Carbohydrate Polymers 86 (2011) 208–213

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Biodegradable IPNs based on oxidized alginate and dextran-HEMA for controlled release of proteins

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ARTICLE INFO

Article history:
Received 28 February 2011
Received in revised form 6 April 2011
Accepted 14 April 2011
Available online 21 April 2011

Keywords:
Polysaccharides
Interpenetrating networks
Oxidized alginate
Sodium periodate
Biodegradability

ABSTRACT

Alginate is a natural polysaccharide that is widely used for biomedical applications because of its biocompatibility and ability to form hydrogels, but its slow and uncontrollable degradation under physiological conditions represents an undesirable feature. To introduce hydrolytically sensitive sites in alginate, this polymer was partially oxidized using periodate. Alginates with different extent of oxidation were characterized for their degradation behavior and ability to form gels in the presence of Ca^{2+} ions. The obtained results showed that the oxidized alginates were indeed degradable under physiological conditions (pH 7.4 and 37 °C) and that their gelling ability was preserved for samples with oxidation degrees up to 5%. IPNs, based on oxidized alginate (1% and 5% oxidation) and dextran-HEMA were prepared, characterized and evaluated for protein release. These IPNs showed properties similar to the IPNs networks composed by native alginate, confirming the suitability of IPNs based on dextran-HEMA and oxidized alginate as *in situ* forming protein releasing hydrogels.

1. Introduction

Hydrogels are hydrophilic three dimensional polymeric networks capable to absorb large amounts of water and are frequently studied for pharmaceutical applications because of their ability to entrap and release drugs or proteins in a controlled manner (Hoare & Kohane, 2008; Pal, Paulson, & Rousseau, 2009). An interesting feature of these materials is the possibility to design *in situ* forming gels for application as drug delivery system or scaffolds for tissue engineering (Van Tomme, Storm, & Hennink, 2008). These materials have a fluid character before and during administration but rapidly solidify due to the formation of a 3-D network at the site of injection. This sol/gel conversion can be triggered by temperature using thermosensitive block/graft copolymers (Klouda & Mikos, 2008; Vermonden, Besseling, van Steenbergen, & Hennink, 2006) or due to chemical reaction of complementary groups, e.g. by Michael addition reactions (Censi, Fieten, di Martino, Hennink, & Vermonden, 2010; Pritchard et al., 2011) or by enzymatic conversions (Jin, Hiemstra, Zhong, & Feijen, 2007). These *in situ* gelling systems have several beneficial properties including the possibility for the site-specific delivery of drugs. Importantly, administration does not need surgical intervention as opposed to "classical" drug-loaded implants which results in a better patient compliance. Critical properties of hydrogels include appropriate mechanical properties, biocompatibility and biodegradability of the overall network as well as a low or absent toxicity of the formed degradation products.

Among the various hydrogels described in literature, hydrogels prepared using polysaccharides are attractive because of the abundance of such polymers, the possibility of chemical derivatization and, in most cases, good biocompatibility (Coviello, Matricardi, Marianecci, & Alhaique, 2007; Dumitriu, 2005; Steinbüchel, 2006).

Recently, we developed an *in situ* forming IPN (Interpenetrated Polymer Network) based on alginate and hydroxyethyl methacrylate derivatized dextran (dex-HEMA) polysaccharides suitable for biomedical applications (Pescosolido et al., 2011). These IPN hydrogels showed tailorable mechanical properties, *in vitro* degradability and good cytocompatibilty. Dex-HEMA is a photo-crosslinkable modified polysaccharide capable to form a hydrogel after radical polymerization of the HEMA groups initiated by a persulphate (Franssen, Vandervennet, Roders, & Hennink, 1999; Van Dijk-Wolthuis, Hoogeboom, Van Steenbergen, Tsang, & Hennink, 1997) or by UV irradiation (Pescosolido et al., 2011; Van-Dijk-Wolthuis, Tsanga, Kettenes-van den Bosch, & Hennink, 1997) while alginate is an anionic polysaccharide that forms physically crosslinked hydrogels in the presence of bivalent cations, with a typical "egg-box" structure (Draget, Skjåk-Bræek, & Smidsrød, 1997; Rees & Welsh, 1977). One of the major concerns for *in vivo* application of a hydro-

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doi:10.1016/j.carbpol.2011.04.033

gel is the bioelimination of the polymer fragments resulting from the network degradation. As a general rule macromolecules with molecular weights lower than 60 kDa can be excreted by the kidney (Venkatachalam & Rennke, 1978).

No hydrolytic or enzymatic cleavage of the alginate chains occurs under physiological conditions although a slow degradation of alginates with high molecular weights ($M < 80 \times 10^5$ Da) was observed in *in vivo* studies (Matthew, Browne, Frame, & Millar, 1995).

A strategy that has been investigated to improve the clearance of alginate is the introduction of hydrolytic labile groups in the polymer backbone (Bouhadir, Lee, Alsberg, Damm, Anderson, & Mooney, 2001). It has been shown that oxidation of alginate with periodate leads to cleavage of the C2–C3 bond in the monosaccharide units thus forming a dialdehyde derivative. This derivative is susceptible to alkaline β-elimination (Scott, Tigwell, Phelps, & Nieduszynski, 1976; Veelaert, de Wit, Gotlieb, & Verhé, 1997), leading to a decrease of the polymer molecular weight.

In order to improve the bioelimination of the degradation products of the IPNs previously developed, we substituted the alginate chains by oxidized alginate chains and we evaluated the influence of the periodate induced alginate oxidation on the physico-chemical properties of the IPNs. In particular, the aim of this study was to assess the use of oxidized alginate as component of biodegradable IPN hydrogels based on alginate and dex-HEMA. The gelling capability of the alginate derivates, the degradation, the mechanical and release properties of the IPNs were evaluated.

2. Experimental

2.1. Materials

Dextran (dex) from Leuconostoc ssp. with M_w 40×10^3, 4-*N*,*N*-dimethylaminopyridine (4-DMAP), and hydroxyethyl methacrylate (HEMA) were Fluka products. Sodium alginate (M_w 6.5×10^5) with 70% L-guluronic acid and 30% D-mannuronic acid, carbonyldiimidazole (CDI) and (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer, sodium periodate, Irgacure 2959 (2-hydroxy-4β-(2-hydroxyetoxy)-2-methyl-propiophenone), myoglobin and BSA were all provided by Sigma–Aldrich, USA. Bradford reagent was a Bio-Rad product. All other chemicals were analytical grade.

2.2. Partial oxidation of sodium alginate

Alginate was partially oxidized with sodium periodate following a modified procedure reported elsewhere (Bouhadir, Hausman, & Mooney, 1999; Gomez, Rinaudo, & Villar, 2007). Briefly, 5.0 g of alginate (25 mmol of repeating sugar units) was dissolved in bidistilled water (300 ml) in a 1000 ml flask, under continuous stirring. Then, an appropriate amount of sodium periodate was added (0.25, 1.25. 2.5 and 25 mmol), corresponding to molar ratios of sodium periodate/repetitive units of alginate of 0.01, 0.05, 0.10 and 1) and the reaction mixture was stirred for 24 h in the dark at 20 °C. The corresponding products are indicated as AO_1, AO_5 and AO_{10}, AO_{100}, respectively, where the subscripted numbers indicate oxidized units expressed as percentage of the total number of monomeric units of alginate. After 24 h, the reaction was quenched by addition of an excess of ethylene glycol (3.5 ml) and the mixture was stirred for another 30 min. Next, 500 ml ethanol and 24 g of NaCl were added to the reaction mixture to precipitate alginate which was subsequently collected by centrifugation and solubilized in distilled water (500 ml). The precipitation process was repeated twice. Finally, the precipitate was washed with 200 ml ethanol and dried at 25 °C under vacuum. For an appropriate comparison, the procedure was also carried out without the addition of sodium periodate (AO_0).

2.3. Dex-HEMA synthesis and characterization

Dex-HEMA was prepared according to a method previously described by Van-Dijk-Wolthuis et al. (1997). Briefly, in the first step HEMA was activated with CDI, leading to HEMA-CI, which, in a second step, was coupled to the hydroxyl groups of dextran. The degree of substitution (DS, i.e., the number of HEMA groups per 100 glucopyranose residues of dextran) was determined by ^{1}H NMR in D_2O using a Gemini 300 MHz spectrometer (Varian Associates Inc., NMR instruments, Palo Alto, CA). Dex-HEMA with DS = 10 was obtained.

2.4. GPC measurements

Molecular weight determinations were done by Gel Permeation Chromatography (GPC). A GPC instrument (Varian), equipped with 2 columns PL Aquagel-OH Mixed-H 7.5 mm × 300 mm, 8 μm, and with a refractive index (Varian) detector was used. $NaNO_3$ 0.1 M, filtered (0.2 μm) and degassed under vacuum, was used as mobile phase; polymer solutions were filtered through 0.45 μm filters before injection. The flow rate was 1 ml/min, while the volume of injection was 200 μl. Pullulan standards (Varian Polymer Laboratories) were used for calibration.

Degradation of alginate and oxidized alginates under physiological conditions was studied as follows. The polymers were dissolved in 100 mM HEPES pH 7.4 (final concentration 0.2%, w/v), the solutions were incubated at 37 °C and samples were withdrawn at different times and analyzed by GPC.

2.5. FTIR analysis

The oxidized alginates were characterized using FTIR analysis. FTIR spectra were recorded with a Perkin Elmer Paragon 1000 spectrophotometer (USA) in the range 4000–400 cm^{-1} using KBr pellets (number of scans 100, resolution of 1 cm^{-1}).

2.6. Hydrogel preparation

IPNs (1 ml) were formed in cylindrical vials (diameter 12 mm) for the swelling/degradation and for the release experiments as follows. Oxidized alginate (0.030 g) was dissolved in 0.6 ml of HEPES buffer (100 mM, pH 7.4). Next, dex-HEMA (0.10 g) was added and, after its dissolution, 0.4 ml $CaCl_2$ solution (0.025 or 0.05 M) containing NaCl (0.385 or 0.776 M) was added. The system was mixed by mechanical stirring leading to the formation of the semi-IPN. Then, after the addition of 9 μl of a concentrated solution (11%, w/v) of the photoinitiator Irgacure, the semi-IPN was irradiated with a mercury lamp (λ range 350–450 nm, light intensity 20 mW/cm^2) for 10 min to polymerize dex-HEMA, resulting in the IPN formation.

For the release studies, the IPNs were loaded with myoglobin or BSA by dissolving 10 mg of the protein in 200 μl of HEPES and adding the resulting solution to the alg-oxidized/dex-HEMA solution.

IPN discs for the rheological measurements were prepared in petri dishes (diameter 40 mm, high 1 mm) instead of the cylindrical vials as above described.

In all the prepared samples the polymer concentrations were kept constant: 3%, w/v, for the oxidized alginate and 10%, w/v for the dex-HEMA.

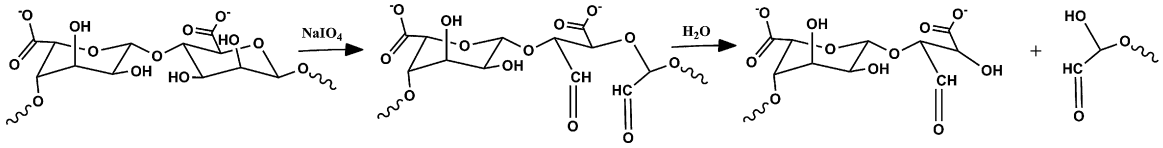

Fig. 1. Periodate oxidation of alginate creates a dialdehyde derivate that is susceptible to hydrolytic scission.

2.7. Rheological measurements

Rheological measurements were performed using a controlled stress Haake RheoStress 300 Rotational Rheometer equipped with a Haake DC10 thermostat. Frequency sweep experiments of alginate solutions (3%, w/v) and hydrogels (AO_0–Ca, AO_1–Ca, AO_5–Ca and AO_{10}–Ca, alginate concentration was 3%, w/v, while Ca^{2+} concentrations were 0.025 and 0.05 M) were carried out using a cone-plate geometry (diameter = 20 mm; cone = 1°) in a frequency range from 0.01 to 10 Hz (in the linear viscoelastic region previously assessed by stress sweep experiments). The frequency sweep experiments of the IPN hydrogels (AO_0–Ca/D, AO_1–Ca/D, AO_5–Ca/D and AO_{10}–Ca/D) were recorded in the same range of frequency setting the gap to 1 mm and using a grained plate–plate geometry (Haake PP35 TI: diameter = 35 mm) in order to reduce the wall slippage (Lapasin, 1995). All measurements were performed in duplicate at 25 °C, using a solvent trap to prevent water evaporation.

2.8. Hydrogel swelling and degradation

IPNs were weighed (1.0 ± 0.3 g) and transferred into preweighed vials. Next, 6 ml of 100 mM HEPES buffer (pH 7.4) with 0.02% NaN_3 was added and the gels were incubated at 37 °C. At defined time interval, after removal of the excess of buffer, the hydrogels were weighed (Pescosolido et al., 2011). After each measurement, 6 ml of fresh buffer was added. The hydrogel swelling is defined as the W_t/W_0 ratio, where W_0 and W_t are the hydrogel weight after preparation and at the time t, respectively. Swelling/degradation experiments were performed in duplicate.

2.9. Protein release

The ability of the studied IPN hydrogels to entrap and release proteins was studied by adding myoglobin (M_w 17,800, van der Waals radius 2.1 nm) (Coviello, Alhaique, Dorigo, Matricardi, & Grassi, 2007) or BSA (M_w 65,000, van der Waals radius 3.6 nm) (Bell & Peppas, 1996) to the alg/dex-HEMA solution followed by preparation of the IPN as described in Section 2.6. The final protein concentration was 10 mg/ml. The obtained cylindrical IPNs were transferred into 20 ml vials and 6 ml of 100 mM HEPES buffer pH 7.4 containing 0.02% NaN_3 was added. The hydrogels were incubated at 37 °C. Samples of the release medium (1 ml) were taken at appropriate time intervals and replaced by an equal volume of fresh buffer. The protein concentration in the samples was spectrophotometrically determined (Perkin–Elmer, lambda 3a, UV–vis spectrometer) using quartz cells (1.0 cm). Myoglobin was detected at 409 nm, while the Bradford protein assay (Bradford, 1976) was used for the BSA determination, according to manufacturer's protocol. Briefly, Bradford reagent (200 μl) was added to the release samples (1 ml) followed by incubation in the dark at room temperature for 15 min. After shaking for 30 s, absorbance was measured at 595 nm. Standard protein solutions were used for calibration.

3. Results and discussion

3.1. Degradation and gelling properties of oxidized alginate

Alginate was oxidized using sodium periodate in order to obtain chains that are hydrolytically labile and that can form low molecular weight fragments during or after degradation of the IPNs based on alginate and dex-HEMA. Periodate is able to split the vicinal diols in alginate, forming the corresponding dialdehyde derivative (Fig. 1) (Scott et al., 1976). The ratio of sodium periodate to the num-

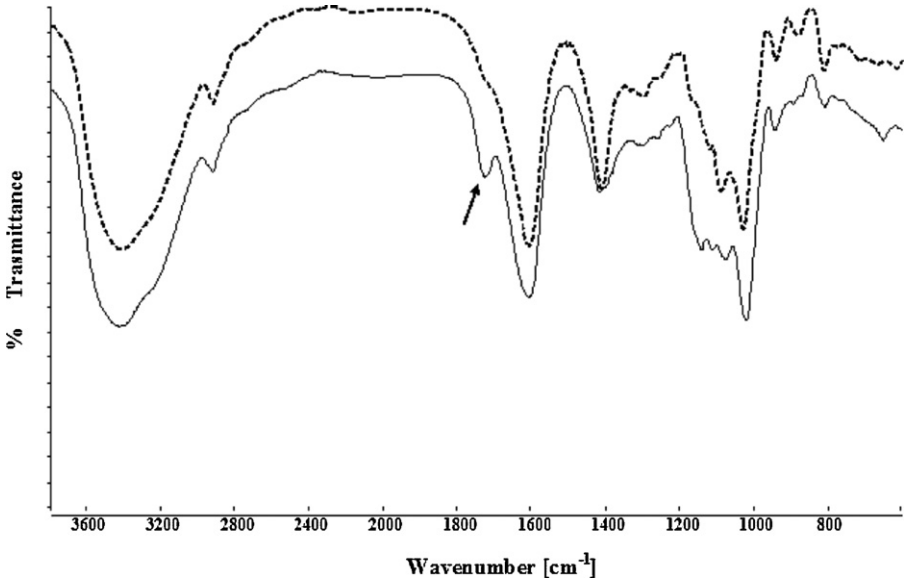

Fig. 2. FTIR spectra of (- - -) alginate (AO_0) and (–) oxidized alginate (AO_{100}). Arrow evidences the vibration band of the aldehyde groups introduced by the oxidation reaction.

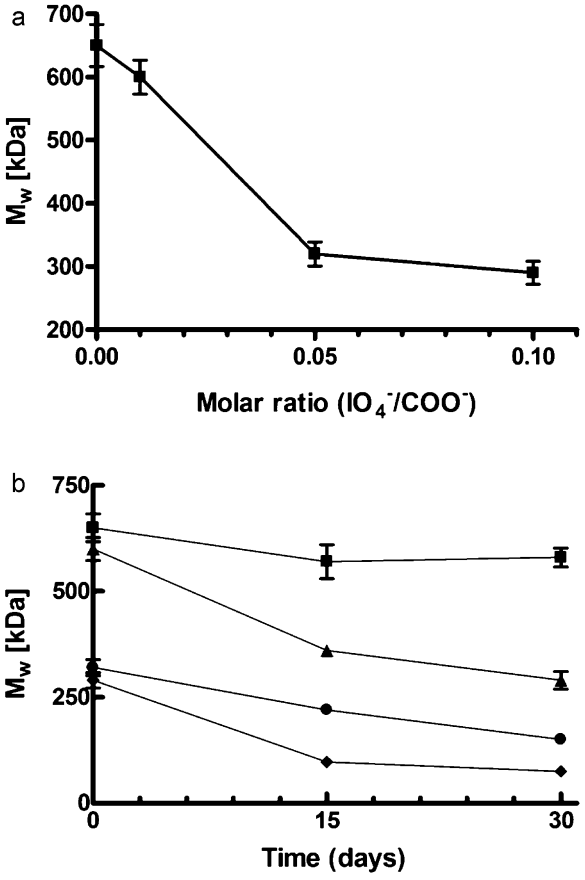

Fig. 3. (a) Molecular weight (M_w) of alginates (GPC analysis) as a function of the molar feed ratio of sodium periodate/repetitive units of alginate. Oxidation was carried out as described in Section 2.2. The M_w/M_n ratio was between 5 and 10. (b) Molecular weight (M_w) of alginates ((■) AO_0) and oxidized alginates ((▲) AO_1; (●) AO_5; (♦) AO_{10}) as obtained from GPC as a function of time. The alginates were dissolved in buffer of pH 7.4 and incubated at 37 °C (Section 2.4).

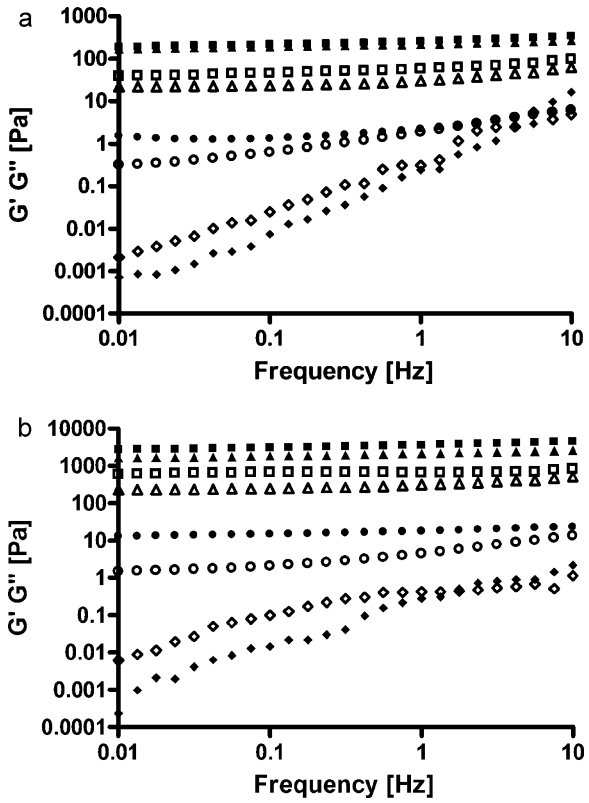

Fig. 4. (a) Frequency sweeps of the hydrogels formed at a low Ca^{2+} content (molar ratio COO^-/Ca^{2+}: 10/1): (■) G′ and (□) G″ of AO_0–Ca; (▲) G′ and (△) G″ of AO_1–Ca; (●) G′ and (○) G″ of AO_5–Ca; (♦) G′ and (◊) G″ of AO_{10}–Ca. (b) Frequency sweeps of the hydrogels formed with high Ca^{2+} content (molar ratio COO^-/Ca^{2+}: 5/1): (■) G′ and (□) G″ of AO_0–Ca; (▲) G′ and (△) G″ of AO_1–Ca; (●) G′ and (○) G″ of AO_5–Ca; (♦) G′ and (◊) G″ of AO_{10}–Ca.

ber of repetitive units of the polysaccharide was varied to obtain alginates with different extent of oxidation (aimed: 1, 5 and 10%). As we aimed for low extents of oxidation in order to retain the Ca^{2+} induced gel forming capability, the analytical methods currently described in literature (IR, NMR, or colorimetric assays using t-butyl carbazate (Bouhadir et al., 1999) were insufficiently sensitive to accurately establish the degree of oxidation. Therefore, in the present work, the degree of oxidation is expressed as the percentage: (moles of periodate/moles of alginate repeating units) × 100, used as feed ratio in the oxidation reaction.

Alginate was oxidized at a molar ratio of sodium periodate/repetitive units of alginate of 1/1 (AO_{100}) to assess the efficacy of the reaction conditions. The aldehyde band at 1725 cm^{-1} in the FTIR spectrum (Fig. 2) clearly confirms that oxidation had occurred.

The M_w of the alginate as obtained from the supplier and AO_0 (an alginate sample that has been exposed to the reaction conditions excluding sodium periodate) were the same, indicating that the polymer chains do not undergo scission under the experimental conditions. On the opposite, during the oxidation process, i.e. in the presence of periodate, the M_w of the polymer chains is reduced. Fig. 3a shows the decrease of M_w of alginate as a function of the molar feed ratio confirming similar previous findings described in the literature where polysaccharides chain scission has been associated with periodate oxidation reactions (Balakrishnan, Lesieur, Labarre, & Jayakrishnan, 2005; Kong, Kaigler, Kim, & Mooney, 2004; Kristiansen, Potthast, & Christensen, 2010).

The degradation of alginates oxidized at different extents was studied by incubating aqueous solutions of these polymers at 37 °C and at pH 7.4 (Fig. 3b). The M_w of AO_0 remained almost constant, confirming that the glycosidic bonds of alginate are not sensitive for hydrolysis under the experimental conditions. Instead, Fig. 3b clearly shows that the molecular weights of the oxidized alginates decreased because of the labile bonds in the main chains resulting from the oxidation reaction (Fig. 1). The M_w of AO_1 decreased from a 600 kDa to 290 kDa (decrease of ~50%) upon incubation for 30 days. The alginate with higher degree of oxidation was, as expected, more sensitive for hydrolysis; after 30 days of incubation the M_w of AO_{10} dropped from 290 to 75 kDa (decrease of ~75%).

Oxidation of alginate influences the capability of this polymer to form gels in the presence of bivalent ions (Bouhadir et al., 2001; Kristiansen, Schirmer, Aachmann, Skjåk-Bræk, Draget, & Christensen, 2009) because the gelling properties, as well as the elasticity of the formed gels, is strictly dependent on the presence of sufficiently large G-blocks to form the rigid "egg-box" structure.

In a previous paper we studied IPNs of alginate calcium and dex-HEMA that were formed at a molar ratio COO^-/Ca^{2+} of 10 (Pescosolido et al., 2011). We therefore studied the hydrogel forming properties of the different oxidized alginates at the same COO^-/Ca^{2+} ratio (Fig. 4a). The rheological properties of the hydrogel composed of alginate with the lowest degree of oxidation (AO_1–Ca) were similar to those of the non-oxidized alginate hydrogel (AO_0–Ca storage modulus of ~300 Pa, $\tan\delta$ ~0.2 at 1 Hz) indicating that this low oxidized alginate fully retained its gelling ability. On the other hand, the G′ of the AO_5–Ca alginate hydrogels was around a factor 100 lower than that of the AO_0–Ca and AO_1–Ca gels showing that this polymer lost to a great extent its gelling

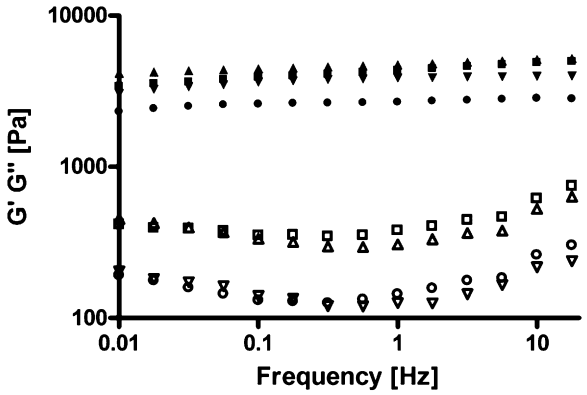

Fig. 5. Frequency sweeps of the IPNs based on (oxidized) alginates and dex-HEMA. The molar ratio COO^-/Ca^{2+} was 5/1, the dex-HEMA and alginate contents were 10% and 3%, respectively. (■) G′ and (□) G″ of AO_0–Ca/D; (▲) G′ and (△) G″ of AO_1–Ca/D; (●) G′ and (○) G″ of AO_5–Ca/D. For comparison also the frequency sweep of the dex-HEMA hydrogel is reported: (▼) G′ and (▽) G″ of D.

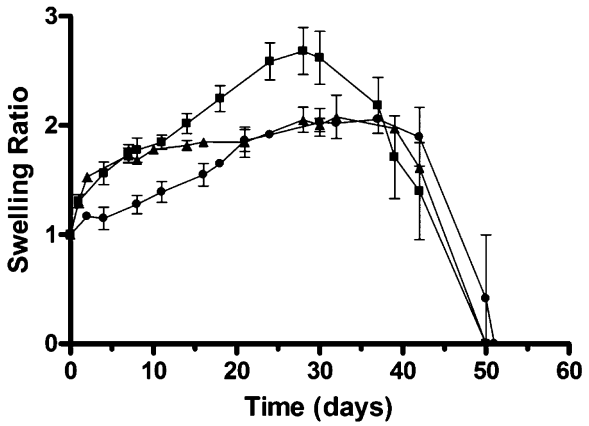

Fig. 6. Swelling/degradation of IPN hydrogels composed of (■) AO_0–Ca/D; (▲) AO_1–Ca/D; (●) AO_5–Ca/D in buffer at 37 °C, pH 7.4.

properties, but still a viscoelastic gel was formed (G′ > G″). On the opposite, the AO_{10}–Ca alginate was unable to form a gel (G′ < G″).

To improve the hydrogel mechanical properties, samples with higher Ca^{2+} content were prepared. Fig. 4b reports the rheological curves of the samples at Ca^{2+} molar ratio COO^-/Ca^{2+}: 5/1. As expected, the higher the Ca^{2+} concentration, the higher was the strength of the AO_0–Ca hydrogel (~3000 versus ~300 Pa). This can be explained by an increase of the "egg-box" content leading to a higher elasticity of the network. Also, the AO_1–Ca sample showed a typical curve of a strong hydrogel, although slightly shifted to lower moduli values compared to that of the AO_0–Ca sample which can be ascribed to its low extent of oxidation.

Fig. 4b also shows that the AO_5 sample is able to form a hydrogel (G′~10 Pa; G′ > G″ for all frequencies). On the contrary, the AO_{10}–Ca sample was still unable to form a gel. This inability to form hydrogel is maintained even at higher Ca^{2+} concentrations (up to molar ratio COO^-/Ca^{2+} of 1/1, rheological curves not shown). Thus "full gels" could still be obtained using alginate with an oxidation degree of up to 5%, confirming the results obtained by Kristiansen (Kristiansen et al., 2009). Because of their gel forming properties, the AO_1–Ca and AO_5–Ca alginates were used for the preparation of IPNs.

3.2. Rheological, degradation and protein release characteristics of alginate/dex-HEMA IPNs

One of the purposes of this work was to investigate the possibility to form an alg/dex-HEMA IPN using the oxidized alginates instead the native polymer, in order to obtain an IPN that degrades leaving low M_w alginate chains that potentially can be eliminated from the human body. Fig. 5 shows that it is indeed possible to form strong IPN hydrogels based on AO_1 and AO_5. The G′ of the gels is comparable with the G′ of the dex-HEMA hydrogel ((▼) D in Fig. 5) and it is almost independent of frequency. Further, G″ is about one order of magnitude smaller than G′ (tan delta ~0.1), confirming that fully elastic hydrogels are formed. The AO_5–Ca/D IPN shows lower G′ than those of the corresponding AO_0–Ca/D and AO_1–Ca/D gels likely due to the weakness of the AO_5–Ca gel (see Fig. 4b).

Swelling and degradation experiments on the IPN hydrogels were performed by incubating samples in HEPES buffer at 37 °C, at pH 7.4 (Fig. 6). All the studied IPNs showed a degradation time of ~50 days, irrespective of the alginate sample used confirming the results obtained in a previous study (Pescosolido et al., 2011), in which it was shown that the swelling and degradation behavior of the IPNs is governed by the chemical degradation of the dex-HEMA network. It is worth noticing that, in the present study, a higher Ca^{2+} concentration is adopted in IPNs formation, with respect to the previous systems. An increase of Ca^{2+} content results in an increase of the amount of "egg boxes" stacking, i.e. the lateral association of two or more "egg-boxes" segments (Grant, Morris, Rees, Smith, & Thom, 1973), thus modifying the network arrangement. Furthermore, both the reduction of the molecular weight and the increased flexibility of alginate chains due to the oxidation reaction increase the ability to form "egg box" stacking (Smidsrød & Painter, 1973). As a consequence, the water uptake by the IPNs is slowed down, shifting the maximum of the swelling toward longer times – from 15 days for the non-oxidized IPNs (Pescosolido et al., 2011) to 30 days for the AO_0–Ca/D. The swelling capability of the IPNs with oxidized alginate samples is also reduced, because of the increase of

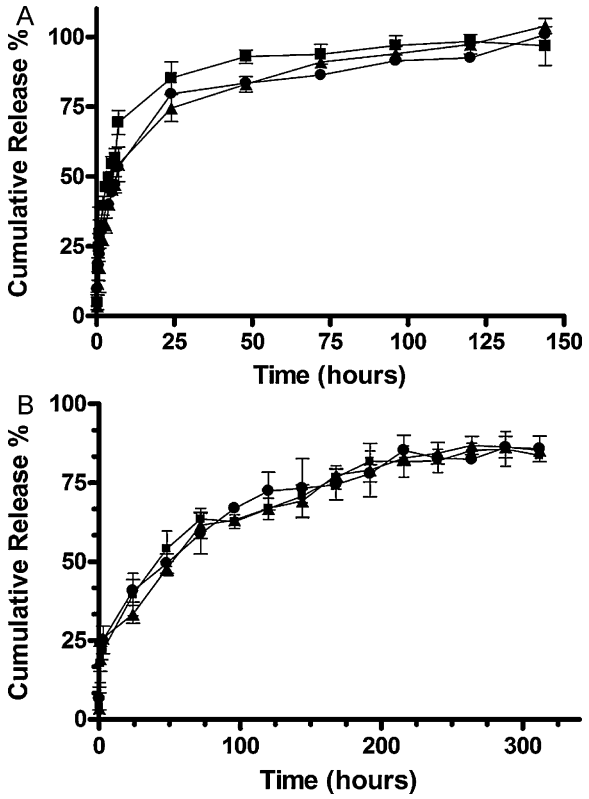

Fig. 7. (a) Cumulative release of myoglobin from the IPNs hydrogels in HEPES buffer 100 mM: (■) AO_0–Ca/D (▲) AO_1–Ca/D (●) AO_5–Ca/D. (b) Cumulative release of BSA from the IPN hydrogels in HEPES buffer 100 mM (■) AO_0–Ca/D (▲) AO_1–Ca/D (●) AO_5–Ca/D.

the network hydrophobicity due to the increase in the "egg boxes" stacking.

The ability of the IPNs to release proteins was evaluated using myoglobin (Fig. 7a) and BSA (Fig. 7b) as model proteins. It was observed that myoglobin and BSA were released in about 100 and 300 h, respectively. The faster release of myoglobin compared to BSA can be explained by the smaller size of the above mentioned proteins (radius is 2.1 nm and 3.6 nm for the myoglobin and BSA, respectively). Moreover, Fig. 7a and b shows that the release kinetics is independent of the IPNs polymer compositions. For the IPN containing AO_5, it can be calculated that the molar ratio of aldehyde groups in the network and lysine residues in BSA is around 1. In principle, the aldehyde groups present in the oxidized alginate chains can react by Schiff base formation with amines (lysine residues) of the proteins. This might results in immobilization of protein molecules in the network which in turn can slow down the release kinetics (when protein-network bonds are reversible) or to incomplete release (when irreversible bonds are formed). Fig. 7a and b shows that almost quantitative release of the proteins occurs evidencing that no permanent protein immobilization occurred. This finding is in accordance with expectations since Schiff bases are normally reversible (Hernández-Molina, Mederos, McCleverty, & Meyer, 2003). Moreover, the release kinetics of the proteins is independent of the degree of alginate oxidation, indicating that the formation and breaking of the Schiff base bonds is relatively fast compared to the diffusion of the protein in the gel.

4. Conclusions

This paper shows that alginate can be oxidized to such an extent that the resulting chains undergo hydrolysis under physiological conditions whereas their ability to form gels in the presence of Ca^{2+} ions was retained. The oxidized alginates, in combination with dex-HEMA, were able to form strong but still degradable IPNs. The IPNs showed a sustained and complete release of myoglobin and BSA. Based on these results, the IPNs described in this paper represent promising *in situ* forming biodegradable hydrogels suitable as protein delivery systems.

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