Carbohydrate Research 344 (2009) 223–228

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

# Synthesis and rheological properties of hydrogels based on amphiphilic alginate-amide derivatives

Frédéric Vallée [a], Christophe Müller [b], Alain Durand [a], Sarah Schimchowitsch [b], Edith Dellacherie [a], Christian Kelche [b], Jean Christophe Cassel [b], Michèle Leonard [a,*]

[a] Laboratoire de Chimie Physique Macromoléculaire, UMR 7568 CNRS—Université de Nancy1, rue Grandville, B.P. 20451, F-54001 Nancy, France
[b] Laboratoire d'Imagerie et de Neurosciences Cognitives, LINC, UMR 7191 CNRS, F-67000 Strasbourg, France

ARTICLE INFO

*Article history:*
Received 24 July 2008
Received in revised form 28 October 2008
Accepted 31 October 2008
Available online 7 November 2008

*Keywords:*
Alginate
Hydrogel
Rheology
Amphiphilic associative polymers

ABSTRACT

New amphiphilic derivatives of sodium alginate were prepared by covalent attachment of dodecylamine onto the polysaccharide via amide linkages at different substitution ratios, using 2-chloro-1-methylpyridinium iodide (CMPI) as coupling reagent. The aim was to limit the progressive loss of associative behaviour which occurs in the case of previously described dodecyl ester alginate derivatives due to hydrolysis of ester bonds. A series of hydrogels was obtained which differed by the amount of attached dodecyl tails. The stability and viscoelastic properties were evaluated and compared to those of hydrogels obtained with alginate esters. The observed differences were discussed in relation to the synthesis procedures. The advantages of amide links are underlined, especially with regard to long-term stability of hydrogels.



## 1. Introduction

Alginate (AA) is a biomaterial widely used in the food industry as thickener and in biotechnological applications including cell encapsulation, protein delivery or tissue engineering. Alginate is a negatively charged polysaccharide obtained from marine algae and various bacteria, with solution properties ranging from viscous to gel-like structures in the presence of divalent cations. It consists of (1→4)-linked β-D-mannuronate (M) and α-L-guluronate (G) residues. The chemical composition and sequence of M and G residues depend on the source from which the alginate has been extracted.

Gelation of alginate is mainly achieved by the exchange of sodium ions with divalent cations such as $Ca^{2+}$, $Cu^{2+}$, $Zn^{2+}$ or $Mn^{2+}$.[1–6] Structural and mechanical properties of calcium-alginate hydrogels can be tuned by adjusting the ionic strength of the gelification medium or the calcium source. However, ionically cross-linked alginate hydrogels lose their initial mechanical strength within a few hours of exposure to physiological buffers. This has been ascribed to the loss of divalent ions from the hydrogels by exchange with calcium chelators or monovalent electrolytes in the surrounding medium.[7,8]

Common approaches to stabilize alginate gels and to control their permeability include freeze-drying processes,[9–11] or complex coacervation with polycations.[12–15] Alginate sponges prepared by calcium gelation, followed by freeze-drying exhibit a macroporous structure appropriate for cell growth and transplantation, and have been reported to be efficient for the reconstruction of tissues, in vitro as well as in animals. Chemical modification of alginate could also enable production of hydrogels with tailor-made properties. Various kinds of cross-linking reagents have been investigated to control parameters such as swelling properties, porosity or stability.[16–19]

In previous papers, we described the synthesis and solution properties of hydrophobically modified alginate-ester derivatives in which dodecyl or octadecyl chains were grafted onto the polysaccharide backbone via ester functions.[20,21] In semi-dilute aqueous solution, intermolecular hydrophobic associations result in the formation of physical networks, the physico-chemical properties of which can be controlled through polymer concentration, hydrophobic chain content and non-chaotropic salts such as sodium chloride. As compared to the $Ca^{2+}$-alginate hydrogel, alginate-ester hydrogels proved to be very stable in the presence of non-gelling cations or calcium-sequestering agents. However, over long periods of time, the gel degrades and looses its mechanical properties due to the hydrolysis of ester linkages. This is a major inconvenience for the preparation of scaffolds designed for long-term applications in tissue engineering.

The current work aims at investigating the synthesis of hydrolytically stable amphiphilic alginate-based hydrogels involving amide functions. The ultimate objective is to obtain hydrogels that would serve as scaffolds for nerve regeneration in the central nervous system. In addition, the shear-thinning character and the thixotropic behaviour of hydrogels obtained with hydrophobically modified associative polymers should allow them to be easily

* Corresponding author. Tel.: +33 03 83 17 52 76; fax: +33 03 83 37 99 77.
*E-mail address:* mleonard@ensic.inpl-nancy.fr (M. Leonard).

0008-6215/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2008.10.029

injected in the lesion and take the exact shape of lesion cavities when shearing is stopped, that is, at rest after injection.

For the polymer synthesis, our approach consisted in the activation of the carboxylate groups of alginate in the presence of 2-chloro-1-methylpyridinium iodide (CMPI) in order to bind an alkylamine (here dodecylamine). This reaction has already been described, using various cross-linking di-amines to prepare alginate or hyaluronate (HA) covalent networks.[18,22–24] In the present work, the main difficulty arises from the poor reactivity of dodecylamine, mostly due to its poor solubility in the solvent used for the reaction. Insights in the formation of the amide bond between alginate and dodecylamine in the presence of CMPI are provided and the properties of alginate derivatives, that is, stability, solubility and rheological behaviour in dilute and semi-dilute solution, are compared to those obtained with other alginate derivatives where hydrocarbon chains are linked via ester groups. For the sake of concision, alginate derivatives with amide bonds between the polysaccharide backbone and alkyl chains will be called 'alginamides', while alginate derivatives involving ester junctions will be called 'alginate esters'.

## 2. Materials and methods

Medium viscosity alginate extracted from *Macrocystis pyrifera* was purchased from Aldrich (France). Its average molar masses, $\overline{M_n}$ = 130,000 and $\overline{M_w}$ = 181,000 g/mol, were determined from size exclusion chromatography–multi-angle laser light scattering (SEC–MALLS) experiments. This sample contained 65% mannuronic acid and 35% guluronic acid ($M/G$ = 1.86), as determined by circular dichroism measurements.

Dodecyl bromide, dodecylamine and tetrabutylammonium hydroxide ($TBA^+OH^-$) (40 wt % soln in water) were purchased from Aldrich. $Me_2SO$ and DMF were obtained from Fluka.

### 2.1. Polymer synthesis

Hydrophobic alkyl chains were linked to the polysaccharide backbone via ester functions as described earlier.[21,25] Substitution ratios were determined by gas chromatography (Shimadzu GC 17 AAF, column SE 30 Chromosorb W-HP, length 2 m; injection temperature 280 °C, column temperature 230 °C; nitrogen flow 25 mL/min) on aliquots (100 mg) first subjected to alkaline hydrolysis (0.08 M NaOH for 4 h at room temperature), followed by toluene extraction of the resulting dodecyl alcohol. The nomenclature used for these polymers is $x$AAC$_{12}$, where $x$ is the substitution ratio (in mol per 100 mol of uronic units) and $C_{12}$ stands for the number of carbon atoms in the alkyl chain.

Hydrophobically modified alginamides $x$AANC$_{12}$ were prepared as follows: $Na^+$ alginate was transformed into its acidic form (EtOH/0.6 M HCl, 4 °C, 30 min), and then neutralized to pH 7 by $TBA^+OH^-$. The TBA–alginate salt (2 g) was dissolved in 200 mL DMF and stirred overnight to allow its complete dissolution. Then CMPI (required amount) and an excess of dodecylamine (1 mol/mol of uronic units) were added at 0 °C. Triethylamine was added into the soln at a conc similar to that of dodecylamine. The soln was kept at 0 °C for 45 min, and then left at room temperature for 20 h. Aq NaCl (2.5 M, 60 mL) was added to the soln in order to exchange $TBA^+$ by $Na^+$ ions. Finally, the polymers were purified by precipitation in 7:1 EtOH-water, washed three times and dried under diminished pressure. The substitution ratio was determined by elemental analysis.

### 2.2. $^1$H NMR and light scattering controls

$^1$H NMR spectra were recorded on a Bruker Avance 300 spectrometer at 300 MHz in $D_2O$. Three characteristic signals corresponding to the alkyl chains were observed between 0.8 and 1.7 ppm, but no reliable quantitative estimation of the substitution ratio proved successful. However, the complete exchange of $TBA^+$ by $Na^+$ ions was ascertained by the absence of the TBA characteristic signal at 3.2 ppm.

SEC–MALLS experiments were performed so as to quantify some possible degradation of the polysaccharide backbone during the synthesis. SEC was performed using a Waters HPLC pump equipped with a serial set of SP 806, 805 and 804 OH pack columns. Elution was carried out with 0.1 M $NaNO_3$ at 0.7 mL/min, and was monitored by MALLS (Wyatt, Mini Dawn, Santa Barbara) and differential refractometry (Waters 410) dual detection.

### 2.3. Determination of sol/gel fractions

Sol/gel fractions were determined as follows: alginate derivatives (100 mg) were dissolved in deionized water (50 mL) for 24 h. The solns were then centrifuged at low speed (5000 rpm, 15 min) to separate the soluble polymer which remained in the supernatant and microgels. The polymer fractions were then collected, freeze-dried and finally weighted for concentration determination. Sol and gel fractions were also analyzed to determine the polymer substitution ratio.

### 2.4. Rheological experiments

Aqueous solns of polymers were prepared by gentle stirring in deionized water for 24 h. Samples were subsequently centrifuged (20 min, 5000 rpm) to get rid of entrapped air bubbles, and were then stored at 4 °C overnight before rheological measurements were performed. Steady shear and dynamic studies were performed at 20 °C on a AR 2000 rheometer (TA instruments) using a parallel plate geometry (diameter 25 mm) or a double Couette geometry, depending on the viscosity of the sample. Oscillatory experiments were performed within the linear viscoelasticity region, where storage ($G'$) and loss ($G''$) moduli are independent of the stress magnitude.

## 3. Results and discussion

### 3.1. Polymer synthesis and characterization

Alginate modification was carried out in dimethylformamide. In all cases, the reaction of dodecylamine with alginate was carried out with a molar excess of dodecylamine with regard to CMPI. The substitution ratio of the modified alginamides, $x$, was determined by elemental analysis, and alginamides are named $x$AANC$_{12}$. Modified alginamides with increasing substitution ratios were obtained by keeping constant the molar ratio of dodecylamine to uronic units, while increasing the molar ratio of CMPI to uronic units. This was chosen because of the limited solubility of dodecylamine in the reaction medium. In order to get a better reproducibility, we chose to keep identical amounts of solvent, dodecylamine and alginate, while varying the added amount of CMPI which is fully soluble in the reaction medium. Figure 1 shows the evolution of the substitution ratio as a function of the molar ratio CMPI/100 uronic units. It appears that the substitution ratio can be controlled up to about 20% by varying the amount of added CMPI.

Modified alginate-esters in which dodecyl chains were attached via ester groups were prepared following the previously described procedure.

$x$AANC$_{12}$ solutions were not optically clear compared to $x$AAC$_{12}$ solutions, whatever the polymer concentration. This suggests differences between the chemical structure of AANC$_{12}$ and AAC$_{12}$

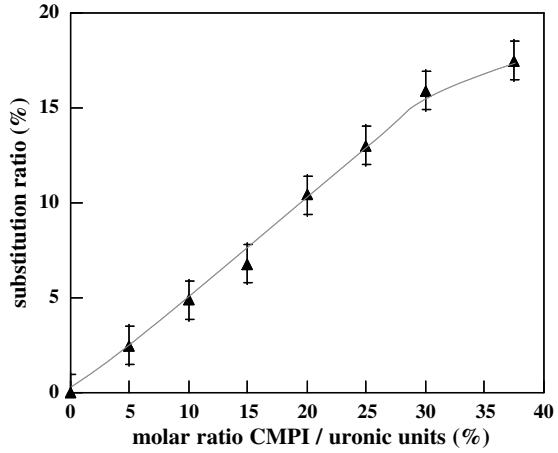


**Figure 1.** Evolution of the substitution ratio (=number of grafted $C_{12}$ chains/100 uronic units) as a function of the molar ratio CMPI/100 uronic units. (dodecylamine/100 uronic unit = 100). Error bars correspond to errors made on the substitution ratios, as determined by NMR spectroscopy.

polymers. The amount of macroscopic aggregates (microgels) in $x$AANC$_{12}$ solutions was determined by performing solubility measurements in dilute solution (0.2 g polymer/L).

The dependence of the sol/gel fraction on the amount of CMPI is given in Figure 2. As can be seen, the soluble fraction decreases when the amount of CMPI used for the synthesis increases. This cannot be attributed to the presence of an increasing number of grafted alkyl chains since, for comparison, AAC$_{12}$ were totally soluble in water in this range of substitution ratios under similar test conditions. In addition, the amount of grafted alkyl chains on the polymer in the sol fraction was in all cases not significantly different from that determined in the gel fraction. Since the synthesis procedure kept constant the molar ratio of dodecylamine while increasing the molar ratio of CMPI, it seems reasonable to suggest that cross-linked esters are formed in AANC$_{12}$ samples, resulting from the nucleophilic attack of alginate hydroxyl groups on the CMPI-activated carboxylic groups of alginate, in spite of the fact that dodecylamine was added in excess to the reaction medium, and that the number of covalent cross-links increases with the amount of added CMPI. As a conclusion, the formation of inter–intramolecular ester bonds may be partly favoured by the low solubility of dodecylamine in DMF, compared to the amine or diamine used by Barbucci et al.[22,23] For instance, Young et al. prepared HA films stabilized by inter- and intramolecular ester links using only CMPI in water.[24]

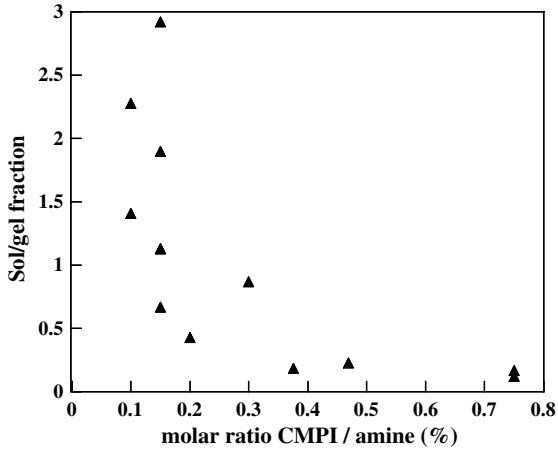


**Figure 2.** Dependence of sol/gel fraction on the molar ratio CMPI/100 uronic units.

### 3.2. Rheological behaviour of aqueous solutions of alginamides and alginate esters

The rheological characterization of a material generally comprises both studies in the flow and oscillatory modes.

Experiments in the oscillatory regime were performed with various amphiphilic alginamide derivatives, and the results were compared with those corresponding to the non-modified polymer. As expected for non-modified AA, the slopes of $G'$ and $G''$ versus $\omega$ (log/log coordinates) were close to 2 and 1, respectively (results not shown), in agreement with theoretical predictions. Figure 3a shows typical mechanical spectra for solutions of alginamide derivatives $x$AANC$_{12}$ (10 g/L) at different substitution levels x. A classical behaviour was observed, with $G'$ and $G''$ increasing with the substitution ratio due to the formation of hydrophobic associations in the aqueous system. With polymers at low substitution ratios, viscous solutions were obtained, with the loss moduli $G''$ higher than the storage moduli $G'$, both being frequency dependent. These solutions exhibit typical viscoelastic behaviour. However, $G'$ and $G''$ do not vary as $\omega^2$ and $\omega$, as usually observed for a Maxwell fluid. As an example, the slopes of $G'$ and $G''$ versus $\omega$ (log/log coordinates) are 1.25 and 0.9, respectively, for the 3AANC$_{12}$ solution at 10 g/L. This phenomenon is generally attributed to the superimposition of both the disentanglement of the polymer chains and the disengagement of the apolar segments from hydrophobic associations.[26]

With polymers at substitution ratios higher than 9%, hydrogels were obtained, characterized mainly by an elastic behaviour, that is, $G'$ is higher than $G''$ and is frequency independent.

In the flow mode, the non-modified alginate solution, at 10 g/L, exhibited typical shear thinning behaviour with a Newtonian plateau in the range of lower shear rates (1–100 $s^{-1}$, curve not shown). Its zero shear viscosity is quite low, that is, 0.08 Pa s. After treatment with HCl, as in the first step of the synthesis, its average molar masses decreased from $\overline{M_n}$ = 130,000 g/mol and $\overline{M_w}$ = 181,000–82,000 and 115,000 g/mol, respectively. Consistently, the zero shear viscosity decreases to 0.03 Pa s for a 10 g/L aqueous solution. Figure 3b shows viscosity-shear stress plots for the different polymer solutions. For all samples, a viscosity plateau is observed at low shear stress. As expected, the higher the substitution ratio, the higher the zero shear viscosity. For polymers with substitution ratio higher than about 10%, the flow curves display at intermediate shear stress an inflection point in the shear thinning region with two critical stress levels. A similar behaviour has already been observed.[27,28] These results may indicate that the network structure is a function of the applied stress, such as a shear induced structuring through hydrophobic junctions. It can also be seen as the mark of important heterogeneities in the nature of hydrophobic junctions between alginate chains. This result is in contrast to the rheological behaviour of alginate ester solutions which exhibit classical properties of amphiphilic polymers where the decrease in viscosity with shear rate obeys a power law and is approximately $-0.9\dot{\gamma}$. For comparison, Figure 4 shows the viscosity versus the shear stress for 15AANC$_{12}$ and 15AAC$_{12}$ solutions.

Another significant difference was observed between the rheological behaviour of solutions of AANC$_{12}$ and AAC$_{12}$ with various substitution ratios $x$. Figure 5a and b displays the variation of the low shear viscosity $\eta$ (first Newtonian plateau) and $G'$, respectively, versus $x$ (for polymer concentration set at 10 g/L). For $x$ values lower than 13–15, the increase of $\eta$ and $G'$ is more pronounced for AANC$_{12}$ solutions than for AAC$_{12}$ solutions at the studied concentration.

To confirm the presence of the covalent ester cross-links in alginamides, some alginate derivatives were synthesized using 50 mol CMPI/100 mol uronic units and ethanolamine (100 mol/100 mol uronic units) or no amine instead of dodecylamine. Ethanolamine

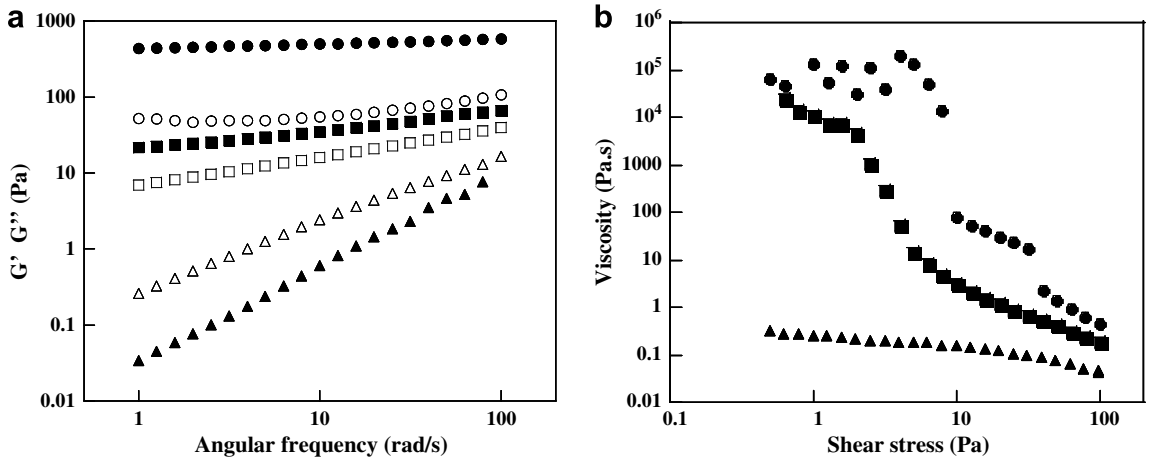


**Figure 3.** (a) Dynamic moduli of $x$AANC$_{12}$ samples at various substitution ratios: $x$ = 3 (▲G′, ΔG″); $x$ = 9 (■G′, □G″); $x$ = 20 (●G′, ○G″). Polymer concentration:10 g/L in deionized water; (b) steady shear viscosity versus shear stress for $x$AANC$_{12}$: $x$ = 3▲, $x$ = 9■, $x$ = 20●. Polymer concentration:10 g/L in deionized water.

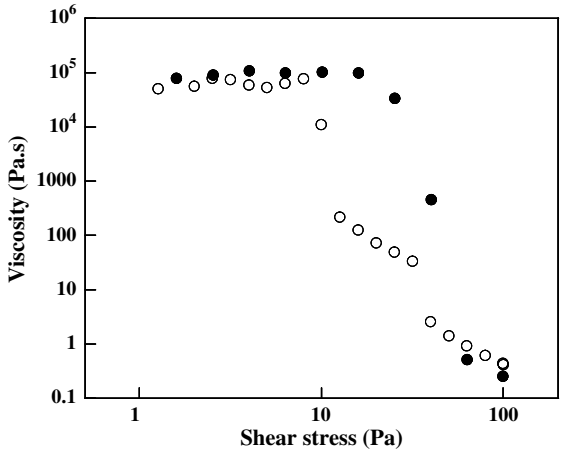


**Figure 4.** Steady shear viscosity versus shear stress for 15AANC$_{12}$ (○) and 15AAC$_{12}$ (●). Polymer concentration:10 g/L in deionized water.

was chosen in order to obtain an alginamide derivative without associative properties. The influence of ester cross-links on the polymer (respectively, AAN-100ethanolamine-50CMPI and AA-50CMPI) properties was then investigated. Figure 6 shows the storage and loss moduli of these polymer solutions. High $G'$ and $G''$ values were obtained for AA-50CMPI reflecting a great number of ester cross-links in the absence of competitive amine. By comparison, the increase in moduli of AAN-100ethanolamine-50CMPI is less pronounced. Nevertheless, this last result shows that the formation of ester cross-links cannot be totally avoided during the reaction.

### 3.3. Comparison of hydrolytic stabilities of hydrophobically modified alginamides and alginate-esters hydrogels

The stability of 6AANC$_{12}$ and 6AAC$_{12}$ towards hydrolysis in cerebrospinal fluid-like medium (pH 7.4, ionic composition: 128 mM NaCl, 3 mM KCl, 1.3 mM $CaCl_2$, 1 mM $MgCl_2$, 21 mM $Na_2HPO_4$, 3 mM $NAH_2PO_4$) was then investigated and compared to that of alginate ester derivatives over a two-month period. The substitution ratio of 6AAC$_{12}$ decreases from 6 to 2.5 during this period of time, while no change was observed for 6AANC$_{12}$. For both polymers, the magnitude of loss and storage moduli decreases, this being related to the hydrolysis of the ester bonds (Table 1). Under these conditions, no significant degradation of the alginate chains was observed, as determined by SEC–MALLS experiments.

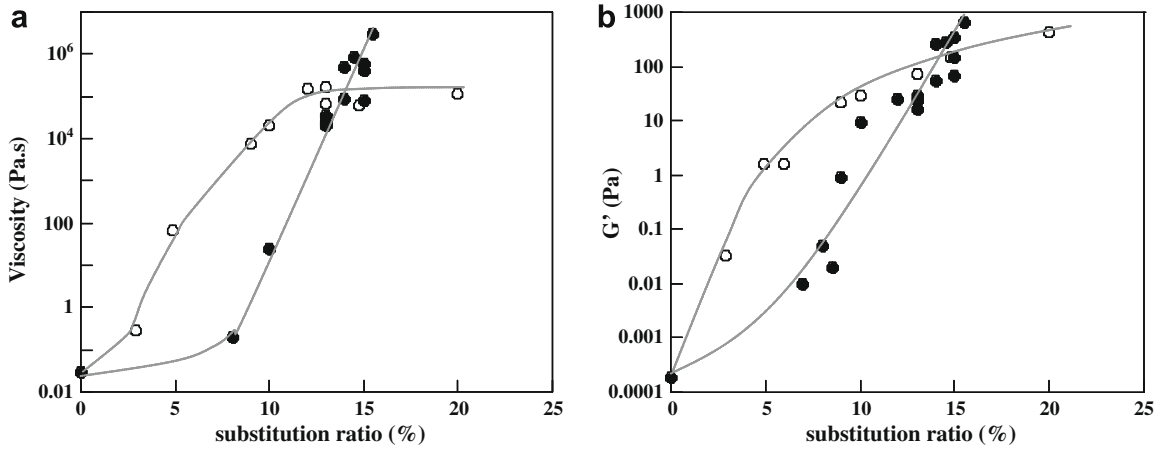


**Figure 5.** (a) Dependence of low shear viscosity on the substitution ratio for AA-NC$_{12}$ (○) and AA-C$_{12}$ (●) series. Polymer concentration:10 g/L in deionized water; (b) dependence of storage modulus on the substitution ratio for AA-NC$_{12}$ (○) and AA-C$_{12}$ (●) series. Polymer concentration:10 g/L in deionized water.

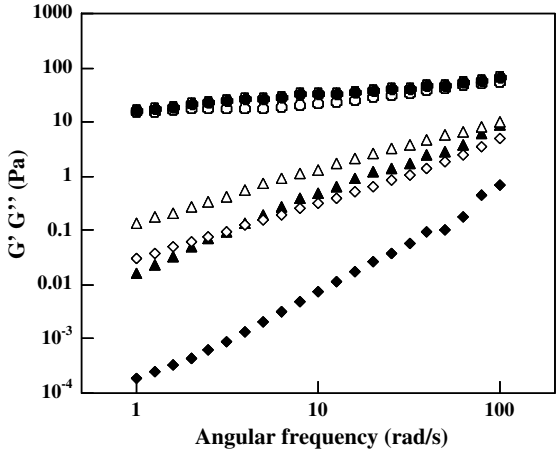


**Figure 6.** Storage and loss moduli of non-modified AA (♦G′, ◇G″), AAN-100ethanolamine-50CMPI (▲G′, ΔG″) and AA-50CMPI (●G′, ○G″). Polymer concentration:10 g/L in deionized water.

**Table 1**
Dynamic moduli G′ and G″ (angular frequency = 1 rad $s^{-1}$, oscillatory stress = 1 Pa) and substitution ratio *x* of $AANC_{12}$ and $AAC_{12}$ samples (30 g/L) before and after incubation at 37 °C in cerebrospinal fluid-like medium pH 7.4

| Polymer | *t* (days) | G′ (Pa) | G″ (Pa) | *x* (%) |
|---|---|---|---|---|
| $AANC_{12}$ | 0 | 109 | 63 | 6 |
| $AANC_{12}$ | 60 | 22 | 12 | 6 |
| $AAC_{12}$ | 0 | 400 | 120 | 6 |
| $AAC_{12}$ | 60 | 7 | 8 | 2.5 |

$AANC_{12}$ hydrogels that would serve as scaffold for nerve regeneration should be stable over relatively long periods of time. In this respect, the hydrolysis of ester functions in basic media was investigated. It is obvious that the rheological properties of $AANC_{12}$ solutions after hydrolysis of ester cross-links could be easily modulated by varying the polymer concentration and the level of substitution. Figure 7 shows the flow curve of AA-25CMPI before and after hydrolysis in NaOH, $10^{-3}$ M, for 48 h. After hydrolysis, the viscosity of the ester-linked derivative (AA-25CMPI) decreased by several orders of magnitude, and the properties were quite similar to those of the non-modified alginate (after treatment with HCl, as in the first step of the synthesis) in terms of solubility, molecular weight and rheological behaviour. When hydrolysis is carried out at pH

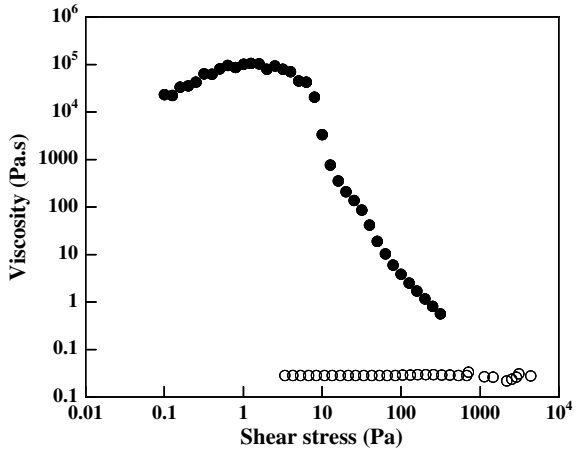


**Figure 7.** Steady shear viscosity of AA-25CMPI before (●) and after hydrolysis in $10^{-3}$ M NaOH (○). Polymer concentration: 10 g/L in deionized water.

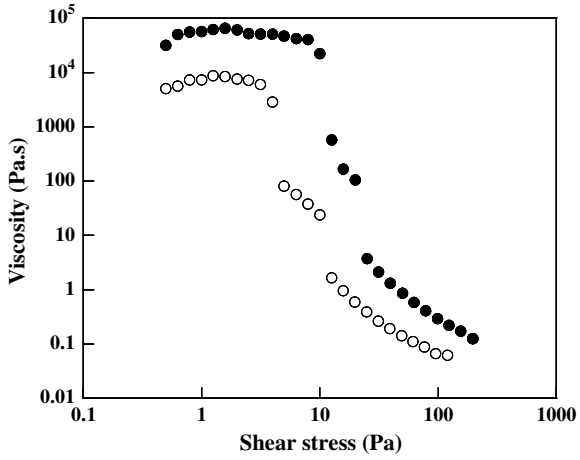


**Figure 8.** Steady shear viscosity of $13AANC_{12}$ before (●) and after hydrolysis in $10^{-3}$ M NaOH (○). Polymer concentration:10 g/L in deionized water.

**Table 2**
Dynamic moduli G′ and G″ (angular frequency = 1 rad $s^{-1}$, oscillatory stress = 1 Pa) of $13AANC_{12}$ (10 g/L in deionized water) before and after hydrolysis

| Polymer | G′ (Pa) | G″ (Pa) |
|---|---|---|
| $13AANC_{12}$ | 124 | 16 |
| Hydrolysed-$13AANC_{12}$ | 19 | 3 |

higher than 11, both amide linkages and polysaccharide backbone started to degrade.

$AANC_{12}$ were then subjected to $10^{-3}$ M NaOH hydrolysis. As an example, Figure 8 displays the flow curves of $13AANC_{12}$ before and after hydrolysis. G' and G" values are given in Table 2. After hydrolysis, $13AANC_{12}$ is totally soluble in water under dilute conditions, and the gel obtained at 10 g/L is not optically turbid. Compared to the non-hydrolyzed sample, the low shear viscosity and the critical stress—at which the first deviation from Newtonian flow occurs—decrease, which can be easily explained by the hydrolysis of ester cross-links. For the same reason, the dynamic moduli also decrease. However, one should notice that an inflection point remains in the shear thinning region, suggesting differences in alginate chains conformation between amide and ester derivatives. As ester derivatives were synthesized in homogeneous medium while amide derivatives were obtained under heterogeneous conditions—due to the poor solubility of dodecylamine in DMF—one should consider possible differences in alkyl chain distribution along the polysaccharide backbone.

## 4. Conclusion

Hydrophobically modified alginamides ($AANC_{12}$) were prepared by covalent fixation of dodecyl chains onto the alginate backbone using 2-chloro-1-methylpyridinium iodide (CMPI) as coupling reagent.

Dilute and semi-dilute solution properties evidenced heterogeneities in $AANC_{12}$ hydrogels compared to $AAC_{12}$ hydrogels. In dilute solution, the solubility of alginamides was strongly reduced compared to that of esters derivatives. In semi-dilute regime, viscous solutions or viscoelastic hydrogels were obtained in both cases, depending on the substitution ratio of polymers, but rheological characterizations suggest differences between the structure of $AANC_{12}$ network and that of $AAC_{12}$ network. This phenomenon was explained by a cross-linking reaction between hydroxyl and carboxyl groups of alginate in the presence of CMPI, leading to for-

mation of intra/intermolecular ester links. However, after hydrolysis of these links, the rheological properties of $AANC_{12}$ remained significantly different from those of $AAC_{12}$, suggesting differences in the polysaccharide chains conformation, possibly due to differences in the distribution of alkyl chains along the polymer backbone.

From in vitro degradation at 37 °C over a two-month period, we showed that alginamide derivatives were more stable than alginate ester derivatives ($AAC_{12}$). Biological tests on these materials are now in progress.

## References


1. Draget, K. I.; SkjakBraek, G.; Smidsrod, O. *Int. J. Biol. Macromol.* **1997**, *21*, 47–55.
2. Haug, A.; Smidsrod, O. *Acta Chem. Scand.* **1965**, *19*, 341–351.
3. Kuo, C. K.; Ma, P. X. *Biomaterials* **2001**, *22*, 511–521.
4. Nunamaker, E. A.; Purcell, E. K.; Kipke, D. R. *J. Biomed. Mater. Res. Part A* **2007**, *83*, 1128–1137.
5. Smidsrod, O.; Haug, A. *Acta Chem. Scand.* **1972**, *26*, 2063–2074.
6. Wang, C.; Liu, H.; Gao, Q.; Liu, X.; Tong, Z. *Carbohydr. Polym.* **2008**, *71*, 476–480.
7. Gombotz, W. R.; Wee, S. F. *Adv. Drug Deliv. Rev.* **1998**, *31*, 267–285.
8. LeRoux, M. A.; Guilak, F.; Setton, L. A. *J. Biomed. Mater. Res.* **1999**, *47*, 46–53.
9. Shapiro, L.; Cohen, S. *Biomaterials* **1997**, *18*, 583–590.
10. Yen, C. N.; Lin, Y. R.; Chang, M. D. T.; Tien, C. W.; Wu, Y. C.; Liao, C. J.; Hu, Y. C. *Biotechnol. Prog.* **2008**, *24*, 452–457.
11. Zmora, S.; Glicklis, R.; Cohen, S. *Biomaterials* **2002**, *23*, 4087–4094.
12. Baruch, L.; Machluf, M. *Biopolymers* **2006**, *82*, 570–579.
13. Lucinda-Silva, R. M.; Evangelista, R. C. *J. Microencapsulation* **2003**, *20*, 145–152.
14. Orive, G.; Tam, S. K.; Pedraz, J. L.; Hallé, J. P. *Biomaterials* **2006**, *27*, 3691–3700.
15. Tam, S. K.; Dusseault, J.; Polizu, S.; Ménard, M.; Hallé, J. P.; Yahia, L. *Biomaterials* **2005**, *26*, 6950–6961.
16. Bu, H.; Kjøniksen, A. L.; Knudsen, K. D.; Nyström, B. *Biomacromolecules* **2004**, *5*, 1470–1479.
17. Kulkarni, A. R.; Soppimath, K. S.; Aminabhavi, T. M.; Dave, A. M.; Mehta, M. H. *J. Controlled Release* **2000**, *63*, 97–105.
18. Leone, G.; Torricelli, P.; Chiumiento, A.; Facchini, A.; Barbucci, R. *J. Biomed. Mater. Res. Part A* **2007**, *84*, 391–401.
19. Moe, S. T.; Skjåk-Braek, G.; Smidsrød, O. *Food Hydrocolloid* **1991**, *5*, 119–122.
20. Leonard, M.; Rastello de Boisseson, M.; Hubert, P.; Dalançon, F.; Dellacherie, E. *J. Controlled Release* **2004**, *98*, 395–405.
21. Rastello de Boisseson, M.; Leonard, M.; Hubert, P.; Marchal, P.; Stequert, A.; Castel, C.; Favre, E.; Dellacherie, E. *J. Colloid. Interface Sci.* **2004**, *273*, 131–139.
22. Barbucci, R.; Rappuoli, R.; Borzacchiello, A.; Ambrosio, L. *J. Biomat. Sci. Polym. Ed.* **2000**, *11*, 383–399.
23. Barbucci, R.; Consumi, M.; Magnani, A. *Macromol. Chem. Phys.* **2002**, *203*, 1292–1300.
24. Young, J. J.; Cheng, K. M.; Tsou, T. L.; Liu, H. W.; Wang, H. J. *J. Biomater. Sci. Polym. Ed.* **2004**, *15*, 767–780.
25. Pelletier, S.; Hubert, P.; Lapicque, F.; Payan, E.; Dellacherie, E. *Carbohydr. Polym.* **2000**, *43*, 343–349.
26. Jenkins, R.D.; Silebi, C.A.; El-Aasser, M.S. In *Polymers as rheology modifiers*, Glass JE, editor, ACS Symposium Series: New York, 1991, Vol. 462, pp 222–223.
27. English, R. J.; Gulati, H. S.; Jenkins, R. D. *J. Rheol.* **1997**, *41*, 427–443.
28. Tirtaatmadja, T.; Tam, K. C.; Jenkins, R. D. *Macromolecules* **1997**, *30*, 3271–3282.