polymer communications

# Intermolecular associations in hydrophobically modified derivatives of propyleneglycol alginate

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A partially esterified derivative of sodium alginate was substituted by various long-chain alkylamines. The resulting amphiphilic polymers exhibit intermolecular hydrophobic associations in semidilute aqueous solution, evidenced by rheological measurements and fluorescence spectroscopy experiments.

**(Keywords: alginate; intermolecular associations; fluorescence spectroscopy)**

### *Introduction*

The concept of polymeric surfactants emerged some 40 years ago, with the study by Strauss and Jackson on 'polysoaps' derived from polyvinylpyridine[1]. Since then, and especially during the last decade, much effort has been dedicated to the synthesis and the physicochemical characterization of hydrophobically associating water-soluble polymers, owing, for instance, to their importance as thickening agents in oil recovery and latex paint systems[2,3].

The synthetic pathways leading to such derivatives are either the copolymerization of hydrophilic and hydrophobic monomers in an adequate ratio, or the chemical modification of water-soluble polymeric precursors. The former approach involves various monomers derived principally from styrene[4], maleic anhydride[5] and, above all, acrylic acid[6–11], whereas the latter route is mainly employed to introduce pendent hydrophobic substituents onto naturally occurring macromolecules, such as polysaccharides. Since the first article by Landoll[12] in 1982, hydrophobically modified derivatives of cellulose have been studied extensively and, very recently, Akiyoshi *et al.* have described the formation of nanoparticles, resulting from the self-organization of hydrophobic aggregates of cholesterol-substituted pullulan[13].

In the present article, we report our preliminary results on the physicochemical study of new amphiphilic polysaccharide derivatives, obtained by attachment of long-chain alkylamines onto a partially esterified derivative of sodium alginate.

The alginate salts (copolymers of mannuronic acid and guluronic acid), which are found in the cell walls of brown seaweed, differ from most other polysaccharides in that they exhibit a sol–gel transition when simply submitted to modifications of their ionic environment (substitution of $Na^+$ by divalent cations such as $Ca^{2+}$). Such $Ca^{2+}$ alginate gels or beads are widely used in a variety of biomedical or biotechnological applications involving the physical entrapment of cells, vaccines, hybridomas, etc.[14].

In addition to the physicochemical characterizations carried out in aqueous solution, these hydrophobically substituted alginate derivatives will also be investigated in the gel state in the near future, both for theoretical reasons and because of the potential improvements they may bring to existing biomedical applications.

### *Experimental*

The starting derivative was provided by Protan (Drammen, Norway). This compound, propyleneglycol alginate (PGA, batch L25-A), is produced on an industrial scale by treatment of alginic acid with propylene oxide. As claimed by the supplier, esterification involves more than 70% of the available carboxylic groups (*Figure 1*).

The amphiphilic derivatives (PGA-$C_n$, $n=8$, 12, 14) were prepared by the procedure of Yalpani and Hall[15]. It consists of the nucleophilic displacement of the ester by the amine under consideration. The reaction was carried out for 15 min in freshly distilled anhydrous dimethylformamide, at room temperature, with a 5/1 amine/sugar unit molar ratio. The resulting polymers were purified by precipitation in absolute ethanol, followed by extensive washings with, successively, absolute ethanol, dioxane and acetone, until a reliable constant nitrogen analysis was obtained. In the resulting PGA-$C_n$, about 9% of the total saccharide units were substituted.

Polymer solutions were prepared from ultrapure water (Milli-Q water purification system, Millipore) under vigorous stirring for 24 h. The solutions thus obtained were allowed to stand for 12 h before measurements were performed.

Viscosity measurements were carried out in the semidilute regime at low shear rate ($\dot{\gamma}=0.06\ s^{-1}$) with a Contraves LS-30 viscometer or, when the high viscosity of the most concentrated solutions made it necessary, with a RFS-2 Rheometrix apparatus. The temperature (25°C) was controlled to within 0.1°C.

Fluorescence emission spectra of pyrene ($1.1\times10^{-6}$ M) added to the polymer solutions were recorded in the range 350–500 nm on a SPEX Fluorolog 2 spectrometer. The excitation wavelength was 335 nm.

### *Results and discussion*

The very weak chemical reactivity of alginate causes difficulty in the introduction of hydrophobic side-chains by mild activation of the directly available hydroxyl or

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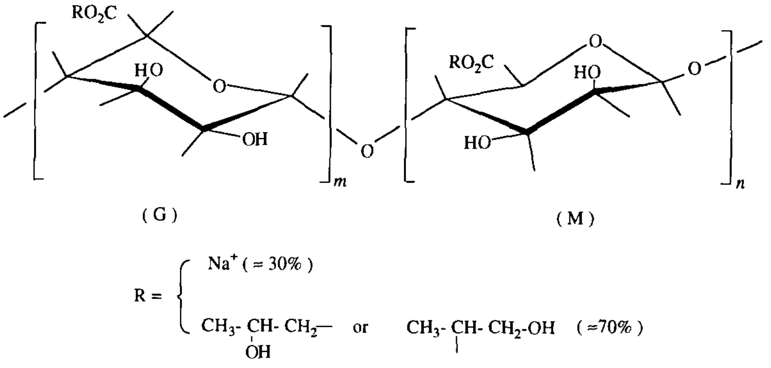

**Figure 1** Schematic representation of PGA, showing mannuronic (M) and guluronic (G) acids, substituted by $Na^+$ (30%) and propyleneglycol (70%)

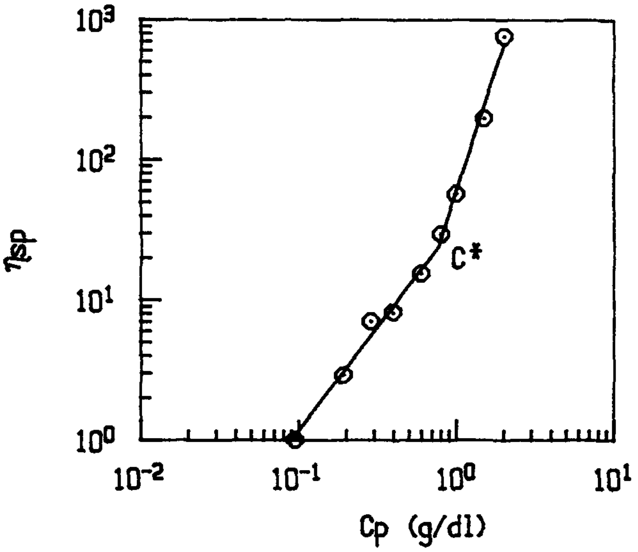

**Figure 2** Determination of the overlap concentration, $C^*$, of the starting PGA in pure water

carboxyl groups. Stronger coupling methods could be applied, but with the associated risk of causing depolymerization or yielding intermediary activated species, which can rearrange and finally remain on the polysaccharide backbone. Both situations would lead to erroneous interpretation of the influence of hydrophobic interactions on the physicochemical properties of the resulting amphiphilic derivative.

In contrast, a large variety of amines have been reported to react at ambient temperature with propyleneglycol esters of alginate (PGA) to give amides in high yields[15]. In most of these commercially available industrial derivatives, residual non-esterified carboxylate groups are sufficient for the behaviour of the polymer in aqueous solution to remain typically that of polyelectrolytes. On the other hand, however, the mechanical quality of $Ca^{2+}$ alginate gels is highly dependent on the density of carboxylate groups available on the polysaccharide chain. It may therefore be necessary, with this type of precursor, to perform a partial and controlled hydrolysis of the esters' functions[16], subsequent to the introduction of long-chain alkylamines, when mechanically high-quality gels are sought.

Owing to the mild conditions involved, this synthetic pathway was selected to introduce hydrophobic alkyl chains onto alginate. Under the conditions used (see Experimental section), it afforded the various desired derivatives (PGA-$C_n$, $n = 8$, 12, 14), where approximately 9% (mol/mol) of the saccharide units were substituted.

The general behaviour of hydrophobically modified polyelectrolytes in aqueous solution is primarily dependent on the balance between coulombic repulsions due to the ionic charges borne by the macromolecule and the attractive interactions between the immobilized hydrophobic moieties. Of course, such an antagonism does not exist in the presence of additional low molecular weight electrolytes since, in this case, electrostatic repulsions are screened.

Besides this effect of ionic strength, the balance between electrostatic repulsions and hydrophobic attractions is also strongly related to the polymer concentration. In this respect, a critical concentration, the overlap concentration $C^*$, corresponding to the transition point between dilute and semidilute regimes, plays an important role. Below this overlap concentration, macromolecular chains are isolated and the system behaves as a mixture of repulsive coils exhibiting little or no interdependency. In the case of hydrophobically modified polyelectrolytes, the only hydrophobic associations liable to occur in dilute solutions are therefore of an intramolecular nature. These lead to more compact, shrunken conformations, resulting in lower intrinsic viscosities, $[\eta]$, and higher Huggins coefficient, $k_H$, when compared to those of the unmodified parent polymer. Such behaviour at low concentration has been discussed earlier[17] with PGA-$C_{12}$ and has also been demonstrated with solutions of other associating macromolecules[18,19].

Above $C^*$, all ionic charges are in close proximity to counterions and are therefore screened. Accordingly, even in pure water, repulsive effects are minimized, hydrodynamic volumes can interpenetrate and coils overlap. In this semidilute regime, hydrophobically modified polymers are therefore expected to develop interactions of principally intermolecular nature, leading to high molecular weight pseudo-reticulated structures of very high viscosity.

*Figure 2* shows a log-log plot of the specific viscosity ($\eta_{sp}$) of the starting PGA, *versus* its concentration in pure water. It allows determination of the overlap concentration of this polymer, which is around $0.77\ g\ dl^{-1}$. Under the same conditions, a value of $C^* = 0.40\ g\ dl^{-1}$ is found for PGA-$C_{12}$ whereas for PGA-$C_{14}$ $C^* \approx 0.33\ g\ dl^{-1}$ (results not shown). For the three PGA-$C_n$ polymers, the plot of log $\eta_{sp}$ *versus* (log $C$) has a slope slightly greater than 1 in the dilute regime (1.48, 1.31 and 1.73 for PGA, PGA-$C_{12}$ and PGA-$C_{14}$, respectively), in agreement with theory. Above $C^*$ in the semidilute regime, the specific viscosity of PGA is proportional to $C^{3.46}$, in good agreement with general observations ($\eta_{sp}$ is proportional to $C^{3-5}$ for polymers in good solvents[20]). In contrast, specific viscosities of PGA-$C_{12}$ and PGA-$C_{14}$ vary as $C^{6.7}$ and $C^{10.5}$, respectively.

This behaviour, generally signifying preferential polymer–polymer associations, is confirmed in *Figure 3*, which shows the log–log plot of $\eta_{sp}$ *versus* polymer concentration for the various PGA-$C_n$ derivatives in pure water. For a given substitution ratio (9% mol/mol), the influence of the length of the immobilized alkyl chain is spectacularly evidenced. $C_8$ substitution does not have the effect of increasing the viscosity of this derivative, which is somewhat lower even than that of the parent PGA, presumably owing to either some depolymerization or to a modification of its conformational structure. Light scattering measurements are to be carried out, and should allow discrimination between these two hypotheses. In contrast, the rheological behaviours of PGA-$C_{12}$ and PGA-$C_{14}$ start diverging from that of the parent PGA at their respective overlap concentrations, and considerably enhanced viscosities are obtained. For instance, at the same polymer concentration (0.8%), the specific viscosities of PGA-$C_{12}$ and PGA-$C_{14}$ are increased by almost two and four orders of magnitude, respectively, compared to that of PGA. As already claimed for other hydrophobically modified water-soluble polymers[7,21] this behaviour is very likely due to the establishment of intermolecular hydrophobic associations between immobilized dodecyl or tetradecyl chains, which may ultimately result in the formation of some physically crosslinked gel-like structure.

Pyrene is a strongly hydrophobic polyaromatic hydrocarbon with low solubility in water. Its fluorescence spectrum presents a fine structure, where the relative peak intensities are highly influenced by the polarity of the solvent molecules by which this probe is surrounded. The ratio of the fluorescence intensity of the highest energy vibrational band ($I_1$) to that of the third highest energy band ($I_3$) correlates with the solvent polarity: $I_1/I_3$ is around 0.6 in hydrocarbon solvents, 1.1 in ethanol and around 1.6 in water[22].

This dependence of fluorescence vibrational fine structure has been widely used to investigate the formation of hydrophobic microdomains in micro-heterogeneous aqueous systems such as micelles[23] or amphiphilic copolymers[24–26]. As a matter of fact, owing to its low solubility in water and its strong hydrophobic character, this polyaromatic probe is preferentially solubilized in the hydrophobic regions formed by these systems, and its fluorescence spectrum is accordingly modified.

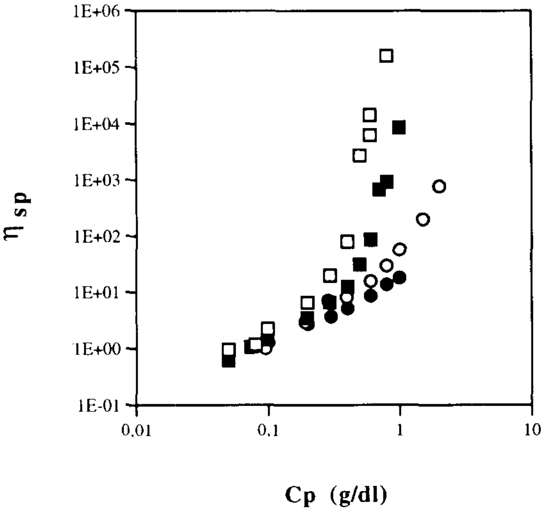

**Figure 3** Specific viscosity of PGA (○), PGA-$C_8$ (●), PGA-$C_{12}$ (■) and PGA-$C_{14}$ (□), in pure water, *versus* polymer concentration ($C_p$) (shear rate $\dot{\gamma} = 0.06\ s^{-1}$)

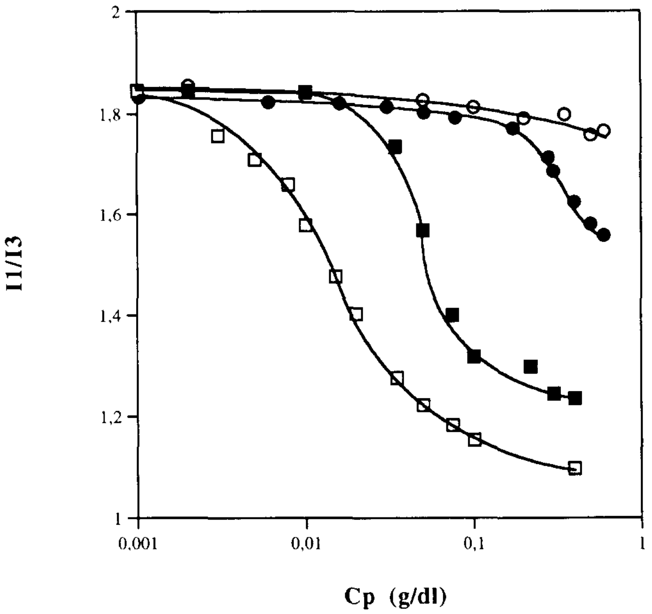

**Figure 4** Ratio of the intensity of fluorescence vibrational bands ($I_1/I_3$) of pyrene ($C = 1.1 \times 10^{-6}$ M) in the presence of PGA (○), PGA-$C_8$ (●), PGA-$C_{12}$ (■) and PGA-$C_{14}$ (□), *versus* polymer concentration ($C_p$), in pure water

*Figure 4* shows the variation of $I_1/I_3$ with polymer concentration for the starting PGA and the three hydrophobically modified PGA-$C_n$ derivatives. The presence of increasing amounts of either PGA or PGA-$C_8$ in the solution hardly affects the fine structure of pyrene's fluorescence spectrum. The $I_1/I_3$ ratio remains in the range 1.6–1.8, indicating the essentially aqueous nature of the environment probed. In agreement with the conclusions drawn from rheological measurements, the octyl side-chains on PGA-$C_8$ do not associate, or associate too little to create a hydrophobic region able to accommodate the large pyrene probe and therefore protect it from the aqueous environment.

However, the situation is quite different with PGA-$C_{12}$ and even more so with PGA-$C_{14}$. For polymer concentrations as low as 0.02% for PGA-$C_{12}$, and almost one order of magnitude lower for PGA-$C_{14}$, $I_1/I_3$ progressively decreases, to reach values (1.25 for PGA-$C_{12}$ and 1.1 for PGA-$C_{14}$) close to those experienced, for instance, with sodium dodecyl sulfate[23]. Similarly to these low molecular weight surfactants, which organize in micelles above their critical micellar concentration, PGA-$C_{12}$ and PGA-$C_{14}$ associate hydrophobically via their alkyl side-chains, thus creating microdomains able to shelter the pyrene molecules. Since the effect observed is enhanced upon increasing the polymer concentration, this association is concluded to be intermolecular in nature.

These results are in agreement with the conclusions drawn from viscometric measurements. However, the decrease of fluorescence ratio $I_1/I_3$ occurs at concentrations far below those observed for the viscosity increase. Although, as detected by pyrene, hydrophobic domain ordering starts at lower concentration, the bridging of domains, leading to a network exhibiting enhanced viscosity, occurs at a much higher concentration.

Before starting the physicochemical characterization of these hydrophobically modified derivatives in the gel state, other techniques such as size exclusion chromatography and light scattering will be used to determine the dimensions of the hydrophobic microdomains. In addition, transmission electron microscopy will allow them to be visualized.

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