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Thermal depolymerization of chitosan chloride

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Abstract

The thermal depolymerization of chitosan chloride in solid state has been examined. Depolymerization was followed by measuring the apparent viscosity and intrinsic viscosity. The initial rate constants were determined from the intrinsic viscosity data and were found to increase markedly with increasing degree of acetylation, F_A, showing that the F_A is an important parameter for the rate of thermal degradation. The activation energies of the three chitosan chlorides with degrees of acetylation, $F_A = 0.02$, $F_A = 0.16$ and $F_A = 0.35$ were determined to be 114 ± 11 kJ/mol, 112 ± 5 kJ/mol and 109 ± 5 kJ/mol, respectively.

The rate of degradation was found not to be dependent on the presence of oxygen. On the other hand, the initial rate constant for chitosan chloride prepared by freeze-drying of a solution at pH 4 was about 30 times greater than that of a sample freeze-dried at pH 6, showing that the pH of the chitosan is important for its ability to degrade. ^{1}H and ^{13}C NMR spectroscopy of the thermally degraded chitosan with $F_A = 0.35$ was used to identify the specificity in the reaction. The rate of acid hydrolysis of the glycosidic bond in chitosan solutions was recently (Proceedings of the 1st International Conference on Chitin and Chitosan (1997), 168) found to be in the order **A**–**A** ≈ **A**–**D** ≫ **D**–**A** ≈ **D**–**D**, which appeared also to be valid for thermal depolymerization of chitosan. The NMR spectra also indicated that hydrolysis of the *N*-acetyl bond (de-*N*-acetylation) at the new reducing ends occurs in addition to the cleavage of the glycosidic bond.

The results reported herein show that acid hydrolysis is the primary mechanism involved in the thermal depolymerization of chitosan chlorides in the solid state and that cleavage of the **A**–**A** and **A**–**D** linkages is mainly responsible for the degradation in the range of acetyl content investigated here.

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

1. Introduction

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). Chitosan is the *N*-deacetylated derivative of chitin, which consists mainly of β(1 → 4)-linked 2-acetamido-2-deoxy-β-D-glucopyranose (GlcNAc; **A**-unit). It has been shown that the **A**- and **D**-monomers are randomly distributed along the chitosan chain (Vårum, Anthonsen, Grasdalen & Smidsrød, 1991a,b).

Chitosan is, like other polysaccharides, susceptible to a variety of degradation mechanisms, including oxidative–reductive free radical depolymerization and acid-, alkaline- and enzymatic-catalysed hydrolysis. Degradation of polysaccharides occurs via cleavage of the glycosidic bonds. To control depolymerization of chitosan is useful in order to control properties like viscosity, solubility and biological activity. Especially for biomedical applications, high quality polymers of known molecular weight are mandatory as well as knowledge of the stability of chitosan over time in solution and in the dry state (shelf life). Roberts (1992) and Vårum and Smidsrød (1997) have reviewed results on the degradation of chitin and chitosan.

The degradation of chitosan has been mostly described for chitosan solutions. The aim of this study was to examine the thermal degradation of chitosan in solid form. In addition, information regarding the stability of chitosan chlorides at various storage temperatures would be obtained. It has been reported by others (Alonso, Peniche-Covas & Nieto, 1983; Köll, Borchers & Metzger, 1991; Peniche-Covas, Argülles & San Romàn, 1993), that decomposition (release of material) of chitosan starts at 200°C. Little is known about the thermal degradation mechanism of chitosan in solid state before decomposition takes place.

In this study, chitosan chlorides were thermally degraded at 60, 80, 105 and 120°C. Degradation was followed by measuring the apparent viscosity and intrinsic viscosity. Analysis of depolymerization kinetics was based on the

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intrinsic viscosity data. The initial degradation rates of chitosans with different degrees of acetylation have been determined and compared in order to determine possible specificities in the thermal degradation. Specificity refers to the identity of the sugar units adjacent to the bond that is cleaved. NMR spectroscopy was also used to find the specificity in the reaction as demonstrated by Vårum, Holme, Izume, Stokke and Smidsrød (1996). The activation energies of the chitosans were determined to get some information about possible degradation mechanisms. Furthermore, the influence of oxygen and H^+ ions on the initial degradation rates was investigated.

2. Materials and methods

2.1. Chitosans

Chitosan chlorides (PROTASAN™ chloride) were manufactured by Pronova Biomedical a.s. (Oslo, Norway). The chitosans were characterized by 400 MHz proton NMR spectroscopy (Vårum et al., 1991a) and by their intrinsic viscosities (Draget, Vårum, Moen, Gynnild & Smidsrød, 1992). The degrees of acetylation were determined to be $F_A = 0.02$, $F_A = 0.16$ and $F_A = 0.35$, and the intrinsic viscosities, $[\eta]$, were 690, 800 and 610 ml/g, respectively. ($F_A = 0.02$, 0.16 and 0.35 correspond to $DA = 98$, 84 and 65%, respectively. DA (%) is the characteristic of the degree of deacetylation given by Pronova Biomedical a.s.) Solutions of 1% (w/w) of the chitosan samples had pH values of 5.0 ± 0.1.

2.2. Thermal degradation

The degradation experiments were carried out by placing chitosan chloride powder in a drying oven maintaining a constant temperature (60, 80, 105 and 120°C), and then removing samples at various time intervals. In all cases, containers of a size of more than ten times the volume of the powder have been used, thus assuring a sufficient amount of oxygen present. The same ratio between the container and the sample volume were used in all the experiments that were directly compared. The samples were stored in air-tight flasks in a freezer (−18°C) before they were analyzed.

The chitosan sample with $F_A = 0.16$ was used for the experiments studying the effect of oxygen and H^+ ions on the degradation.

In the experiment studying the effect of oxygen on degradation, the chitosan chloride was placed in a steel chamber that was a part of an apparatus where extremely hypoxic conditions (<4 ppm O_2) could be obtained (Pettersen, Oftebro & Brustad, 1973). Thermal degradation of chitosan chloride at 105°C was followed with and without nitrogen gas (99.9999%) flow through the steel chamber.

In order to study the influence of H^+ ions on the degradation, the pH of three solutions of 1% (w/w) chitosan chloride was adjusted with HCl and NaOH to pH 4, 5 and 6. The resulting samples were then lyophilized by freeze-drying. The samples were subsequently thermally degraded at 105°C.

2.3. Viscometry

The apparent viscosity of 1% (w/w) chitosan solutions was measured at 20°C using a Brookfield Digital Rheometer with spindle rotation set at 20 rpm. The intrinsic viscosities $[\eta]$ were determined in a Schott–Gerate Ubbelohde viscometer as described by Draget et al. (1992). All chitosan solutions were filtered through 0.8 μm Millipore filters before determining $[\eta]$.

2.4. Determination of the rate of thermal degradation and activation energy

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

$$\frac{1}{\overline{\mathrm{DP}_{\mathrm{n},t}}} = \frac{1}{\overline{\mathrm{DP}_{\mathrm{n},0}}} + kt \tag{1}$$

where $\overline{\mathrm{DP}_{\mathrm{n},t}}$ and $\overline{\mathrm{DP}_{\mathrm{n},0}}$ are number average degrees of polymerization, at times t and 0, respectively, and k the rate constant for bond cleavage. As $\overline{\mathrm{DP}_{\mathrm{n}}}$ is proportional to the molecular weight ($\overline{M_{\mathrm{n}}} = M_0\overline{\mathrm{DP}_{\mathrm{n}}}$), Eq. (1) indicates that the inverse of the molecular weight should increase linearly with the depolymerization time:

$$\frac{1}{\overline{M_{\mathrm{n}}}} - \frac{1}{\overline{M_{\mathrm{n},0}}} = \frac{k}{M_0} \times t \tag{2}$$

The decrease in molecular weight is therefore uniquely defined by the rate constant k.

For a linear single stranded polymer such as chitosan, combination of Eq. (2) and Mark–Houwink–Sakurada (MHS) equation yields:

$$\frac{1}{[\eta]^{1/a}} - \frac{1}{[\eta]_0^{1/a}} = k't \tag{3}$$

where $[\eta]$ is the intrinsic viscosity and $k' = k/(M_0K^{1/a})$; k the rate of bond cleavage, M_0 the monomer weight, and K and a are MHS parameters. K and a for chitosans can be estimated as described by Anthonsen, Vårum and Smidsrød (1993). The rate constant k' for degradation is obtained simply by plotting $1/[\eta]^{1/a}$ versus degradation time and converted to k by the equation above. The degradation rate (k) can be used to find the activation energy by taking the natural logarithm of the Arrhenius equation:

$$\ln k = \ln A - E_{\mathrm{a}}/\mathrm{R}T \tag{4}$$

where E_{a} is the activation energy, R the gas constant and T the absolute temperature. A plot of $\ln k$ versus $1/T$ gives a straight line whose slope is equal to $-E_{\mathrm{a}}/\mathrm{R}$.

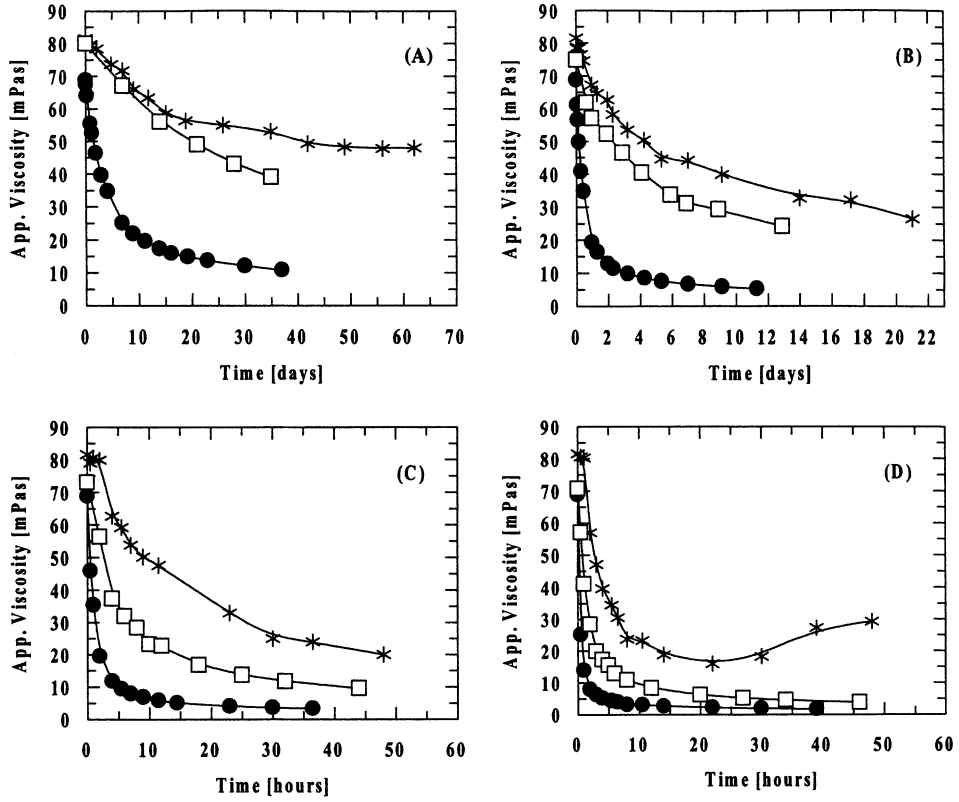

Fig. 1. Apparent viscosity of 1% solutions of thermally degraded chitosan chlorides with (*) $F_A = 0.02$, (□) $F_A = 0.16$ and (●) $F_A = 0.35$ vs. degradation time at (A) 60°C, (B) 80°C, (C) 105°C and (D) 120°C. The degradation time for (A) and (B) is given as days, while for (C) and (D) it is given as hours.

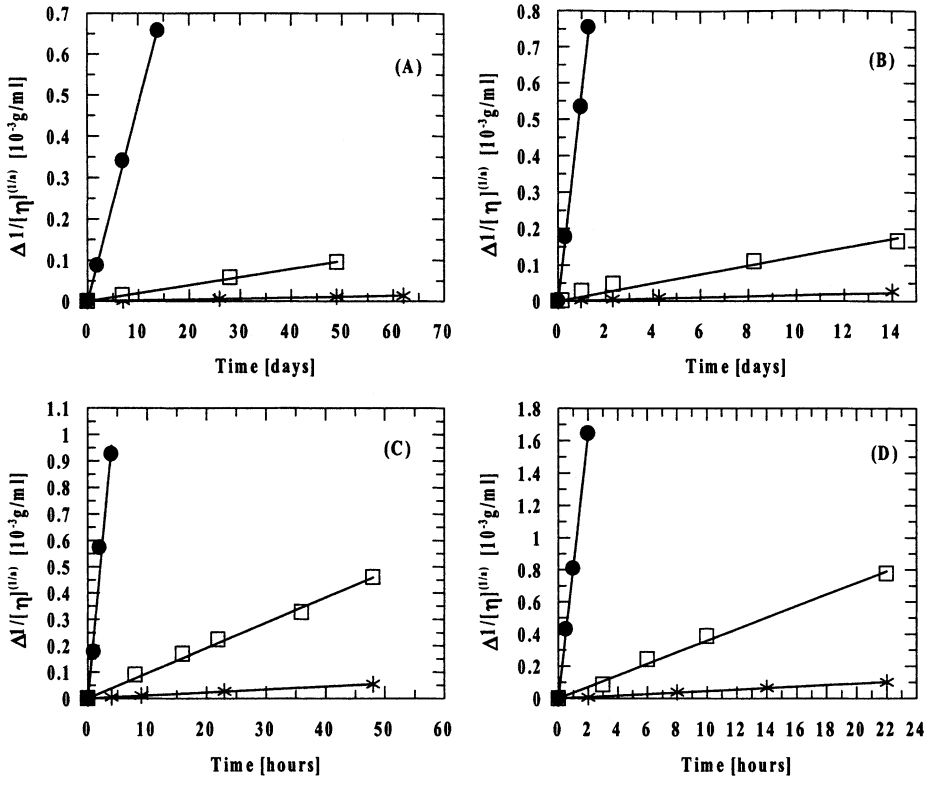

Fig. 2. Time course of thermal degradation of chitosan chlorides with (*) $F_A = 0.02$, (□) $F_A = 0.16$ and (●) $F_A = 0.35$ at (A) 60°C, (B) 80°C, (C) 105°C and (D) 120°C. The degradation time for (A) and (B) is given as days, while for (C) and (D) it is given as hours.

2.5. Nuclear magnetic resonance spectroscopy

The number average degree of polymerization, $\overline{DP_n}$, of thermally degraded chitosan chlorides ($F_A = 0.35$) was determined by 400 MHz ^{1}H NMR spectroscopy using a Varian Mercury BB instrument by determining the reducing end resonance of H-1 compared to the internal H-1 resonance (Ishiguro, Yoshie, Sakurai & Inoue, 1992). The calculation of the intensity of the reducing end resonance was based on an anomeric equilibrium α/β of 60:40 (Domard, Gey & Taravel, 1991; Tsukada & Inoue, 1981). The ^{1}H NMR spectra were also used to identify the reducing ends and the fraction of the acetylated residues on the reducing ends. Thermally degraded chitosan chloride ($F_A = 0.35$) was analyzed by ^{13}C NMR spectroscopy using a Varian Mercury BB (400 MHz) instrument in order to determine the identity of the non-reducing ends as described by Vårum et al. (1996).

3. Results and discussion

3.1. Time course of thermal degradation of chitosan chlorides of different chemical composition

Degradation of chitosan chlorides at four temperatures, 60, 80, 105 and 120°C, was followed by viscometry. In Fig. 1, the apparent viscosity of 1% solutions of thermally degraded chitosan chlorides with $F_A = 0.02$, $F_A = 0.16$ and $F_A = 0.35$ is plotted as a function of the degradation time. As can be seen from the figure, the viscosity decreases exponentially initially and then decreases at a slower rate over an extended period of time. This is only a consequence of 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 degradation of the chitosan chlorides increased, as expected, with increasing temperature, with the exception of the three last measurements of chitosan chloride with $F_A = 0.02$ at 120°C. Some insoluble material was observed in these solutions that may have contributed to the increase in the apparent viscosity. The formation of insoluble material can be explained by interchain cross-link formation involving free amino groups and reducing ends (Roberts & Taylor, 1989). It is also seen from Fig. 1 that the rate of degradation increases when the F_A increases.

Fig. 2 shows $\Delta 1/[\eta]^{(1/a)}$ plotted against the time of degradation of chitosan chlorides with $F_A = 0.02$, $F_A = 0.16$ and $F_A = 0.35$ at 60, 80, 105 and 120°C. The pH of 1% (w/w) solution of the chitosan chlorides was five. This time course of degradation demonstrates that the initial degradation rates of chitosan are dependent on their chemical composition (F_A), and increases with increasing degree of acetylation. This must be caused by some specificity in the degradation mechanism. The initial degradation rate constants for the chitosan chlorides at each temperature were obtained from the plots of $\Delta 1/[\eta]^{(1/a)}$ versus time, and are given in Table 1. The data in Table 1 show that the degradation rate of the chitosan with $F_A = 0.35$ and $F_A = 0.16$ was around ten and two times, respectively, greater than for the chitosan with $F_A = 0.02$ at the same temperature. These data demonstrate that chitosan chlorides with a low-degree of acetylation are more thermally stable than chitosan chlorides with a high-degree of acetylation.

Table 1
Initial degradation rates (k) of chitosan chlorides with different degrees of acetylation (F_A) at four temperatures

Temperature [°C]	Degradation rate [10^{-6} h^{-1}]		
	$F_A = 0.02$	$F_A = 0.16$	$F_A = 0.35$
60	0.25	0.54	3.5
80	1.6	3.4	42
105	29	63	420
120	120	240	1450

3.2. Kinetics of thermal degradation of chitosan chlorides

The activation energy for thermal depolymerization was obtained by plotting the natural logarithm of the degradation rate versus $1/T$, as shown in Fig. 3. The Arrhenius plots are linear, indicating that the degradation mechanism is the same within the temperature interval tested (60–120°C). The activation energies, E_a, for the three chitosan chlorides were calculated from the slopes, $-E_a/R$, in Fig. 3, and the results are given in Table 2. The E_a values for the different chitosan chlorides are identical within the experimental errors, indicating that the degradation mechanism is the same for all the samples.

3.3. Degradation mechanism

Potential mechanisms for temperature-induced degradation of chitosan are oxidative–reductive degradation (ORD) and acid catalyzed hydrolysis. The presence of molecular oxygen during the thermal degradation of chitosan may lead

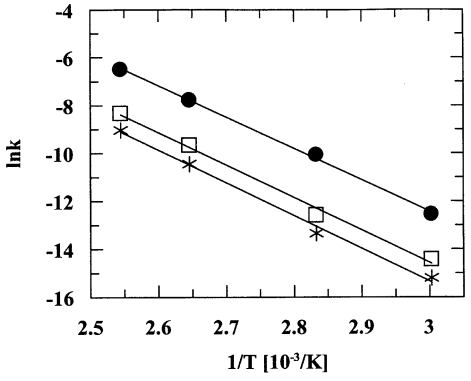

Fig. 3. Natural logarithm of the degradation rate vs. $1/T$ for chitosan chlorides with (*) $F_A = 0.02$, (□) $F_A = 0.16$ and (●) $F_A = 0.35$.

Table 2
Activation energy (E_a) of chitosan chlorides with different degrees of acetylation (F_A)

F_A	Activation energy, E_a [kJ/mol]
0.02	114 ± 11
0.16	112 ± 5
0.35	109 ± 5

to ORD. 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, and the protonated amino group of chitosan may probably also act as a proton donor in the catalysis.

Nordtveit, Vårum and Smidsrød (1994) studied the degradation of chitosans by an ORD mechanism, and found that the rate of degradation was independent on the degree of acetylation. As distinct from their result, the rate of thermal degradation found in this study is dependent on the F_A, indicating that the ORD mechanism does not dominate the thermal degradation in the present study. To confirm this, thermal degradation was performed in the presence and in the absence of oxygen. Fig. 4 shows $\Delta 1/[\eta]^{(1/a)}$ plotted against the time of degradation of a chitosan chloride with $F_A = 0.16$ for experiments carried out in air and in a nitrogen atmosphere at 105°C. The results demonstrate that the removal of oxygen does not affect the degradation rate. Consequently, the ORD mechanism is not responsible for the thermal degradation of the chitosan chlorides. The results are in agreement with the studies done by Lim, Khor and Ling (1999), who found that atmospheric gaseous composition was not a critical factor for the rate of heat-induced reactions of chitosan.

The other mechanism proposed for thermal degradation of chitosan is acid hydrolysis. Experiments reported by Vårum and Smidsrød (1997) showed that the rate of degradation of chitosans by acid hydrolysis increases with

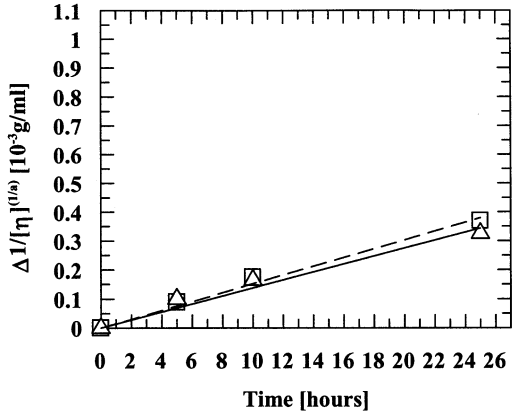

Fig. 4. Time course of thermal degradation of chitosan chloride (F_A = 0.16) at 105°C carried out in (- -□- -) air and in a (– △ –) nitrogen atmosphere.

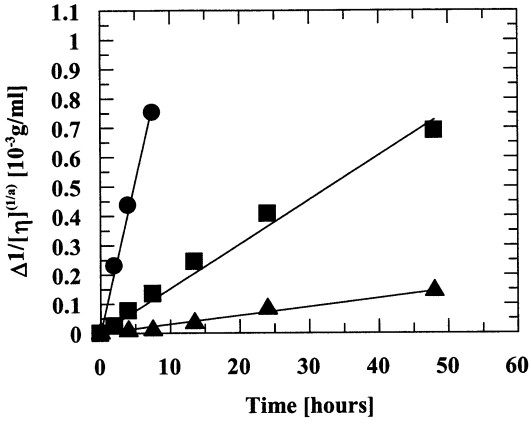

Fig. 5. Time course of thermal degradation of chitosan chloride (F_A = 0.16) with different pH at 105°C. (▲) pH 6.0, (■) pH 5.0, (●) pH 4.1.

increasing degree of acetylation, which is in accordance with our results for thermal degradation. However, the initial degradation rates did not increase in proportion to F_A (see Table 1), as demonstrated by Vårum and Smidsrød (1997). They also concluded that the rates of acid hydrolysis of the glycosidic linkages in chitosans are of the order **A–A** ≈ **A–D** ≫ **D–A** ≈ **D–D**. The two first linkages were hydrolyzed about three orders of magnitude faster than the other two (Vårum, Ottøy & Smidsrød, 2000). To further investigate the role of acid hydrolysis in thermal degradation, the influence of the pH of the chitosan was also studied. Chitosan chloride with $F_A = 0.16$ was dissolved in deionized water and the pH was adjusted to 4, 5 and 6 before the samples were lyophilized by freeze-drying and thermally degraded at 105°C. The results are shown in Fig. 5, where $\Delta 1/[\eta]^{(1/a)}$ is plotted against the time of degradation. The initial rate of degradation is strongly dependent on the pH of the chitosan chloride. The initial degradation rate constants for the chitosan chloride at each pH are given in Table 3. The initial rate constant for chitosan chloride at pH 4 was about 30 times greater than at pH 6. Fig. 6 shows that the initial degradation rate constant of chitosan at pH 4–6 increased with the H^+ concentration in the 0.8 power. These results demonstrate that the concentration of H^+ ions in chitosan (measured as the pH of a 1% (w/w) chitosan chloride solution) is important for the time course of the thermal degradation of chitosan, and that acid hydrolysis is the primary mechanism involved.

Acid hydrolysis of the glycosidic linkages involves both protonation of the glycosidic oxygen and addition of water to yield the reducing sugar end group (BeMiller, 1967). The rate-determining step is the formation of the activated complex which is a cyclic carbonium–oxonium ion after the protonation. The chitosan powders used for the degradation studies all had a water content of about 10%. Although some water disappeared during the degradation, measurements of dry matter content indicated that the water content was never reduced below 4–5%. Compared to the low concentration of H^+ ions and thereby the activated complex

Table 3
Initial degradation rates (k) at temperature 105°C of chitosan chlorides ($F_A = 0.16$) freeze-dried from solutions with different pH values

pH	Degradation rate [10^{-6} h^{-1}]
4.1	660
5.0	99
6.0	20

in the chitosan, the water is probably present in excess enabling the acid hydrolysis to occur.

^{13}C NMR spectroscopy of the thermally degraded chitosan with $F_A = 0.35$ was used to study the specificity in the reaction as demonstrated by Vårum et al. (1996). Fig. 7 shows part of the ^{13}C NMR spectrum (carbons 4 and 5) of the thermally degraded chitosan ($F_A = 0.35$), showing the new non-reducing ends at 79.0 ppm (**D**-units) and 78.5 ppm (**A**-units). The degree of acetylation calculated from the non-reducing ends is 0.38, nearly the same as the degree of acetylation of the chitosan ($F_A = 0.35$). That means there is no specificity for the unit following the linkage that is cleaved, in agreement with the specificity found for acid hydrolysis (Vårum & Smidsrød, 1997).

Our data from the thermal degradation of chitosan chlorides of different chemical composition have shown that the degradation mechanism has a specificity for **A**-units. ^1H NMR spectroscopy was used to further investigate the specificity. The depolymerization of chitosans by acid hydrolysis is found to be specific in the sense that the protons attack the glycosidic oxygen following the non-charged **A**-units (Vårum & Smidsrød, 1997). ^1H NMR spectroscopy of the thermally degraded chitosan with $F_A = 0.35$ showed an unexpected resonance corresponding to the new reducing ends of **D**-units. As previously reported by Rupley (1964), hydrolysis of the *N*-acetyl bond (deacetylation) may occur in addition to the hydrolysis of the glycosidic bond. ^1H NMR spectroscopy was used to determine $\overline{DP_n}$ of thermally degraded chitosan chlorides ($F_A = 0.35$) and their F_A, calculated both from internal and end signals and calculated only from the new reducing ends. The results are given in Table 4. There were no significant changes in F_A of chitosan chloride before and after the thermal degradation. Looking at the F_A calculated only from the new reducing ends, the ratio between **A**-units and **D**-units at the new reducing ends decreased by depolymerization. Deacetylation was confirmed by proton spectrum of thermally degraded chitosan ($F_A = 0.35$) at pD of 3 and 6, showing the presence of acetate (data not shown). Acetate has a pK_a value at 4.7 and will therefore give a characteristic upfield shift in the proton spectrum when adjusting the pD from 3 to 6. Hydrolysis of the *N*-acetyl bond of the non-reducing ends was not observed with ^{13}C NMR. The new reducing ends of chitosan in the solid form seem to be more available for deacetylation than the non-reducing ends and the internal units. Vårum and Smidsrød (1997) studied the acid hydrolysis of chitosan in solution and found that the new reducing ends are clearly dominated by **A**-units. However, they have demonstrated that the rate of deacetylation and the rate of hydrolysis are of equal magnitudes in dilute acid solution (0.1 M HCl). Our results indicate that hydrolysis of the *N*-acetyl bond occurs in addition to the cleavage of the glycosidic bond, with specificity for the new reducing ends. The rate of acid hydrolysis of the glycosidic bond in chitosan solution was found by Vårum and Smidsrød (1997) to be in the order **A**–**A** ≈ **A**–**D** ≫ **D**–**A** ≈ **D**–**D**, and this is also consistent with our NMR data for thermal depolymerization of chitosan.

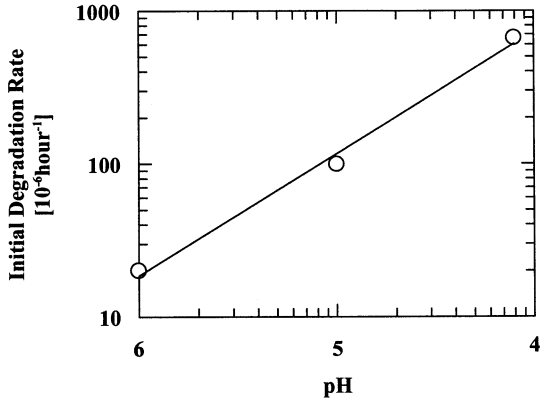

Fig. 6. Initial degradation rates (logarithmic scale) vs. pH for chitosan chloride ($F_A = 0.16$) at 105°C. The straight line in the figure is given by $k = 1.1[H^+]^{0.8}$.

Samples of chitosan chloride degraded at 105°C were analyzed by size exclusion chromatography coupled to a multi-angle laser light scattering detector (SEC–MALLS). The results indicated that the molecular weight decreased with increasing degradation time and 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 thermal degradation of chitosan chlorides is a random process. Because **A**- and **D**-monomers are randomly distributed along the chain, the binary heteropolysaccharide (chitosan) will be randomly degraded in spite of the fact that the degradation is specific for cleaving the glycosidic bond after an **A**-monomer.

The activation energies determined for the chitosan chlorides with $F_A = 0.02$–0.35 were identical (114–109 kJ/mol) within experimental errors. Moggridge and Neuberger (1938) determined the activation energies, E_a, for acid hydrolysis of the glycosidic linkage of model compounds such as methyl-2-acetamido-2-deoxy-β-D-glucopyranose and methyl-2-amino-2-deoxy-β-D-glucopyranose to be 28.4 (119 kJ/mol) and 36.0 kcal/mol K (151 kJ/mol), respectively. Methyl-2-acetamido-2-deoxy-β-D-glucopyranose showed an E_a for acid hydrolysis that corresponds to the E_a previously found for acid hydrolysis of glycosides (BeMiller, 1967) and the results found in this study for chitosan chlorides. Moggridge and Neuberger (1938) obtained a difference in E_a between the deacetylated unit and the acetylated unit, which is in apparent disagreement

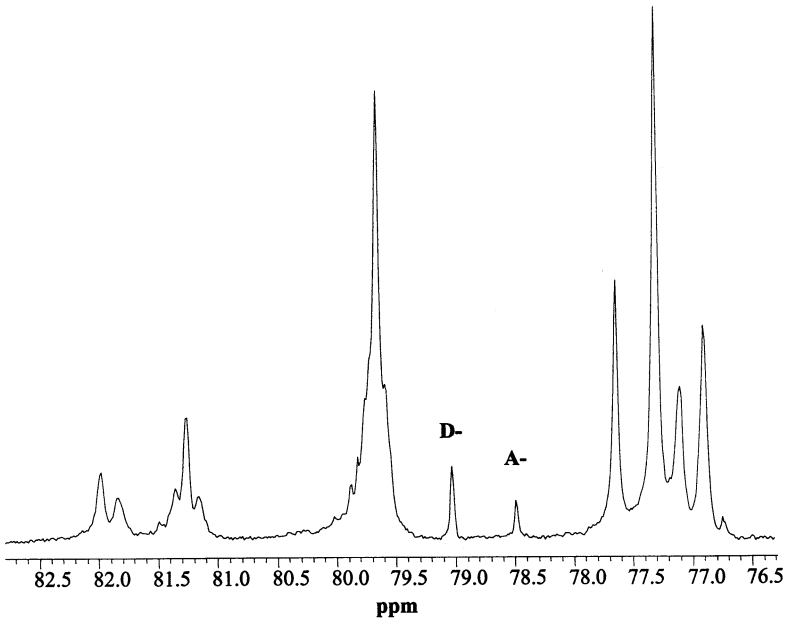

Fig. 7. Part of the ^{13}C NMR spectrum (carbons 4 and 5) of the thermally degraded chitosan ($F_A = 0.35$).

with the data presented here. However, since only initial degradation rates have been determined in this paper, it was estimated that even at the highest temperatures only 15% of the linkages adjacent to **A**-units (in chitosan with $F_A = 0.02$) had been cleaved in the range of degradation used to estimate the rate constant. The slow degradation of the chitosan with the lowest degree of acetylation is therefore mainly due to the low content of acetyl groups, resulting in lower collision frequencies between the catalytic protons and the **A**–**A** and **A**–**D** glycosidic linkages. The contribution from hydrolysis of the **D**–**A** and **D**–**D** linkages is probably negligible. Vårum et al. (2000) estimated that these two linkages were cleaved in solution at 60°C by a rate approximately three order of magnitude lower than the **A**–**A** and **A**–**D** linkages which is in harmony with the present analysis.

Vårum et al. (2000) have determined the E_a for acid hydrolysis of chitosan to be around 130 kJ/mol for the **A**–**A** and **A**–**D** linkages. The E_a is about 20 kJ/mol higher than the data presented in this paper. This may be due to a difference between acid hydrolysis of chitosan in solution and in the solid state. The reason why the rate of acid degradation of chitosan appears to be more temperature dependent in a solution than in a solid state is not clear, but both a loss of water at higher temperatures and lower thermal mobility in the solid state may contribute to the effect.

Table 4
Degree of polymerization, $\overline{DP_n}$, of thermally degraded chitosan chlorides ($F_A = 0.35$) and their degree of acetylation, F_A, calculated both from internal and end signals and calculated only from the new reducing ends

$\overline{DP_n}$	F_A	$F_{A,\ new\ red.\ ends}$
58	0.34	0.32
42	0.34	0.28
30	0.33	0.23
19	0.32	0.16

4. Conclusions

The initial rate constant of thermal degradation of chitosan chlorides in the solid state was found to increase markedly with the degree of acetylation, and increase with the H^+ concentration in the pH range of 4–6,when pH is defined as the pH of 1% (w/w) chitosan salt solution. The rate of degradation was found not to be dependent upon the presence of oxygen. The rate of thermal depolymerization of the glycosidic bond in chitosan appeared to be in the order **A**–**A** ≈ **A**–**D** ≫ **D**–**A** ≈ **D**–**D**, which is also valid for the rate of acid hydrolysis of chitosan in solution. Our data also indicated that some hydrolysis of the *N*-acetyl bond occurs in addition to the cleavage of the glycosidic bond, with high specificity for the new reducing ends. The activation energies of the three chitosan chlorides with $F_A = 0.02$, $F_A = 0.16$ and $F_A = 0.35$ were determined to be 114 ± 11, 112 ± 5 and 109 ± 5 kJ/mol, respectively.

Our data suggest that acid hydrolysis is the primary mechanism involved in the thermal depolymerization of chitosan chlorides in the solid state.

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