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Molecular weight manipulation of chitosan I: kinetics of depolymerization by nitrous acid

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Abstract

The kinetics of the depolymerization of chitosan in dilute aqueous HCl solutions by nitrous acid were studied. The rate of depolymerization is independent of the molecular weight of chitosan, first order with respect to the concentrations of both nitrous acid and glucosamine moieties, not catalysed by either hydrogen or chloride ions, and Arrhenius temperature dependent. Chitosan exhibits significantly decreased reactivity as the degree of deacetylation of the polymer increases. These results are consistent with a depolymerization reaction mechanism in which the rate-limiting step is nitrosation of the unprotonated amine by nitrous acidium ion.

Keywords: Chitosan; Nitrous acid; Molecular weight

1. Introduction

Chitosan, a copolymer derived from the abundant natural polymer chitin, is composed of 2-amino-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-glucose units. Chitosan and its derivatives have been used in a wide variety of applications [1], but the effectiveness of these materials has been found to be dependent upon their molecular size. Thus, the efficacy of chitosan to coagulate pollutants [2], accelerate burn healing [3], lower blood cholesterol levels [4], improve crop yields [5], enhance drug dissolution [6] and control viscosity [7] depends on this characteristic. Chitosan must be available with a molecular weight (MW) range of 3–4 orders of magnitude in order to satisfy the requirements of its various applications.

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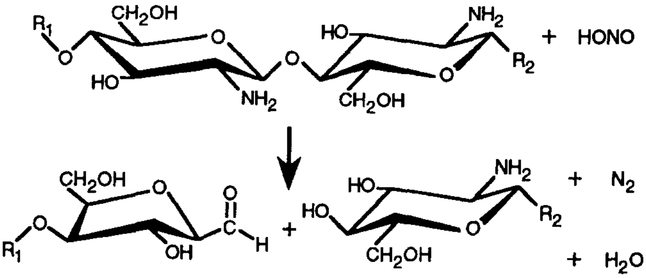

Fig. 1. Overall stoichiometry in the depolymerization of chitosan with nitrous acid.

An economical, rapid, well-understood, and easily controlled method of producing chitosan of a preselected size would greatly assist the technical and commercial utilization of this abundant polymer. Among the variety of chemical and non-chemical methods available to depolymerize chitosan [8], nitrous acid (HONO) offers several advantages. The reaction is selective, rapid, and easily controlled. In addition, the transformation of carbohydrate amines with HONO has long been used to study the structure of sugars [9,10], and, as a consequence, the stoichiometry and reaction products are well established [11-13]. The overall stoichiometry of the reaction between chitosan and HONO is shown in Fig. 1. Nitrosating species attack the amine groups, but not the *N*-acetyl moieties, and subsequently cleave the β-glycosidic linkages. One mole of HONO is consumed per mole of amine group reacted, and a 2,5-anhydro-D-mannose unit is formed at the reducing end of the cleaved polymer. Although a myriad of deamination products are often observed with carbohydrate amines, no side reactions have been observed with chitosan [14,15]. In general, the deamination reaction is very rapid, even in mildly acidic solutions at room temperature [12,16]. Furthermore, because chitosan is soluble in dilute acids, the reaction with HONO is homogeneous. Although depolymerization of chitosan with HONO has been previously examined [8,17-22], this publication reports the first systematic investigation of the depolymerization kinetics.

2. Experimental

Purification and characterization of chitosan.—Commercial chitosan samples were provided by Protan, Inc., Commack, NY (crab samples C52 and C81), Maripol Systems, Inc., Minneapolis, MN (squid samples C54 and C69), and Hercules, Inc., Wilmington, DE (shrimp sample C85). Unless otherwise noted chitosan samples were purified by dissolution in 1% (w/w) aq AcOH solutions, filtered through a non-woven polyester filter above a fine wire mesh, and precipitated with methanolic ammonia (70% methanol and 30% aq NH_4OH). The solids obtained were washed, at least three times with deionized water and then with acetone or methanol, before being air-dried, ground, and dried at 30-40°C under reduced pressure. The end groups of chitosan samples C54, C69, and C81 were reduced [23] with $NaBH_4$ prior to final purification.

Table 1
Characteristics of chitosan samples

Chitosan sample	dd (%) [a]	Amine content (mequiv/g)	LVN (cm^3/g) [b]
C52	52 ± 1	2.87 ± 0.06	–
C54	54.0 ± 1.0	2.99 ± 0.06	1,210 ± 15
C69	68.8 ± 1.0	3.96 ± 0.07	1,070 ± 20
C81	81 ± 3	4.79 ± 0.21	855 ± 40
C85	85 ± 2	5.08 ± 0.14	1,250 ± 20

[a] Degree of deacetylation.
[b] Limiting viscosity number.

The copolymer compositions of the various samples were measured by first-derivative UV spectroscopy [24] with a Perkin–Elmer Lambda Array 3840 spectrophotometer, calibrated with 2-acetamido-2-deoxy-α-D-glucose. The derivative absorbance maxima (192–195 nm) of chitosan solutions (40–150 mg/L) in aq AcOH (0.010 M) were measured at several (1–5) different concentrations, with replicate determinations (3–12) at each concentration. FTIR spectroscopy [25] was also utilized for determination of degree of deacetylation (dd) with films, cast from chitosan solutions (1.0%), neutralized with methanolic ammonia, and washed with water and methanol before final drying. The relative MWs of the samples were estimated from values of the limiting viscosity number, LVN. Kinematic viscosities of the solvent (0.10 M acetic acid and 0.20 M NaCl) and a number of chitosan solutions (5–10) of variable concentration (0.1–0.5 g/L) were measured at 25.0 ± 0.05°C with an Ubbelohde dilution capillary viscometer [26]. The measured characteristics of the chitosan samples are summarized in Table 1.

Potentiometric titration of chitosan for pK_a *determination.*—Chitosan samples (C54, C69, and C85) were analyzed by the procedure of Domard [27]. Replicate experiments (2–6) were conducted by dissolution of chitosan (0.6–1.0 mM amine) in aq HCl solution (2 mM) and titration with aq NaOH (20–50 mM) at 25°C. The amine pK_a value was computed [28] as a function of degree of ionization, pH, ionic strength, and mean ionic activity coefficient for the protonated amine.

Depolymerization of chitosan.—Stirred solutions (0.50–1.50% w/w; 40 mL) of chitosan in aq HCl (50.0–125 mM) maintained at selected temperatures were treated with a freshly prepared KNO_2 solution (1.0 mL) at the same temperature. The viscosity of the chitosan solutions rapidly decreased, and evolution of nitrogen gas was evident. At various times, aliquots (1–5 mL) of the mixture were pipetted into NaOH solutions (1.2–1.8 g/L) at 0°C to stop the reaction and precipitate the chitosan. The resulting suspension (pH 11–12) was immediately filtered through a glass fibre filter on top of a cellulose filter. The filtrate was analysed for its nitrite ion content [29], and an average concentration was calculated for replicate samples (2–4). Data were typically acquired until over 90% of the initial HONO had been consumed in the reaction.

Comparable experiments were performed in the absence of chitosan to characterize the combined kinetics of HONO decomposition and vaporization. Experiments were performed with KNO_2 (1.00 mM initially) in an aq HCl solvent (50.0 mM) at 25–65°C.

3. Basis of the measurement technique

Experimental design considerations.—Important experimental variables include chitosan and HONO concentrations, chitosan and solvent compositions, pH and temperature. Preliminary experiments [8] were performed to identify the extent to which these key factors could be varied. Aqueous HCl was selected as the solvent acid because it solubilizes chitosan samples over a wide range of copolymer compositions and hydrolyses chitosan slowly so as not to compete with the rapid rate of depolymerization by HONO [8]. In addition, because HONO is a relatively weak acid, a low pH (< 2) is necessary in order to convert the nitrite ion into HONO [28], but the pH cannot be so low as to render chitosan insoluble. A target pH range of $\sim 1-2$ was selected, and the experiments were designed so that [HCl] > [chitosan amine groups] ≫ [HONO]. An excess of hydrogen ions relative to amine groups buffers the pH of the solutions, while the low HONO:amine ratio significantly simplifies the data analysis and improves experimental accuracy. The range available for varying chitosan concentration is limited because solutions can be very viscous and difficult to mix effectively above concentrations of 1.5–2%. Preliminary experiments suggested that quantitative data can be acquired at, or somewhat above, room temperature, but at temperatures > 55°C the reaction rate is too rapid to measure accurately. Thus, the experimental design incorporated a relatively narrow temperature range of 25–55°C.

Nitrous acid disappearance as a measure of reaction rate.—Measurement of the rate of consumption of HONO offers several advantages over alternatives [30] for quantification of the reaction kinetics. HONO can be easily converted to nitrite ion, which can be accurately measured [29]. Moreover, the consumption of HONO by the deamination of chitosan is of known 1:1 stoichiometry, and determination of reactant consumption is much more accurate than direct measurement of polymer MW. Another advantage of the HONO technique is that identical protocols can be used to measure both the kinetics of chitosan depolymerization and the stability of HONO solutions.

For a batch reactor, the overall rate of disappearance, R, of HONO at concentration, C, at time, t, can be regarded as the sum of the rate of depolymerization, R_C, and the rates of other reactions and mechanisms involving HONO, R_N, so that

$$R = -\frac{dC}{dt} = R_C + R_N. \tag{1}$$

Clearly, the quantitative application of this method requires that the disappearance of HONO be mainly attributable to the depolymerization reaction and that side reactions involving HONO be minimized.

Apparent decomposition characteristics of nitrous acid.—The chemistry of HONO solutions is complex [31,32], and their instability has been documented [33–39]. Although the value of the Henry's Law constant for HONO is relatively small [40], volatilization from aqueous solution could contribute significantly to the overall rate of disappearance. An initial study [8] suggested that the assumption that the rate of chitosan depolymerization is much greater than the combined rate of disappearance by decomposition, oxidation, and volatilization is justifiable in most instances, but the two rates are

Table 2
Summary of the nitrous acid decomposition study

Temp. (°C)	Solution pH [a]	HONO lost (%) [b]	Apparent rate constant k_N (1/min) [c]
25	1.43	46	$6.68 \pm 0.41 \times 10^{-3}$
35	1.47	84	$1.47 \pm 0.04 \times 10^{-2}$
45	1.50	72	$2.81 \pm 0.04 \times 10^{-2}$
50	1.59	94	$3.15 \pm 0.12 \times 10^{-2}$
65	1.70	83	$6.91 \pm 0.29 \times 10^{-2}$

[a] 50 mM HCl solvent.
[b] Measured % of initial nitrite ion lost during the experiment.
[c] 95% confidence interval.

comparable under certain conditions. A small correction in quantifying the depolymerization kinetics is therefore required.

Measurement of the overall disappearance of HONO from solution lumps together several effects; in this study, the term ‘apparent decomposition’ refers to the combined effects of HONO decomposition, oxidation, and vaporization. The apparent rate of HONO decomposition in 50 mM HCl solvent was studied over a range of temperatures of 25–65°C; first-order behaviour was observed in all experiments, consistent with previous results [8]. At 25°C half of the initial HONO disappeared in just over 100 min.

For a constant volume batch reaction following first-order kinetics,

$$R_N = -\frac{dC}{dt} = k_N C, \tag{2}$$

and

$$\ln\left(\frac{C}{C_0}\right) = -k_N t, \tag{3}$$

where k_N represents the apparent first-order rate constant for HONO decomposition, and C_0, the initial HONO concentration. The data were fitted to Eq. (3) by least-squares linear regression analysis, and the derived rate constants are summarized in Table 2. The applicability of an Arrhenius temperature dependence was examined, but it was found to be unsuitable because so many effects are lumped into the apparent decomposition kinetics.

Due to the importance of acid–base equilibria, the kinetics will likely be influenced by the acidity of the solution, at least until the pH is low enough so that complete conversion of nitrite ion into HONO is achieved. Calculations [28] based on the pK_a of HONO (3.1) indicate that below pH ~ 2 at 25°C this conversion is essentially complete. Since the pH values for experiments in 50 mM HCl were 1.4–1.7, the kinetics should be independent of pH. This conclusion is corroborated by the work of Hughes et al. [41] on the decomposition of HONO in perchloric acid solutions that showed the rate was not catalysed by hydrogen ion once conversion was complete. In our study, the HONO decomposition behaviour was sufficiently characterized by the experiments in 50 mM HCl solution so that results can be directly compared to the chitosan depolymerization experiments, where nearly identical conditions were maintained.

Table 3
Experimental conditions and results of the kinetic studies of chitosan depolymerization

Exp. code	Chitosan sample	Temp. (°C)	Chitosan concentration		$[HONO]_0$ (mM)[b]	[HCl] (mM)	pH range	χ (%)[c]	k_C (1/min)[d]
			wt%	[amine] (mM)[a]					
1	C69	25	0.976	38.7	0.779	50	2.29	98	0.0385 ± 0.0054
2	C69	25	0.976	38.7	0.783	100	1.27	71	0.0402 ± 0.013
3	C69	25	0.976	38.7	0.194	60	1.84	96	0.0400 ± 0.0046
4	C69	25	0.976	38.7	3.90	60	1.8	96	0.0463 ± 0.0024
5	C69	35	0.976	38.7	0.196	50	2.24	97	0.120 ± 0.014
6	C69	35	0.976	38.7	0.197	100	1.28	82	0.133 ± 0.018
7	C69	35	0.976	38.7	3.89	50	2.3–2.6	98	0.120 ± 0.006
8	C69	35	0.976	38.7	3.89	100	1.31	94	0.123 ± 0.008
9	C69	35	0.976	38.7	0.778	60	1.87	99	0.131 ± 0.010
10	C69	35	0.976	38.7	0.781	60	1.86	96	0.130 ± 0.014
11	C69	35	0.976	38.7	0.778	60	1.85	98	0.124 ± 0.010
12	C69	35	0.976	38.7	0.776	60[e]	1.86	98	0.119 ± 0.006
13	C69	35	0.500	19.8	0.404	50	1.67	84	0.0542 ± 0.0070
14	C69	35	0.732	29.0	0.595	75	1.50	89	0.0944 ± 0.0036
15	C69	35	1.40	55.4	1.12	120	1.38	98	0.211 ± 0.009
16	C69	40	0.980	38.8	0.781	60	1.84	99	0.241 ± 0.018
17	C69	45	0.976	38.7	3.87	60	1.9	98	0.372 ± 0.008
18	C69	50	0.977	38.7	1.94	60	2.05	99	0.666 ± 0.021
19	C69	55	0.978	38.7	1.93	60	2.10	97	0.975 ± 0.070
20	C52	35	0.612	[f]	0.696	100	1.25	42	[f]
21	C54	35	0.500	15.0	0.321	60	1.52	81	0.0620 ± 0.0037
22	C54	35	0.850	25.4	0.549	80	1.41	94	0.130 ± 0.004
23	C54	35	1.21	36.2	0.779	110	1.48	99	0.205 ± 0.015
24	C54	35	1.50	44.9	[g]	60	–	–	–
25	C81	35	0.832	39.8	0.784	100	1.34	95	0.0539 ± 0.0021
26	C85	35	0.743	37.7	0.784	100	1.30	94	0.0221 ± 0.0012
27	C85	35	1.06	54.1	1.12	120	1.23	97	0.0331 ± 0.0013
28	C85	35	1.20	61.0	1.25	80	2.6	84	0.0392 ± 0.0065
29	C85	35	1.50	76.2	1.57	125	1.23	55	[h]

[a] Initial amine concentration.
[b] Initial HONO and KNO_2 concentrations.
[c] Final extent of reaction of nitrous acid.
[d] 95% confidence interval reported.
[e] Also contained 1.0 M NaCl.
[f] Chitosan not fully dissolved, so no reliable data available.
[g] Polymer solution too viscous for magnetic stirring; no data taken.
[h] Data not included since final extent of reaction was too low.

4. Depolymerization kinetics of chitosan

Chitosan samples of various dd values were dissolved in aqueous HCl of differing concentrations and reacted with HONO at various initial concentrations over a range of temperatures. The reaction conditions investigated and the results obtained are summarized in Table 3.

Typical kinetics are illustrated in Fig. 2, where the fraction of HONO remaining is shown as a function of time after addition of KNO_2 to the acidic chitosan solutions, for

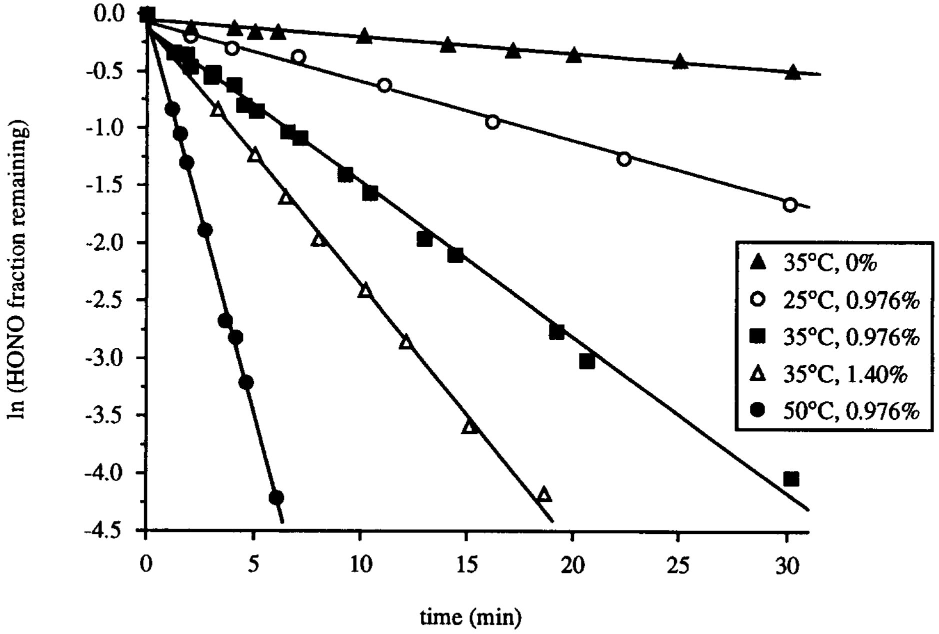

Fig. 2. Effect of reaction temperature and chitosan concentration (wt%) on the observed disappearance of HONO in chitosan sample C69 solutions (Expts 4, 11, 15, and 18). 0% chitosan refers to apparent decomposition of HONO under identical experimental conditions.

experiments at different reaction temperatures and chitosan concentrations. The results demonstrate that HONO is consumed by reaction with chitosan at a much greater rate than it decomposes or vaporizes. The reaction rate also increases with reaction temperature. Furthermore, because the rate of disappearance of HONO increases with chitosan concentration, a bimolecular reaction mechanism is suggested. The linearity of plots of the logarithm of HONO fraction remaining versus time, for these and all other experiments, demonstrates that the kinetics are first order with respect to HONO, despite the wide range of experimental conditions investigated. Similar results were observed with chitosan samples with other copolymer compositions (54–85% dd). As the reaction proceeds, the MW drops dramatically, by as much as three orders of magnitude in some experiments. Even with this large drop in MW, the reaction continues to show first-order kinetics with respect to HONO, confirming that the depolymerization kinetics are independent of the size of the chitosan substrate.

Linear regression analysis of the natural logarithm of concentration–time data was used to determine the apparent first-order rate constants, which include the effects of both chitosan depolymerization and HONO decomposition. Since both chitosan depolymerization kinetics and apparent HONO decomposition characteristics are first order with respect to HONO concentration (Fig. 2), the expression for the overall rate of disappearance of HONO (R) may be written as

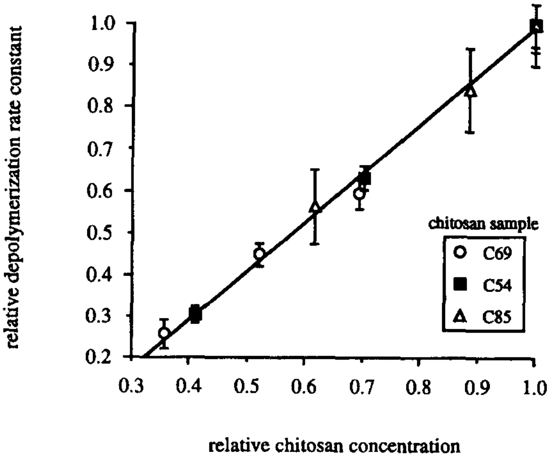

Fig. 3. Effect of amine concentration on the observed depolymerization rate for chitosan samples of different copolymer compositions. Data are normalized as described in the text.

$$R = -\frac{dC}{dt} = kC = (k_N + k_C)C. \tag{4}$$

The apparent rate constant, k, is therefore the sum of the rate constants for depolymerization, k_C, and apparent decomposition, k_N. Thus, values of k_C can be determined from experimental values of k and k_N, as summarized in Table 3. In general, since the values of k_C are significantly larger than k_N, the uncertainties of the derived values of k_C are relatively small. Also, results from replicate runs (Expts 9–12) illustrate the reproducibility of the data.

The effect of the various experimental parameters on the depolymerization kinetics can be examined by comparing values of k_C. Nitrosation is generally the rate-limiting step in the reaction of amines and HONO [42], so the reaction rate is expected to be proportional to the concentration of amine groups. Experimental conditions were chosen such that the concentration of 2-amino-2-deoxy-D-glucose (glucosamine) units was in great excess compared to the HONO concentration. The concentration of amine groups can therefore be considered to be essentially constant over the course of the experiments. Thus, the value of k_C includes the effect of the concentration of chitosan amine groups, as suggested in Fig. 2. This is verified in Fig. 3 where the relative change in k_C is shown as a function of the relative increase in the concentration of glucosamine units for three chitosan copolymer compositions. Separate experiments were conducted at different amine concentrations (3–4) for each of these three chitosan samples. The relative values of k_C for a given dd value were then calculated by dividing k_C at a given amine concentration by its value at the highest amine concentration in the series of 3–4 experiments. In this way normalized data from all chitosan samples can be plotted simultaneously. The data, shown in Fig. 3, fall on a single straight line with a slope very close to unity. The small deviation is most probably due to the importance of the competing HONO decomposition mechanism [Eq. (1)] at lower chitosan concentrations.

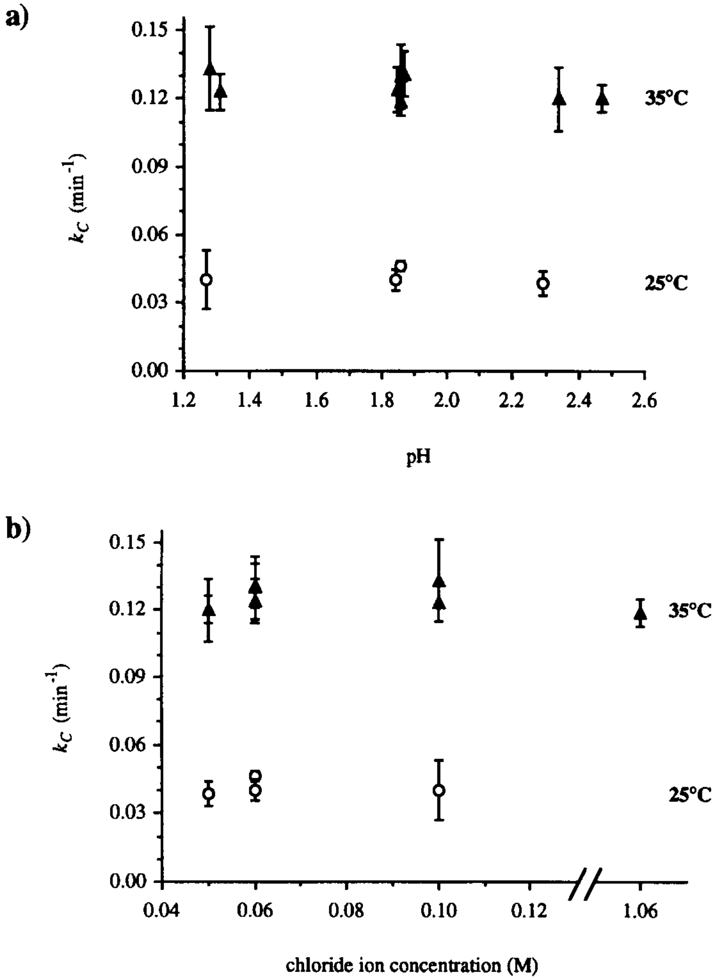

Fig. 4. Effect of (a) hydrogen-ion and (b) chloride-ion concentration on the chitosan depolymerization rate constant for sample C69 solutions (38.7 mM amine) at 25 and 35°C.

Thus, at a given copolymer composition, the value of k_C is directly proportional to amine concentration, which confirms that the overall rate of depolymerization is proportional to amine concentration.

In contrast to the effects of HONO and chitosan concentration, the depolymerization kinetics were relatively insensitive to the solvent acid composition. In Fig. 4(a) the dependence of the depolymerization rate constant on hydrogen-ion concentration is presented. Over the pH range 1.2–2.4, k_C is independent of pH. Because this range represents a 16-fold increase in hydrogen-ion concentration, it may be concluded that the deamination reaction is not catalysed by hydrogen ion. Some amine reactions with HONO in HCl solvents are catalysed by chloride ion [13], but the data shown in Fig.

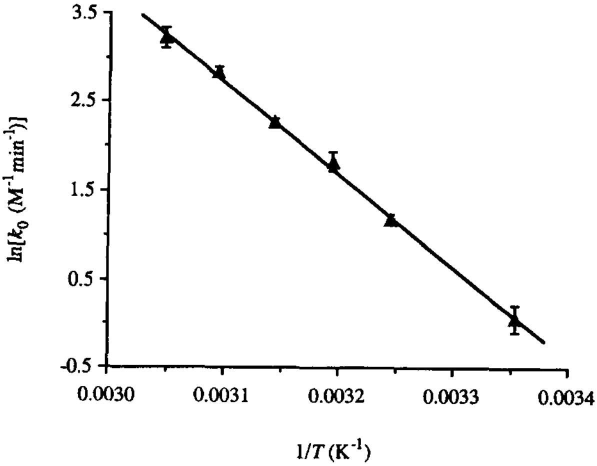

Fig. 5. Arrhenius plot of the logarithm of the observed depolymerization rate constant for sample C69 vs. inverse temperature.

4(b), where k_C is plotted against chloride ion concentration, demonstrate the absence of this effect. No increase in reaction rate was observed even when 1.0 M NaCl was added (Expt 12).

The amine groups on chitosan are basic (pK_a ~ 6.5), so virtually all are protonated in solutions containing excess HCl. Thus, the depolymerization rate expression can be written in terms of the observable protonated amine and HONO concentrations, as

$$R_C = k_C[\text{HONO}] = k_0[\text{GNH}_3^+][\text{HONO}], \qquad (5)$$

where R_C represents the reaction rate and, k_0, the observed depolymerization rate constant. The value of k_0 is then only a function of chitosan composition and temperature, and for each chitosan sample its value can be easily computed from the relationship $k_0 = k_C/[\text{GNH}_3^+]$. Consequently, the suitability of an Arrhenius temperature dependence for k_0 was assessed by the use of

$$k_0 = k_0^* \exp\left[-\frac{E_A}{RT}\right], \qquad (6)$$

where the parameters are the activation energy, E_A and frequency factor, k_0^*. This model holds very well over the relatively narrow temperature range of 25–55°C for the results from chitosan sample C69 (Fig. 5). By application of the method of least-squares with reparameterization [43], E_A was determined to be 87.1 ± 1.5 kJ/mol, while ln[k_0^* (1/M min)] was 35.2 ± 1.2. This value of E_A is close to that determined for both the homogeneous acid hydrolysis of chitosan (82 kJ/mol [44]) and the heterogeneous alkaline hydrolysis of chitosan (92 kJ/mol [45]), but is significantly lower than the value reported for the heterogeneous acid hydrolysis of chitin (124 kJ/mol [1]).

In addition to the effect of temperature, the data in Fig. 6 show that the value of k_0 decreases significantly as the dd value of chitosan increases. These results can be

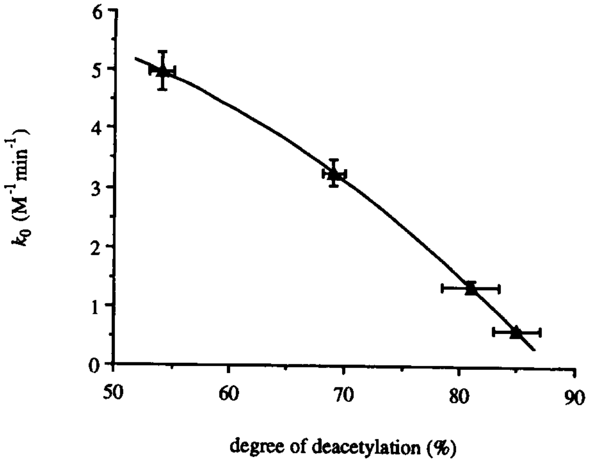

Fig. 6. Effect of the degree of deacetylation of chitosan on the observed depolymerization rate constant at 35°C.

understood by hypothesizing an appropriate reaction mechanism. Since the observed kinetics are bimolecular, nitrosation must be the rate-limiting step in the reaction; a conclusion that is consistent with findings for nitrosation of low-molecular-weight aromatic and aliphatic amines [42]. Moreover, it is well established [13] that it is the highly reactive substances originating from HONO that attack the amine groups, not the HONO itself. Nitrosation-limited reactions have been identified for a number of attacking species, the most common of which are nitrosyl chloride (NOCl), dinitrogen trioxide (N_2O_3), and nitrous acidium ion ($H_2NO_2^+$).

Because the measured chitosan depolymerization kinetics are first order with respect to HONO and not second order, N_2O_3 cannot be the attacking electrophile [41]. Likewise, since NOCl attack would be catalysed by chloride ion, which is inconsistent with the observed kinetics, NOCl cannot be involved. However, attack by $H_2NO_2^+$ would be first order in HONO concentration and independent of both hydrogen and chloride-ion concentration. All the expected trends for nitrosation by $H_2NO_2^+$ are consistent with the data observed for chitosan depolymerization, and the intermediacy of this entity has been postulated in the HONO degradation of amine-containing compounds [46–50]. The kinetics for nitrosation by nitrosonium ion (NO^+) and $H_2NO_2^+$ are not easily distinguishable [15], but the $H_2NO_2^+$ mechanism is favoured at low solution acidities such as those used in the chitosan depolymerization experiments.

Since the unprotonated amine is a far more reactive nucleophile than the protonated form [41,42,51], it is reasonable to postulate a bimolecular nitrosation mechanism with the reaction of $H_2NO_2^+$ and unprotonated amine groups, GNH_2, on chitosan as the rate-limiting step. The bimolecular rate expression for this mechanism is then defined as

$$R_C = k_f[H_2NO_2^+][GNH_2], \qquad (7)$$

where k_f represents the fundamental nitrosation rate constant. Unfortunately, low $H_2NO_2^+$ concentrations have not been directly measured. However, by employing a commonly applied approach for identification of nitrosation mechanisms [42], and assuming that equilibrium is attained, the $H_2NO_2^+$ and unprotonated amine concentrations can be related to measurable quantities. The equilibrium relationships for the formation of $H_2NO_2^+$ and the dissociation of protonated amine groups are defined by Eqs (8) and (9), respectively, as

$$\mathrm{HONO} + \mathrm{H}^+ \overset{K_\mathrm{E}}{\rightleftharpoons} \mathrm{H_2NO_2^+} \tag{8}$$

and

$$\mathrm{GNH_3^+} \overset{K_\mathrm{A}}{\rightleftharpoons} \mathrm{GNH_2} + \mathrm{H}^+ \tag{9}$$

By substitution of these equilibrium relationships into Eq. (7), it follows that

$$R_\mathrm{C} = k_\mathrm{f} K_\mathrm{A} K_\mathrm{E}[\mathrm{HONO}][\mathrm{GNH_3^+}]. \tag{10}$$

Thus, the rate expression for the proposed mechanism has been restated in terms of experimentally observable concentrations. By comparing Eqs (5) and (10), it is clear that the observed and fundamental rate constants are related by

$$k_0 = k_\mathrm{f} K_\mathrm{A} K_\mathrm{E}. \tag{11}$$

In addition, the measured values of k_C, shown in Table 3, can be related to the more fundamental constants by the expression

$$k_\mathrm{C} = k_\mathrm{f} K_\mathrm{A} K_\mathrm{E}[\mathrm{GNH_3^+}] = k_0[\mathrm{GNH_3^+}]. \tag{12}$$

The experimental data are thereby rationalized by the proposed mechanism, and it may be concluded that the depolymerization of chitosan involves a rate-limiting nitrosation step in which the unprotonated amine of chitosan is attacked by nitrous acidium ion. Such a mechanism has apparently not previously been documented for chitosan or other amine-containing polymers, although it has been postulated for the deamination of acetyltryptophan [46] as well as for aliphatic hydroxylamine compounds [47,48].

Although the effect of copolymer composition on the observed rate constant, k_0, was established in Fig. 6, evaluation of the effect of this property on the fundamental rate constant, k_f, which is a measure of the inherent reactivity the nucleophilic amine, provides additional insight. By combining the measured values of k_0 with the equilibrium constants, k_f can be computed. The pK_a values of the amine units on chitosan have been determined to be a strong inverse function of the dd value, and this effect is even more pronounced when the extent of amine protonation is increased [27]. Values of the pK_a of the amine groups on chitosan (extrapolated to 100% amine protonation to mimic reaction conditions) are shown in Fig. 7. Unfortunately, because of low $H_2NO_2^+$ concentrations, the value of the equilibrium constant for the formation of $H_2NO_2^+, K_\mathrm{E}$, has never been determined. Consequently, absolute values of k_f cannot be directly computed. An alternative approach is to compare the relative values of k_f for samples of differing copolymer compositions by use of the ratio

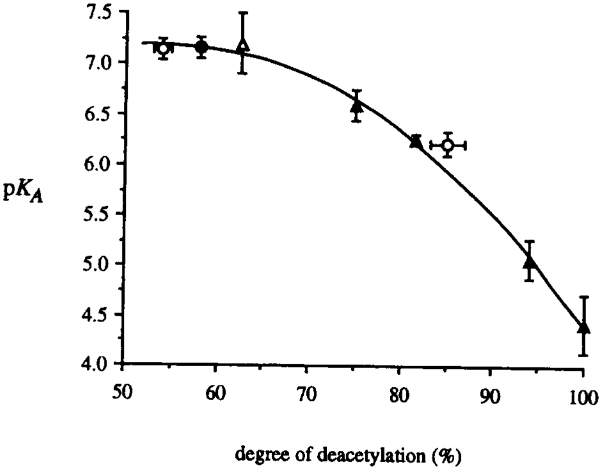

Fig. 7. Effect of the degree of deacetylation of chitosan on the pK_a values of its amine groups. Results of Domard [27] (▲), Terayama [55] (△), Muzzarelli [56] (●) and this work (○) extrapolated to 100% amine protonation.

$$\text{relative reactivity} = \frac{(k_f)_{dd}}{(k_f)_{54}} = \frac{(k_0/K_A)_{dd}}{(k_0/K_A)_{54}}. \qquad (13)$$

The subscripts after the parentheses refer to percentage dd values. Although any dd value could be used as a basis for comparison, a 54% dd chitosan sample (C54) was chosen because it exhibited the maximum reactivity and has the lowest dd value that is still soluble in dilute HCl solutions. Experimental values of k_0/K_A are listed in Table 4 for chitosan samples with four different copolymer compositions, and the relative reactivity of the chitosan amine groups with $H_2NO_2^+$ is shown in Fig. 8. The dd value has a pronounced effect on the inherent reactivity of the amine groups, which is even more significant than its effect on k_0. An increase in the dd value from 54 to 85% resulted in a 100-fold decrease in the fundamental reactivity of the amine, while the observed rate of depolymerization decreased by only a factor of eight.

A basis for this dramatic change in amine reactivity is suggested by the data in Fig. 7, which show that the pK_a value of the amine units on chitosan drops from 7.2 to 4.4 as the dd value increases from 54 to 100%. For ionic reactions, the pK_a value is an

Table 4
Comparison of chitosan depolymerization rate constants

Chitosan sample	k_0 (1/M min)	Amine pK_a	k_0/K_A (1/M^2 min)
C54	4.97 ±0.32	7.14±0.10	$6.86 \pm 1.4 \times 10^7$
C69	3.26 ±0.22	6.93±0.10	$2.77 \pm 0.59 \times 10^7$
C81	1.35 ±0.08	6.28±0.10	$2.57 \pm 0.51 \times 10^6$
C85	0.614±0.039	5.97±0.10	$5.73 \pm 1.2 \times 10^5$

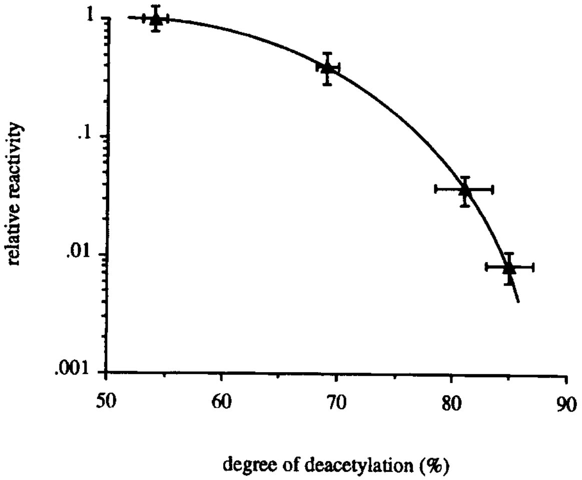

Fig. 8. Effect of the degree of deacetylation of chitosan on the fundamental nitrosation rate constant relative to the value at 54% dd (35°C).

excellent measure of nucleophilic reactivity [52], and it has been correlated with the nitrosation rates of aliphatic and aromatic amines [53]. In other investigations, a unit decrease in the pK_a value resulted in an order of magnitude decrease in the reactivity of aromatic amines [51], while the reactivity of aliphatic amines was observed to decrease by a factor of 50 with a change in pK_a from 10.8 to 9.2 [54]. Thus, the decreased nitrosation rate of chitosan with an increase in the dd value appears to be due to a reduction in the nucleophilicity of the amine moieties.

The simplicity of the kinetics of the depolymerization of chitosan with nitrous acid is remarkable, and the reaction can be recommended for the reliable manipulation of the molecular size of the aminopolysaccharide.

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