ELSEVIER

Carbohydrate Polymers 43 (2000) 343–349

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

Amphiphilic derivatives of sodium alginate and hyaluronate: synthesis and physico-chemical properties of aqueous dilute solutions

S. Pelletier[a], P. Hubert[a], F. Lapicque[b], E. Payan[b], E. Dellacherie[a,*]

[a]*Laboratoire de Chimie-Physique Macromoléculaire, UMR CNRS-INPL 7568, Groupe ENSIC, BP 451, 54001 Nancy, France*
[b]*Laboratoire de Pharmacologie et de Physiopathologie Articulaires, UMR CNRS-UHP 7561, Faculté de Médecine, BP 184, 54505 Vandoeuvre-lès-Nancy, France*

Received 12 July 1999; received in revised form 17 January 2000; accepted 19 January 2000

Abstract

This paper reports on the synthesis and the physico-chemical characterisation of various amphiphilic derivatives of two natural polysaccharides, sodium alginate and sodium hyaluronate, in which a rather small proportion of the carboxylic groups (≤10% mol) was esterified by long alkyl chains ($C_{12}H_{25}$ or $C_{18}H_{37}$).

The derivatives thus prepared were characterised by gas chromatography, ^{1}H and ^{13}C n.m.r. spectroscopy and size exclusion chromatography coupled to a multi-angle laser light scattering detection. The tendency of these water-soluble compounds to hydrophobic association in aqueous solutions was evidenced firstly in dilute regime using capillary viscometry as well as fluorescence spectroscopy in the presence of a molecular probe, 1,1-dicyano-(4′-*N*,*N*-dimethylaminophenyl)-1,3-butadiene.

Keywords: Associative polysaccharides; Hyaluronate; Alginate; Fluorescence; Viscometry

1. Introduction

Sodium alginate (Na–AA, Fig. 1) and sodium hyaluronate (Na–HA, Fig. 1) are regarded as biocompatible, non-toxic, non-immunogenic and biodegradable—at least for Na–HA—polymers, which make them attractive candidates for biomedical applications. HA is the polysaccharide found in cartilage. In addition, it has already been proposed for various health applications, in particular, in the treatment of joint diseases (Balazs, Leschiner, Larsen & Band, 1993; Goa & Benfield, 1994). Calcium alginate gels have been successfully used to encapsulate and maintain cells in tissue culture (Smidsrød & Skjåk-Bræk, 1990).

Aqueous solutions of Na–HA or Na–AA do not form hydrogels, except if the polymer molecular weight and/or concentration are tremendously high. A way of achieving such properties, is to transform the polymers into amphiphilic derivatives so that they can associate hydrophobically in aqueous solution.

Following our previous experience in this area with other polysaccharides, i.e. propylene glycol alginate (Sinquin, Hubert & Dellacherie, 1994; Sinquin, Hubert, Marchal, Choplin & Dellacherie, 1996a; Sinquin, Houzelle, Hubert, Choplin, Viriot & Dellacherie, 1996b) and pectin (Fischer et al., 1998), the present work aims at preparing water-soluble hydrophobically associating derivatives of Na–HA and Na–AA. The final objective is to design some associative biocompatible polymers capable, under certain conditions, of leading to biomaterials (hydrogels or fluids) useful for cartilage repair. A wide range of synthetic polymeric substitutes have already been proposed (Oxley, Corkhill, Fitton & Tighe, 1993) but either they do not give suitable rheological properties, or they lead to the reconstruction of fibrous cartilage because of a poor chondrocyte colonisation, or they are not truly biocompatible.

This paper reports on the synthesis of amphiphilic derivatives of Na–HA and Na–AA by substitution of long alkyl chains on the parent polysaccharides. The characterisation was carried out by size exclusion chromatography coupled with a multi-angle laser light scattering detection, gas chromatography and ^{1}H and ^{13}C n.m.r. spectroscopy. Some information on hydrophobic association was obtained by dilute solution capillary viscometry and fluorescence spectroscopy in the presence of a molecular rotor as a probe. The associative properties in semi-dilute solutions will be described in a following paper.

* Corresponding author. Tel.: +33-383-17-52-21; fax: +33-383-37-99-77.

E-mail address: Edith.Dellacherie@ensic.inpl-nancy.fr (E. Dellacherie).

0144-8617/00/$ - see front matter
PII: S0144-8617(00)00188-0

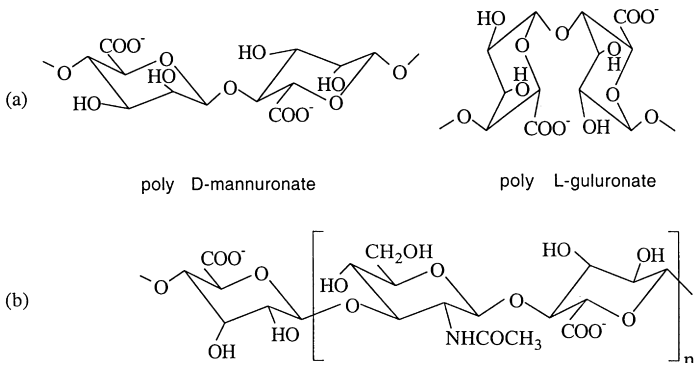

Fig. 1. Chemical structures (a) of mannuronate and guluronate found in alginate and (b) of hyaluronate.

2. Materials and methods

2.1. Materials

Sodium alginate (Na–AA) from *Macrocystis Pyrifera* (medium viscosity) was purchased from Sigma (France). Sodium hyaluronate (Na–HA) was obtained from Acros Organics (France).

HPLC grade dimethylsulfoxide, analytical grade toluene, 1-brom-octadecan, octadecanol, tetrabutylammonium-hydroxide solution in water (40% w/w), sodium chloride, sodium azide and deuterium oxide were obtained from Aldrich (France). Absolute ethanol, hydrochloric acid and sodium hydroxide (Normapur™ A.R.) were purchased from Prolabo R.P. (France). Sulphonic acid resin Dowex 50*8, dodecanol, tetradecanol and 1-brom-dodecan were obtained from Fluka (France). Extra pure sodium nitrate was purchased from Merck (France). Water was ultrapure (Milli Q Waters purification system, Millipore).

The molecular rotor 1,1-dicyano-(4′-*N*,*N*-dimethyl-aminophenyl)-1,3-butadiene (DMAC) (Fig. 2) was synthesised by a Knoevenagel's reaction between 4-(*N*,*N*-dimethyl-amino)-cinnamaldehyde and malononitrile with a 84% yield after crystallisation from ethyl acetate.

2.2. Methods

Substitution ratios of hydrophobically modified polymers were determined by gas chromatography measurements after alkaline hydrolysis. An aliquot of the polymer (typically 100 mg) was hydrolysed for 4 h in 0.4 N NaOH at room temperature. Toluene (3 ml) containing an internal reference (tetradecanol) was added and the mixture was vigorously stirred (Vortex). After centrifugation (3000*g*, 15 min), the organic phase containing the internal reference and the released dodecanol or octadecanol (according to whether C_{12} or C_{18} substituted derivatives were being prepared) was pipetted off and analysed by gas chromatography by comparison with a calibration curve (column SE30, 2m, injection temperature 280°C, column temperature 210°C (dodecanol) or 230°C (octadecanol)).

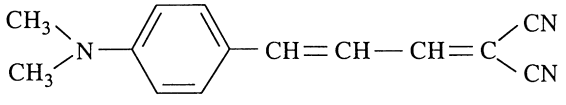

Fig. 2. Chemical structure of the molecular rotor, 1,1-dicyano-(4′-*N*,*N*-dimethylamino-phenyl)-1,3-butadiene (DMAC).

^{1}H and ^{13}C n.m.r. spectra were run in D_2O on a Bruker AC 200P, at 200.13 MHz and 50.327 MHz, respectively.

Size exclusion chromatography (SEC) was performed using a Waters HPLC Model 590 pump with a serial set of OH Pack SP 806, 805, 804 columns (Shodex) and a OH Pack SBG as the guard column. The injection volume was 200 μl. The flow-rate was 0.7 ml/min. The eluent was a 0.1 N $NaNO_3$ solution containing NaN_3 as a bactericide (0.4 g/l) and was filtered (0.1 μm, Millex VC, Millipore, France) prior to use. The polymer solutions were prepared by dissolution in the same eluent and were left under stirring for 24 h. They were filtered (Millex GSWP, 0.22 μm, Millipore, France) immediately before injection, in order to remove possible aggregates. The eluent was continuously monitored by multi-angle laser light scattering (MALLS) detection, using a Mini-Dawn (Wyatt Technology Corporation, USA) unit equipped with a He–Ne laser operating at 690 nm and by differential refractometry (Waters Model 410). d*n*/d*c* was measured in 0.1 N $NaNO_3$ using an Optilab 903 refractometer (Wyatt Technology Corporation, USA) calibrated with a solution of known specific refractive index increment (NaCl, d*n*/d*c* = 0.174).

Viscometric measurements at high dilution were carried out with an Ostwald-type automatic capillary viscometer (0.46 mm diameter) (Viscologic TI.1, Sematech, France) thermostated at 37 ± 0.05°C. Polymer solutions were prepared by dissolution in NaCl solutions of various ionic strengths, previously filtered on Millex HA 0.45 μm (Millipore, France). They were stirred for 24 h before dilution to appropriate concentrations, and then for a further 24 h.

Fluorescence spectroscopy studies were carried out with polymer solutions prepared by dissolution in 0.15 N NaCl, previously filtered on Millex GSWP 0.22 μm (Millipore, France). They were left under stirring for 18 h before the fluorescent probe was added, and then for a further 18 h in order to ensure the completion of the probe incorporation in

Table 1
Characteristics of the synthesised Na–AA and Na–HA derivatives

Polymer backbone	Alkyl chain length (n)	Alkyl bromide ratio (%)[a]	Experimental substitution ratio[b] (%)[a]	Reactivity yield[c] (%)	Symbol
Alginate	12	11	8	73	Na–AA–C_{12}-8
Hyaluronate		8.5	5	59	Na–HA–C_{12}-5
Alginate	18	4	1.3	32.5	Na–AA–C_{18}-1.3
Hyaluronate		7	2	28.5	Na–HA–C_{18}-2
		3	1	33	Na–HA–C_{18}-1

[a] Number of alkyl chains per 100 uronic acid units.
[b] Measured by gas chromatography.
[c] Molar ratio of substituted alkyl chains with respect to the initial alkyl bromide.

the hydrophobic microdomains. Fluorescence emission spectra were recorded on a Spex-fluorolog-2 spectrometer equipped with a cell thermostated at 25°C. All samples containing the DMAC rotor (5×10^{-6} M) were excited at 511 nm and the emission spectra recorded in the range 530–650 nm (slit width 1.5 mm, $\Delta\lambda_{1/2}$ 6 nm). In all cases, the excitation generated one single fluorescence peak, the position of which was medium-dependent.

2.3. Synthesis of the amphiphilic derivatives

The synthesis is derived from the procedure previously described by Della Valle and Romeo (1990). Briefly it consists of the reaction, in homogeneous medium (dimethylsulfoxide, DMSO), of an alkyl halide (dodecyl or octadecyl bromide), with the carboxylic groups of the considered polysaccharides, preliminarily transformed into their tetrabutylammonium (TBA) salts. The long alkyl chains are thus linked to the polysaccharide backbone via ester functions.

Na–AA and Na–HA were first transformed into their acidic forms. This step was carried out either by treatment with a strongly acidic cation exchange sulphonic resin (Dowex 50*8) or by reaction with ethanolic HCl (ethanol/0.6 N HCl, 4°C, 15 h). The TBA salts of HA or AA acids were then prepared by neutralisation of the polysaccharide acidic form with TBA hydroxide. After freeze-drying, they were dissolved in DMSO (1% w/v), the alkyl bromide (C_{12} or C_{18}) was introduced at adequate stoichiometry and the mixture was left to react for 24 h under stirring. It was then dialysed for several days against double distilled water containing sodium azide (400 mg/l) used both as a bactericide and to ensure the exchange of TBA^+ by Na^+ ions, and for one more day against bidistilled water. The final products were obtained after freeze-drying.

3. Results and discussion

3.1. Characterisation of the amphiphilic derivatives

The substituted alkyl chains were detected by ^{1}H n.m.r., by the presence of three characteristic signals between 0.8 and 1.7 ppm (spectra not shown). The exchange of TBA^+ by Na^+ was ascertained by the absence of TBA characteristic signal at 3.2 ppm [$(CH_3–CH_2–CH_2–\mathit{CH_2})_4N^+$] in a region totally devoid of any other signal in the native polysaccharides. No peaks corresponding to the solvents used for the synthesis (DMSO, ethanol) were found.

Owing to the broadening of the representative signals of the polymer chain, very commonly observed for molar masses over 80,000 g/mol (Vårum, Anthonsen, Grasdalen & Smidsrød, 1991), the substitution ratios could not be determined by ^{1}H n.m.r. To overcome this difficulty, a preliminary acid degradation of the polysaccharide backbone was performed. Under these conditions, all ^{13}C signals of native Na–AA or Na–HA were perfectly assigned. However, the acidic treatment resulted in the partial cleavage of long chain esters. Unfortunately, the low solubility in D_2O of long chain alcohols thus released made their quantitative determination unreliable.

Alternatively the content in alkyl chains was calculated using gas chromatography according to the procedure described in Section 2. Low substitution ratios (1–2% for instance for C_{18} derivatives) could be measured, provided that known amounts of dodecanol (or octadecanol) were included in the extraction solvent (toluene + internal reference) after the alkaline hydrolysis treatment. The results of these measurements are shown in Table 1. It can be seen that the reactivity yields of dodecyl bromide were around 75 and 60%, for AA and HA, respectively, while with octadecyl bromide, it was approximately 30% for both polysaccharides.

The measurement of the average molar masses of the final derivatives (Na–AA–C_n and Na–HA–C_n) by SEC-MALLS was impossible owing to their strong retention on the stationary phases. The effect of the different reaction steps on the polymer chain length was then evaluated by measuring the average molar masses of the various intermediates. The results are given in Table 2. Na–AA_{ctrl} and Na–HA_{ctrl} presented in this table, were prepared in order to investigate the intrinsic effects of experimental conditions (solvents, dialysis, freeze-drying) on the properties of the final products, irrespective of the hydrophobic substitution itself.

First of all, the acidic treatment of both polysaccharides,

Table 2
Average molar masses of the intermediate derivatives of the amphiphilic polysaccharides

Symbols	Na–AA	Na–AA_{dow} [a]	Na–AA_{HCl} [b]	Na–AA_{ctrl} [c,b]	Na–HA	Na–HA_{dow} [a]	Na–HA_{HCl} [b]	Na–HA_{ctrl} [c,a]
$\overline{M_w}$ [d] (kg/mol)	250	210	140	93	480	460	17	440
$\overline{M_w}/\overline{M_n}$ [d]	2.4	2.4	2.1	1.9	2	1.7	–	1.7

[a] Acidification by the Dowex treatment then neutralisation with NaOH.
[b] Acidification by EtOH/HCl treatment then neutralisation with NaOH.
[c] Control samples having undertaken all the reaction steps except the addition of the alkyl bromide.
[d] Determined by SEC-MALLS.

whatever the route involved (EtOH/HCl or Dowex resin), was not harmless, since $\overline{M_w}$ was decreased. Whereas both pathways led to a limited decrease with Na–AA (some 40% decrease by EtOH/HCl and 20% by Dowex resin), in contrast Na–HA was strongly degraded by EtOH/HCl. According to the literature, this degradation would consist of glycosidic $1 \rightarrow 4$ bond cleavage, without ring opening (Grasdalen, Larsen & Smidsrød, 1979; Tokita & Okamoto, 1995).

Moreover, some additional degradation did definitely occur after the initial acidic treatment specially in the case of AA. Thus it is seen that under the conditions of the following steps (DMSO, dialysis, freeze-drying), $\overline{M_w}$ decreased from 140,000 g/mol (Na–AA_{HCl}) down to 93,000 g/mol (Na–AA_{ctrl}). It is not possible to know whether this degradation occurs upon contact with DMSO in the final reaction, during dialysis or during the freeze-drying process as already described (Tokita, Ohshima & Okamoto, 1997).

3.2. Physico-chemical properties in the dilute regime

3.2.1. Viscometry

Table 3 shows the values of some viscometric parameters in 0.15 N NaCl, for the parent polysaccharides and various amphiphilic derivatives.

First of all, the $[\eta]$ (intrinsic viscosity) values of controls (Na–HA_{ctrl} and Na–AA_{ctrl}) are appreciably lower than those of Na–HA and Na–AA. This result was to be expected, owing to the degradation occurring during the synthesis, as evidenced by SEC-MALLS. The k_H (Huggins coefficient) values are in the range 0.3–0.8, which is characteristic of interactions between macromolecular coils and good solvents.

Secondly, for all hydrophobically modified derivatives, $[\eta]$ is lower than that of the corresponding native or control homologues. On the other hand, the k_H values are far higher. These results are as expected. In the dilute regime, the intramolecular hydrophobic interactions between the long alkyl chains are favoured/reinforced in the presence of 0.15 N NaCl, which results in more compact conformations, compared to those of the unmodified polysaccharides. However, taking into account the very high values of k_H, which indicates polymer–polymer intermolecular interactions, the assumption that even at high dilution, some intermolecular associations are existing cannot be rejected, which would mean that $[\eta]$ does not correspond to the completely isolated macromolecule.

It is noteworthy that the covalent attachment of long alkyl chains, at nearly identical substitution ratios, leads to much more compact structures for HA derivatives than for the corresponding AA homologues. For instance, the substitution of HA by C_{18} chains (Na–HA–C_{18}-1) results in a dramatic intrinsic viscosity decrease (from 820 to 170 ml/g) whereas a similar chemical modification on AA (Na–AA–C_{18}-1.3) gives a much more modest effect (from 695 to 520 ml/g). This difference could be interpreted in terms of the intrinsic rigidity of the parent polysaccharide backbones. In this respect, the relative stiffness of both polysaccharides was investigated, following the empirical method developed by Smidsrød and Haug (1971).

Fig. 3 shows the variation of $[\eta]$ of both polysaccharides as a function of $I^{-1/2}$, I being the ionic strength corresponding to various NaCl concentrations (in the range 0.017–0.34 N). The straight lines thus obtained can be described by the empirical law of Smidsrød and Haug (1971): $[\eta]_I = [\eta]_\infty + S(I)^{-1/2}$ where $[\eta]_I$ is the intrinsic viscosity at the ionic strength I and $[\eta]_\infty$ at infinite ionic strength. The slope S is related to the Smidsrød's flexibility parameter B, according to: $B = S([\eta]_{0.1\ M})^{-1.3}$. The higher the value of B is, the more flexible is the polymer chain.

For Na–HA, it was found that $B = 0.07$ and for Na–AA,

Table 3
Viscometric parameters of the intermediate derivatives of the amphiphilic polysaccharides

	Na–AA	Na–AA_{ctrl}	Na–AA–C_{18}-1.3	Na–HA	Na–HA_{ctrl}	Na–HA–C_{12}-5	Na–HA_{18}-1
$[\eta]$ [a] (ml/g)	920	695	520	930	820	270	170
k_H [b]	0.65	0.45	3.7	0.4	0.35	5.7	4.5

[a] Intrinsic viscosity.
[b] Huggins coefficient. 0.15 N NaCl, 37°C. Symbols as in Table 2.

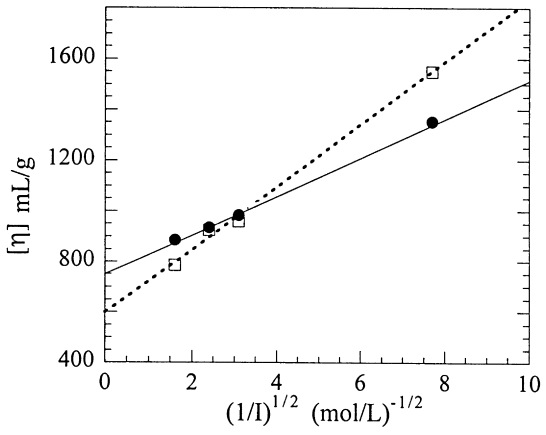

Fig. 3. Variation of the intrinsic viscosity [η] with ionic strength I of polysaccharide aqueous NaCl solutions. 37°C. ● Na–AA; □ Na–HA.

$B = 0.04$, in good agreement with the results of Smidsrød and Haug (1971) and those of Fouissac, Milas, Rinaudo and Borsali (1992). The relative stiffness of Na–HA was attributed to the presence of intramolecular hydrogen bonds, via H_2O bridges, between the acetamido and the carboxylate groups of the macromolecule (Heatley & Scott, 1988). The rigidity of alginate is due to the steric hindrance of the guluronate blocks which hampers the free rotation around the glycosidic linkage. This steric hindrance is not suppressed after alkyl chain substitution and the macromolecule remains relatively inflexible. In contrast, it can be assumed that the substitution of HA carboxylate groups by alkyl chains limits the possibility of intramolecular hydrogen bonds, which results in a greater flexibility compared to that of HA and of the amphiphilic AA derivatives. Accordingly, a HA amphiphilic chain can accommodate itself more easily with internal foldings imposed by intramolecular hydrophobic associations than the AA amphiphilic chain, which can hardly bend into very compact conformations.

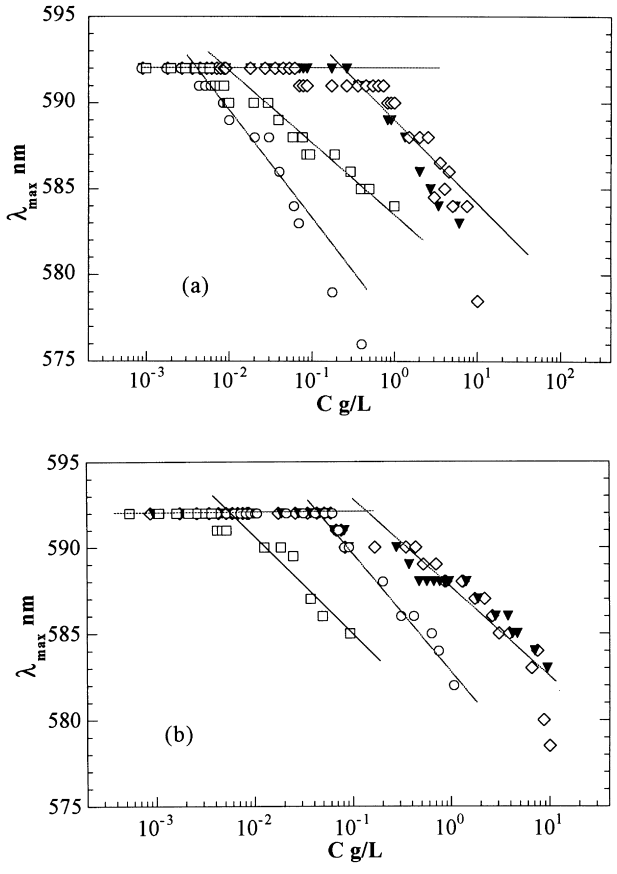

Fig. 4. Variation of the maximum fluorescence emission wavelength, λ_{max}, of DMAC, with the concentration of various polymers. [DMAC] = 5×10^{-6} M in 0.15 N NaCl, 25°C. (a) Alginate: ◇ Na–AA; ▼ Na–AA_{ctrl}; ○ Na–AA–C_{12}-8; □ Na–AA–C_{18}-1.3. (b) Hyaluronate: ◇ Na–HA; ▼ Na–HA_{ctrl}; ○ Na–HA–C_{12}-5; □ Na–HA–C_{18}-2. Symbols as in Tables 1 and 2.

3.2.2. Fluorescence spectroscopy

Fluorescence spectroscopy has been shown to be useful in the study of the organised structures of amphiphilic polymers in water. Several reviews on this topic are available (Winnik, 1993; Winnik, Regismond & Goddard, 1996). Undoubtedly, pyrene has been, by far, the most investigated fluorescence probe, and the variations in fluorescence spectra are mainly associated with polarity changes in the microenvironment of the probe.

The fluorescence study in the present article involves a molecular rotor, DMAC (Fig. 2). With this type of probe, after illumination with a source of appropriate wavelength, de-excitation occurs by two main pathways: radiative decays such as fluorescence, and non-radiative ones, such as, essentially, internal rotations of the molecule. This latter process depends strongly on the cohesion of the probe microenvironment: the more structured the hydrophobic microdomains that are formed, the more viscous is the microenvironment and the higher is the fluorescence quantum yield (Fischer et al., 1998). In fact, fluorescence becomes all the more prevalent as the increase in viscosity in the microenvironment hampers the dissipation of energy by internal rotations or isomerisations of the probe (Loufty, 1986; Rettig, 1994). On the other hand, similarly to pyrene, DMAC also witnesses polarity changes in its microenvironment. In fact, the wavelength corresponding to the maximum fluorescence emission λ_{max} decreases with the microenvironment polarity.

Fig. 4 shows the variation of λ_{max} vs C (polymer concentration) and Fig. 5 the variation vs C, of the ratio between the fluorescence quantum yield Φ_f of DMAC in polymer solution and its fluorescence quantum yield Φ_{f0} in the solvent, for various AA and HA derivatives.

First of all, for the controls, the variations of λ_{max} as well as those of Φ_f/Φ_{f0} are superimposed on those corresponding to the parent polymers, whatever the polysaccharide. The effects observed on the various fluorescence features with amphiphilic derivatives are therefore only due to the hydrophobic modification itself and not to the other experimental conditions. On the other hand, Fig. 4 shows that for both

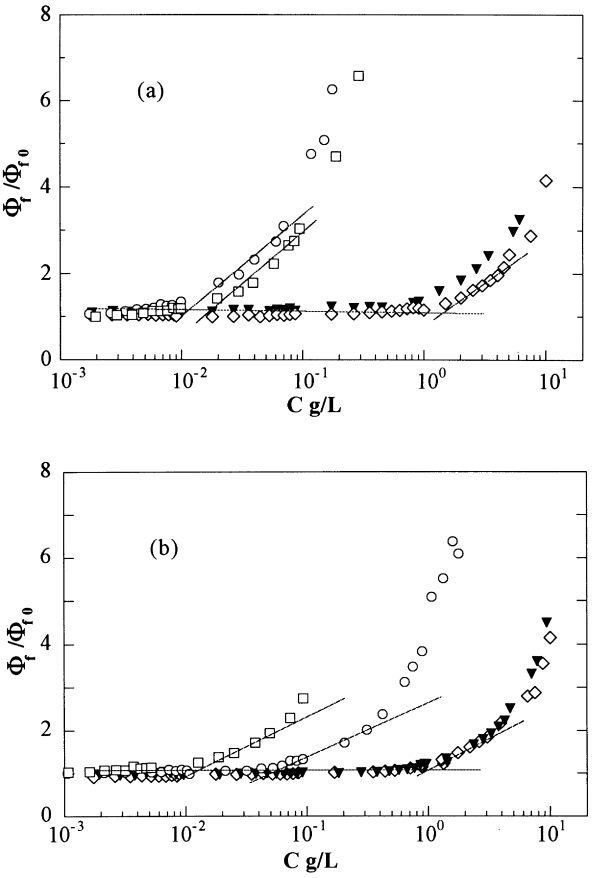

Fig. 5. Variation of the ratio between the fluorescence quantum yield Φ_f of DMAC in polymer solution and its fluorescence quantum yield Φ_{f0} in the solvent, with the concentration of various polymers. All conditions and symbols as in Fig. 4.

unmodified polysaccharides, after a plateau at low C values, the λ_{max} value starts decreasing when C reaches about 0.2–0.4 g/l for Na–AA and about 0.1–0.2 g/l for Na–HA. This decrease can be related to the fact that the probe environment is richer and richer in polysaccharide hydroxyl groups as C increases, and for high enough C values, λ_{max} becomes of the same order of magnitude as that obtained for the same probe in alcohols (Benjelloun, Brembilla, Lochon, Adibnejad, Viriot & Carré, 1996).

As expected, the fixation of pendent long alkyl chains on the polysaccharide backbone causes the λ_{max} value to start diminishing at lower concentrations than for the unmodified polysaccharides, indicating some change in the medium polarity due to the apolar parts of the macromolecule. The approximate polymer concentrations at which λ_{max} begins to decrease for the various polymers ($C_{polarity}$), are shown in Table 4. From these data and from Fig. 4, it can be deduced that Na–AA–C_{12}-8, is more hydrophobic than Na–AA–C_{18}-1.3, while Na–HA–C_{18}-2 is more hydrophobic than Na–HA–C_{12}-5.

The existence of hydrophobic microdomains in the aqueous solutions of amphiphilic polymers, can also be visualised in Fig. 5, that shows the variation of Φ_f/Φ_{f0} with C. For the parent polymers, Φ_f/Φ_{f0} starts increasing at $C \sim 1$–2 g/l for Na–AA and $C \sim 1$ g/l for Na–HA. This increase can be attributed to the increase in the macroscopic viscosity of the solutions. For the hydrophobically modified polymers, the concentration at which Φ_f/Φ_{f0} begins to increase, is much lower than that obtained for the parent polymers. The approximate values of this concentration ($C_{cohesion}$) for the various polymers are reported in Table 4 and the same order of hydrophobicity as that obtained from the λ_{max} values, is observed. It is to be seen that for each amphiphilic polymer, $C_{cohesion}$ is of the same order of magnitude as $C_{polarity}$. This agreement is not observed in all cases and for example for amphiphilic pectins (Fischer et al., 1998) a difference of one or two orders of magnitude was obtained. These latter results mean that the medium polarity decreases well before alkyl chains actually organise in structured stiff microdomains, which can be understood considering that the studied pectin was highly methylated (about 80%). In this case, both the methyl and alkyl groups contributed to the decrease in the solution polarity when the amphiphilic pectin concentration increased, whereas only the long alkyl chains were responsible for the formation of hydrophobic structured microdomains.

4. Conclusion

The covalent immobilisation of long alkyl chains (C_{12} and C_{18}) on the backbones of both alginate and hyaluronate affords amphiphilic derivatives, whose hydrophobically associative character was evidenced, in dilute regime, by capillary viscometry and fluorescence spectroscopy measurements using a non-usual molecular rotor, 1,1-dicyano-(4′-*N*,*N*-dimethylaminophenyl)-1,3-butadiene.
This analysis confirmed that for a given polymer concentration, the longer the alkyl chain, the stronger the

Table 4
Approximate critical polymer concentrations for various amphiphilic polysaccharides. (Values determined by fluorescence spectroscopy in the presence of DMAC as the probe. 25°C, 0.15 N NaCl)

	Na–AA	Na–AA–C_{12}-8	Na–AA–C_{18}-1.3	Na–HA	Na–HA–C_{12}-5	Na–HA_{18}-2
$C_{polarity}$ [a]	0.1–0.3	0.003–0.005	0.008–0.01	0.1–0.2	0.04–0.06	0.005–0.009
$C_{cohesion}$ [b]	1–2	0.01	0.02	1	0.05–0.06	0.01

[a] Corresponding to the λ_{max} decrease.
[b] Corresponding to the Φ_f/Φ_{f0} increase.

hydrophobic interactions. The same conclusion could be drawn when, for a given chain length, its content on the polymeric backbone was increased. It also allowed to show that a good connection existed between the polarity induced by the alkyl chains and the "microviscosity" of the hydrophobic domains.

The associative properties in water, were also evidenced in the semi-dilute regime by a rheological study both in the flow and oscillatory modes. The detailed results of this study carried out on all the synthesised amphiphilic polysaccharides, will be published in a forthcoming paper.

Acknowledgements

The authors thank Dr M.L. Viriot and Dr M.C. Carré for their help in the fluorescence studies with the molecular rotor and Dr A. Vicherat for the n.m.r. analysis.

References

Balazs, E. A., Leschiner, E., Larsen, N. E., & Band, P. (1993). In C. G. Gebelein, *Biotechnological polymers* (p. 41). Lancaster, Pa, USA: Technomic Publishing Company Inc.

Benjelloun, A., Brembilla, A., Lochon, P., Adibnejad, M., Viriot, M. L., & Carré, M. C. (1996). *Polymer*, *37*, 879.

Della Valle, F., & Romeo, A. (1990). US Patent, No. 4, 965, 353.

Fischer, A., Houzelle, M. C., Hubert, P., Axelos, M. A. V., Geoffroy-Chapotot, C., Carré, M. C., & Dellacherie, E. (1998). *Langmuir*, *14*, 4482.

Fouissac, E., Milas, M., Rinaudo, M., & Borsali, R. (1992). *Macromolecules*, *25*, 5613.

Goa, K. L., & Benfield, P. (1994). *Drugs*, *47*, 536.

Grasdalen, H., Larsen, B., & Smidsrød, O. (1979). *Carbohydrate Research*, *68*, 23.

Heatley, F., & Scott, J. E. (1988). *Biochemical Journal*, *254*, 489.

Loufty, R. O. (1986). Photophysical and photochemical tools. In M. F. Winnik, *Polymer science* (p. 429). Dordrecht: Reidel.

Oxley, H. R., Corkhill, P. H., Fitton, J. H., & Tighe, B. J. (1993). *Biomaterials*, *14*, 1064.

Rettig, W. (1994). *Topics in current chemistry*. Berlin: Springer (p. 253).

Sinquin, A., Hubert, P., & Dellacherie, É. (1994). *Polymer*, *35*, 3557.

Sinquin, A., Hubert, P., Marchal, P., Choplin, L., & Dellacherie, É. (1996a). *Colloids and Surfaces A*, *112*, 193.

Sinquin, A., Houzelle, M. C., Hubert, P., Choplin, L., Viriot, M. L., & Dellacherie, É. (1996b). *Langmuir*, *12*, 3779.

Smidsrød, O., & Haug, A. (1971). *Biopolymers*, *10*, 1213.

Smidsrød, O., & Skjåk-Bræk, G. (1990). *Tibtech*, *8*, 71.

Tokita, Y., & Okamoto, A. (1995). *Polymer Degradation and Stability*, *48*, 269.

Tokita, Y., Ohshima, K., & Okamoto, A. (1997). *Polymer Degradation and Stability*, *55*, 159.

Vårum, K. M., Anthonsen, M. W., Grasdalen, H., & Smidsrød, O. (1991). *Carbohydrate Research*, *211*, 17.

Winnik, F. M. (1993). *Chemical Reviews*, *93*, 587.

Winnik, F. M., Regismond, S. T. A., & Goddard, E. D. (1996). *Colloids and Surfaces A.*, *106*, 243.