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Carbohydrate Polymers 54 (2003) 431–438
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Thermal depolymerization of alginate in the solid state

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Received 7 March 2003; accepted 4 April 2003

Abstract

The mechanism of thermal depolymerization of alginate in the solid state has been investigated. Depolymerization at elevated temperatures of two commercial highly purified alginates, one with high content of guluronic acid (G) and another with high content of mannuronic acid (M) was followed by measuring the apparent viscosity and the intrinsic viscosity. The initial rate constants were determined from the intrinsic viscosity data, and no significant difference between the G-rich ($F_G = 0.63$) and M-rich ($F_G = 0.43$) alginate was found. The activation energies of the G-rich alginate and the M-rich alginate were determined from the initial rate constants to be 114 ± 6 kJ/mol and 126 ± 12 kJ/mol, respectively.

The rate of depolymerization was not affected by the presence of oxygen, showing that the oxidative–reductive depolymerization mechanism is not responsible for the thermal depolymerization. The initial rate constants for alginates prepared by freeze-drying of solutions with pH between 3.8 and 9.5, were pH dependent. The depolymerization was found to be catalyzed simultaneously by protons and hydroxide ions. These catalytic effects were negligible between pH 5 and pH 8. The catalytic constants for the OH^- were identical for the M-rich and G-rich alginate. However, the catalytic constant for the H^+ was about 2 times greater for the M-rich alginate than for the G-rich alginate. This suggests that the M-rich alginate is more susceptible to acid hydrolysis than the G-rich alginate in the solid state, as also was found for alginate in solution when the mechanism of intramolecular acid catalysis prevailed. ^1H and ^{13}C NMR spectroscopy of the thermally degraded alginates were used to identify new non-reducing ends obtained from the depolymerization. New non-reducing ends from β-elimination caused by alkaline hydrolysis were clearly identified in the spectra obtained from alginates with pH between 4.3 and 9.5, when pH is defined as the pH of 1% (w/w) alginate solution prepared from a given freeze dried sample.

The results reported herein indicate that acid hydrolysis and alkaline hydrolysis are the primary mechanisms involved in the thermal depolymerization of highly purified alginate in the solid state.

Keywords: Depolymerization; Thermal degradation; Acid hydrolysis; β-Elimination; Activation energy; NMR spectroscopy

1. Introduction

Alginate is a linear binary copolymer consisting of (1 → 4)-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues. The relative amount of the two uronic acid monomers and their sequential arrangement along the polymer chain vary widely, depending on the origin of the alginate. The uronic acid residues are distributed along the polymer chain in a pattern of blocks, where homopolymeric blocks of G residues (G-blocks), homopolymeric blocks of M residues (M-blocks) and blocks with alternating sequence of M and G units (MG-blocks) co-exist (for a review see: Moe, Draget, Skjåk-Bræk, & Smidsrød, 1995).

Depolymerization of polysaccharides occurs via cleavage of the glycosidic bonds. The glycosidic linkages of alginates are susceptible to a variety of degradation mechanisms, including oxidative–reductive free radical depolymerization (ORD-reaction), acid-, alkaline- and enzymatic-catalyzed hydrolysis. The depolymerization of alginate in solution has been mostly described, in contrast to alginate in solid form. Smidsrød, Haug, and Larsen (1963) showed that the presence of oxygen affects the stability of non-purified alginates due to the presence of phenolic reducing substances which give rise to the ORD-reaction. Recently, thermal depolymerization of highly purified chitosan chloride in solid form was investigated (Holme, Foros, Pettersen, Dornish, & Smidsrød, 2001), and the rate of degradation was found to be independent on the presence

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doi:10.1016/S0144-8617(03)00134-6

of oxygen. The aim of this study was therefore to examine the thermal depolymerization of highly purified alginate as powder or freeze dried material, in order to get a better knowledge of the parameters affecting its stability and how to control a thermal depolymerization to obtain a desirable molecular weight or viscosity.

Oates and Ledward (1990) reported that alginate undergoes extensive decomposition when it is exposed to temperatures above 250 °C. In this study, alginates were thermally degraded at moderate temperatures (60–120 °C) to investigate depolymerization without decomposition taking place. Depolymerization was followed by measuring the apparent viscosity and the intrinsic viscosity. Depolymerization kinetics was analyzed using intrinsic viscosity data. The activation energies for the depolymerization of the alginates were determined to get information about possible depolymerization mechanisms. Furthermore, the influence of oxygen, H^+ and OH^- ions on the initial depolymerization rates was investigated. NMR spectroscopy was used to identify the new non-reducing ends in order to identify the mechanism responsible for the cleavage of the glycosidic bonds.

2. Materials and methods

2.1. Alginates

Ultra pure sodium alginates (PRONOVA™ UP) with high content of guluronic acid (G), isolated from *Laminaria hyperborea*, and with high content of mannuronic acid (M), isolated from *Macrocystis pyrifera*, were manufactured by FMC BioPolymer AS (Oslo, Norway). The fraction of G-units, F_G, was determined by 400 MHz proton NMR spectroscopy using the assignments published by Grasdalen (1983) and Grasdalen, Larsen, and Smidsrød (1979). The intrinsic viscosities were determined as described by Draget, Vårum, Moen, Gynhild, and Smidsrød (1992). The G-rich alginate, PRONOVA™ UP G, had a guluronic acid content of 63 % ($F_G = 0.63$) and intrinsic viscosity of 850 ml/g. The M-rich alginate, PRONOVA™ UP M, had a guluronic acid content of 43 % ($F_G = 0.43$) and intrinsic viscosity of 710 ml/g. The pH values of 1(w/w) % solutions of the alginates were 6.6 ± 0.1.

2.2. Thermal depolymerization

The thermal depolymerization experiments were performed in a drying oven held at a constant temperature of 120, 105, 80 or 60 °C. A sufficient amount of alginate powder was used to ensure that removal of alginate samples did not considerably affect the ratio of alginate volume to container volume. Also, the container was more than ten times the volume of the alginate powder, to make certain that a sufficient amount of oxygen was present. Samples were removed at various time intervals and stored in airtight flasks at −18 °C before characterization. Apparent viscosities of all samples were determined, and intrinsic viscosities of selected samples were measured to determine the rate constant of depolymerization at each temperature.

In the experiment used to study the effect of oxygen on depolymerization, the alginate powder was placed in a tight steel container that was part of an apparatus that ensured extreme hypoxic conditions (<4 ppm O_2) (Pettersen, Oftebro, & Brustad, 1973). Thermal depolymerization of alginate was followed at hypoxic conditions at 105 °C with continuous flow of nitrogen gas (99,9999%). As a control experiment the same apparatus and temperature was used, with flowing of air trough the system. Intrinsic viscosities were determined for samples taken at selected time-intervals.

The G- and M-rich alginate with different pH-values were prepared to study the influence of pH on the rate of depolymerization. Alginates with pH values of 3.8, 4.3, 5.2 and 5.9 were produced by adjusting a 1 % (w/w) solution to the correct pH-value with HCl or NaOH, followed by freeze-drying. Alginates with pH 6.6 were made by freeze-drying 1% (w/w) solutions of the alginates. Alginates with pH 8.0 and 9.5 were made in phosphate buffer (pH 8, 0.063 M Na_2HPO_4/0.0035 M NaH_2PO_4) and carbonate buffer (pH 9.5, 0.046 M $NaHCO_3$/0.0040 M Na_2CO_3) solution before freeze-drying. The freeze-dried alginate powder was depolymerized at 105 °C and samples were taken at selected time intervals to determine the rate constant of depolymerization at the different pH-values. Alginate samples containing buffer were dialyzed and freeze-dried and the remaining samples were adjusted to neutrality during dissolution of the sample prior to analyzing.

2.3. Viscometry

Apparent viscosity of 1 (w/w)% alginate solutions was measured at 20 °C using a Brookfield Digital Rheometer with spindle rotation set to 20 rpm. The intrinsic viscosities, $[\eta]$, were determined in a Schott-Geräte Ubbelohde viscometer as described by Draget et al., 1992. All alginate solutions were filtered through 0.8 μm Millipore filters prior to determination of intrinsic viscosity.

2.4. Molecular weight determination

The weight average molecular weight, M_w, and the distribution of the molecular weights in the alginate samples were determined by Size Exclusion Chromatography with Multiple Angle Laser light Scattering (SEC-MALS). TSK guard column was used in series with 3 TSK gel PW_{XL} columns (G6000 PW_{XL} G5000 PW_{XL} and G3000 PW_{XL}). The mobile phase was 0.05 M Na_2SO_4/0.01 M EDTA at a flow rate of 0.5 ml/min. Absolute concentrations of alginate at discrete intervals were determined using a calibrated Waters 2410 refractive index detector, and the corresponding

absolute molecular weights were determined using a DAWN DSP multiple angle laser light scattering detector (Wyatt Technology Corporation). The raw data were collected and processed to determine M_w and the number average molecular weight, M_n, using Astra software from Wyatt Technology Corp. The molecular weight distribution was characterized by the polydispersity index, M_w/M_n.

2.5. Determination of the rate of thermal depolymerization and activation energy

A random depolymerization of a single stranded polymer obeys the following equation (Tanford, 1961):

$$\frac{1}{\overline{DP}_{n,t}} = \frac{1}{\overline{DP}_{n,0}} + kt \quad (1)$$

where $\overline{DP}_{n,t}$ and $\overline{DP}_{n,0}$ are number average degrees of polymerization, at times t and 0, respectively, and k is the rate constant for bond cleavage. For a linear single stranded polymer such as alginate, combination of Eq. (1) and Mark–Houwink–Sakurada (MHS) equation yields:

$$\frac{1}{[\eta]^{1/a}} - \frac{1}{[\eta]_0^{1/a}} = \Delta 1/[\eta]^{1/a} = k't \quad (2)$$

where $k' = k/(2M_0K^{1/a})$. M_0 is the molecular weight of a single monomer residue and $[\eta]$ and $[\eta]_0$ are intrinsic viscosities at time t and time 0, respectively. K and a are constants from the MHS equation $[\eta] = KM^a$. The MHS-constants were determined specifically by measuring intrinsic viscosity and weight average molecular weight for selected depolymerized samples of the G-and M-rich alginates. The MHS-constants determined are given in Table 1. The rate constant, k' was found by plotting $\Delta 1/[\eta]^{1/a}$ as a function of t and converted to k through the equation above.

The rate constant for depolymerization, k, can be used to find the activation energy by taking the natural logarithm of the Arrhenius' equation:

$$\ln k = \ln A - E_a/RT \quad (3)$$

where E_a is the activation energy, R the gas constant, A the frequency factor and T the absolute temperature. A plot of $\ln k$ as a function of $1/T$ gives a slope equal to $-E_a/R$.

2.6. Nuclear magnetic resonance (NMR) spectroscopy

Alginate with various pH values was depolymerized at 120 °C to a number average degree of polymerization, $\overline{DP}_n < 30$, prior to characterization by ^{1}H and ^{13}C NMR, using a 400 MHz Varian Mercury BB NMR spectrometer (Varian Inc.). Triethylenetetramine hexaacetic acid (TTHA) was used in the NMR tube as chelator to prevent traces of divalent cations from interacting with the alginate. New reducing and non-reducing ends were identified using the assignments published by Grasdalen, Larsen, and Smidsrød (1981) and Heyraud et al. (1996).

^{1}H NMR spectroscopy was performed at 80 °C using 90° pulse angles and a repetition time of 6 s. 64 transients were collected over 4000 Hz (10 ppm) with 32k data points. ^{1}H chemical shifts were measured relatively to TMSP at −0.01 ppm (pH 5.5) according to recommendations by Wishart et al. (1995).

^{13}C NMR spectroscopy was performed at 80 °C with full ^{1}H decoupling at 100.59 MHz using 90° pulse angles with a repetition time of 2 s. 64k data points were collected over 21,000 Hz (210 ppm) with approx. 30,000 transients. ^{13}C chemical shifts were measured relatively to C1 of G-blocks at 101.84 ppm, in accordance with Heyraud et al. (1996). The experimental conditions for ^{13}C NMR may not be quantitative with respect to ^{13}C end signals, which typically have longer $T1$ relaxation times than carbons within the polymer chain.

Table 1
MHS constants of PRONOVA™ UP alginates

Alginate	K (ml mol/g^2)	a
PRONOVA™ UP M ($F_G = 0.43$)	1.4×10^{-2}	0.92
PRONOVA™ UP G ($F_G = 0.63$)	1.1×10^{-2}	0.93

3. Results and discussion

3.1. Depolymerization rates and activation energies

The thermal degradation of M-rich and G-rich alginates was followed by viscometry at four temperatures. In Fig. 1, the apparent viscosity of 1% solutions of the thermally degraded alginates with $F_G = 0.43$ and $F_G = 0.63$ is plotted as a function of the degradation time. The figure shows that the viscosity decreases nearly as a hyperbola by time. The same trends of viscosity versus depolymerization time were reported on chitosan salt by Holme et al. (2001), and is due to the fact that a fixed number of chain breaks in a population of long molecules leads to a relative decrease in viscosity which is larger than what is obtained for the same number of breaks in a population of shorter chains. The rate of depolymerization of the alginates increased, as expected, with increasing temperature. As can be seen from Fig. 1, the time courses of the depolymerization of the M-rich and G-rich alginates are similar.

The initial depolymerization rate constants for the M-rich and G-rich alginates at four temperatures were obtained from the plots of $\Delta 1/[\eta]^{1/a}$ versus time, and are given in Table 2. The pH of 1% solution of the alginates was 6.6 ± 0.1. At temperatures of 80, 105 and 120 °C, the initial rates of the M-rich alginate was around 1.5 times that of the G-rich alginates. This may be due to the effect of a higher value of the MHS parameter K used for the M-rich alginate. The difference in the K constant (given in Table 1) between the two alginates was found to be within the experimental

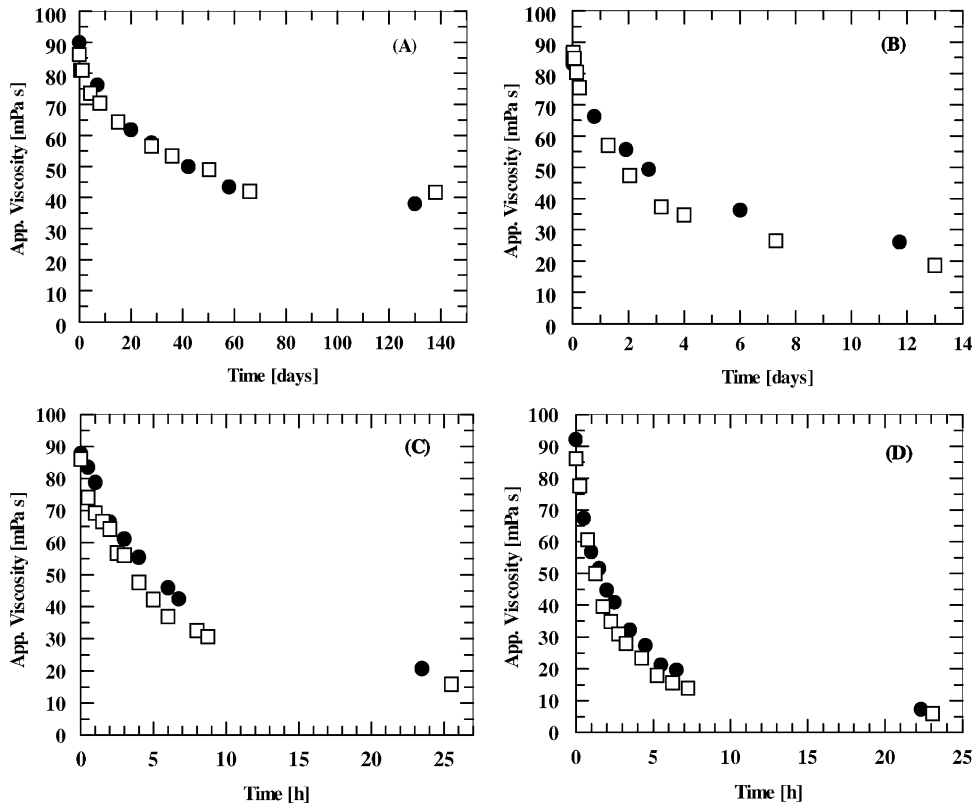

Fig. 1. Apparent viscosity of 1% solutions of thermally degraded alginate with (□) $F_G = 0.43$ and (●) $F_G = 0.63$ versus depolymerization time at (A) 60, (B) 80, (C) 105 and (D) 120 °C. The depolymerization time for (A) and (B) is given as days, while for (C) and (D) it is given as hours.

errors. Consequently, there is no significant difference in the thermal stability of the M-rich and the G-rich alginate samples (pH 6.6 in 1% solution).

The Arrhenius plots were linear, indicating that the depolymerization mechanism is the same within the temperature interval tested (60–120 °C). The E_a values for the two alginates are identical within the experimental errors (Table 3) with an average value of 120 kJ/mol, indicating that the depolymerization mechanism is the same for both samples. The use of *a* and *K* values determined by others (Martinsen, Sjåk-Bræk, Smidsrød, Zanetti, & Paoletti, 1991) in the calculations was found not to influence the value of the activation energy. The activation energy for the depolymerization of aqueous solutions of alginate was determined to 29 kcal/mol for acid hydrolysis (Smidsrød et al., 1963), 27 kcal/mol for alkaline hydrolysis/β-elimination (Haug, 1964) and for ORD 7 kcal/mol (presence of hydroquinon) and 19 kcal/mol (presence of ascorbate) (Smidsrød et al., 1963). These values correspond to 122 kJ/mol for acid hydrolysis, 113 kJ/mol for β-elimination and 29–80 kJ/mol for ORD mechanism. The activation energy found for the thermal depolymerization of chitosan chloride by Holme et al. (2001) was 109–114 kJ/mol where the primary mechanism was found to be acid hydrolysis. The activation energies determined here for the highly purified alginates samples suggest that acid- and/or alkaline catalyzed hydrolysis are the primary mechanisms for the depolymerization.

Table 2
Initial depolymerization rates (*k*) of the M-rich and G-rich alginate at four temperatures

Temperature (°C)	Initial depolymerization rate (10^{-6} h^{-1})	
	$F_G = 0.43$	$F_G = 0.63$
60	0.41	0.62
80	13	9.3
105	150	100
120	460	320

3.2. Depolymerization mechanism

Potential mechanisms for temperature-induced depolymerization of alginate are oxidative–reductive degradation (ORD) and acid- and alkaline catalyzed hydrolysis. Molecular oxygen is an active ingredient for ORD, and is

Table 3
Activation energy (E_a) of the M-rich alginate ($F_G = 0.43$) and G-rich alginate ($F_G = 0.63$)

F_G	Activation energy, E_a (kJ/mol)
0.43	126 ± 12
0.63	114 ± 6

present during the thermal degradation in this study. Protonation of the glycosidic oxygen is the first step of an acid hydrolysis. The catalytic protons may be present in the water contained in the samples (Holme et al., 2001), and the carboxyl group of alginate may also act as a proton donor in the catalysis (Smidsrød, Haug, & Larsen, 1966). Catalytic hydroxyl ions may also be present in the water contained in the samples, leading to alkaline hydrolysis (Haug, Larsen, & Smidsrød, 1967).

The depolymerization of alginate by ORD has been studied in detail by Smidsrød et al. (1963) and Smidsrød, Haug, and Larsen (1965). ORD involves a series of free radical reactions which ultimately leads to chain scission. Autooxidable compounds like ascorbate, sulphites and phenols may initiate ORD, and transition metal ions act as catalysts. Therefore, optimum stability with respect to ORD should be obtained for highly purified alginates with low content of phenols and transition metal ions. To confirm this, thermal degradation was performed in the presence and in the absence of oxygen. Fig. 2 shows $\Delta 1/[\eta]^{1/a}$ plotted against the time of degradation of M-rich alginate ($F_G = 0.43$) carried out in air and in a nitrogen atmosphere at 105 °C. The results demonstrate that the removal of oxygen does not affect the thermal depolymerization of the alginates, showing that the ORD mechanism is not responsible for the depolymerization.

Oates and Ledward (1990) have reported a much greater depolymerization rate of a M-rich alginate than a G-rich alginate in the solid state. The pH of 1% solution of the samples was not given, but is supposed to be around neutrality. In their study, the M-rich alginate was obtained from *Ascophyllum nodosum* and the G-rich alginate from *Laminaria hyperborea*. The difference in depolymerization rates of M-rich and G-rich alginates observed by Oates and Ledward (1990), which is not seen in the study herein, may be due to an ORD mechanism because of the presence of varying amounts of reducing phenolic compounds in not highly purified alginates. *Ascophyllum nodosum* is known to have a much higher content of phenols than *Laminaria hyperborea* (Smidsrød et al., 1963).

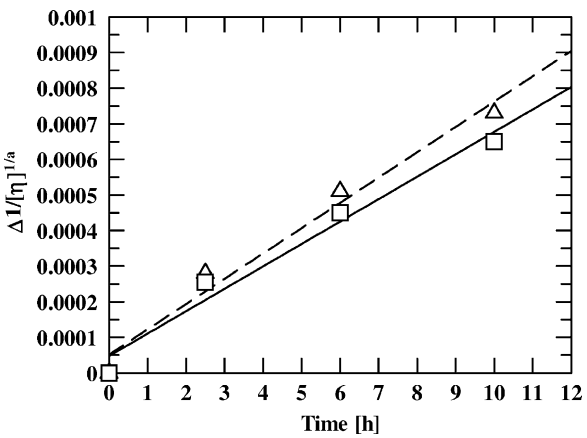

Fig. 2. Time course of thermal depolymerization of M-rich alginate ($F_G = 0.43$) at 105 °C carried out in (–△–) nitrogen atmosphere and in (-□-) air.

The other mechanism proposed for thermal depolymerization of alginate is acid hydrolysis. Data reported by Smidsrød et al. (1966) show that below circa pH 1, the depolymerization rate is directly proportional to the concentration of protons. However, at pH-values near the pK_a value of alginates, the depolymerization is less dependent on the pH. In this range the protonated (–COOH) form of M and G contributes as a proton donor to an intramolecular catalysis, and the rate of depolymerization is approximately 4 times higher for M-blocks than for G-blocks (Smidsrød, Larsen, Painter, & Haug, 1969). Smidsrød et al. (1969) have also shown that the non-reducing ends after an acid hydrolysis is dominated by the M-residue. To further investigate the role of acid hydrolysis in thermal degradation, the influence of pH of the alginates was studied. The M-rich and G-rich alginates were dissolved in deionized water and the pH was adjusted to 3.8, 4.3, 5.2, 5.9 and 6.6 before the samples were lyophilized by freeze drying and thermally degraded at 105 °C. The initial depolymerization rate constants for the alginates at pH 5.2 and 6.6 were nearly the same, showing no pH dependence. But the initial depolymerization rate constant for the alginates at pH 3.8 was around 5 and 8 times greater than at pH 5.2 for the G-rich and M-rich alginate, respectively. These results are showing pH dependence below pH 5, and are also confirming the data reported by Smidsrød et al. (1969) that the M-rich alginate is more susceptible for acid hydrolysis around the pK_a value of the alginates than the G-rich alginate. Consequently, the acid hydrolysis seems to be involved in the depolymerization.

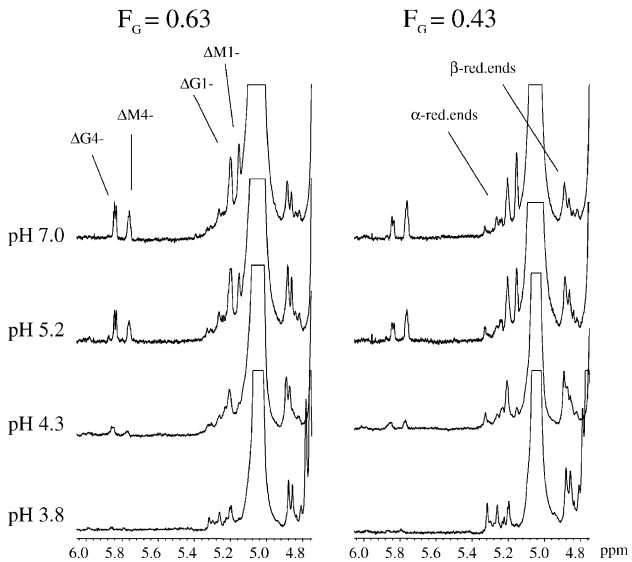

Fig. 3. ^{1}H NMR spectra of thermal degraded M-rich alginate ($F_{G=}$0.43) and G-rich alginate ($F_{G=}$0.63) at various pH-values. The most relevant signals are pointed out. △ refers to the unsaturated non-reducing end from the β-elimination reaction. G and M refer to the neighbour unit of the △. The numbers refer to the carbon atom.

Thermally degraded samples at the different pH-values were analyzed by ^{1}H NMR to study the end groups obtained from the depolymerization. Fig. 3 shows a part of the ^{1}H NMR spectra of thermal degraded M-rich alginate ($F_G = 0.43$) and G-rich alginate ($F_G = 0.63$) at different pH. The ^{1}H NMR assignments of the reducing ends and non-reducing ends obtained from both acid hydrolysis and alkaline hydrolysis (β-elimination) are given by Heyraud et al. (1996). All the spectra except the two spectra from alginates at pH 3.8, display unsaturated non-reducing ends from a β-elimination at 5.7 ppm (ΔM4-) and 5.8 ppm (ΔG4-). These results indicate that also β-elimination, caused by alkaline hydrolysis, is involved in the thermal depolymerization of the alginates. It is notable that β-elimination is presented at acidic pH down to pH 4.3. However, this is in agreement to the studies done by Haug et al. (1963) who found β-elimination in alginate solutions at pH 4.5.

Furthermore, alginate samples at higher pH was prepared and investigated in the same way as the samples at lower pH to find the role of alkaline hydrolysis in the thermal degradation. Fig. 4 shows the initial depolymerization rates of M-rich alginate ($F_G = 0.43$) and G-rich alginate ($F_G = 0.63$) at 105 °C plotted against the pH of 1% (w/w) solution of the alginates. As can clearly be seen from the figure, the depolymerization is affected by both protons and hydroxide ions, which is in agreement with the suggestions from the activation energy results. The catalytic effect is negligible between pH 5 and 8. One may express the first order rate coefficient equation given as follows:

$$k = k_0 + k_{H+}[H^+] + k_{OH-}[OH^-]$$

where k_0 is the rate constant of the spontaneous reaction, and k_{H+} and k_{OH-} are the catalytic rate constants for H^+ and OH^-, respectively. The experimental data given as points in Fig. 4 were fitted to the first order rate coefficient equation given above, and the fitted lines are shown as a whole line for the G-rich alginate and a dotted line for the M-rich alginate. The calculated rate constants are given in Table 4. As can be seen from Table 4, the catalytic constants for

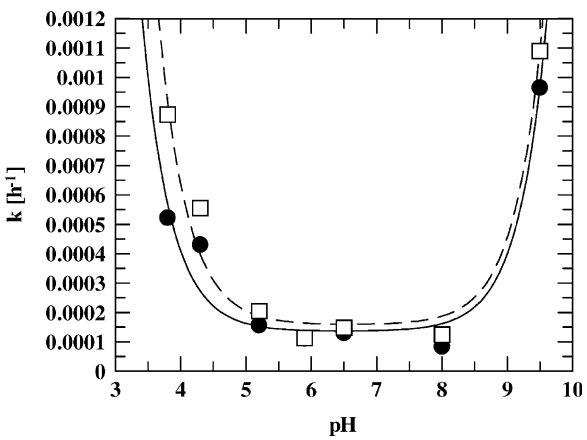

Fig. 4. Initial depolymerization rates versus pH for M-rich alginate (□) ($F_G = 0.43$) and G-rich alginate (●) ($F_G = 0.63$) at 105 °C.

Table 4
k_0 (rate constant of the spontaneous reaction) and k_{H+} and k_{OH-} (catalytic constants) of the thermal depolymerization of the M-rich alginate ($F_G = 0.43$) and G-rich alginate ($F_G = 0.63$)

Rate constants	PRONOVA™ UP M ($F_G = 0.43$)	PRONOVA™ UP G ($F_G = 0.63$)
k_0 (h^{-1})	$1.6 \times 10^{-4} \pm 0.4 \times 10^{-4}$	$1.4 \times 10^{-4} \pm 0.5 \times 10^{-4}$
k_{H+} ($l\ mol^{-1}\ h^{-1}$)	4.8 ± 0.6	2.8 ± 0.7
k_{OH-} ($l\ mol^{-1}\ h^{-1}$)	29 ± 3	26 ± 3

the OH^--catalyzed reaction are identical within the experimental errors suggesting that the β-elimination reaction occurs without any preference between the two monomeric residues. The catalytic constants, k_{H+}, for the M-rich alginate is around 2 times greater than the k_{H+} for the G-rich alginate. These data suggest that there is specificity for chain cleavage near a M-residue in agreement with the specificity found for intramolecular acid hydrolysis of alginate solutions (Smidsrød, 1969). However, if the intramolecular acid hydrolysis is presented in the thermal depolymerization of solid alginate, only a slight pH dependence of the degradation rate should be seen between pH 1 and pH 3. This is not shown in Fig. 4 which is only based on experimental data above pH 3, and in addition the catalytic effect of COOH is not included in the first order rate coefficient equation used for the data fittings.

The spontaneous rate constant k_0 obtained from the intermediate region between pH 5 and 8, may be explained by the water molecule acting as both acid (proton donor) and base (proton acceptor). In both acid hydrolysis and β-elimination mechanisms the catalysis involves the transfer of a proton or hydroxide ion to or from the substrate. It is therefore expected that acids and bases other than H^+ and OH^- (e.g. H_2O) may affect the catalysis, which is then called a general acid–base catalysis (Laidler & Meiser, 1982).

The resolution of the reducing ends in the ^{1}H NMR spectra was not good enough for determination of potential specificity on this unit when the glycosidic bond is cleaved. In addition reducing ends obtained from thermal depolymerization are very unstable and may react further (Oates & Ledward, 1990), and may therefore not give the true picture of the specificity for the depolymerization.

Fig. 5 shows a part of the ^{13}C NMR spectra (carbon 5 on G-residue) of thermally degraded M-rich alginate ($F_G = 0.43$) at various pH-values. The non-reducing ends resulting from the β-elimination and assigned by Heyraud et al. (1996) to be at 67.90 ppm (ΔM2) and 68.05 ppm (ΔG2) can clearly be seen on all the spectra, except the alginates at pH 3.8. The non reducing ends resulting from the acid hydrolysis and assigned by Heyraud et al. (1996) to be at 69.70 ppm (M2) and 69.42 ppm (G5) can clearly be seen, except the alginates at pH 9.5. This investigation is in agreement with the simultaneously effect of acid hydrolysis and β-elimination mechanism on the thermal depolymerization. In addition, Fig. 5 shows a slight increase of the ratio between M- and

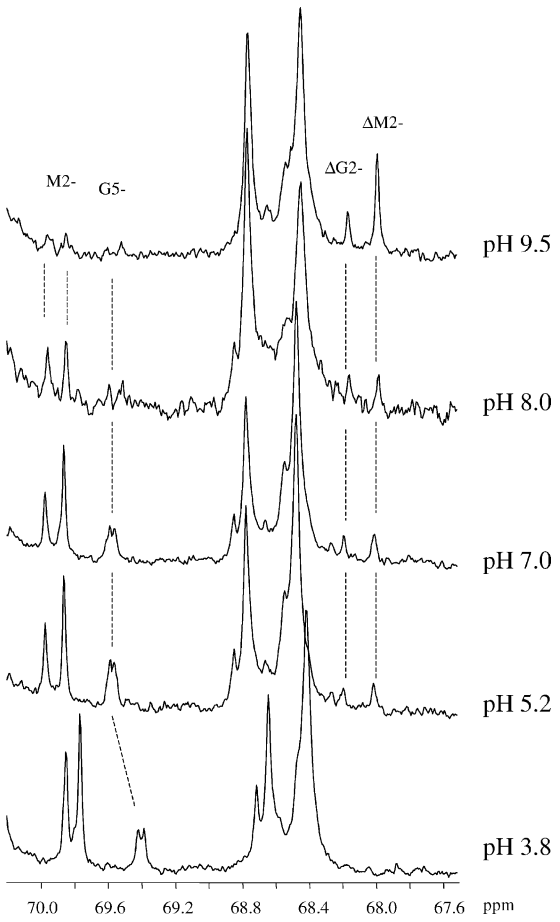

Fig. 5. ^{13}C NMR spectra of thermal degraded M-rich alginate ($F_{G=}$0.43) at various pH-values. The most relevant signals are pointed out. △ refers to the unsaturated non-reducing end from the β-elimination reaction. G and M refer to the neighbour unit of the △. The numbers refer to the carbon atom.

G-residues on the non-reducing ends at pH 3.8 compared to the other pH-values. This is in agreement to the specificity found for intramolecular acid hydrolysis of alginate solutions (Smidsrød, 1969), which favour a M-residue on the unit following the hydrolyzed glycosidic bond.

Molecular weight distribution of alginates degraded at 105 °C were determined by SEC-MALS. The results showed that the polydispersity index stayed close to two (data not shown) during the degradation process. A value of two for the polydispersity index indicates that the thermal degradation is a random process. Because the sequence of blocks are randomly distributed a long the chain, the polymer will be randomly degraded even with some specificity for one of the four different glycosidic bonds.

4. Conclusions

Thermal depolymerization of alginate in the solid state was found to be catalyzed simultaneously by protons and hydroxide ions. The catalytic effect was negligible between pH 5 and 8, when pH is defined as the pH of the sample dissolved in water to a 1% (w/w) solution, suggesting that the depolymerization also occur by a general acid–base catalysis when the water molecule act as a base or as an acid. The M-rich alginate ($F_G = 0.43$) was shown to be more susceptible to acid hydrolysis than the G-rich alginate ($F_G = 0.63$) in the solid state, which is also found for alginate in solution. No specificity was observed for the β-elimination reaction. The initial rate of depolymerization of the M-rich and the G-rich alginate between 5 and 9.5 was not affected by the specificity of acid hydrolysis, and therefore no significant difference in the initial rate constant of the two unlike alginates were observed at these conditions. The rate of depolymerization was found not to be dependent upon the presence of oxygen. The activation energies of the G-rich alginate and the M-rich alginate were determined to be 114 ± 6 and 126 ± 12 kJ/mol, respectively.

Our data suggest that acid hydrolysis and β-elimination, caused by alkaline hydrolysis, are the primary mechanisms involved in the thermal depolymerization of highly purified alginates in the solid state.

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