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Synthesis and characterization of a novel alginate–poly (ethylene glycol) graft copolymer

P. Laurienzo[a,*], M. Malinconico[a], A. Motta[b], A. Vicinanza[a]

[a]*Istituto di Chimica e Tecnologia dei Polimeri, Consiglio Nazionale delle Ricerche, Via Campi Flegrei 34, I-80078 Pozzuoli (Naples), Italy*
[b]*Istituto di Chimica Biomolecolare, Consiglio Nazionale delle Ricerche, Via Campi Flegrei 34, I-80078 Pozzuoli (Naples), Italy*

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Abstract

Sodium alginate grafted with low molecular weight polyethylene glycol (PEG) was prepared by reacting a mono-carboxyl terminated PEG with a sodium alginate modified by inserting a given amount of amine functionalities. The coupling between PEG and alginate was carried out using carbodiimide chemistry in aqueous solutions. Significant reduction of the molecular weight of alginate is recorded, as expected from literature reports. The chosen strategy allowed to graft significant amounts of PEG saving the carboxylic functionalities of alginate, necessary for the cross-linking of the polysaccharide. The chemical structure and chemical–physical properties of alginate derivatives were characterized by FTIR, ^{1}H and ^{13}C NMR, DSC, TGA and wide angle X-ray analysis.

Keywords: Alginate; Polyethyleneglycol; Carbodiimide chemistry; NMR spectroscopy

1. Introduction

Alginate is a natural occurring polysaccharide made of guluronic and mannuronic acids, quite abundant in nature as a structural component in marine brown algae (*Phaeophyceae*) and as capsular polysaccharides in soil bacteria. It instantaneously forms gel-spheres by ionotropic gelation in the presence of divalent ions such as Ca^{2+}, Ba^{2+}, or Sr^{2+}, and for this it is widely used as a gel-entrapment system for the immobilization of cells, and microencapsulation.

The microencapsulation technique has been specifically developed for the oral delivery of proteins, as they are quickly denaturated and degraded in the hostile environment of the stomach. The protein is encapsulated in a core material that, in turn, is coated with a biocompatible, semi permeable membrane, which controls the release rate of the protein while protecting it from biodegradation. Alginate gel can act as core materials in this application, while poly(ethylene glycol) (PEG), which exhibits properties such as protein resistance, low toxicity and immunogenicity (Merril and Salzman, 1983), together with the ability of preserving the biological properties of proteins (Han, Jeon, Lee, & Yang, 1989; Tu, Lu, Thyagarajan, Wang, Nguyen and Shen, 1993), can act as a coating membrane. PEGs are also used to improve the biocompatibility of polymers. A chitosan/PEG–alginate microencapsulation process (Chen, Chu, Shiao, Hsu, & Fu, 1998), applied to biological macromolecules such as albumin or hirudin, was reported to be a good candidate for oral delivery of bioactive peptides (Chandy, Mooradian, & Rao, 1998).

Gel-entrapments allow suspension cells to be cultivated in several types of bioreactors to achieve high cell densities. Alginate immobilization is viewed as a mild process involving non-toxic components and pH, osmolarity, and temperature suitable for preserving mammalian cells. In addition, alginate polymers meet many requirements for an ideal matrix material in that they are amenable to sterilization and storage, and may be chemically modified with rather simple chemistry. However, the high density of network in alginate matrix limits the cell growth (Rowley, Madlambayan, & Mooney, 1999); moreover, cell anchorage, a strict requirement for survival, is limited on alginate gels, as the hydrophilic nature of polysaccharides is able to discourage protein absorption, which is the first step for cell adhesion at biomaterial surfaces.

* Corresponding author. Tel.: +39 81 8675215; fax: +39 81 8675230.
E-mail address: pielle@ictp.cnr.it (P. Laurienzo).

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Several examples to produce alginate gels with adequate pores dimension for a higher density growth have been described in the literature. A number of gel-entrapment methods have been applied to mammalian cells, such as poly-L-lysine (PLL)–alginate and PEG–alginate systems. For the latter, it is reported (Seifert and Phillips, 1997) that the hydrogel pore size can be varied as a function of PEG concentration, being the PEG a water-soluble polymer, which is released in the surrounding aqueous medium. A surface coating of alginate beads with PEG via glutaraldehyde, which leads to a chemical grafting of PEG onto beads, has been proposed (Chandy et al., 1998), but very few other examples of PEG–alginate copolymers are found in literature.

The purpose of this work is to suggest a protocol for the synthesis of new alginate-*g*-PEG copolymers that retain the gelation characteristics of alginate, since the PEG chemical grafting does not consumes the carboxyl groups, essential for the gelation process. The presence of grafted PEG molecules inside alginate gels will increase the pores dimension and, concurrently, will induce improved cell anchorage. Moreover, the reaction proceeds via an hydrophobization of alginate through insertion of alkylic chains with eight carbon atoms. The hydrophobization of alginate promotes protein absorption and, consequently, cell anchorage. Hydrophobized derivatives of alginate demonstrate amphiphilic properties in aqueous medium and have been widely investigated for a variety of applications, such as enzymes immobilization.

2. Experimental

2.1. Materials

High viscosity alginate sodium salt (61% mannuronic and 39% guluronic acids, viscosity about 14,000 cps at 2% solution and 25 °C (273 K), $M_w \sim 200{,}000$) of brown algae origin was supplied by Sigma.

Polyethylenglycole monomethyl ether (mPEG, M_w 2000) was supplied by Aldrich. Sodium periodate ($NaIO_4$), sodium cianoborohydride ($NaCNBH_3$), octyl amine ($C8–NH_2$), succinic anhydride (SA), pyridine, 1-ethyl-dimethylaminopropyl-carbodiimide (EDC), were Aldrich reagent grade and used without any purification.

1,4-Dioxane (Fluka analytical grade) was dried over neutral alumina and stored under nitrogen. Water was distilled just before use. Hexane (Fluka analytical grade) was used without purification.

2.2. Preparation of acid end-functionalized PEG (PEG–COOH)

10.00 mg of mPEG dissolved in 20 mL of 1,4-dioxane were treated with 600 mg of succinic anhydride and 0.48 mL of pyridine as catalyst. The reaction was carried out under nitrogen stream for 24 h at 60 °C. The PEG–COOH was recovered by precipitation in cold hexane, washed twice and finally dried under vacuum.

2.3. Preparation of the 2,3-dialdehydic alginate (AA–CHO)

A sodium alginate solution (1% w/v, 100 cm^3 H_2O) was treated with a mixture of 1-propanol (25 mL) and 0.25 M $NaIO_4$ (4 mL), theoretically sufficient to produce a 20% oxidation ratio. Experiments were carried on in the dark at 4 °C for 24 h. Ethylene glycol was then added (1 mL) to reduce the excess periodate. The reaction solution was then extensively dialyzed against distilled water and subsequently freeze-dried (yield 94–95%).

2.4. Preparation of 2,3-dioctyl amine alginate (AA–NHR)

To a solution of 2.00 g of dialdehydic alginate dissolved in 100 mL of phosphate buffer (pH 7), 0.46 g of $NaCNBH_3$ were added. The solution was then mixed with 50 mL of methanol containing 0.83 mL of octyl amine. The ratio between amine and aldehydic groups on alginate was about two. The reaction proceeded for 12 h with stirring at room temperature and the final solution was poured in methanol. The product was recovered as a white fine powder after centrifugation, then dissolved again, extensively dialyzed against water and finally recovered by freeze-drying.

2.5. Preparation of alginate-co-polyethylene glycol graft copolymer (AA-g-PEG)

To a solution of 2.13 g of PEG–COOH dissolved in 50 mL of water, 50 mg of EDC were added under vigorous stirring. After 2 h, a solution containing 1.25 g of 2,3-dialkyl amine alginate in 30 mL of water was added. The resultant solution was left to react for 4 d. The product was recovered by freeze-drying and then repeatedly washed with chloroform to remove unreacted PEG (final yield = 50%).

2.6. Techniques

Viscosity measurements were carried out with an Ubbelhode viscometer immersed in a thermostatic bath at 25 °C. Alginate solutions at $c = 0.25$ g/dL in water containing NaCl 0.1 M were analyzed.

The infrared spectra were recorded using a Perkin–Elmer System 2000 FTIR spectrophotometer on polymers powder compressed into KBr disks with an average acquisition of 32 scans (resolution = 4 cm^{-1}).

NMR spectra, recorded at the NMR Service of Istituto di Chimica Biomolecolare del CNR (Pozzuoli, Italy), were acquired at 25 and 60 °C on a Bruker DRX-600 operating at 600.13 MHz for ^1H and 150.90 MHz for ^{13}C, and on a Bruker DPX-300 operating at 75.46 MHz for ^{13}C. The DRX-600 was equipped with an inverse TCI CryoProbe

fitted with a gradient along the *Z*-axis, while the DPX-300 was equipped with a standard dual probe. Samples were prepared by dissolving appropriate amounts of products in 0.5 mL of $^{2}H_2O$. Deuterated water was obtained from Aldrich. Proton spectra were referenced to internal sodium 3-(trimethylsilyl-(2,2,3,3-2H4)propionate, while 8 μL of methanol were added as reference for carbon spectra. One- and two-dimensional NMR spectra were performed by using standard or in-house modified Bruker microprograms.

Calorimetric analysis was performed on 10–15 mg of sample using a Mettler DSC-30 differential scanning calorimeter (DSC) at a heating rate of 20°/min.

Thermogravimetric analysis (TGA) was carried out on a Perkin Elmer Diamond Thermogravimetric/Differential Thermal Analyzer under nitrogen flow at a heating rate of 10°/min from 40 to 500 °C.

Wide-angle X-ray (WAXS) powder patterns were recorded at room temperature on a Philips PW 1711 diffractometer using a Ni-filtered Cu Kα radiation.

3. Results and discussion

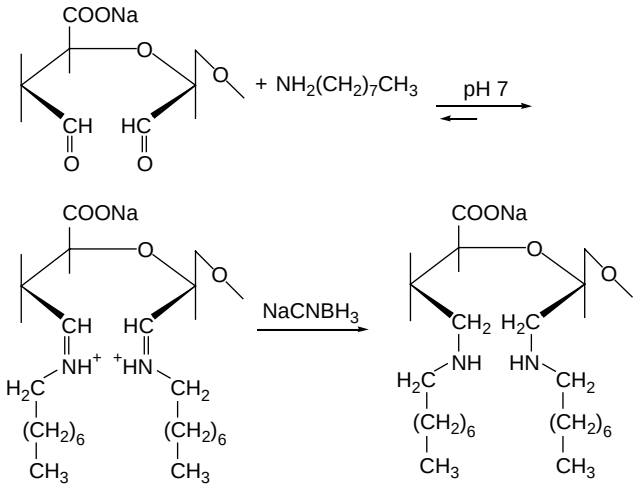
3.1. Synthesis of the aldehydic alginate

The introduction of aldehydic groups onto sodium alginate was the selected approach to activate the polysaccharide for successive chemical modifications. Compared with the activation of hydroxyl or carboxyl groups directly available on the alginate backbone, this approach offers some advantages:

(i) aldehydic groups are more reactive than hydroxyl ones;
(ii) the synthesis of esters directly onto the alginate carboxyl groups may lead to their consumption, and, as a consequence, difficulties in the sol-gel transition process may arise.

To form reactive intermediates containing aldehyde functional groups, sodium alginate was oxidized with sodium periodate. The reaction was carried out as already described (Andresen et al., 1977, Carrè, Delestre, Hubert, & Dellacherie, 1991; Kang et al., 2002). Exclusion of light during oxidation was essential for the limitation of side reaction. It is possible to control the alginate degree of oxidation by varying the concentration of the oxidant. In the conditions here described, a 20% degree oxidation (i.e. 20 uronic acid units oxidized out of 100 uronic acids units in the alginate sample) is reported (Kang et al., 2002). We assume that this value of degree oxidation was obtained too in our experimental conditions.

A striking decrease of the intrinsic viscosity was found (0.34 vs. 8.4 dL/g of the starting polymer). Such a decrease can be partially attributed to an increase in the whole chain flexibility (Smidsrod and Painter, 1973): it is well known



Scheme 1. Mechanism of synthesis of 2,3-dioctylamine alginate.

(Scott, Tigwell, Phelps, & Nieduszynski, 1976) that the oxidation by metaperiodate leads to a depolymerization of the chain, even when the oxidation is carried out in the dark, at low temperature and in the presence of 1-propanol. The oxidation is still more degradative when the content of alternating mannuronic (M) and guluronic (G) blocks is high, as chain-scission preferentially takes place at atypical sugar units in the alginate molecule. Although we have no information on the distribution of sequences of M and G sugar units in our sample, we can hypothesize a high content of MG blocks respect to homogeneous blocks, which are less sensitive to hydrolysis.

3.2. Synthesis of the alginamine

The subsequent reductive amination was performed with octylamine by using $NaCNBH_3$ as reducing agent, as it is more reactive and selective than the frequently employed sodium hydroborate ($NaBH_4$). The advantage of $NaCNBH_3$ is in that the reduction of imine intermediate groups by $CNBH_3^-$ anion is rapid at pH 6–7, while the carbonyl reduction into the corresponding alcohol is negligible in this pH range (Scheme 1).

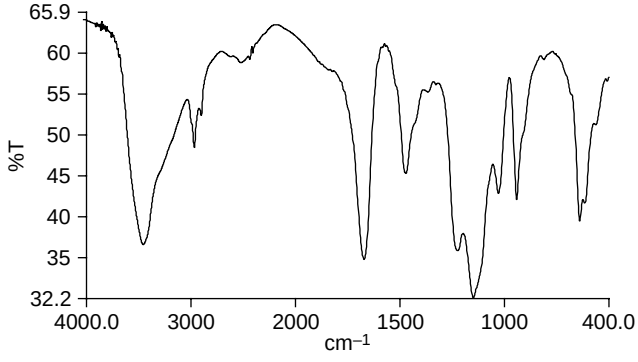
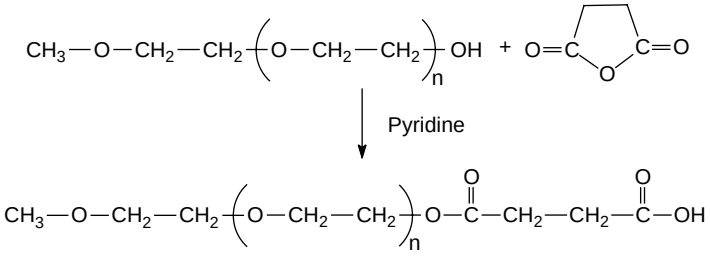


Fig. 1. FTIR spectrum of AA–NHR (2927, 2862 cm^{-1}: C–H streching band).



Scheme 2. Functionalization of PEG.

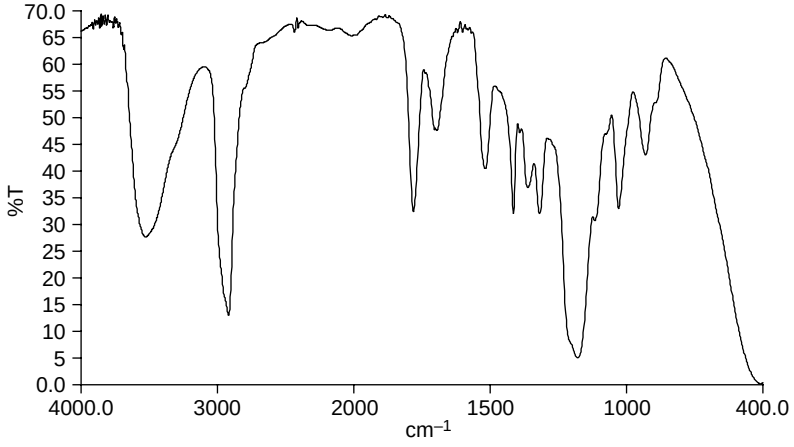


Fig. 2. FTIR Spectrum of PEG–COOH (1736–1710 cm^{-1}: C=O ester–acid streching band).

The FTIR spectrum of the product is shown in Fig. 1, where bands at 2928 and 2862 cm^{-1} are attributable to the alkyl groups of octyl amine and support the modification of the oxidized alginate.

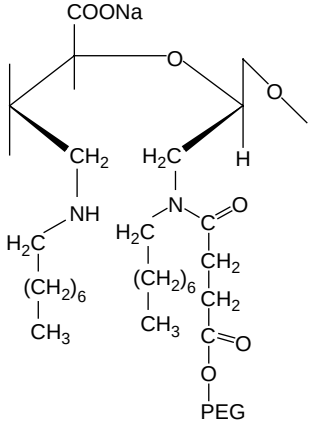
The intrinsic viscosity was found to be the same of the starting oxidized alginate (0.34 dL/g). This is convincing, as the reductive amination does not produce further depolymerization of the chain. A decrease in water solubility (maximum solubility reached: 0.1%) was found, related to the role played by the introduction of hydrophobic side chains onto alginate. The situation may in fact be considered in terms of competition between repulsive electrostatic interactions of the carboxylic groups, which result in fully expanded structure with high interactions with water molecules, and hydrophobic side-chains attractive interactions, which conversely produce shrinkage and expulsion of solvatating water molecules. It is, in fact, reported (Kang et al., 2002) that at high concentrations ($>1\%$) the hydrophobic interactions prevail over Coulombic repulsions, and therefore, aggregation of the alkylic chains occurs, with formation of hydrophobic intermolecular regions.

3.3. Chemical coupling of PEG onto alginamine

Monohydroxyl terminated PEG was previously end-functionalized through reaction with succinic anhydride in dioxane solution (Scheme 2), following a slight modification of a reported procedure (Shuai and Tan, 1997).

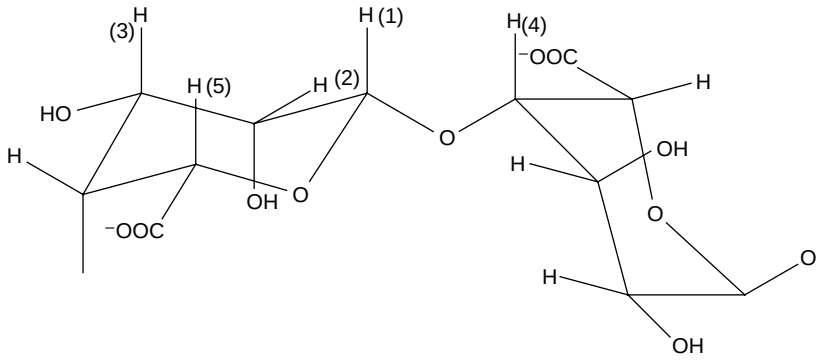
The product was characterized by FTIR spectroscopy (Fig. 2), where the new ester–acid band at 1736–1710 cm^{-1} is evident.

The final step utilizes aqueous carbodiimide chemistry (Kunioka and Furusawa, 1997; Rowley et al., 1999) to covalently couple the PEG–COOH to alginate. Usually, the carbodiimide chemistry involves the coupling between a primary amine and a carboxyl group. In this case, we verify the efficacy of EDC also in the coupling between a secondary amine (on the alginate molecule) and the carboxyl group on PEG. EDC, a water-soluble carbodiimide, was used (Scheme 3). EDC was added at a ratio 0.25:1



Scheme 3. Schematic representation of alginate-*g*-PEG copolymer.

Table 1
The ^{1}H NMR and ^{13}C NMR chemical shifts of alginate blocks



	H-1	H-2	H-3	H-4	H-5
M	4.72	3.94	3.76	3.93	3.83
G	5.10	4.05	4.19	4.24	4.76
	C-1	C-2	C-3	C-4	C-5
M	102.0	65.0	72.0	78.0	72.0
G	101.5	65.0	69.0	80.0	67.0

to PEG–COOH, in order to minimize common side reactions occurring when EDC is used in large amounts. EDC is commonly most reactive at acidic pH (i.e. 4–5). However, in our case, a neutral pH was chosen for covalent incorporation of PEG onto alginate, as the addition of HCl lead to a lower amount of PEG in the final product, presumably due to the tendency of alginate to segregate in form of micelles at acid pH.

A rough but still reliable estimation of the grafting degree as obtained by selective solvent extraction of unreacted PEG gives a value of 20% by weight.

Preliminary attempts to verify the gelation ability of AA/PEG were carried out by dropping a solution of the copolymer in 2% $CaCl_2$ water solution. Visually, it was observed an immediate formation of microparticles. It is to be stressed that in similar conditions, the starting AA would give rise to macroscopic beads. We attribute the different behavior to the effect of grafted PEG chains, which can in part increase the water dispersibility of cross-linked alginate and in part also obstacle the network formation.

3.4. NMR analysis

The products were characterized by NMR spectroscopy. The sequential structure of alginate was investigated by using one-dimensional ^{1}H and ^{13}C, two-dimensional homonuclear COSY and TOCSY, and heteronuclear ^{1}H–^{13}C HSQC experiments (Berger and Braun, 2004). Alginate is composed of mannuronic (M) and guluronic (G) acids in the form of a homopolymeric (MM- or GG-blocks), and heteropolymeric sequence (MG- or GM-blocks). By comparison with the chemical shift data reported (Tako et al., 2001), we were able to identify the alginate proton and carbon signals, and the results are reported in Table 1. The values refer to the most abundant species of each unit, mannuronic or guluronic acids, but from the anomeric signals we were able to follow most of the heteropolymeric sequences, and they will be discussed elsewhere.

The ^{1}H and ^{13}C NMR chemical shifts of the intermediate product AA–CHO were compared with starting alginate. As expected, the spectra (not shown) are completely different from those of alginate because of the M and G ring opening at C2–C3 bond during periodate oxidation. In the AA–CHO carbon and the HSQC spectra, we were not able to identify the signal of the aldehydic carbon, most likely because of the hemiacetal formation by the free aldehyde groups. Similarly, in IR spectra the C=O band of the aldehydic group was not observed (Kang et al., 2002).

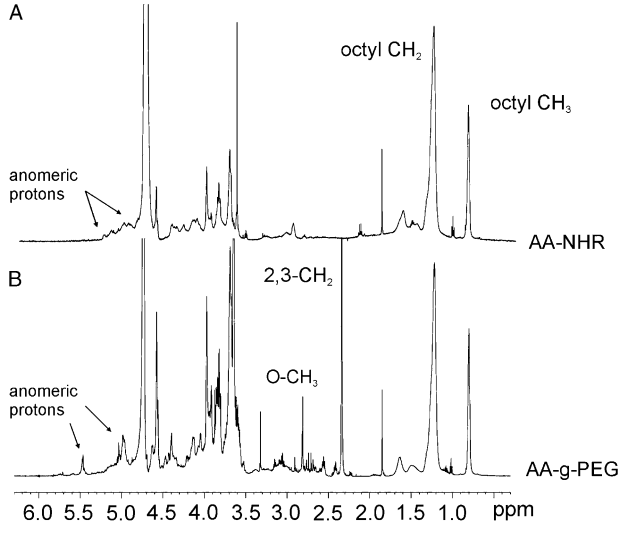
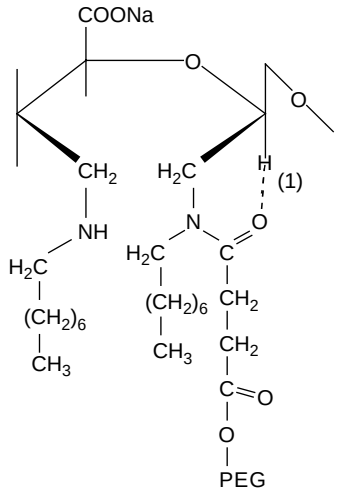


Fig. 3. 600-MHz proton spectra of AA–NHR and AA-*g*-PEG.



Scheme 4. Hydrogen bond in the structure of alginate-*g*-PEG copolymer.

Fig. 3 shows the 600-MHz proton spectrum of the AA–NHR, which is obtained as a product of the reaction between AA–CHO and octyl amine. The presence of new proton peaks affords immediate evidence of covalent bond formation between AA–CHO and the octyl amine. In particular, additional peaks are observed at 0.87 and 1.29 ppm, which were, respectively, assigned to the methyl and methylene protons of the octyl chain. The corresponding carbon signals were identified, as a further confirmation, in a HSQC spectrum. It exhibited signals relative to the presence of the alkyl carbon atoms at 26.8 and 34.7 ppm, respectively.

A comparison between the ^{1}H spectra of AA–NHR (Fig. 3(A)) and AA-*g*-PEG (Fig. 3(B)) gives a clear evidence of the presence of a chemical bond between PEG and modified alginate. The two proton peaks resonating at 3.36 (terminal O–CH_3) and 2.66 ppm (CH_2 at positions 2 and 3) in the PEG are observed upfield-shifted at 2.81 and 2.34 ppm, respectively, in AA-*g*-PEG. Furthermore, the methyl and methylene protons of the octyl chain are also upfield-shifted at 0.80 and 1.22 ppm, respectively. The anomeric proton region is also affected by the addition of PEG to alginate. In the AA–NHR, the anomeric protons are all clustered in the region 5.3–4.9 ppm (Fig. 3(A)), but upon addition of PEG they group in two well-separated regions at 5.47 and 4.97 ppm (Fig. 3(B)). We attribute such a spectral change to the formation of a hydrogen bond between the anomeric proton of the open alginate rings and the amidic carbonyl of the attached PEG, forming a stable six-membered ring. It is important to underline that the splitting in two groups of the anomeric protons is due to the covalent bond formation in the final product AA-*g*-PEG and not to the opening of the rings in the alginate. In fact, in AA–NHR the mannuronic and guluronic rings are already open because of the periodate oxidation before the octyl amine insertion. However, it is only in AA–PEG that the formation of the hydrogen bond induces differentiation of the anomeric protons (Scheme 4).

3.5. DSC characterization

The DSC thermogram of the AA-*g*-PEG final product is shown in Fig. 4. In the first run, two sharp endotherms (at 86.7 and 132.3 °C) are detected, and can be attributed, as

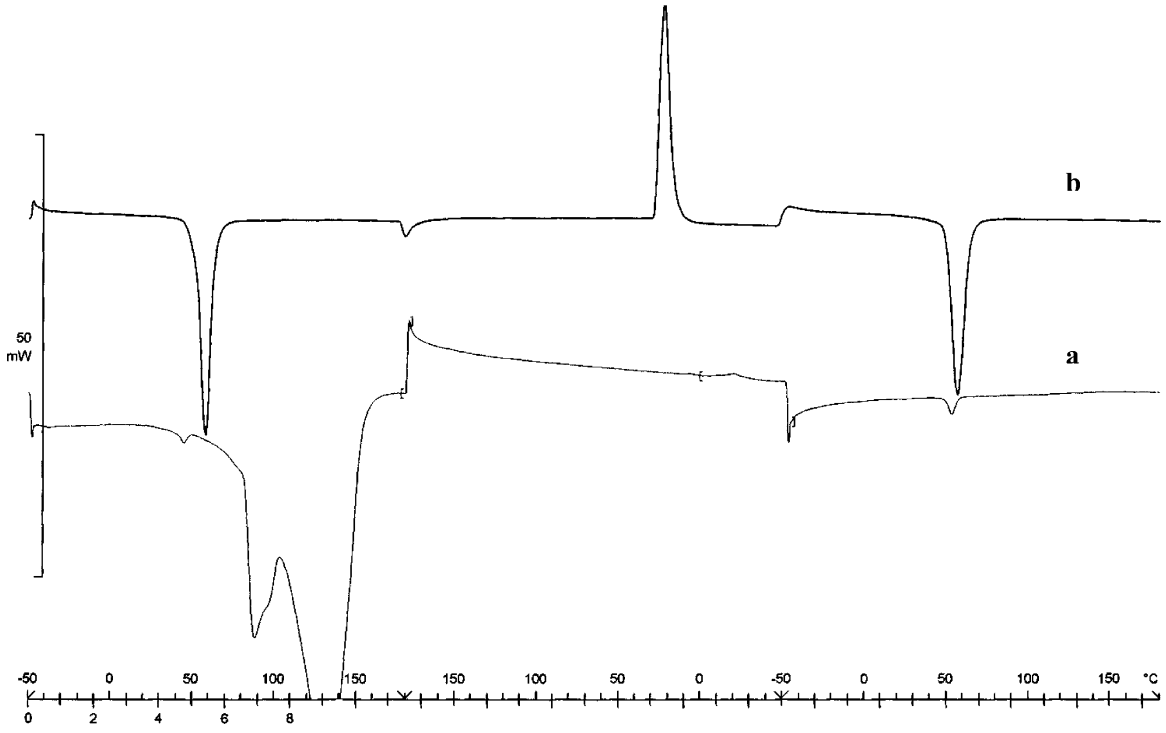


Fig. 4. DSC thermograms of AA-*g*-PEG (a) and of PEG–COOH (b).

discussed in the next paragraph, to the evolution of water linked to the copolymer by different kind of physical bonds. The melting of grafted PEG chains is evident both in first and second run. Calorimetric data show a slight decrease of the melting temperature from 55 °C (pure PEG–COOH) to 52 °C in AA-*g*-PEG copolymer, compatible with a reduced mobility of PEG chains after grafting reaction, which disturbs the crystallization process. Moreover, absolute evaluation of grafting degree of AA-*g*-PEG by means of DSC is not possible, as the amount of crystallisable PEG likely is only a fraction of total grafted PEG.

3.6. TGA characterization

Thermogravimetric analysis was performed on AA–NHR intermediate and on final AA-*g*-PEG copolymer. The corresponding curves are shown in Fig. 5. We know (Russo, Giuliani, Immirzi, Malinconico & Romano, 2004) that alginates release water at different temperatures, depending on the different interactions of water with the polysaccharide. Three different kinds of interactions of water can be identified in the case of plain alginate: the first one, is free water that is released in the 40–60 °C region; the second one, in the region 80–120 °C, is water linked through hydrogen bonds; and finally, water more tightly linked through polar interactions with carboxylate groups is released up to 160 °C.

Fig. 5(a) suggests that in the AA–NHR sample all the water (around 25% b.w.) is released in the range 40–100 °C; that maybe due to the fact that the amines are competitive with water in interactions with carboxylate groups, so only free water (66.5 °C) and water linked through hydrogen bonds (87 °C) are detected. The polymer starts to degrade around 200 °C.

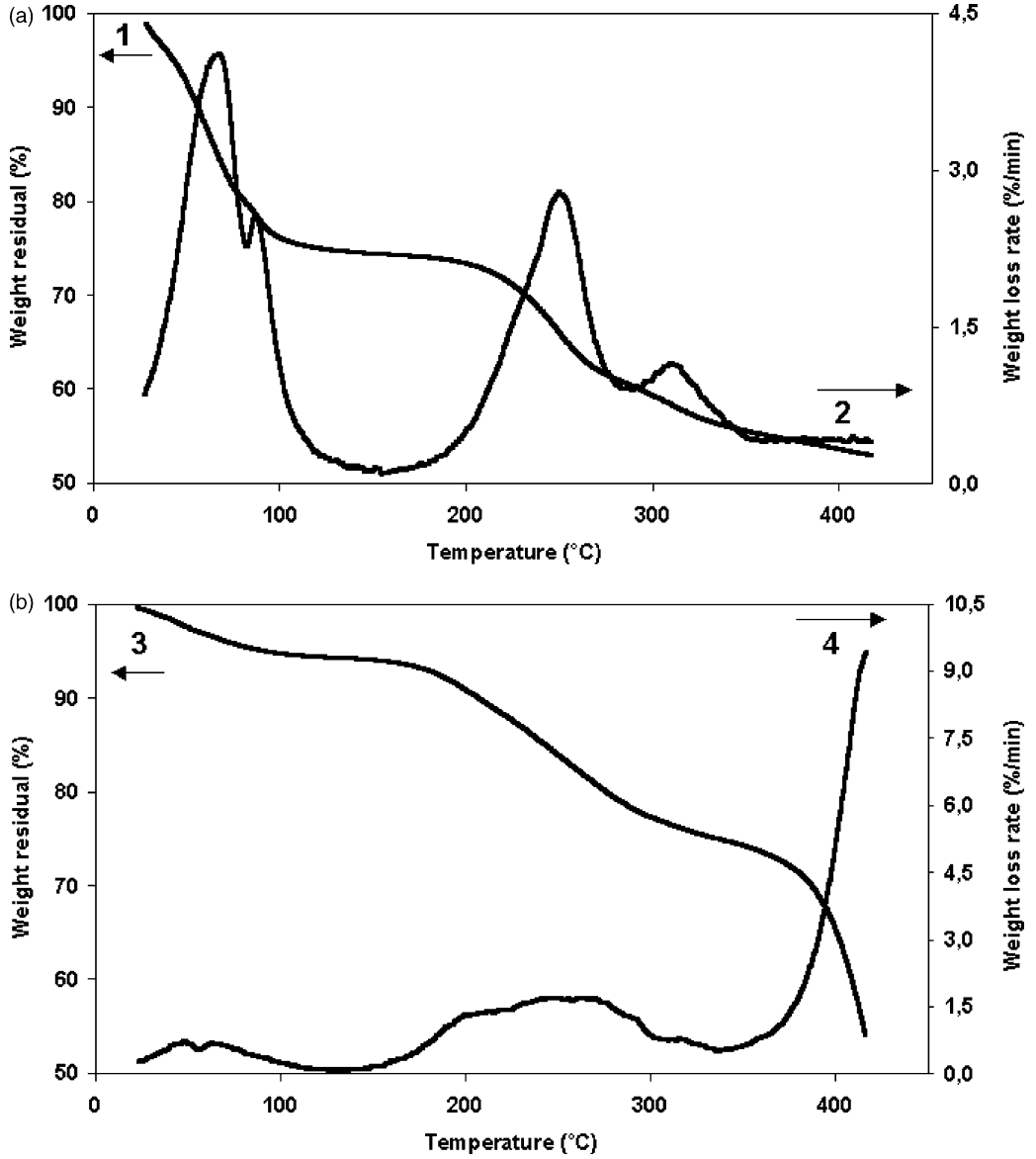


Fig. 5. TGA and DTA curves of AA–NHR (a) and AA-*g*-PEG (b).

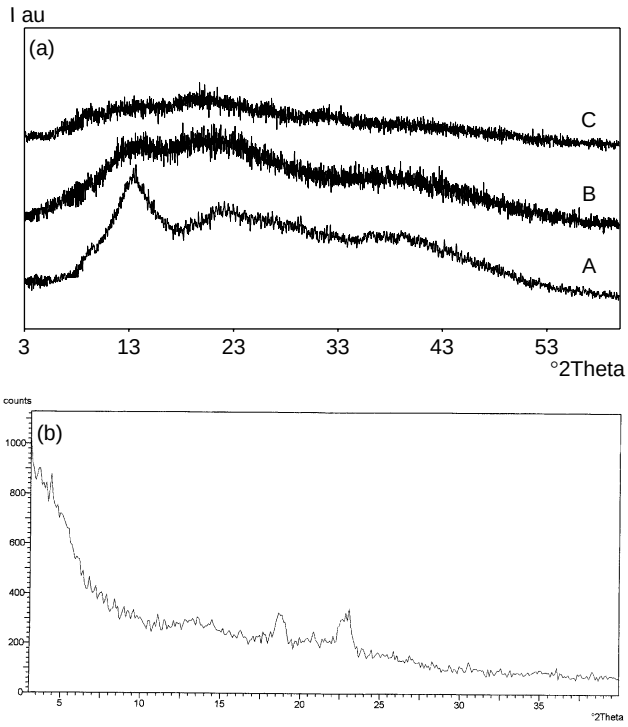


Fig. 6. (a) X-ray diffraction profiles of alginate and intermediates of synthesis: (A) Alginate, (B) AA–CHO, (C) AA–NHR. (b) X-ray diffraction profile of AA-*g*-PEG.

The AA-*g*-PEG shows a continuous weight loss from 40 up to 130 °C, as amines were consumed by the reaction with PEG–COOH, and carboxylate groups are again free to interact with water. The total amount of released water is only 10% b.w., in agreement with the reduced hydrophylicity of AA-*g*-PEG with respect to amine alginate. At higher temperatures, the modified alginate shows two different mechanisms of degradation, typical of AA and PEG chains: the first degrades from 200 to 300 °C, while the more thermally stable PEG chains start to degrade at 300 °C.

3.7. WAXS characterization

WAXS spectra of alginate and synthetic intermediates are reported in Fig. 6(a). The alginate shows a small, rather broad peak at 13.59° (2ϑ) related to a long-range chain order, due to the strong hydrogen and polar intra- and intermolecular interactions. This peak completely disappears in AA–CHO and AA–NHR samples, as a consequence of the substitution of part of hydroxyl groups with aldehydic functionalities or alkylic chains, respectively, which completely destroys the organization of the chains of starting alginate.

In Fig. 6(b) the WAXS spectrum of the AA-*g*-PEG sample is shown. The peaks at 19.2 and 23.1° (2ϑ) are typical of the PEG polymer (Lucke, Tessmar, Schnell, & Gopferich, 2000), and confirm the persistence of residual crystallinity of PEG macromolecules grafted onto alginate.

4. Conclusions

A novel alginate–PEG graft copolymer, which retains the characteristics of gelation, was prepared. To covalently graft PEG macromolecules onto the alginate backbone, a method involving several steps, each well known and characterized, was used. Since, PEG is a high biocompatible polymer, the alginate–PEG copolymers are promising candidates for any application in which alginate gels with higher biocompatibility and pores dimension are required, as for gel entrapment devices and microencapsulation techniques. Moreover, it is conceivable to assume that water retention of hydrophilic, modified alginate should be higher than starting alginate upon cross-linking with divalent cations.

More work is in progress to check the influence of different PEG molecular weights on gelation ability and biochemical properties.

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References

Andresen, I. L., Painter, T., & Smidsrod, O. (1977). *Carbohydrate Research*, *59*, 563–567.

Berger, S., & Braun, S. (2004). *200 and more basic NMR experiments: A practical course*. Weinheim: Wiley-VCH.

Carrè, M. C., Delestre, C., Hubert, P., & Dellacherie, E. (1991). *Carbohydrate Polymers*, *16*, 367–379.

Chandy, T., Mooradian, D. L., & Rao, G. H. R. (1998). *Journal of Applied Polymer Science*, *70*, 2143–2153.

Chen, J. P., Chu, I. M., Shiao, M. Y., Hsu, B. R., & Fu, S. H. (1998). *Journal of Fermentation and Bioengineering*, *86*, 185–190.

Han, D. K., Jeong, S. V., & Kim, Y. H. (1989). *Journal of Biomedical Materials Research, Applied Biomaterials*, *23*, 211–215.

Kang, H. A., Jeon, G. J., Lee, M. Y., & Yang, J. W. (2002). *Journal Chemical Technology Biotechnology*, *77*, 205–208.

Kunioka, M., & Furusawa, K. (1997). *Journal of Applied Polymer Science*, *65*, 1889–1896.

Lucke, A., Tessmar, J., Schnell, E., Schmeer, G., & Gopferich, A. (2000). *Biomaterials*, *21*, 2361–2370.

Merril, E. W., & Salzman, E. W. (1983). *Journal American Society for Artificial Internal Organs*, *6*, 60–66.

Rowley, J. A., Madlambayan, G., & Mooney, D. J. (1999). *Biomaterials*, *20*, 45–53.

Russo, R., Giuliani, A., Immirzi, B., Malinconico, M., & Romano, G. (2004). *Macromolecular Symposia*, *218*, 241–250.

Scott, J. E., Tigwell, M. J., Phelps, C. F., & Nieduszynski, I. A. (1976). *Carbohydrate Research*, *47*, 105–117.

Seifert, D. B., & Phillips, J. A. (1997). *Biotechnology Progress*, *13*, 569–576.

Shuai, X., & Tan, H. (1997). *Journal of Applied Polymer Science*, *66*, 1891–1898.

Smidsrod, O., & Painter, T. (1973). *Carbohydrate Research*, *26*, 125–132.

Tako, M., Kiyuna, S., Uechi, S., & Hongo, F. (2001). *Bioscience, Biotechnology, and Biochemistry*, *65*, 634–657.

Tu, R., Lu, C. L., Thyagarajan, K., Wang, E., Nguyen, H., Shen, S., et al. (1993). *Journal of Biomedical Materials Research*, *27*, 3–7.