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Kinetics and mechanisms of depolymerization of alginate and chitosan in aqueous solution

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Abstract

The kinetics and mechanisms of depolymerization of aqueous chitosan and alginate solutions at elevated temperatures have been investigated. Chitosan salts of different degree of acetylation (F_A), type of counterions (-glutamate, -chloride) and degree of purity were studied. One commercially available highly purified sodium alginate sample with high content of guluronic acid (G) was also studied. Furthermore, the influence of oxygen, H^+ and OH^- ions on the initial depolymerization rates was investigated. Depolymerization kinetics was followed by measuring the time courses of the apparent viscosity and the intrinsic viscosity. The initial rate constants for depolymerization were determined from the intrinsic viscosity data converted to a quantity proportional to the fraction of bonds broken. The activation energies of the chitosan chloride and chitosan glutamate solutions with pH close to 5 and the same degree of acetylation, $F_A = 0.14$, were determined from the initial rate constants to be 76 ± 13 kJ/mol and 80 ± 11 kJ/mol, respectively.

The results reported herein suggest that the stability of aqueous chitosan and alginate solutions at pH values 5–8 will be influenced by oxidative–reductive depolymerization (ORD) as the primary mechanism as long as transition metal ions are presented in the samples. Acid – and alkaline depolymerization will be the primary mechanisms for highly purified samples.

Keywords: Chitosan; Alginate; Polyelectrolytes; Depolymerization; Stability; Activation energy; Thermal degradation

1. Introduction

Polyelectrolytes such as alginate and chitosan can be utilized as hydrophilic drug carriers and as matrix materials. In addition, these polysaccharides have potential as rate controlling excipients in drug release systems. These and other applications of biopolymers have gained interest within the pharmaceutical and biomedical field. Such applications require highly purified biopolymers which are well characterized and documented, including knowledge of the stability over time (shelf life) (Skaugrud, Hagen, Borgersen, & Dornish, 1999). It is important to know how to control the degradation of the products – both in solution and in the dry state.

Alginate and chitosan, like other polysaccharides, are susceptible to a variety of degradation mechanisms, including oxidative–reductive free radical depolymerization and acid-, alkaline- and enzymatic-catalyzed degradation. The degradation occurs via cleavage of the glycosidic bonds which results in depolymerization of the polysaccharide. Generally, the degradation rates depend on the concentrations of reactants and temperature.

Alginate is a natural anionic polysaccharide obtained by extraction from marine brown algae. 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

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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) coexist (for review see: Moe, Draget, Skjåk-Bræk, & Smidsrød, 1995).

Chitosan is a natural cationic polysaccharide obtained by the *N*-deacetylation of chitin, a product found in the shells of crustaceans. Chitosan is a linear binary copolymer consisting of β(1 → 4)-linked 2-acetamido-2-deoxy-β-D-glucopyranose (GlcNAc; A-unit) and 2-amino-2-deoxy-β-D-glucopyranose (GlcN; D-unit). It has been shown that the A- and D-monomers are randomly distributed along the chitosan chain (Vårum, Anthonsen, Grasdalen, & Smidsrød, 1991a, 1991b).

Depolymerization of alginate in solution has previously been investigated on non-purified alginates, and it has been shown that the presence of oxygen affects the stability due to the presence of phenolic reducing substance which gives rise to the ORD reaction (Smidsrød, Haug, & Larsen, 1963). For chitosan it has been shown that the rates of acid hydrolysis of the glycosidic bonds are of the order A–A ~ A–D >> D–A ~ D–D, which means that chitosan influenced by acid hydrolysis is more stable with a lower degree of acetylation (Vårum, Ottøy, & Smidsrød, 2001). Thermal depolymerization of alginate (Holme, Lindmo, Kristiansen, & Smidsrød, 2003) and chitosan chloride (Holme, Foros, Pettersen, Dornish, & Smidsrød, 2001) in solid form has previously been investigated, and the rate of depolymerization was found to be independent on the presence of oxygen. The data suggested that acid hydrolysis and β-elimination, caused by alkaline conditions, are the primary mechanisms involved in the thermal depolymerization of alginate in the solid state, and that thermal depolymerization of chitosan in the solid state was mainly driven by acid hydrolysis. The aim of this study was therefore to examine whether the results from the depolymerization study of the polyelectrolytes in dry form were applicable to the same polyelectrolytes in solution. The activation energies for the depolymerization of aqueous chitosan salt solutions were determined to get information about possible depolymerization mechanisms. Furthermore, the effect of variables such as oxygen, pH, F_A, counterions and transition metal ions were investigated.

2. Materials and methods

2.1. Biopolymer samples

Sodium alginate and chitosan salt samples of different purity were provided by FMC BioPolymer AS (Sandvika, Norway) and are listed in Table 1. The chemical composition was determined by 400 MHz proton NMR spectroscopy. The assignments published by Grasdalen (1983) and Grasdalen, Larsen, and Smidsrød (1979) were used for the determination of fraction of guluronic acid in alginate, F_G. The degree of acetylation, F_A, in chitosan was determined as described in Vårum et al. (1991a). The apparent viscosity, η, of 1% (w/w) solution was measured at 20 °C using a Brookfield digital rotational viscosimeter with a spindle rotation set at 20 rpm. The intrinsic viscosities [η] were determined at 20 °C using a Schott–Gerate Ubbelohde viscometer as described by Draget, Vårum, Moen, Gynnild, and Smidsrød (1992). pH was measured (Radiometer Copenhagen, PHM 92 Lab pH meter) in a 1% (w/w) polymer solution at 20 °C.

2.2. Thermal depolymerization

Thermal depolymerization experiments were performed in a drying oven held at a constant temperature of 22.5, 36, 60 and 80 °C. The sodium alginate and chitosan salt samples were dissolved in purified water to give 1% (w/w) solutions by shaking (100 rpm) over night at refrigerated temperature (2–8 °C). The day after, the polymer solution was heated up on a hotplate while stirring until the temperature for the depolymerization experiment was reached. The polymer solution was thereafter divided into preheated Pyrex glass bottles (100 ml). Each bottle was filled with 100 g polymer solution and was closed by a screw cap. There was some airspace between the meniscus and the cap. One of the Pyrex bottle was put immediately on ice to obtain the viscosity of the starting sample, the other bottles were placed in a preheated oven. Samples were removed at various time intervals and put on ice to stop the depolymerization process. Shortly after the cooling, the samples were brought to 20 °C in order to measure the apparent viscosity and pH. The rest of the sample was freeze dried and kept at −18 °C before further characterization. Intrinsic viscosity and weight average molecular weight of selected samples were determined.

Table 1
Sodium alginate and chitosan salt samples characteristics

Parameters	Chitosan chlorides				Chitosan glutamate	Sodium alginate
Chemical composition	$F_A = 0.02$	$F_A = 0.05$	$F_A = 0.14$	$F_A = 0.35$	$F_A = 0.14$	$F_G = 0.63$
Apparent viscosity η, [mPas]	81	143	147	74	99	120
Intrinsic viscosity [η], [ml/g]	690	~830[a]	800	610	640	850
pH (1% (w/w) solution)	5.0	5.2	5.0	5.0	4.8	6.7

[a] Determined from the correlation between apparent viscosity and intrinsic viscosity for the chitosan chloride, $F_A = 0.02$.

In the experiment with oxygen availability during the whole depolymerization process, an open beaker with chitosan chloride solution (1% (w/w)) was placed on a hotplate and heated up to 80 °C. Mechanical stirrer was used in addition to magnetic stirrer to both ensure air in the solution and that the polymer did not dry at the surface of the beaker. Samples were removed at various time intervals and handled as in the other experiment using Pyrex bottles. The weight of the beaker was controlled, due to evaporation. Purified water was added to keep the chitosan chloride concentration constant during the experiment.

The alginate solutions (1% (w/w)) with different pH values were prepared to study the influence of pH on the rate of depolymerization. Alginate solution with pH value of 4.3 was made by adjusting a 1% (w/w) solution to the correct pH value with 1 M HCl using a mechanical stirrer. Alginate with pH value of 8 was made in phosphate buffer (pH 8, 0.063 M Na_2HPO_4/0.0035 M NaH_2PO_4) by dissolving alginate into a 2% solution and further mixing it with equal volume of double concentrated phosphate buffer, pH 8. Alginate samples containing buffer were dialyzed and freeze dried before measuring the intrinsic viscosity (in 0.1 M NaCl). One alginate solution with a concentration of 5% (w/w) at pH 4.3 was prepared in order to compare the time course of the thermal depolymerization with the 1% (w/w) solution. Intrinsic viscosity measurements were performed on selected samples.

2.3. Molecular weight determination

The weight average molecular weight, M_w, and the distribution of the molecular weights in the chitosan 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}, G5000PW_{XL} and G3000 PW_{XL}). The mobile phase was 0.2 M CH_3COONH_4 (pH 4.5) at a flow rate of 0.5 ml/min. Absolute concentrations of chitosan 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 molar mass in each volume element was considered monodisperse, and the molar mass was determined from a Zimm representation of a Debye plot by linear extrapolation to zero angle. 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 log M–V data of each sample was checked for linearity within the RI peak. The molecular weight distribution was characterized by the polydispersity index, M_w/M_n.

2.4. Transition metal determination

Fe, Cu and Zn were determined by ICP-AES and 1 g samples of chitosan and alginate were pretreated stepwise by nitric acid and hydrogen peroxide at 110 °C before ICP-AES analysis.

2.5. Determination of the rate of 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 chitosan and 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 the intrinsic viscosities at time t and time 0, respectively, and K and a are constants from the MHS equation $[\eta] = KM^a$. The MHS-constants for chitosan were determined specifically by measuring intrinsic viscosity and weight average molecular weight for selected depolymerized samples, as shown in Fig. 1. The MHS-constants determined from Fig. 1 are given in Table 2, together with the MHS-constants for alginate determined by Holme et al. (2003). Referring to Berth and Dautzenberg (2002), the same MHS-constants can be used for all chitosan samples with different degree of acetylation. The MHS parameters are applicable within the molecular weight range of the depolymerized samples used for the calculation of k (Vold, Kristiansen, & Christensen, 2006). The rate constant, k' was found by plotting $\Delta 1/[\eta]^{1/a}$ as a function of t and converting to k through the equation above. Correlations between intrinsic viscosity and apparent viscosity were established based on several measure-

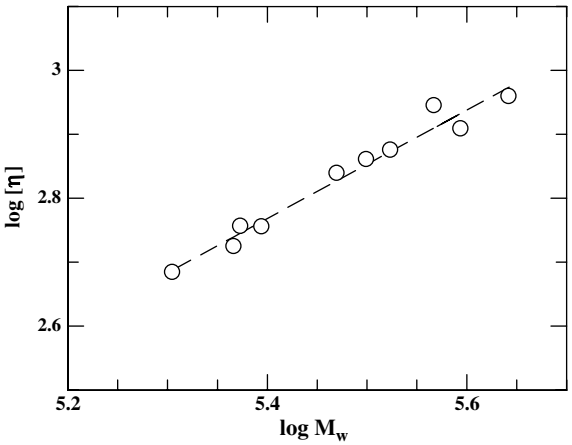

Fig. 1. log $[\eta]$ at 0.1 M ionic strength versus log M_w for chitosan chloride (Mark Houwink Sakurada plot).

Table 2
MHS-constants of chitosan and alginate determined by intrinsic viscosity and SEC-MALS measurements

Sample	K (ml mol/g^2)	a
Alginate ($F_G = 0.63$)[a]	1.1×10^{-2}	0.93
Chitosan ($F_A = 0.14$)	1.6×10^{-2}	0.85

[a] Reference Holme et al. (2003).

ments of depolymerized samples. The correlations were used to calculate the intrinsic viscosity from an apparent viscosity measurement. These correlations were established because the apparent viscosity assay is more sensitive and has a better precision than both the intrinsic viscosity and the SEC-MALS assay to monitor very low degree of scissions within the molecular weight range applied in this study. In addition the apparent viscosity assay is easier and quicker to perform. The correlation between the intrinsic viscosity and the apparent viscosity of the chitosan sample ($F_A = 0.35$) is shown in Fig. 2, and the correlation equations for the different polymer samples are given in Table 3.

The rate constant for depolymerization, k, can be used to find the activation energy by the use of 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.

3. Results and discussion

3.1. Time course of depolymerization of chitosan glutamate and – chloride

The depolymerization of 1% (w/w) solutions of chitosan chloride and chitosan glutamate with $F_A = 0.14$ was followed by viscometry at four temperatures, 22.5, 36, 60 and 80 °C. Fig. 3 shows the apparent viscosity of the 1% solutions as a function of depolymerization time. The same

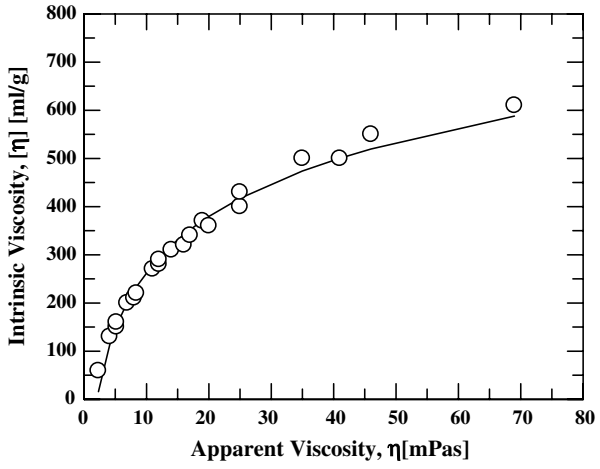

Fig. 2. Intrinsic viscosity ([η], ml/g) versus the apparent viscosity (η, mPas) of 1% (w/w) solution of chitosan chloride samples with $F_A = 0.35$.

Table 3
Correlation between intrinsic viscosity ([η], ml/g) and apparent viscosity (η, mPas) based on several measurements of depolymerized samples

Sample	Correlation equation[a]	
	Trendline	R-squared value
Sodium alginate ($F_G = 0.63$)	$[\eta] = 200.21 \ln(\eta) - 174.17$	$R^2 = 0.958$
Chitosan chloride ($F_A = 0.02$)	$[\eta] = 240.64 \ln(\eta) - 363.19$	$R^2 = 0.992$
Chitosan chloride ($F_A = 0.14$)	$[\eta] = 217.81 \ln(\eta) - 288.25$	$R^2 = 0.977$
Chitosan chloride ($F_A = 0.35$)	$[\eta] = 168.22 \ln(\eta) - 124.38$	$R^2 = 0.984$

[a] Microsoft Office Excel 2003 was used to obtain the equation for the correlation by using a transformed regression model, logarithmic type.

trends of viscosity versus depolymerization time are seen for the chitosan chloride and the chitosan glutamate. The rate of depolymerization of the chitosan salts increased, as expected, with increasing temperature. As an example, the viscosity obtained after 1 day at 80 °C for the chitosan chloride is obtained first after about 3.5 months at 36 °C. The starting viscosity of the chitosan salts at $t = 0$ varies at the different temperatures, due to some depolymerization during the heating of the solution before it was placed in the oven at given temperature. Fig. 4 shows $\Delta 1/[\eta]^{1/a}$ plotted against time of depolymerization of both the chitosan chloride and the chitosan glutamate solutions at 80 °C. The pH was recorded during the degradation and varied within ±0.1 of the initial values. Fig. 4 shows that there is no significant difference in the thermal stability of the chitosan chloride and the chitosan glutamate, suggesting that the type of counterion has no influence on the rate of depolymerization when compared at the same pH of the solution. However, a curvature of the time course of the chitosan salt solutions is noticeable. Fig. 4 shows that there is an initial rate constant which is gradually decreased and followed by a slower rate constant, suggesting an influence of two degradation mechanisms. Previous studies on the thermal depolymerization of chitosan chloride in solid state have shown only linear plots of $\Delta 1/[\eta]^{1/a}$ versus time (Holme et al., 2001, Fig. 5B).

3.2. Time course of depolymerization of chitosan chlorides with variable F_A

Fig. 5A shows $\Delta 1/[\eta]^{1/a}$ plotted against the time of degradation of two replicates of 1% (w/w) solutions of chitosan chlorides with $F_A = 0.02$, $F_A = 0.14$ and $F_A = 0.35$ at 80 °C. The results from a similar plot obtained by Holme et al. (2001) on thermal depolymerization of chitosan chloride in solid state are also given for comparison (Fig. 5B). These time courses of degradation demonstrate that the degradation rates of chitosan solutions are similar for $F_A = 0.02$ and $F_A = 0.35$, but smaller for $F_A = 0.14$ and no systematic trend with respect to F_A is seen. This is not the case for chitosan in solid form and in strong acid solutions (Vårum et al., 2001) where the degradation rates increase markedly with increasing degree of acetylation. The chitosans with $F_A = 0.02$ and $F_A = 0.35$, are taken

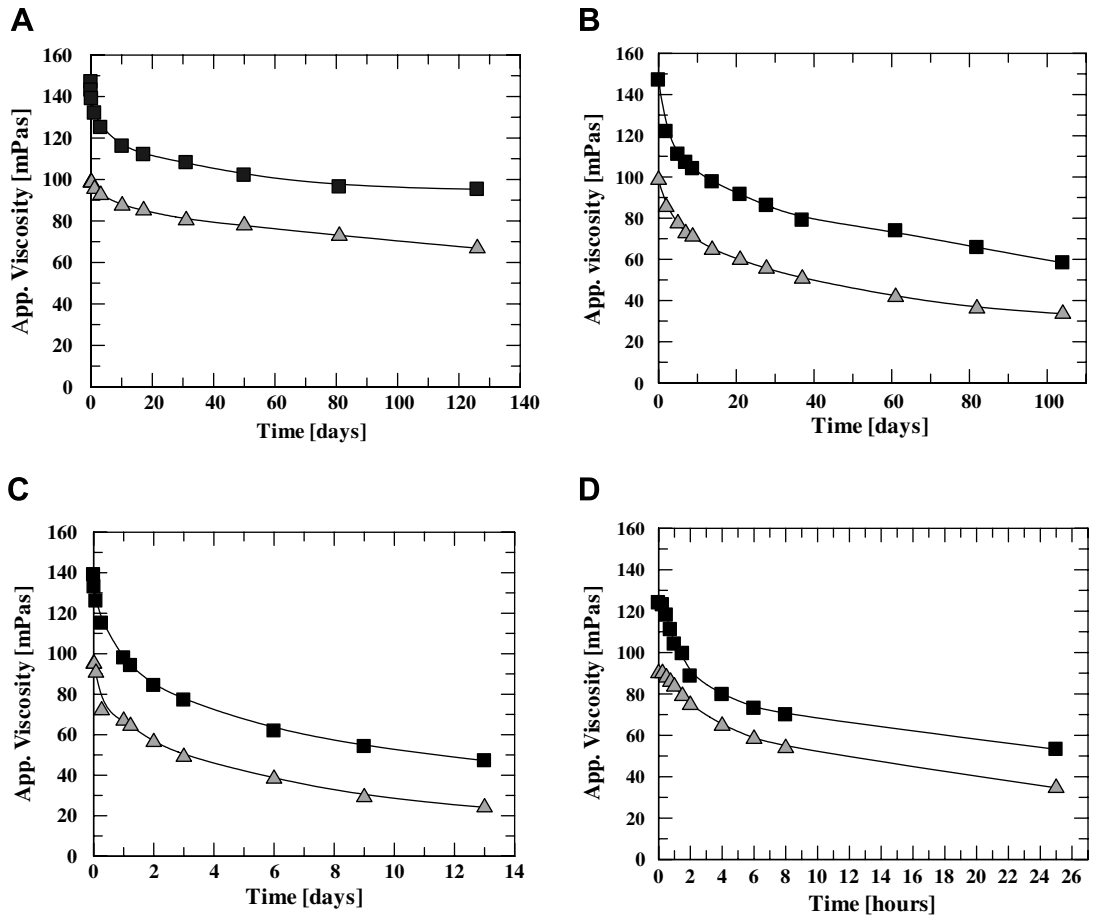

Fig. 3. Apparent viscosity of thermally degraded 1% (w/w) solutions of chitosan glutamate (▲) and chitosan chloride (■) with $F_A = 0.14$ versus degradation time at (A) 22.5 °C, (B) 36 °C, (C) 60 °C and (D) 80 °C. The degradation time for (A), (B) and (C) are given in days, while for (D) it is given as hours.

from the same batches that were used for thermal degradation of chitosan in the solid state (Holme et al., 2001). In addition, there is a difference in the curvature of the degradation plots. The plot, $1/[\eta]^{1/a}$ versus the time of degradation of 1% (w/w) solutions of chitosan chlorides, has also been plotted with $[\eta]$ based on $[\eta]$ measurements of the degraded samples (data not shown). The same curvature was seen as for the plot based on $[\eta]$ estimated values obtained from the apparent viscosity; consequently the viscosity decrease is caused by a depolymerization mechanism rather than dissolution of aggregates.

The degradation of the chitosan chloride solutions was followed by apparent viscosity measurements for 3–7 days, and there was no further change in the degradation rate beyond what is presented in Fig. 5A. The curvatures for chitosan chloride solutions also suggest that there are different depolymerization mechanisms causing the degradation. Acid hydrolysis has been shown to be the primary mechanism for the depolymerization of chitosans as solid form (Holme et al., 2001). The depolymerization of chitosan by acid hydrolysis is found to be specific in the sense that protons attack the glycosidic oxygen following the non-charged A units (Vårum & Smidsrød, 1997). As previously reported (Rupley, 1964; Vårum & Smidsrød, 1997), hydrolysis of *N*-acetyl bond (deacetylation) may occur in addition to the hydrolysis of the glycosidic bond. No significant changes in F_A and pH of the chitosan chloride before and after the thermal degradation were observed in the present work (data not shown). The results reported by Vårum et al. (2001) is not directly comparable since Vårum et al. (2001) performed the acid hydrolysis at pH values below 2 as compared to pH 5 in the presented work.

The depolymerization rate of the ORD reaction has previously been shown to be independent on F_A (Nordtveit, Vårum, & Smidsrød, 1994). Since some oxygen was present above the solutions in the capped flasks, the ORD reaction could be a potential mechanism in the present case. Oxygen is consumed during the ORD reaction and the limited amount of oxygen in the flasks could cause the curvature in the degradation time courses. In addition, the curvature is seen for different degrees of acetylation ($F_A = 0.02$, $F_A = 0.14$, $F_A = 0.35$) with no systematic trend, which suggests no specificity with respect to the type of glycosidic bond cleaved.

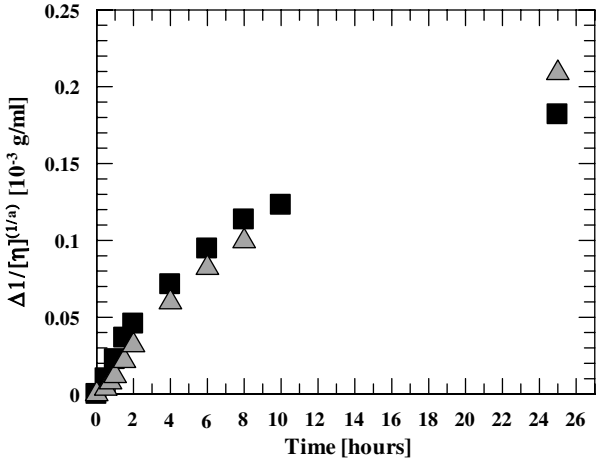

Fig. 4. Time course of thermal degradation of 1% (w/w) solutions of chitosan glutamate (▲) and chitosan chloride (■) with $F_A = 0.14$ at 80 °C.

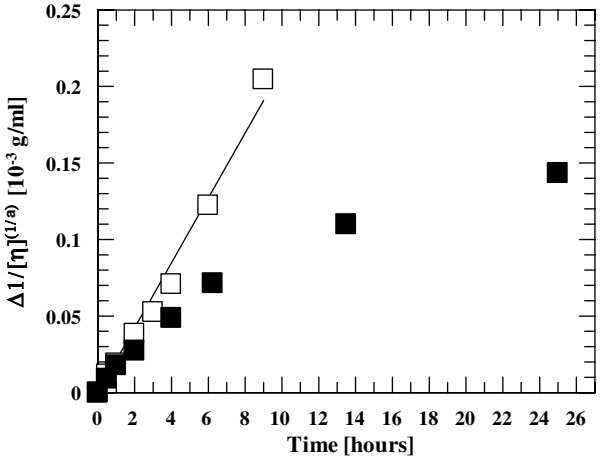

Fig. 6. Time course of thermal degradation of 1% (w/w) solution of chitosan chloride ($F_A = 0.14$) kept in covered bottle (■) and kept with stirring (□) at 80 °C.

3.3. The influence of oxygen on the degradation rates

In order to obtain data with unlimited supply of oxygen a degradation study was performed on a 1% (w/w) solution of chitosan chloride with $F_A = 0.14$ at 80 °C in an open beaker placed on a hotplate and heated up to 80 °C. Mechanical stirrer was used to ensure saturation of air in the solution during the depolymerization. The results are shown in Fig. 6 together with the results obtained from the thermal degradation experiment on the same chitosan in covered bottles. The results demonstrate that when oxygen is present in excess during the degradation period, there is no curvature in the plot of $\Delta 1/[\eta]^{1/a}$ versus degradation time. This suggests that the ORD mechanism is responsible for the initial depolymerization of the chitosan salt solutions. Nordtveit et al. (1994) demonstrated that the viscosity of chitosan solution decreased rapidly in the presence of hydrogen peroxide (H_2O_2) and $FeCl_3$. Furthermore, they found no dependence on F_A, which is in agreement with our results showing no systematic trend with respect to F_A. However, the different depolymerization rates observed for the chitosan chloride tested has to be explained (see Fig. 5A). Alginate has been shown to be degraded in the presence of different reducing compounds (Smidsrød et al., 1963) explained by the ORD mechanism in the following steps: (1) Formation of peroxide occurs with oxidation of reducing compounds. (2) Decomposition of peroxide is catalyzed by transition elements (e.g. Fe^{2+}) with the formation of free radicals including OH radical. (3) Reaction between radicals and the polymer chain leads to depolymerization of the polymer. In this study of thermal depolymerization of chitosan chloride, no reducing compounds were added, and no impurities of reducing compounds are found to be aroused during the process of pure chitosan salts. It is well known that there is a reducing end in the carbohydrate polymer chain and Larsen and Smidsrød (1967) demonstrated the reducing power of this end group. This reducing end may possibly be oxidized. The straight line of the depolymerization rate when oxygen is presented in excess (Fig. 6), shows

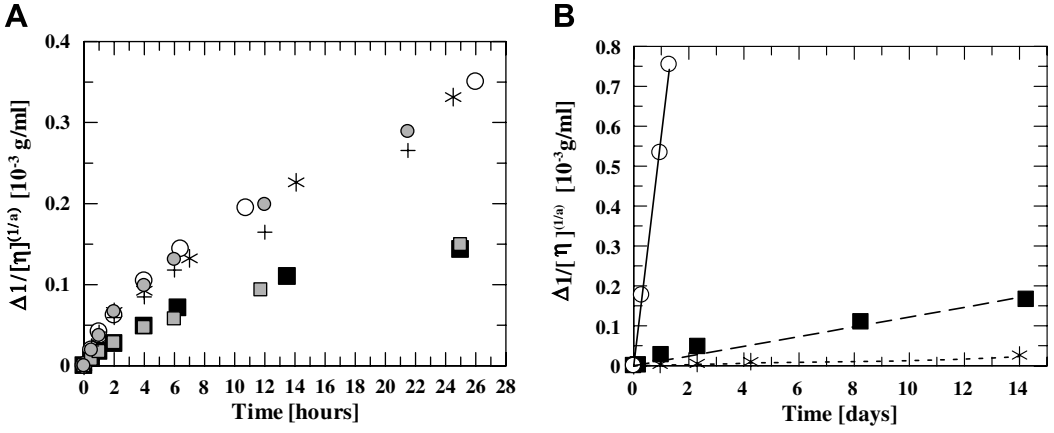

Fig. 5. Time course of thermal degradation of (A) 1% (w/w) solution and (B) solid form of chitosan chlorides with (∗)/(+) $F_A = 0.02$, (■)/(▩) $F_A = 0.14$ and (○)/(●) $F_A = 0.35$ at 80 °C. Data of chitosan chlorides in solid form are taken from Holme et al. (2001).

an independence of the degree of polymerization and a first order reaction. The ORD reaction probably produces new reducing ends with a rate proportional to the rate of oxidation of the existing reducing chain ends.

3.4. The influence of transition metal ions on the degradation rate

Traces of the transition elements Fe, Cu and Zn in the chitosan and alginate samples used in the study were analyzed by ICP-AES and the results are given in Table 4. With reference to Fig. 5A it can clearly be seen that the chitosan chlorides with the largest depolymerization rates, are the ones that have the highest content of trace elements. These results support the theory of ORD being the primary mechanism for the depolymerization of chitosan salts in solution. Another experiment was done on a more purified chitosan chloride ($F_A = 0.05$) with lower trace metal ions content (see Table 4) to compare the time course with the chitosan chlorides given in Fig. 5A. Fig. 7 shows that the chitosan chloride with $F_A = 0.05$ has a lower degradation rate than the chitosan chloride with $F_A = 0.14$, as distinct from Fig. 5A which shows that the chitosan chloride with $F_A = 0.02$ has a higher degradation rate than the chitosan chloride with $F_A = 0.14$. This clearly demonstrates again that the amount of transition metal ions in chitosan samples strongly affects the rate of thermal depolymerization of chitosan in solutions.

Fig. 7 shows that the initial rate constants are similar for the chitosan with $F_A = 0.05$ and the chitosan with $F_A = 0.14$. The slower depolymerization rate following the initial depolymerization, is 1.3 times larger for the chitosan with $F_A = 0.14$ than for the chitosan with $F_A = 0.02$. This may be due to acid hydrolysis prevailing during this period (Vårum et al., 2001).

Thermal degradation of 1% solutions of an alginate sample with high content of guluronic acid (G) has been studied. Alginate solutions of different pH values were examined, and the time courses are given in Fig. 8. Fig. 8 shows linear plots for the different alginate solutions as opposed to chitosan salt solutions (see Fig. 5A). The content of trace metal ions was also determined for this alginate sample and is given in Table 4, and was shown to be below the limit of quantification. This means that there are no or neglectable amounts of transition metal ions that may catalyze an ORD mechanism. The degradation rate of alginate has also shown to be influenced by the presence of oxidizeable phenolic compounds of brown algae (Smidsrød et al., 1963). The alginate used in this study is highly purified and contains neglectable amounts of phenolic compounds (data not shown).

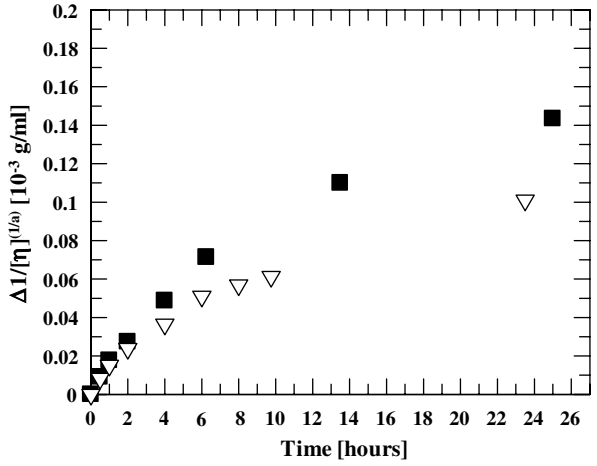

Fig. 7. Time course of thermal degradation of 1% (w/w) solution of chitosan chlorides with (▽) $F_A = 0.05$ and (■) $F_A = 0.14$ at 80 °C. Data of chitosan chloride $F_A = 0.14$ is taken from Fig. 5A.

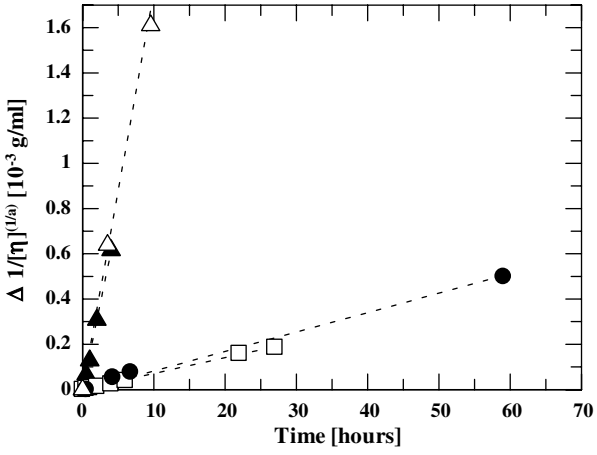

Fig. 8. Time course of thermal degradation of 1% (w/w) solution of sodium alginate with (▲) pH 4.3, (□) pH 6.6 and (●) pH 8.0 and 5% (w/w) solution of sodium alginate with (Δ) pH 4.3.

The rate of thermal depolymerization of alginate in solution increased with decreasing pH of the solution within the pH range of pH 4.3–6.6 (see Fig. 8), suggesting acid hydrolysis as an important mechanism. An ORD is also affected by the hydroxide ions in a polymer solution, and will increase with increasing pH (Smidsrød, Haug, & Larsen, 1965). Such dependence can not be seen in

Table 4
Content of transition metal ions in sodium alginate and chitosan chloride samples

Elements	Chitosan chlorides				Sodium alginate
	$F_A = 0.02$	$F_A = 0.05$	$F_A = 0.14$	$F_A = 0.35$	$F_G = 0.63$
Fe [ppm]	47	5.5	42	80	<2.7
Cu [ppm]	19	1.5	3.9	9.9	<1
Zn [ppm]	6.6	<1	1.3	3.1	<1

Fig. 8, where the initial rate constants at pH 6.6 and 8.0 are identical, in agreement with the observation done by Holme et al. (2003) for alginate in solid state. Holme et al. (2003) explained the pH independence by the water molecule acting as both acid (proton donor) and base (proton acceptor), and that the depolymerization was caused by both acid hydrolysis and β-elimination mechanisms caused by alkaline conditions. Fig. 8 shows also that the thermal depolymerization of an alginate solution of 5% (w/w) is identical to an alginate solution of 1% (w/w) at pH 4.3.

3.5. Activation energies

The results from the degradation experiments of the chitosan chloride and chitosan glutamate with the same degree of acetylation, $F_A = 0.14$, at the four different temperatures are presented in Fig. 9 as a plot of the natural logarithm of the initial rate constants as a function of the inverse of the absolute temperature (Arrhenius plot). The activation energies calculated from the slopes of the lines for the chitosan glutamate and the chitosan chloride were 80 ± 11 kJ/mol and 76 ± 13 kJ/mol, respectively. The values are the same within the experimental error of determination. This activation energy should then represent the activation energy for cleavage of the glycosidic bonds by the ORD mechanism, since this mechanism has been shown to be dominating in the initial thermal depolymerization of chitosan salt solutions. The activation energy for the depolymerization of chitosan salt in solutions is significantly lower than the activation energy obtained for acid hydrolysis of partially acetylated chitosans (Vårum et al., 2001), which has been found to be about 130 kJ/mol. Holme et al. (2001) determined activation energies around 110 kJ/mol for thermal degradation of chitosan chlorides in the solid state, which was found to primarily be caused by acid hydrolysis. Smidsrød et al. (1963) determined activation energy for ORD to be 29 kJ/mol with the presence of ascorbic acid (good radical generator), and 79 kJ/mol with the presence of hydroquinone. This was based on depolymerization of alginate in solution. Chang, Tai, and Cheng (2001) have done kinetic degradation studies of 1% chitosan solution (dissolved in acetic acid) by hydrogen peroxide and report activation energy of 88.5 kJ/mol, which is almost identical to the activation energy reported herein. Generally, the activation energies found in the literature are found to be lower for ORD than for acid hydrolysis. Due to this fact, the activation energies found from the initial depolymerization rates of the thermal degradation of chitosan salt solutions are of values close to activation energies given for the ORD mechanism.

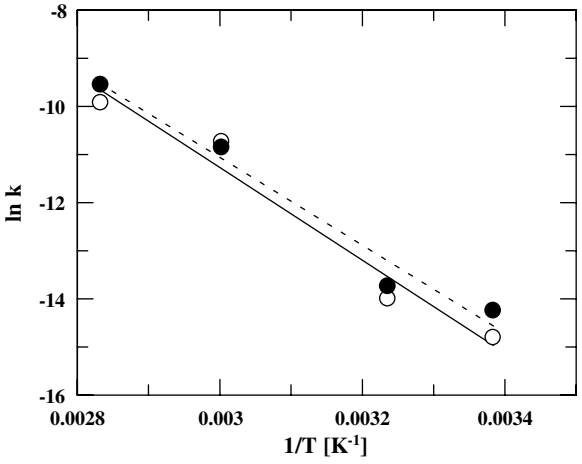

Fig. 9. Natural logarithm of the initial depolymerization rates versus 1/T for (○) chitosan glutamate and (●) chitosan chloride with $F_A = 0.14$.

Holme et al. (2001) did not observe any effect of the ORD mechanism on the thermal depolymerization of chitosan salt in the solid form, as opposed to what is observed for the thermal depolymerization of chitosan salt in aqueous solution. Based on the transition metal ions content in the chitosans used for these studies, there is a long average distance between the transition metal ions along the polymer chain (about 1/3000 monomer). According to the ORD mechanism, the transition metal ions catalyze the formation of hydroxyl radicals which are further responsible for the depolymerization of the polymer chain. To initiate this reaction, a reducing compound must be oxidized and close to a transition metal ion. The reason for no influence of ORD on the thermal depolymerization in the solid state might be the long average distance between the hydroxyl radicals due to low collision frequencies between the transition metal ion and the reducing end, coupled with their short life time and a lower thermal mobility in the solid state.

This indicates that difference in the polymer state may lead to differences in the mechanisms responsible for

Table 5
Weight average molecular weight and polydispersity index of depolymerized chitosan chloride and chitosan glutamate samples

Chitosan chloride			Chitosan glutamate		
$\Delta 1/[\eta]^{(1/a)}$ [10^{-3} g/ml]	M_w[a] [g/mol]	Polydispersity index M_w/M_n	$\Delta 1/[\eta]^{(1/a)}$ [10^{-3} g/ml]	M_w[a] [g/mol]	Polydispersity index M_w/M_n
0	408 000	1.85	0	485 000	2.00
0.07	369 000	1.92	0.05	434 000	1.85
0.11	326 000	1.91	0.10	349 000	1.81
0.23	261 000	1.83	0.24	274 000	1.75
0.28	257 000	1.80	0.37	223 000	1.85

[a] The weight average molecular weight is given as chitosan acetate.

thermal depolymerization of the polymer, probably due to the difference in dynamics of the states. In solution a catalytic ion will be in dynamic ion-exchange equilibrium with other ions in solution and therefore able to travel along and among the flexible chains.

The data from the depolymerization of chitosan salt in aqueous solution suggest that the ORD mechanism is prevailing in the first phase and acid hydrolysis in the second phase. There will be a period where both mechanisms exist and the rate and the length of the first phase will depend on the amount of trace metal ions and reducing ends and this will further affect the following time course, and it is therefore not straight forward to interpret the second slower phase.

3.6. Molecular weight distribution during the thermal depolymerization

Molecular weight distribution of thermal depolymerized samples of chitosan chloride and chitosan glutamate was determined by SEC-MALS. The results are given for selected samples with different degree of depolymerization in Table 5. The results showed that the polydispersity index stayed close to two during the depolymerization process, which indicate a random depolymerization.

4. Conclusions

Thermal depolymerization of aqueous chitosan and alginate solution at pH values at 5–8 was found to be influenced by ORD as the primary mechanism as long as transition metal ions are presented in the samples, and acid – and alkaline depolymerization as the primary mechanisms for highly purified samples. This data suggest that the stability of aqueous chitosan and alginate solutions will be affected by impurities as transition metal ions and the presence of oxygen, and also the pH of the solution.

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