aqueous polymer solution. The resulting mixtures were stirred for a further 24 h at 40 °C

and then allowed to stand for at least 24h at room temperature before measurements were performed. For F4 measurements, the polymers were dissolved in $LiNO_3$ 0.1 M, under stirring for 48h at 40 °C and then filtered through 0.45 or 8 μm type membrane (*Millipore*).

### 2.4. Elementary analysis

The experimental concentrations of carbon and nitrogen were measured using Total Organic Carbon and Total Nitrogen analyzer (TOC–VCSN and TNM1, Shimadzu, Japan) to determine the experimental grafting rates. Theoretical concentration of carbon can be defined by Eqs. (1) and (2):

$$\%C = [(\text{Carbon weight})/\text{molar mass}).100] \quad (1)$$

$$\%C = \frac{[n_{C1}.MC.(1-\tau_{PCL}-\tau_{EDCI})]+[n_{C2}.M_C ldot \tau_{PCL}]+[n_{C3}.M_C.\tau_{EDCI}]}{[M_0.(1-\tau_{PCL}-\tau_{EDCI})]+[\text{Mg}.\tau_{PCL}]+[M_{AA-EDCI}.\tau_{EDCI}]}.100 \quad (2)$$

where $n_{C1}$, number of carbon in an alginate unit ($n_{C1}$ = 6) ;$n_{C2}$, number of carbon in a grafted alginate unit ($n_{C2}$ = 34 for PCL 530 and 77.2 for PCL 1250) ;$n_{C3}$, number of carbon in an "activated" alginate unit ($n_{C3}$ = 14) ;$M_0$, molar mass of an alginate unit (175 g mol$^{-1}$) ;Mg, molar mass of a grafted alginate unit (Mg = 688 g mol$^{-1}$ for PCL 530 and 1408 g mol$^{-1}$ for PCL 1250) ; $M_{AA\text{-}EDCI}$, molar mass of an "activated" alginate unit (331 g mol$^{-1}$)$\tau_{PCL}$, grafting rate of PCL (mol%); $\tau_{EDCI}$, rate of «activated» but non grafted alginate unit.

$\tau_{EDCI}$ was determined from the experimental concentration of nitrogen: (Eq. (3))

$$\%N = \frac{[n_N.M_N.\tau_{EDCI}]}{M_{AA-EDCI}} \cdot 100 \quad (3)$$

The experimental grafting rate $\tau_{PCL}$ can be calculated from Eq. (2) and (3).

### 2.5. Infrared spectroscopy (FTIR)

The IR spectra were obtained thanks to a spectrophotometer 2000 AFT–FTIR with an ATR MKII Golden gate system (Specac, UK). Angle of incidence: 45, monoreflexion system, 10 co-added scan, diamond crystal.

### 2.6. Viscosity measurements

The viscosity measurements were performed in the Newtonian domain using a Contraves LS30 viscometer. The apparatus was equipped with concentric cylinder geometry of outer radius of 12 mm and gap width of 0.5 mm. In this configuration, shear rates in the range of 0.01–100 s$^{-1}$ were attainable. The temperature around the couette geometry was kept constant at 25 °C thanks to the circulation of water from a controlled temperature bath. All measurements have been conducted in the Newtonian regime.

The measurements of the intrinsic viscosity [$\eta$] corresponding, for neutral polymers, to the extrapolation to infinite dilution of the Flory–Huggins equation:

$$\eta_{red} = \eta_{sp}/C_p = [\eta] + k_H[\eta]^2 C \quad (4)$$

(Where $C_p$ is the polymer concentration and $\eta_{sp}$ the specific viscosity of the solution) is one of the most frequently used methods to obtain an accurate image of the expansion state.

$k_H$ is the Huggins constant, depending on the nature of the polymer/polymer interactions in solution. This constant gives information on the solvation state, i.e. on interactions between the coil and the solvent considered. For a random coil polymer, a common value of 0.3–0.8 is found for $k_H$ and larger values indicate associations (Charpentier et al., 1997). Because of the polyelectrolyte nature of alginate and PCL-*g*-alginate, the addition of a screening salt is required to mask the polyelectrolyte effect. For all the samples, precursor as well as hydrophobically modified derivatives, it was found a NaCl concentration as low as 0.1 M is sufficient to screen the electrostatic long range repulsions and to reach linearity.

Critical concentrations $C_{Cr}$ (delimiting dilute and semi-dilute regime) have been determined using the Utracki–Shima representation (Utracki & Shima, 1963) that is the bi-logarithmic plot of the zero-shear specific viscosity as a function of polymer concentration (Eq. (5)).

$$\log(\eta_{sp}) = \text{p}.\log(C_p) + \text{constant} \quad (5)$$

The $C_{Cr}$ values, and consequently the transition from dilute to semi-dilute solution, correspond to a break in the plot.

### 2.7. SEC and F4/MALLS

The principle of these methods was widely described elsewhere (Duval, Le Cerf, Picton, & Muller, 2001; Picton, Bataille, & Muller, 2000; Simon et al., 2003). For Flow Field Flow fractionation (F4), macromolecules are eluted from the lower to the higher size contrary to Size Exclusion Chromatography (SEC). The F4 is a Universal Fractionator model F-1000, from PostNova, 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 ($\overline{Mw}$ cut off = 10,000 g mol$^{-1}$). The linear channel flow rate ($F_L$) is regulated with an intelligent pump HPLC flom 301, Japan, 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.

Carrier is $LiNO_3$ 0.1 M (used because less corrosive than NaCl) + $NaN_3$ 0.02%, 0.1 μm filtered and degassed (ERC-413, Erma-CR Inc., Japan). For all measurements the following conditions were respected: $F_L$ = 0.3 mL min$^{-1}$, $F_C$ = 1 mL min$^{-1}$ for 5 min, a decay of 10 min (exponential factor −0.5) until 0.2 mL min$^{-1}$, a plateau at 0.2 mL min$^{-1}$ for 10 min then a last plateau at 0.05 mL min$^{-1}$ until the end of analysis.

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 of SEC 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) and eluted at 0.6 mL min$^{-1}$ flow rate (Flom HPLC pump 301). Both F4 and SEC are coupled on-line with MALLS. The MALLS photometer, a DAWN-EOS from Wyatt Technology inc. (Santa Barbara, USA) is equipped with a K5 cell and a Ga-As laser ($\lambda$ = 690 nm). QELS modulus from Wyatt Technology is connected to the photodiode N°13 (angle 111°). The collected data were analyzed using the Astra V-4.90 software package. The concentration of each eluted fraction has been determined with the differential refractive index (DRI). Refractive index increment d$n$/dC was 0,140 mL g$^{-1}$.

## 3. Results and discussion

### 3.1. Characterization of the PCL-g-alginates

#### 3.1.1. FTIR spectra

Grafting of PCL onto alginate was investigated via FTIR spectrum (Fig. 4A, B and C for alginate, PCL and a sample of PCL-*g*-alginate, respectively).

The IR spectrum of the PCL-*g*-alginate copolymer shows the characteristic –C=O stretching vibration of PCL chains at 1722 cm$^{-1}$ that doesn't appear in alginate spectrum. The ester functional group formed via the reaction between the –OH group

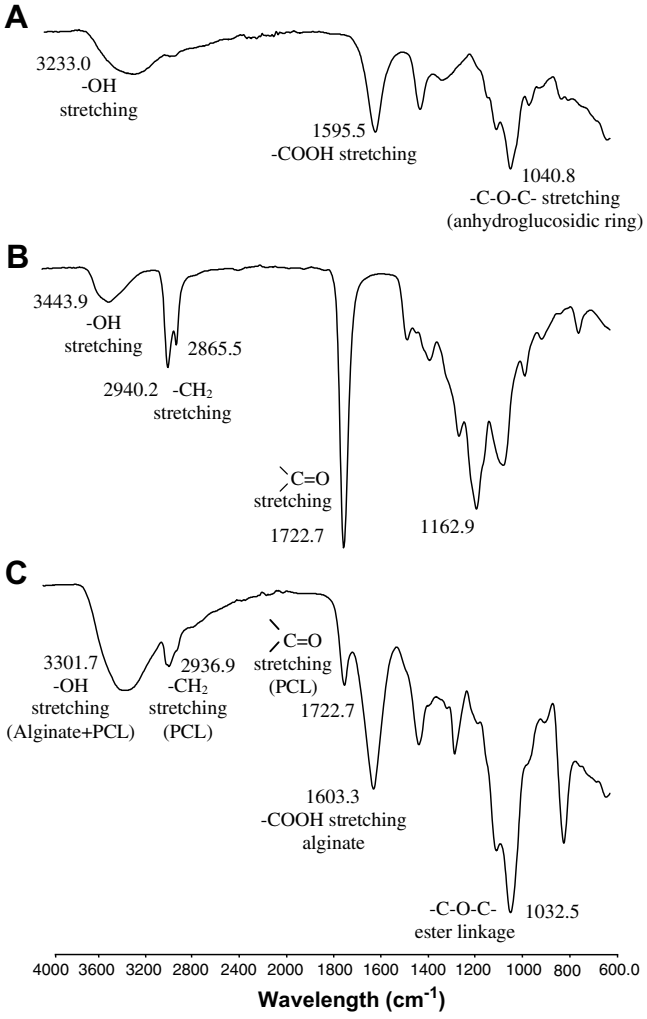


**Fig. 4.** FTIR spectra of pure alginate (A), PCL (B) and the final copolymer PCL-*g*-alginate (C).

of PCL and the "activated" carboxylic acid of "activated" alginate appears at 1032.5 $cm^{-1}$. This strong band is characteristic of –C–O–C– stretching absorbance.

Furthermore, the FTIR spectrum of PCL-*g*-alginate shows peak between 3200 and 3700 $cm^{-1}$ (–OH absorbance) that are much more intense than the stretching absorbance at 3000–3600 $cm^{-1}$ observed separately for the both components. All these results implied significantly the successful grafting of the PCL chains onto the alginate backbone.

### 3.1.2. Grafting content of PCL in alginate-graft-PCL copolymers

The characteristics of samples from different grafting reaction, realized by varying the feed amounts of EDCI and PCL chains, are listed in Table 1. The experimental grafting rates confirm the occurrence of the grafting of PCL chains, and it is also important to notice that the amount of residual "activated" functions is lower than 2% (data not shown).

The presence of carboxylate functions along the backbone of copolymer synthesized allows them to be water soluble even at high polymer concentrations. Moreover, Sinquin et al. (1993) reported that in the case of a partially esterified derivative of alginate, a rate superior to 70% of non-esterified sodium carboxylate functions remains sufficient to retain not only the typical viscosimetric behaviour exhibited by polyelectrolytes in dilute pure water solution but, above all, the ability of alginate to undergo a sol-gel transition upon treatment with $Ca^{2+}$.

### 3.2. SEC and F4/MALLS characterization

Fractionation of polymer is usually performed using size exclusion chromatography (SEC). However SEC analysis is generally difficult for amphiphilic polymers due to interactions with the stationary phase. By using flow field flow fractionation (F4), such effects can be avoided (Duval et al., 2001). Before any measurements, it is prealably necessary to compare the efficiency of F4 method to the SEC method. Consequently, "activated" alginate precursor (AA-EDCI) was analyzed using both SEC and F4 methods. The obtained results are reported in the first part of Table 2 and proved that both methods lead to number and weight average molar masses ($\overline{Mn}$ and $\overline{Mw}$, respectively) in good agreement. Therefore it can be firstly argued that any degradation occurs during the "activation" step of alginate and F4 can be used for analyzing amphiphilic alginate. The "activated" alginate AA-EDCI will be used as our reference for the following.

As an example we have reported the fractionation profiles (both Differential Refractive Index (DRI) and Light scattering (LS) at 90° responses) together with the molar masses distributions of "activated" alginate and 530–15 in $LiNO_3$ 0.1 M (Fig. 5). These fractograms are representative of the trend of almost overall profiles obtained for PCL-*g*-alginate derivatives. On contrary to the precursor, the LS response of 530–15 evidences two populations whereas only one population (the first one eluted at low elution volume) appears thanks to the DRI response. Thus, the whole material is represented in the first population whereas the second population eluted at high elution volumes, shows only intensive and saturated LS signal. This second population clearly demonstrates the presence of very few but large aggregates in the case of modified alginate. This behaviour has also been observed for carboxymethylpullulane grafted with octyl side chains (Simon et al., 2003). The characteristics of overall samples ($\overline{Mn}$, $\overline{Mw}$, Ip, and Rh (hydrodynamic radius)) have been extracted from the first population, which constitutes almost the whole analyzed fraction, and are reported in Table 2. It is important to notice that only about 70% of the modified sample was detected because of loss after filtration.

Significant differences can be observed between AA-EDCI precursor and 530–15. We notice a translation of DRI response toward higher elution volumes for the modified sample compared to the precursor. According to the fractionation of F4 (from low to high particles in size), it appears that the modified sample presents higher size than the precursor (see Rh values in Table 2). This is related to an increase of molar masses (Table 2 and Fig. 5). These results indicate the presence of large aggregates of 530–15 due to associative behaviour, even in dilute solutions. This trend has also been observed for the 1250-× samples (see Table 2) and we can also notice an increase of Rh and molar masses, when PCL substitution rate increases (*x*). The aggregation trend (i.e. intermolecular association) is clearly more pronounced for the 1250-×

**Table 2**
Characteristics of alginate, "activated alginate" (from both SEC/MALLS and F4/MALLS) and hydrophobically modified alginate (from F4/MALLS) according to the DRI response

| Sample | $\overline{Mn}$ (g $mol^{-1}$) | $\overline{Mw}$ (g $mol^{-1}$) | Ip | Rh (nm) |
|---|---|---|---|---|
| $Alginate_{(SEC)}$ | 194,000 | 342,000 | 1.76 | 62 |
| $AA\text{-}EDCI_{(SEC)}$ | 207,000 | 353,000 | 1.71 | 63 |
| $AA\text{-}EDCI_{(F4)}$ | 205,000 | 358,000 | 1.75 | 64 |
| 530–4 | 207,000 | 390,000 | 1.88 | 61 |
| 530–8 | 210,000 | 425,000 | 2.02 | 63 |
| 530–15 | 290,000 | 618,000 | 2.13 | 85 |
| 1250–3.5 | 248,000 | 524,000 | 2.15 | 92 |
| 1250–7.5 | 342,000 | 733,000 | 2.14 | 115 |
| 1250–13.5 | 387,000 | 993,000 | 2.56 | 128 |

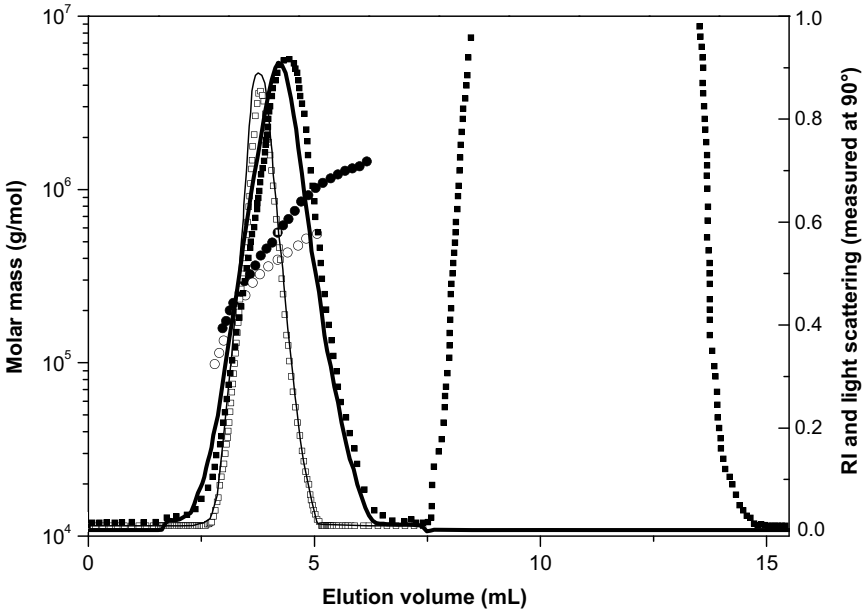


**Fig. 5.** Elution profiles from refractive index (full lines) and light scattering (dotted lines) of "activated" alginate (black hairline and □), 530–15 (black bold line and ■) together with molar mass distributions of "activated alginate" (○), 530–15 (●) and determined by F4/MALLS in $LiNO_3$ 0.1 M, $C_p$ = 2 g/L.

samples than for the 530-× ones, and consequently this phenomenon seems more favourable when the length of grafted PCL increases.

The conformation of such modified polymer in salt media (0.1 M $LiNO_3$) can also be discussed. When we compare the influence of the substitution rate in PCL for the 530-× samples (Fig. 6), it clearly appears that for a fixed elution volume (i.e. a fixed hydrodynamic volume), the molar mass increases when substitution rate increases in agreement with a more compact structure. This is confirmed by the values of the obtained molar masses and Rh for 530-× samples (not really far from those of the precursor), may evidence strong intramolecular PCL interactions. Comparison of two PCL-*g*-alginates characterized by a same grafting rate but with various length of PCL (i.e. 530–8 and 1250–7.5) is shown in the Fig. 7. Except for the highest molar masses (above 5 mL), for which there is the occurrence of large aggregates, the same reasoning done just above for the Fig. 6 leads to evidence that the 530-× modified samples are more compact than the 1250-x ones. This result seems to indicate that intramolecular associations are much favourable when the length of associative groups decreases.

### *3.3. Viscometric properties of PCL-g-alginates in dilute and semi-dilute aqueous solutions*

The particular behaviour of PCL-*g*-alginates in salt media has been also studied by viscometric measurements. Experiments

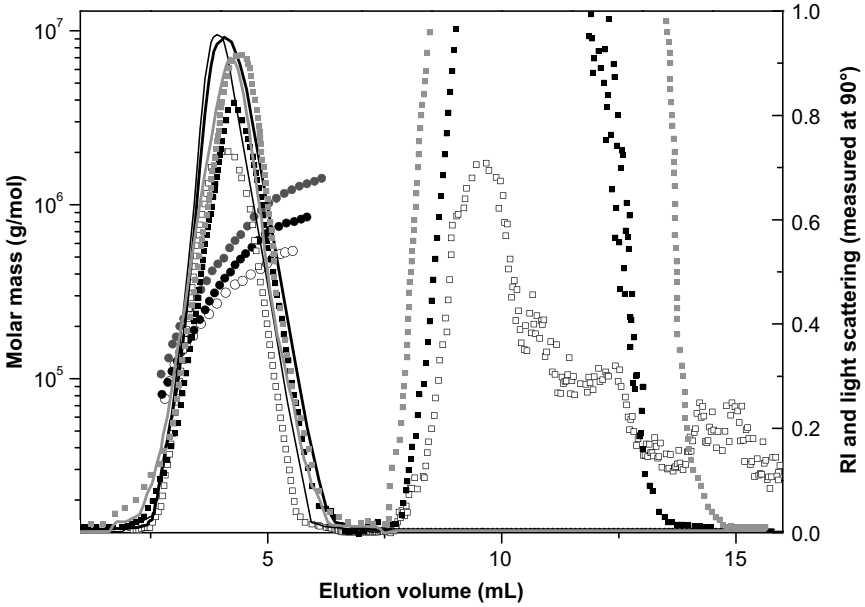


**Fig. 6.** Elution profiles from refractive index (full lines) and light scattering (dotted lines) of 530–4 (black hair line and □), 530–8 (black bold line and ■) and 530–15 (grey bold line and ■) together with molar mass distributions of 530–4 (○), 530–8 (●) and 530–15 (●) determined by F4/MALLS in $LiNO_3$ 0.1 M, $C_p$ = 2 g/L.

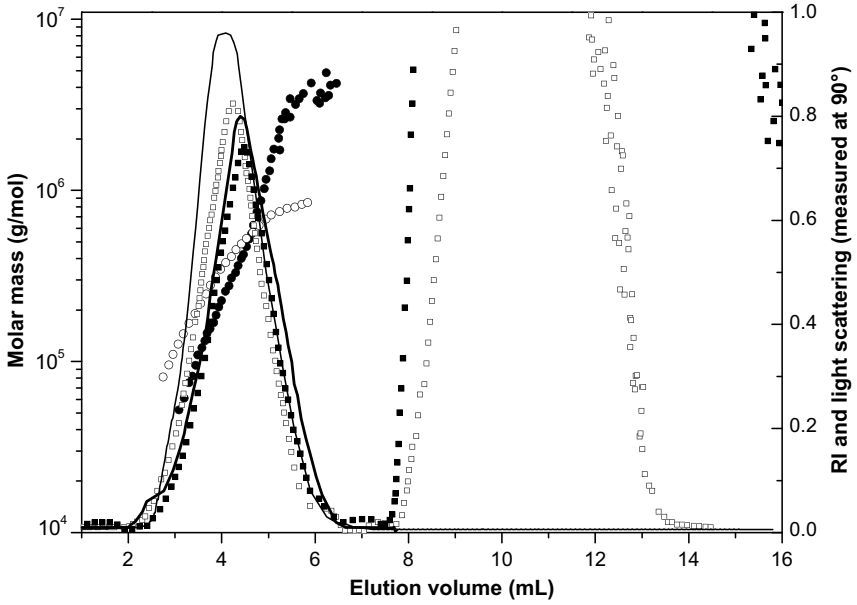


**Fig. 7.** Elution profiles from refractive index (full lines) and light scattering (dotted lines) of 530–8 (hair line and □) and 1250–7.5 (bold line and ■) together with molar mass distributions of 530–8 (○) and 1250–7.5 (●) determined by F4/MALLS in $LiNO_3$ 0.1 M, $C_p$ = 2 g/L.

were performed in both water and salt (NaCl 0.1 M) media. Figs. 8 and 9 display the variation of specific viscosity ($\eta_{sp}$) in water (a/) and NaCl 0.1M (b/) as a function of polymer concentration ($C_p$), for the precursor (alginate) and hydrophobically modified alginate 530-× and 1250-×, respectively.

*3.3.1. Dilute regime*

At low polymer concentrations, in both water and NaCl 0.1 M, the viscosities of PCL-*g*-alginate are slightly lower compared to the precursor alginate. Classically, such results are explained by the occurrence of intramolecular interactions resulting in a decrease of hydrodynamic radius of the copolymer coil (collapse). It must be noted that intramolecular interactions prevail in dilute regime in pure water despite electrostatic repulsions which are clearly evidenced for alginate in pure water (increase of viscosities for the lowest concentrations). These intramolecular associations are favored in presence of salt, thanks to screening of charges as evidenced by the values of intrinsic viscosity determined in NaCl 0.1 M for the whole samples which are widely lower compared to those of the unmodified parent polymer (Table 3). In addition, the Huggins coefficients of modified samples are always higher than that of the precursor, indicating a strong association trend correlated logically with the increase of both rate and length of PCL chains. This behaviour in dilute regime and salt media is well-known and has been often reported (Charpentier et al., 1997; Glinel et al., 1999). It is also important to notice that these results, and consequently the behaviour of PCL-*g*-alginate in very dilute regime (extrapolated to infinite dilution), cannot be correlated with F4/MALLS experiments realized at 2 g/L.

*3.3.2. Semi-dilute regime*

In water, the viscosity of all PCL-*g*-alginate samples gradually increases above a critical concentration. In semi-dilute regime, the viscosity of amphiphilic alginates is always higher than that of the precursors. Hydrophobic intermolecular associations between PCL moieties become more probable, giving rise to a network structure of connected polymer chains. In water, the viscosity gradually increases with the hydrophobe content and/or the length

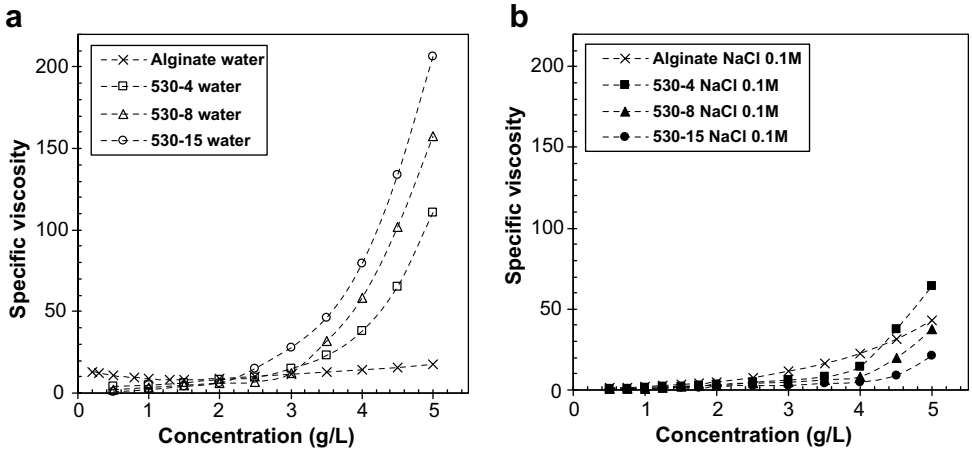


**Fig. 8.** Specific viscosity versus polymer concentration for alginate and PCL-*g*-alginate ($M_{PCL}$ = 530 g $mol^{-1}$) in water (a/) and NaCl 0.1 M (b/) at 25 °C.

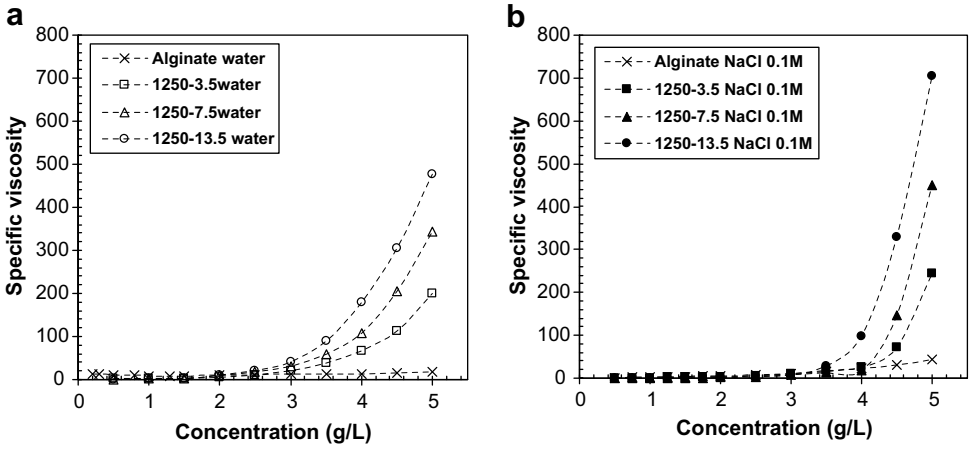


**Fig. 9.** Specific viscosity versus polymer concentration for alginate and PCL-*g*-alginate ($M_{PCL}$ = 1250 g mol$^{-1}$) in water (a) and NaCl 0.1 M (b) at 25 °C.

**Table 3**
Results of viscosimetric measurements

| Sample | [$\eta$] (mL/g) (NaCl) | $k_H$ |
|---|---|---|
| Alginate | 1050 | 0.36 |
| 530–4 | 834 | 0.68 |
| 530–8 | 686 | 0.84 |
| 530–15 | 464 | 1.01 |
| 1250–3.5 | 740 | 0.75 |
| 1250–7.5 | 563 | 0.88 |
| 1250–13.5 | 370 | 1.15 |

of PCL chains. This phenomenon is more marked when the length of associative PCL groups is increased (i.e. for 1250-x samples than for 530-× samples). This is also a classical behaviour for associative polyelectrolyte polymers as reported in the literature (Sinquin et al., 1993; Volpert et al., 1998).

*In NaCl 0.1 M, the viscometric behaviour, seems more interesting and appears clearly different according to the length of the PCL chains. For 1250-× derivatives (Fig. 9), viscosity drastically increases with increasing concentration and/or grafting rate. For the higher 1250-× concentration (i.e. 5 g/L) the viscosity is always higher in salt than in pure water. Consequently, intermolecular associations are reinforced by screening of charges. In contrast, for 530-× derivatives (Fig. 8), viscosities are lower or very slightly higher than those of alginate precursor in the range of the considered concentration. This behaviour is really different with respect to that of usual associative polymer. Therefore, it seemed of interest to study the transition between dilute and semi-dilute solution, identified by the critical concentration $C_{Cr}$ above which the viscosity sharply increased with the polymer concentration. Values of $C_{Cr}$ together with the slope p (Table 4) have been obtained thanks to the Utracki and Shima representation (i.e. log $\eta_{sp}$ vs. log $C$) (Utracki & Shima, 1963). For a non associative polymer, the slope is approximately equal to 1–2 for dilute regime, 3–5 for semi-dilute regime and higher than five for concentrated regime (Joly, Le Cerf, Chappey, Langevin, & Muller, 1997).

**Table 4**
Results of $C_{Cr}$ and values of the slope p for alginate and PCL-*g*-alginate in water and NaCl 0.1 M

| Sample | $C_{Cr}$ (water) (g/L) | $C_{Cr}$ (NaCl) (g/L) | $p_1$ (water) | $p_2$ (water) | $p_1$ (NaCl 0.1 M) | $p_2$ (NaCl 0.1 M) |
|---|---|---|---|---|---|---|
| Alginate | 5.08 | 6.3 | 2 | 4 | 1.2 | 2.5 |
| 530–4 | 3.43 | 3.62 | 1.2 | 4.5 | 1.4 | 5.3 |
| 530–8 | 2.63 | 3.98 | 1.2 | 4.7 | 1.6 | 6.2 |
| 530–15 | 2.21 | 4.36 | 1.1 | 5.1 | 1.4 | 7.1 |
| 1250–3.5 | 3.18 | 4.22 | 1.5 | 5 | 1.4 | 9.1 |
| 1250–7.5 | 2.51 | 3.49 | 1.3 | 5.4 | 1.5 | 9.7 |
| 1250–13.5 | 1.95 | 2.74 | 1.2 | 6.7 | 1.6 | 11.3 |

As a confirmation, the critical concentrations $C_{Cr}$ of modified samples are always lower than for alginate, indicating the establishment of hydrophobic interactions. The presence of salt leads to a decrease of $C_{Cr}$ for the modified samples. This can be explained by a competition between both sterical space occupation of coil and associative connection on one hand, repulsive and associative forces on the other hand (Simon et al., 2003). Electrostatic repulsions (in pure water) favoured the opening of compact structures. The connection of hydrophobic moieties in pure water (i.e. decrease of $C_{cr}$) is favoured when increases both the amount and the length of PCL groups (Table 4). By comparison, screening electrostatic repulsions (ionic strength) leads to more compact aggregates than in water, which induce an increase of $C_{Cr}$ (Table 4). Above all, as a confirmation of the previous observation (Figs. 8 and 9), it is particularly interesting to notice that $C_{Cr}$ increases with $x$ for the 530-× samples, while the contrary is observed for the 1250-× samples. As shown in a previous work (Simon et al., 2003), the transition between dilute and semi-dilute regime is particularly complex for such associative polyelectrolyte systems. This is largely due to the competition between intra and intermolecular hydrophobic association. Here, the transition from intramolecular to intermolecular association appears more difficult for lower lengths of PCL group. This result is fully confirmed by the values of p slopes (Table 4).

This important conclusion in the semi-dilute range is also in agreement with the F4/MALLS results which indicated a much more compact structure of the 530-× samples (i.e. intramolecular associations are favoured) compared to the 1250-× samples. This result appears particularly interesting because it indicates that in salt media (i.e. when electrostatic repulsions are neutralized), the simple variation of length of PCL chains allows to obtain a classical behaviour for $M_{PCL}$ = 1250 g mol$^{-1}$ (intermolecular hydrophobic interactions are predominant) or an opposed and non-classical behaviour for $M_{PCL}$ = 530 g mol$^{-1}$ (intramolecular hydrophobic interactions are predominant).

## 4. Conclusion

Amphiphilic derivatives of sodium alginate were prepared by covalent fixation of PCL pendant chains onto the polysaccharidic backbone via ester links. These polymers exhibit viscometric properties markedly different from those of the parent polymer and

resulting from intra and intermolecular hydrophobic associations. In water, the enhancement of viscosity observed is in good agreement with the common behaviour of amphiphilic polyelectrolyte and viscosity increases logically with increasing of grafting rate and/or length of PCL chains.

In salt media, the aggregation behaviour is very different according to the length of PCL chains. For a PCL chain of 1250 g $mol^{-1}$, as expected, intermolecular hydrophobic associations are predominant and lead to the formation of aggregates that strongly increase viscosity of its solutions. At higher concentrations, these samples should have particular rheological properties. On contrary, for a short PCL chain ($M$ = 530 g $mol^{-1}$), an opposed and non-classical behaviour has been observed. Intramolecular associations are predominant, even in semi-dilute regime, and lead to a compact structure which hindered the connexion of macromolecules, by intermolecular associations, when concentration increases. This phenomenon induces the formation of hydrophobic clusters that could be of great interest for the elaboration of controlled release matrices of hydrophobic drugs. However, a slightly aggregative behaviour was observed by increasing grafting rate and/or concentration and it will be necessary to study the evolution of these "clusters" at higher concentrations.

The rheological behaviour of PCL-*g*-alginates at higher concentration will be reported in near studies.

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