Carbohydrate Polymers 86 (2011) 1595–1601

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Periodate oxidized alginates: Depolymerization kinetics

Kåre A. Kristiansen, Henrik Berg Tomren [1], Bjørn E. Christensen *

NOBIPOL, Department of Biotechnology, Norwegian University of Science and Technology (NTNU), Sem Saelands veg 6/8, NO-7491 Trondheim, Norway

ARTICLE INFO

Article history:
Received 18 May 2011
Received in revised form 23 June 2011
Accepted 24 June 2011
Available online 2 July 2011

Keywords:
Alginate
Periodate oxidation
Degradation
β-Elimination
Biomaterial
Tissue engineering

ABSTRACT

Limited periodate oxidation (0–8%) of alginates results in enhanced degradability, extending the range of applications of alginates as biomaterials, e.g. in tissue engineering. Oxidation produces dialdehydes that are highly sensitive to alkaline β-elimination, even under physiological conditions (pH 7.4, 37 °C). Although all periodate is consumed during limited oxidation, not all of the resulting dialdehydes are equally degradable, leading to a characteristic level off in molecular weight upon prolonged degradation. A significant fraction (20–50%) of the oxidized residues were resistant even at pH 10.4. We tentatively assign this finding to the presence of particularly stable intramolecular hemiacetals known to form in periodate oxidized alginates (POA). The enhanced degradation of POA persists into the gel state, allowing tailoring of more biodegradable alginate gels. The activation energy for β-elimination of dialdehydes was 88 kJ/mol compared to 113 kJ/mol for unoxidized residues, presumably reflecting the absence of strain in the non-cyclic transition state of the former.

1. Introduction

Alginates are widely studied and applied as matrices in tissue engineering because of their mild gelling properties, biocompatibility and the possibility to tailor the biomaterial by accurately varying macromolecular parameters such as composition and molecular weight distribution (Augst, Kong, & Mooney, 2006; de Vos et al., 2009; Donati & Paoletti, 2009). Alginates are polysaccharides derived from brown algae, although recent advances point towards production also in bacteria (Remminghorst & Rehm, 2006). They are linear (unbranched) polysaccharides containing 1,4-linked β-D-mannuronic acid (M) and its 5-epimer 1,4-linked α-L-guluronic acid (G) (Fig. 1), and are often classified as binary copolymers (Draget & Taylor, 2011). However, the content and distribution of G residues is governed not by a classical (sequential) co-polymerisation, but by epimerisation at the polymer level of the transiently produced mannuronan. Alginates applied in tissue engineering have typically high G contents (F_G = 0.6–0.7) and long G-blocks to facilitate gelling with calcium ions.

Implanted biomaterials are in many cases designed to dissolve or disintegrate at a predetermined rate. Ca-alginate gels disintegrate by Ca–Na exchange (Shoichet, Li, White, & Winn, 1996) but the dissolved alginate chains are not degraded in the human body (Alshamkhani & Duncan, 1995), presumably due to the lack of alginate degrading enzymes in mammals. The biodegradability of alginate may be enhanced by limited periodate oxidation, taking advantage of the susceptibility of periodate oxidized polysaccharides to alkali-catalysed β-elimination, which takes place even at physiological pH (Bouhadir et al., 2001; Kristiansen, Potthast, & Christensen, 2010). In alginates, oxidation may occur between C2 and C3 in both M and G residues, leading to dialdehydes, which easily degrade in alkali (Fig. 1). The degree of oxidation needs to be low (1–5%) to avoid significantly reduced gelation ability (Gomez, Rinaudo, & Villar, 2007).

Some features of the reported degradation behaviour of periodate oxidized alginates have recently been questioned (Kristiansen et al., 2010). For example, the degradation kinetics (decrease in molecular weight) is not in agreement with the reported degree of oxidation. In their study, Bouhadir et al. (Bouhadir et al., 2001) observed a level-off of the degradation rate with increasing extent of degradation. This was attributed to a 'change of chain rigidity during degradation, observed as an increase in the MHS exponent'. A change in rigidity upon depolymerisation is consistent with the wormlike chain behaviour of alginates (Vold, Kristiansen, & Christensen, 2006). Additionally, selective consumption of the highly flexible oxidized resides due to selective β-elimination, will stiffen the molecules. It is, however, unclear how such macroscopic changes might influence the rate of β-elimination. In our opinion, a major contribution to the level-off behaviour could well be linked to the chemical properties of oxidized residues, which are known to form intramolecular hemiacetals (Painter & Larsen, 1970).

* Corresponding author. Tel.: +47 73593327; fax: +47 73591283.
E-mail address: b.christensen@biotech.ntnu.no (B.E. Christensen).
[1] Present address: FMC BioPolymer AS/NovaMatrix, Industriveien 33, NO-1337 Sandvika, Norway.

0144-8617/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2011.06.069

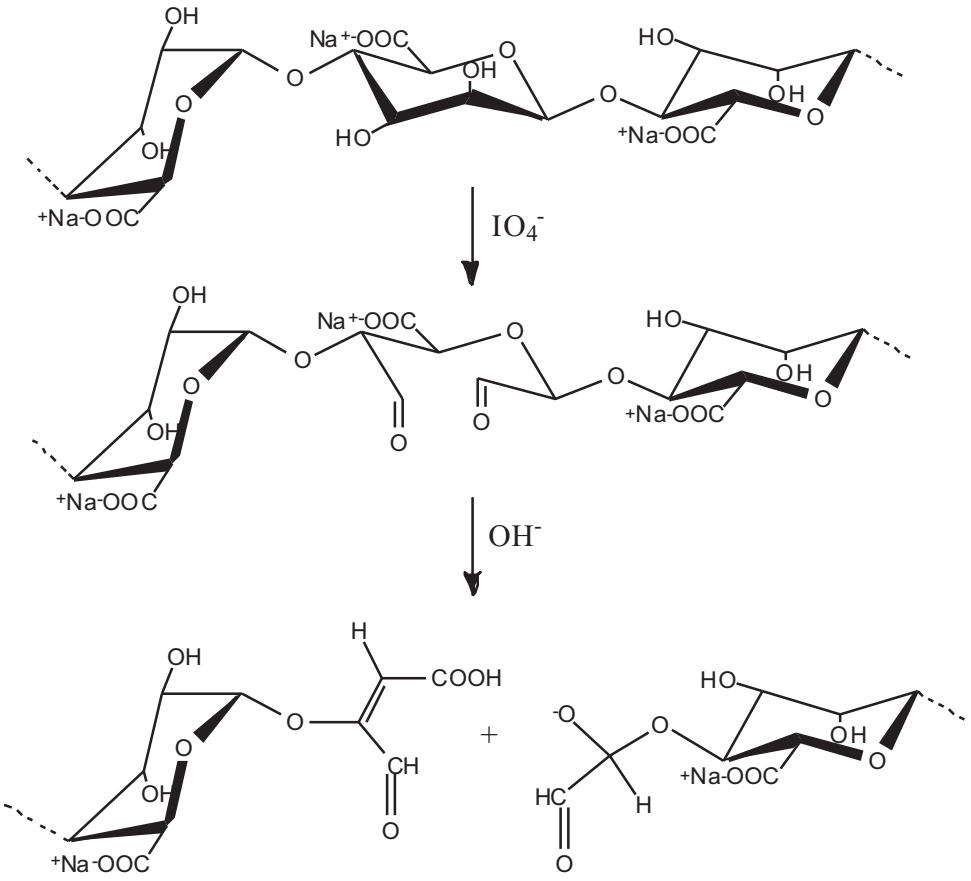

Fig. 1. The chemistry of periodate oxidation and subsequent alkaline β-elimination of alginate (. . .GMM. . . fragment shown). Both G (1,4-linked α-L-guluronic acid) and M (1,4-linked β-D-mannuronic acid) can be oxidized.

Here we present novel data for the degradation of periodate oxidized alginates (POA). Compared to previous work we extend the range of oxidation degrees, pH ranges, alginate types (including homopolymeric mannuronan), temperatures, and degradation times (molecular weight ranges). We also compare the degradation in solution compared to the gel state. We apply a simple kinetic model to determine the effective degree of oxidation. In total, the data provide new insights into the mechanism of periodate oxidation of alginates, and the degradation behaviour. We conclude that not all oxidized residues do form alkali-labile linkages.

2. Degradation of periodate oxidized polysaccharides

With the introduction of very labile bonds (by oxidation) we assume the degradation behaviour is determined by the amount of oxidized residues and the rate constants of oxidized (k_{ox}) and unoxidized (k_A) residues. We further assume the reaction is in both cases *pseudo* first order with respect to the reacting residues:

$$-\frac{dn_{ox}}{dt} = k_{ox}n_{ox} \quad \text{(oxidised residues)} \tag{1}$$

$$-\frac{dn_A}{dt} = k_A n_A \quad \text{(unoxidised residues)} \tag{2}$$

where n denotes the number of residues at a given instant (t). Separation of the variables and integration from $t = 0$ to t, and $n = n_0$ to n yields (suffixes omitted):

$$\frac{n}{n_0} = e^{-kt} \tag{3}$$

Thus, the number of remaining (uncleaved) linkages decreases exponentially. We further assume, as a first approximation, that the rate constants are independent of the chemical composition of the alginate, although the theory can easily be expanded to account for the M/G profile. It is convenient to use the fractions of chain cleavage (α) for each of the two processes in the calculations. It is further practical to introduce DP_n, the standard abbreviation for to the number average degree of polymerization, which is equivalent to the number average monomers per chain, rather than the molecular weight:

$$\alpha_{ox,t} = \frac{(n_{ox,0} - n_{ox,t})}{n_{ox,0}} = 1 - e^{-k't} \quad (k' = k_{ox}) \tag{4}$$

$$\alpha_{A,t} = \frac{(n_{A,0} - n_{A,t})}{n_{A,0}} = 1 - e^{-k''t} \quad (k'' = k_A) \tag{5}$$

$$\alpha_0 = \frac{1}{DP_{n,0}} \quad (DP_n \text{ at } t = 0) \tag{6}$$

where $n_{ox,0}$ and $n_{ox,t}$ refer to the number of remaining (uncleaved) oxidized residues at $t = 0$ and t, respectively, whereas $n_{A,0}$ and $n_{A,t}$ are the corresponding numbers of remaining unoxidized residues. The degree of oxidation (D_{ox}) is defined as the fraction of oxidized residues:

$$D_{ox} = \frac{n_{ox}}{n_{ox} + n_A} = \frac{n_{ox}}{n_0} \tag{7}$$

The total degree of chain scission (α_{tot}), which equals DP_n^{-1}, depends on the degree of oxidation and is simply:

$$\alpha_{tot} = \frac{1}{DP_{n,t}} = \alpha_0 + D_{ox}\alpha_{ox,t} + (1 - D_{ox})\alpha_{A,t}$$

$$= \frac{1}{DP_{n,0}} + D_{ox}(1 - e^{-k't}) + (1 - D_{ox})(1 - e^{-k''t}) \quad (8)$$

The term α_0 (=$1/DP_{n,0}$) must be added because of the finite DP at the start of the degradation. By measuring the change in DP_n during the degradation, the rate constants and D_{ox} can be determined by fitting the experimental data for different degrees of oxidation and different reaction times. If the reaction is dominated by cleavage of the oxidized residues ($k_{ox} \gg k_A$), which is often the case, the equation simplifies to:

$$\alpha_{tot} = \frac{1}{DP_{n,0}} + D_{ox}(1 - e^{-k't}) \quad (9)$$

For the initial part of the degradation ($t \to 0$) we can use the following approximation for the slope ($d\alpha/dt$) of Eq. (9):

$$\left(\frac{d\alpha}{dt}\right)_{t\to 0} = D_{ox}k_{ox} \quad (10)$$

For long degradation times ($t \to \infty$), the oxidized residues will be effectively consumed. The general equation then reduces to:

$$\frac{1}{DP_{n,t\to\infty}} = \frac{1}{DP_0} + D_{ox} + (1 - D_{ox})(1 - e^{-k''t}) \quad (11)$$

And if further $D_{ox} \gg 1/DP_{n,0}$, then

$$\frac{1}{DP_{n,t\to\infty}} = D_{ox} + (1 - D_{ox})(1 - e^{-k''t}) \quad (12)$$

Moreover, if k_A is sufficiently small, then $(1 - e^{-k''t}) \approx 0$ and consequently, $1/DP_{n,t}$ asymptotically approaches D_{ox}.

DP_n can normally be easily determined by size-exclusion chromatography with on-line multi-angle light scattering detection (SEC-MALLS). However, SEC-MALLS chromatograms of polysaccharides often show an upturn in M at high elution volumes. This complicates the data processing, and in particular influences the determination of M_n, and hence, DP_n. Since both starting materials and degraded samples have polydispersities (M_w/M_n) close to 2, we generally use the more accurate M_w values and the relation $M_n = M_w/2$ unless specifically stated.

3. Experimental

3.1. Materials and chemicals

Mannuronan and alginate from *L. hyperborea* and *L. digitata* were obtained from FMC Biopolymer, Drammen, Norway. Alginate containing 90% guluronic acid ($F_G = 0.90$) was obtained by epimerising mannuronan *in vitro* with the C-5 epimerase AlgE6 according to previous work (Kristiansen, Schirmer, et al., 2009). Pullulan was supplied by Hayashibara, Japan. All other chemicals were obtained from commercial sources and were of analytical grade.

3.2. Periodate oxidation

Periodate oxidation was performed as described previously (Kristiansen, Schirmer, et al., 2009; Vold et al., 2006). In brief, alginate or pullulan were dissolved in MQ-water (deionized water purified with the MilliQ system from Millipore (Bedford, MA, USA)) at a concentration of 8.89 mg/mL. MQ-water and 10% (v/v) *n*-propanol (free radical scavenger) were added before degassing with N_2. Finally, 0.25 M sodium meta-periodate (degassed) was added to obtain P_0 (moles of added periodate per mole AGU) of 0.02, 0.04 or 0.08. The final concentration of polysaccharide was usually 4.45 mg/mL. Reaction vials were kept in the dark at 4 °C, and left for 48–72 h until no residual periodate could be detected by titration with thiosulphate as described by Painter (1988). Kinetic experiments were performed as described above with $P_0 = 0.2$ and different concentrations of alginate (0.4–4 mg/ml) at 4 °C or ambient temperature.

3.3. Degradation experiments

McIlvaine buffer (Na_2HPO_4/citric acid) was used for degradation at pH 3.0 and 5.4, and carbonate buffer pH 10.3 or 10.4 for alkaline degradation. Polysaccharides were dissolved in MQ-water and buffer was added so that the ionic strength (I) equalled 0.10 M. The polysaccharide concentration was 3 mg/mL. In all cases samples were placed into the water bath immediately after addition of buffer/acid, and neutralized using dilute HCl or NaOH.

In some cases alkaline degradation at pH 10.4 (7.5 mg/mL in 0.0625 M carbonate buffer) took place in the autoinjector unit of the SEC-MALLS setup, followed by injection at regular intervals.

3.4. Preparation and degradation of Ca^{2+}-alginate/Ca^{2+}-POA gels

75 mg of alginate was dissolved in 5 mL MQ-grade water. $CaCO_3$ was added as solid powder, and distributed evenly in solution. Further, D-glucono-δ-lactone (GDL) was dissolved in 2.5 mL MQ-water, and added to the solution. Final concentrations were 10 mg/mL and 20 mM $CaCO_3$/40 mM GDL (Gel I) or 40 mM $CaCO_3$/80 mM GDL (Gel II). This solution was divided in equal amounts into three vials (three replicas). In cases where gelation took place almost instantly after the addition of GDL, alginate/$CaCO_3$ solution was distributed into vials before addition of GDL. In the last step of preparation, solutions were left for 48 h in order for the gel to set.

Degradation experiments were carried out by transferring the cylindrical gels (2.5 ml) to vials containing 25 mL of 0.1 M McIlvaine buffer pH 7.4 pre-heated to 60 °C. The vials were capped and placed in an oven at 60 °C. Following incubation (0–72 h), the gels were subsequently dissolved by adding EDTA to a final concentration of 0.1 M, transferred to dialysis membranes, and dialysed against MQ-water until the conductivity was <4 μS. Dissolution and dialysis were performed at 4 °C. Finally, samples were freeze dried, and analysed by SEC-MALLS as described below.

3.5. SEC-MALLS

Measurements were carried out at ambient temperature on an HPLC system consisting of a solvent reservoir, on-line degasser, HPLA isocratic pump, autoinjector, guard column, and three columns (serially connected) of TSK G-5000PWXL, 4000 PWXL, and 3000PWXL. In some experiments, only one or two of these columns were used. The column outlet was connected to a Dawn DSP or Heleos multiangle laser light scattering photometer (Wyatt, USA) ($\lambda_0 = 633/658$ nm) followed by Optilab DSP differential refractometer (P-10 cell). The mobile phase used was 0.05 M Na_2SO_4/0.01 M EDTA, pH 6. The flow rate was 0.5 mL/min, and injection volume 20–250 μL. Samples were filtered (pore size 0.45 μm) prior to injection. The refractive index increment (dn/dc) and the second virial coefficient (A_2) were set to 0.150 ml/g and 0.005 ml mol g^{-2}, respectively (Vold et al., 2006). Data from the light scattering detector and differential refractometer were collected, and processed using Astra (v. 5.3.4.14 or v.4.72) software (Wyatt, USA). In certain cases data were exported and further processed in an Excel spreadsheet.

4. Results

4.1. Kinetics of oxidation

The rate of oxidation is known to depend on the type of alginate (Painter & Larsen, 1970). The uptake of periodate (moles of periodate consumed per mol of alginate monomers, P_t) at room

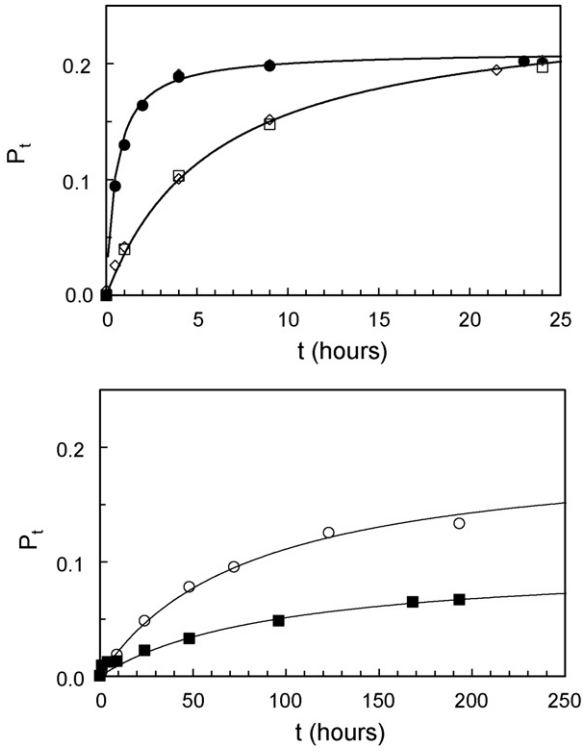

Fig. 2. Uptake of periodate (moles per alginate monomer) (P_t) at room temperature for alginate from *L. digitata* (♦, 4 mg/ml, ◊, 1 mg/ml), *L. hyperborea* stipe (●, 4 mg/ml) and mannuronan (□, 4 mg/ml, ○, 1 mg/ml, ■, 0.4 mg/ml). Lines are drawn as a 'guide to the eye'.

temperature for 20% oxidation ($P_0 = 0.2$) was therefore investigated for different alginates and different concentrations (Fig. 2).

Alginates from *L. digitata* ($F_G = 0.41$) and *L. hyperborea* stipe ($F_G = 0.66$) were both rapidly oxidized at 4 mg/ml, reaching the theoretical limit ($P_t = P_0$) in about 10 h. The data were overlapping for these alginates. At lower concentration (1 mg/ml) the rate of oxidation (dP_t/dt) was about 4 times lower (initially), reaching P_0 after about 25 h. This is in accordance with a first order kinetics (with respect to the concentration of alginate). Mannuronan was oxidized about 4 times slower than the *Laminaria* alginates at the same concentration (4 mg/ml) (data happened to overlap those of the latter at 1 mg/ml). Oxidation of mannuronan at 1 and 0.4 mg/ml was very slow (Fig. 2, lower panel), suggesting reaction kinetics of higher order. The rate of oxidation was about 2 times lower at 4 °C (data not shown), corresponding to an activation energy in the range 30 kJ/mol. Oxidation of *Laminaria* alginates with an excess of periodate ($P_0 = 1.2$, room temperature, 48 h) was stoichiometric up to $P_0 = 0.2$ (all added periodate consumed) (data not shown), but levelled off at higher values, probably reflecting the well-known oxidation limit (Larsen & Painter, 1969).

To ensure quantitative oxidation in subsequent work at low P_0 values (0.02–0.08) end titrations of residual periodate were performed. Generally, all periodate was consumed, suggesting the fraction of oxidized residues (dialdehydes) equalled P_0 (Kristiansen, Ballance, Potthast, & Christensen, 2009; Kristiansen, Schirmer, et al., 2009).

4.2. Degradation of partially oxidized alginates

Alginate from *L. hyperborea* ($F_G = 0.66$) was partially oxidized with periodate ($P_0 = 0$–0.08), and subjected to degradation at different pH values and temperatures. The degradation was monitored by SEC-MALLS. A typical example is showed in Fig. 3.

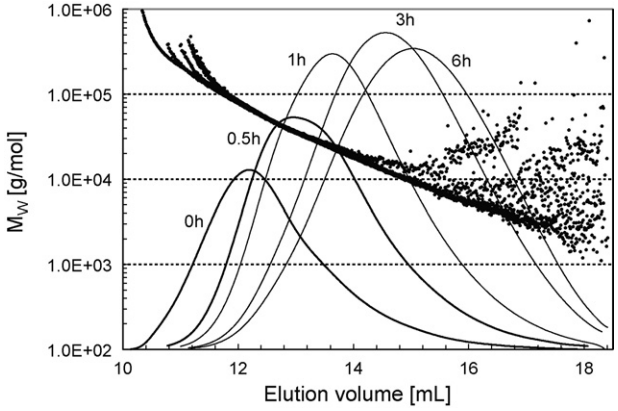

Fig. 3. SEC-MALLS data for alkaline degradation (pH 10.4, 37 °C) of periodate oxidized alginate ($P_0 = 0.02$).

Fig. 4a shows the kinetic plot for change in $1/M_w$ at pH 10.4 and 37 °C for different degrees of oxidation. Both Figs. 3 and 4a illustrate an initially rapid degradation of POA at high pH. Moreover, the initial degradation rate (Eq. (10)) was essentially proportional to the degree of oxidation. The results further demonstrate that the degradation levelled off and was essentially complete after about 8 h. The limiting M_w decreased with increasing P_0, but the values were much higher (roughly factor 1.6–2.5) than those predicted by

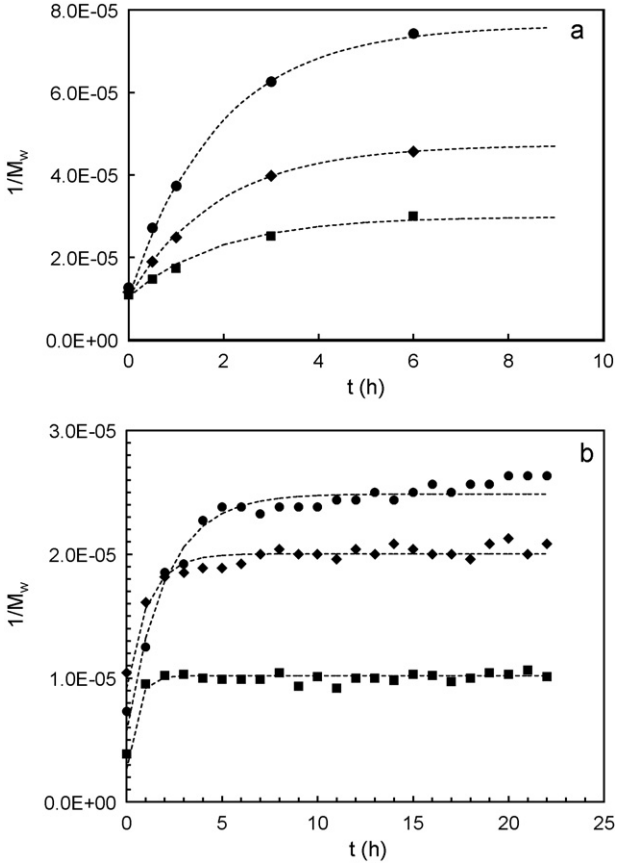

Fig. 4. (a) Experimental (symbols) and fitted (dotted lines) data for the degradation of periodate oxidized alginate (*L. hyperborea stipe*) at pH 10.4, 37 °C ($I = 0.1$ M). Symbols (degree of oxidation): (■) 2%, (♦) 4%, (●) 8%. (b) Experimental (symbols) and fitted (dotted lines) data for the degradation at pH 10.4, 37 °C of periodate oxidized (2%) alginates with different chemical compositions. Symbols: (■) mannuronan, (●) high-G alginate ($F_G = 0.90$) and (♦) *L. hyperborea stipe* ($F_G = 0.66$).

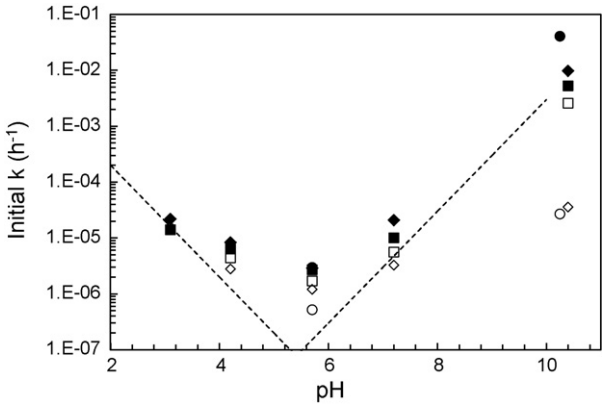

Fig. 5. pH-dependence of the initial rate of depolymerization (k) of periodate oxidized alginate. Oxidation degrees 0% (◊), 2% (□), 4% (■) and 8% (♦). Pullulan 0% (○) and 8% (●) oxidized was included for comparison. Dotted lines with slopes of −1 and +1, are included to illustrate a purely acid catalysed or a purely base catalysed process (see text).

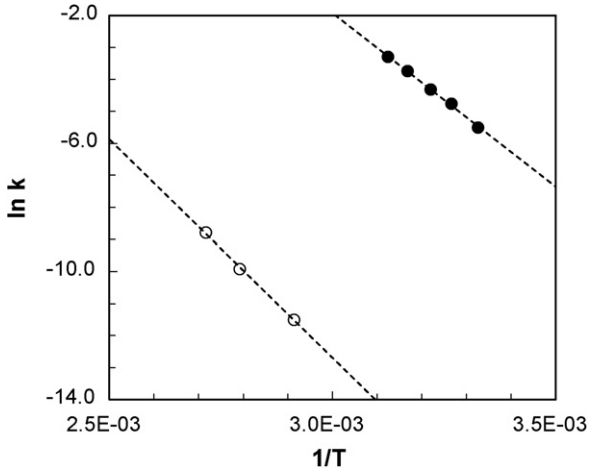

Fig. 6. Arrhenius plots for rate of depolymerisation (k) at pH 10.3 for 0% (○) and 8% (●) periodate oxidized alginates. The corresponding activation energies are 113 and 88 kJ/mol, respectively.

Eqs. (10)–(12) corresponding to quantitative cleavage of oxidized residues.

Since plots of M_w^{-1} versus t were markedly non-linear (Fig. 4a) and tended to approach a level-off value, the experiment (pH 10.4, 37 °C) was repeated with longer degradation times (Fig. 4b). We included three different alginates with different chemical compositions (F_G = 0, 0.66 and 0.90), all with P_0 = 0.02. In this case it is clearly seen that after an initial rapid degradation, little or no degradation takes place after 4–5 h. The level-off values also differ. For mannuronan (F_G = 0), M_w levels off at about 98.000 (DP_n = 248), whereas the high-G alginate and the alginate with F_G = 0.66 level off at an M_w of 38.000 (DP_n of 102) and 50.000 (DP_n of 126), respectively. The data may leave the impression that the extent of degradation depends primarily on the type of alginate. However, the initial DP values are very different in this case, and at such low degrees of oxidation (2%) the $DP_{n,0}$ terms markedly influences the level-off value (according to Eqs. (10) and (11)). Nevertheless, when fitting the data as described above, the fraction of cleaved linkages relative to P_0 were 15% for mannuronan, 38% for F_G = 0.90, and 22% for F_G = 0.66. Hence, there exists a certain dependence on the chemical composition, with a preference for β-elimination of oxidized G residues.

Degradation was further performed for pH values ranging from 3.1 to 10.4 (at 37 °C) for alginate (F_G = 0.66) with P_0 values of 0.02, 0.04 and 0.08. In addition, periodate oxidized pullulan (P_0 of 0 and 0.08) was included as a reference compound. Initial degradation rates were determined according to Eq. (10). Results are shown in Fig. 5.

Optimal stability was observed near pH 6, both for oxidized and unoxidized alginate. At lower pH, the difference between different oxidation degrees gradually decreased, and at pH 3.1 they were practically identical to unoxidized alginate. At high pH the rate of degradation increased rapidly, and became more dependent on P_0. Near physiological conditions (pH 7.2), the alginate with P_0 of 0.08 was degraded 6.4 times faster than the parent, unoxidized sample. At pH 10.4, the oxidized samples were degraded about two orders of magnitude faster than unoxidized alginate, and the relative rates were roughly proportional to P_0. Interestingly, periodate oxidized pullulan (8%) is degraded 2–3 times faster than alginate (8%), both at pH 5.8 and 10.4.

4.3. Activation energy

The activation energy (E_A) for the alkaline β-elimination of oxidized residues (P_0 = 0.08, pH 10.3) was determined according to the Arrhenius equation. The initial rate constant was obtained at different temperatures. Results (Arrhenius plots) are shown in Fig. 6. The corresponding activation energies were 113 and 88 kJ/mol for unoxidized and oxidized alginate, respectively.

4.4. Degradation in the gel state

The degradation of oxidized alginate in the gel state (Ca^{2+}-POA gels) at physiological pH (7.4) was investigated using homogeneous gels prepared using internal setting (Draget, Østgaard, & Smidsrød, 1991), as described in Section 3.4. Following degradation at 60 °C, the gels were dissolved by adding EDTA, and dialyzed to recover the sodium alginate, which was analysed by SEC-MALLS to obtain the molecular weights. The estimated degradation rates for depolymerisation of alginate are given in Table 1.

It was observed that the alginate itself is degraded much slower in the gel state than in solution. For POA the situation was different, and only small differences could be observed between degradation in solution and gel I, and also between gel I and the even more dense gel network in gel II.

5. Discussion

5.1. Oxidation pattern and mechanism

Periodate oxidized polysaccharides, including alginates, are well known to be susceptible to β-elimination. A low degree of oxidation (2–8%) allows a significant increase in the degradability under physiological conditions (about one order of magnitude for 8% oxidation). At the same time, limited oxidation still allows gel formation with calcium ions, although the gelling properties are influenced by the oxidation (Gomez et al., 2007). It may also be stressed that the mechanism of degradation is alkaline β-elimination, not hydrolysis as stated elsewhere (Bouhadir et al., 2001).

We present for the first time degradation data for POA covering a pH range from 3.1 to 10.4. Like for degradation of unoxidized alginates (Haug, Larsen, & Smidsrød, 1963), the optimum stability is found close to pH 6. On the acidic side the degradation rate becomes progressively less dependent on P_0, indicating that the oxidized residues are not more susceptible to acid hydrolysis than unoxidized residues. At pH 3 (and below) it is safe to assume that the major degradation mechanism is acid hydrolysis, where the rate of degradation becomes proportional to [H^+] (Fig. 5). The influ-

Table 1
Degradation rates at pH 7.4, 60 °C (I = 0.1 M) for periodate oxidized alginates in solution and in the gel state.

P_0	k ($\times 10^4$ h^{-1})		
	Solution	Gel I (20 mM $CaCO_3$/40 mM GDL)	Gel II (40 mM $CaCO_3$/80 mM GDL)
0	0.61	0.05	0.002
0.02	1.7	1.5	1
0.04	3.3	3	3

ence of alkaline β-elimination becomes influential even around pH 7, as also observed for degradation of alginate in the solid state (Holme, Lindmo, Kristiansen, & Smidsrød, 2003), and dominates completely at pH 10.4, where oxidized alginates are degraded two orders of magnitude faster than unoxidized alginate. According to Fig. 5, the rate constant is roughly proportional to [OH^-] at high pH, in accordance with an alkali-catalysed mechanism. Moreover, where β-elimination dominates (pH 10.4) the degradation rate is proportional to the degree of oxidation (Fig. 4a), as expected.

The plots of M_w^{-1} versus t are not linear for oxidized alginates (Fig. 4), as the rate of degradation decreases progressively, in qualitative agreement with other investigations (Bouhadir et al., 2001). As long as $k_{ox} \gg k_A$, a levelling-off DP corresponding to the degree of oxidation is expected. Such behaviour was observed upon prolonged degradation (Fig. 4b). However, the level-off values are much higher than the theoretical value P_0^{-1} (25 in this case), indicating that only a fraction (20–50%) of the oxidized residues are actually degraded by β-elimination. We have previously shown that for low degrees of oxidation the amount of dialdehydes equals the theoretical amount (P_0) (Kristiansen, Schirmer, et al., 2009). Thus, the results indicate that not all oxidized residues are equally susceptible to β-elimination, and that some turn out to be stable even at pH 10.4. This observation points towards the existence of several types of 'dialdehydes', some of which are relatively stable in alkali. The fact that periodate oxidized alginates do not exist in water as macromolecular dialdehydes, but tend to form intramolecular hemiacetals (Painter & Larsen, 1970), suggest that different hemiacetals have quite variable stabilities.

5.2. Activation energy

Our study provided accurate estimates of the activation energy for β-elimination of both unoxidized and oxidized alginates (113 and 88 kJ/mol, respectively). The former agrees well with estimates for degradation of alginate in the solid state, where β-elimination is a major process even near pH 7 (Holme et al., 2003). Our estimate for oxidized alginate is very different from a value of 37 kJ/mol reported previously (Bouhadir et al., 2001), although we note the substantial non-linearity on the underlying Arrhenius plot. The lower activation energy of oxidized residues seems reasonable in light of their non-cyclic transition state, avoiding the strain involved in producing an unsaturated linkage in a pyranose ring.

5.3. Degradation in the gel state

Degradation of unoxidized alginate chains were strongly retarded in the gel state, indicating that gelation with calcium directly influences the rate of chain degradation at pH 7.4. Since the degradation of alginate is marginally dependent on the ionic strength near pH 7, although strongly dependent near pH 11 (Haug, Larsen, & Smidsrød, 1967), the retardation is not an effect caused by different salt conditions. Due to the widespread use of calcium alginate gels as biomaterials this topic certainly deserves further attention. For oxidized alginates we observed a quite different behaviour, as the degradation rates were approximately the same in the gel state and solution state. In calcium alginate gels junction zones are formed mainly when consecutive guluronic acid residues (G-blocks) on different chains are coming close together, and their contact is mediated by Ca^{2+} ions. When gels are prepared of POA it is likely that the oxidized, and hence flexible, residues cannot be a part of a junction zone. This can to a certain extent explain the lower gel strength of POA gels along with a claimed faster gelling kinetics compared to gels made of unmodified alginate (Kristiansen, Schirmer, et al., 2009). In this way the oxidized residues are not bound tightly into the gel network, and are probably more accessible towards degradation than unoxidized residues. This could offer an explanation to why there is little difference in degradation rate between POA in solution, and POA gels. If this result is interpreted further it might also indicate a looser gel network for POA gels compared to (unoxidized) alginate gels, but further studies must be carried out in order to give more information about the topology of the gel network.

5.4. Conclusions

Limited periodate oxidation (0–20%) of alginates is quantitative as all periodate eventually becomes consumed. The reaction kinetics does not follow simple first- or second-order kinetics. The rate of reaction depends on the type of alginate, with homopolymeric mannuronan being oxidized about 4 times slower than an alginate with 66% guluronic acid. The oxidized residues are very susceptible to alkaline β-elimination, rendering oxidized alginates more degradable than unoxidized alginate under physiological conditions. The degradation pattern, monitored as decrease in molecular weight, deviated from a simple model assuming all oxidized residues being equally degradable. The level-off molecular weight observed suggested that a significant fraction (20–50%) of the oxidized residues were resistant even at pH 10.4. We tentatively assign this finding to the presence of particularly stable intramolecular hemiacetals known to form in periodate oxidized alginates. The enhanced degradation of POA persists into the gel state, allowing tailoring of more biodegradable alginate gels.

Acknowledgements

Eng. Ann-Sissel Ulset is thanked for technical assistance in the laboratory. This work was supported by the Research Council of Norway (Grant nos. 1459457/I20 and 146974/130).

References

Alshamkhani, A., & Duncan, R. (1995). Radioiodination of alginate via covalently-bound tyrosinamide allows monitoring of its fate in-vivo. *Journal of Bioactive and Compatible Polymers*, *10*(1), 4–13.

Augst, A. D., Kong, H. J., & Mooney, D. J. (2006). Alginate hydrogels as biomaterials. *Macromolecular Bioscience*, *6*(8), 623–633.

Bouhadir, K. H., Lee, K. Y., Alsberg, E., Damm, K. L., Anderson, K. W., & Mooney, D. J. (2001). Degradation of partially oxidized alginate and its potential application for tissue engineering. *Biotechnology Progress*, *17*(5), 945–950.

de Vos, P., Bucko, M., Gemeiner, P., Navrátil, M., Svitel, J., Faas, M., et al. (2009). Multiscale requirements for bioencapsulation in medicine and biotechnology. *Biomaterials*, *30*(13), 2559–2570.

Donati, I., & Paoletti, S. (2009). Material properties of alginates. In B. H. A. Rehm (Ed.), *Alginates: biology and applications* (pp. 1–53). Berlin/Heidelberg: Springer.

Draget, K. I., Østgaard, K., & Smidsrød, O. (1991). Homogeneous alginate gels: a technical approach. *Carbohydrate Polymers*, *14*, 159–178.

Draget, K. I., & Taylor, C. (2011). Chemical, physical and biological properties of alginates and their biomedical implications. *Food Hydrocolloids*, *25*, 251–256.

Gomez, C. G., Rinaudo, M., & Villar, M. A. (2007). Oxidation of sodium alginate and characterization of the oxidized derivatives. *Carbohydrate Polymers*, *67*(3), 296–304.

Haug, A., Larsen, B., & Smidsrød, O. (1963). The degradation of alginates at different pH values. *Acta Chemica Scandinavica*, *17*, 1466–1468.

Haug, A., Larsen, B., & Smidsrød, O. (1967). Alkaline degradation of alginate. *Acta Chemica Scandinavica*, *21*, 2859–2870.

Holme, H. K., Lindmo, K., Kristiansen, A., & Smidsrød, O. (2003). Thermal depolymerization of alginate in the solid state. *Carbohydrate Polymers*, *54*, 431–438.

Kristiansen, K. A., Ballance, S., Potthast, A., & Christensen, B. E. (2009). An evaluation of tritium and fluorescence labelling combined with multi-detector SEC for the detection of carbonyl groups in polysaccharides. *Carbohydrate Polymers*, *76*(2), 196–205.

Kristiansen, K. A., Potthast, A., & Christensen, B. E. (2010). Periodate oxidation of polysaccharides for modification of chemical and physical properties. *Carbohydrate Research*, *345*(10), 1264–1271.

Kristiansen, K. A., Schirmer, B. C., Aachmann, F. L., Skjak-Braek, G., Draget, K. I., & Christensen, B. E. (2009). Novel alginates prepared by independent control of chain stiffness and distribution of G-residues: structure and gelling properties. *Carbohydrate Polymers*, *77*(4), 725–735.

Larsen, B., & Painter, T. J. (1969). Periodate-oxidation limit of alginate. *Carbohydrate Research*, *10*(1), 186.

Painter, T. J. (1988). Control of depolymerisation during the preparation of reduced dialdehyde cellulose. *Carbohydrate Research*, *179*, 259–268.

Painter, T., & Larsen, B. (1970). Formation of hemiacetals between neighbouring hexuronic acid residues during periodate oxidation of alginate. *Acta Chemica Scandinavica*, *24*(3), 813.

Remminghorst, U., & Rehm, B. H. (2006). Bacterial alginates: from biosynthesis to applications. *Biotechnology letters*, *28*(21), 1701–1712.

Shoichet, M. S., Li, R. H., White, M. L., & Winn, S. R. (1996). Stability of hydrogels used in cell encapsulation: an in vitro comparison of alginate and agarose. *Biotechnology and Bioengineering*, *50*(4), 374–381.

Vold, I. M. N., Kristiansen, K. A., & Christensen, B. E. (2006). A study of the chain stiffness and extension of alginates, in vitro epimerized alginates, and periodate-oxidized alginates using size-exclusion chromatography combined with light scattering and viscosity detectors. *Biomacromolecules*, *7*(7), 2136–2146.