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Molecular weight manipulation of chitosan II: prediction and control of extent of depolymerization by nitrous acid

G. Graham Allan *, Mark Peyron

Department of Chemical Engineering and College of Forest Resources, University of Washington, Seattle, WA 98195, USA

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Abstract

The molecular size of chitosan can be precisely modified by depolymerization with nitrous acid. The stoichiometry and specificity of the reaction, together with the simplicity of the reaction kinetics, provide the basis for derivation of a theoretical expression that relates the number-average molecular weight (M_n) of chitosan before and after depolymerization to the mass ratio of nitrous acid added to chitosan originally present. The simplicity of the expression and the lack of limiting assumptions in its derivation permits its use for the determination of the amount of nitrous acid required to degrade chitosan to a specified molecular weight or for the estimation of the M_n of high molecular weight chitosan. Optimized experimental conditions are identified for quantitative application of the method.

Keywords: Chitosan; Nitrous acid; Molecular weight; Depolymerization

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1. Introduction

Chitosan and its derivatives have found use in a wide variety of applications [1], but the properties and effectiveness of these materials are dependent upon their molecular size; thus a need is evident for a controlled method of manipulating this characteristic. Chitosan depolymerization by the action of nitrous acid (HONO) is particularly advantageous because the reaction between chitosan and HONO, Fig. 1, has been investigated, and the stoichiometry and reaction products are established [2–4]. Nitrosating species

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* Corresponding author.

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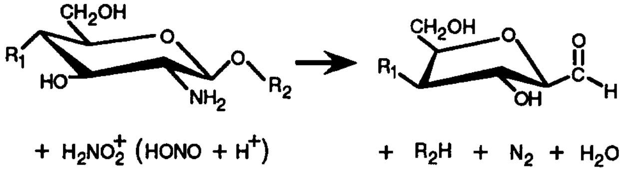

Fig. 1. Overall stoichiometry of the depolymerization of chitosan with nitrous acid.

originating from HONO selectively attack the amine groups and subsequently cleave the β-glycosidic linkages of the polymer chain. 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 [5,6]. In the accompanying paper [7] and in a preliminary communication [8], the kinetics of chitosan depolymerization with HONO have been reported. The specificity of this reaction and the simplicity of the kinetics allows for the derivation of a simple relationship between the M_n of a chitosan sample before and after depolymerization. This affords the opportunity of using depolymerization of chitosan with HONO to quantitatively manipulate the molecular size of the polysaccharide.

2. Experimental

Identical procedures to those reported [1] for the chitosan depolymerization experiments were followed to establish the combined rates of HONO decomposition, vaporization, and oxidation for KNO_2 (1.00 mM initially) in an aqueous HCl solvent (50.0 mM), with a cylindrical reactor with a surface-area-to-solution-volume (S/V) ratio of 0.28 cm^{-1} and at temperatures of 25–65°C. Additional experiments were performed with a 37.5 mM HCl and 12.5 mM HNO_3 solvent at 35°C (S/V ratio 0.28 cm^{-1}). The effect of reducing the S/V ratio from 0.28 to 0.20 cm^{-1}, by reducing the reactor diameter and utilizing a cap on the reaction vessel with only a 1 mm hole for escape of the gaseous products, was also studied.

3. Model derivation

Consider an initial solution of chitosan of number-average molecular weight, M_1, and degree of polymerization, dp_1. For a given copolymer composition the average formula weight of the repeating unit, M_0, is fixed, and the initial number of moles of chitosan polymer chains, N_1, is related to the initial mass of chitosan in solution, m_1, according to

$$N_1 = \frac{m_1}{M_1} = \frac{m_1}{M_0 \mathrm{dp}_1}. \tag{1}$$

Now suppose that n moles of HONO is added to depolymerize chitosan, thereby reducing the number-average degree of polymerization to dp_2 and increasing the number of moles of polymer chains to N_2. The values of dp before and after depolymerization can be related as

$$\frac{\mathrm{dp}_1}{\mathrm{dp}_2} = \frac{N_2}{N_1}. \tag{2}$$

Of the n moles of HONO added to the chitosan solution, n_c moles cleave the chain, n_{eg} react with the amine group on the reducing end of the polymer molecule, n_d decompose or are volatilized and may not react further with chitosan, and n_s undergo side reactions with the polymer, so that

$$n = n_c + n_{eg} + n_d + n_s. \tag{3}$$

According to the 1:1 stoichiometry of the deamination reaction, for every n_c moles that cleave the chain, n_c new moles of shorter polymer molecules are formed. As the reaction proceeds the number of moles of polymer chains in solution increases according to

$$N_2 = N_1 + n_c. \tag{4}$$

Upon substitution of Eq. (4) into Eq. (2), and using the definition in Eq. (1), expressions can be derived for dp_2 and M_2:

$$\frac{1}{\mathrm{dp}_2} - \frac{1}{\mathrm{dp}_1} = \frac{n_c M_0}{m_1} \tag{5}$$

and

$$\frac{1}{M_2} - \frac{1}{M_1} = \frac{n_c}{m_1}. \tag{6}$$

It is therefore possible to determine the M_n or dp_n from the initial molecular weight (MW) and knowledge of the number of moles of HONO that cleave the chitosan chain per unit mass of chitosan.

Since knowledge of the amount of HONO added to the chitosan solution is not sufficient to establish n_c, the relative importance of n_{eg}, n_s, and n_d must be determined. For example, it has been determined that HONO reacts selectively with the amine groups on chitosan; no side reactions with the polymer have been observed even when the depolymerization is conducted in the presence of oxygen [5,6]. Therefore, it may be confidently assumed that $n_s = 0$.

Prediction of n_{eg} is possible by assuming that all amine groups are equally reactive, which is expected for homogeneous depolymerization conditions. Thus, an instantaneous probability that an end group will react, P_{eg}, can be defined based upon the total number of reactable groups (i.e., amine-containing sugar residues) in solution. For every n moles of HONO consumed, $n_c + n_{eg}$ moles of reactable groups are lost via deamination. Because a reaction that cleaves the chain produces a 2,5-anhydro-D-mannose end group, which no longer contains a reactive amine, only reactions of end group amines originally present result in a loss of reactable end groups. The degree of deacetylation, dd, is defined as the fraction of sugar residues composed of glucosamine (2-amino-2-deoxy-Dglucose), so the average number of amine groups per polymer chain is the product of the dd value and the $\mathrm{dp_n}$. Since only one reducing end group is present per chitosan chain, and of those, only a fraction equal to the dd value contain amine groups that may react with HONO, the number of reactable end groups per chain is equal to the dd value. Accordingly, the initial probability that an end group will react is $1/\mathrm{dp_1}$, and this probability can be shown to be constant during the course of the reaction [9].

$$P_{eg} = \frac{\text{number of reactable end groups}}{\text{total number of reactable groups}} = \frac{1}{\mathrm{dp_1}}. \tag{7}$$

The value of n_{eg} can then be expressed as a function of n_c according to

$$n_{eg} = \left(\frac{P_{eg}}{1 - P_{eg}}\right) n_c. \tag{8}$$

For chitosan with even a moderately high MW, the value of P_{eg} is insignificantly small and may be ignored. None the less, this general result is included in the subsequent derivation in the interests of completeness.

In order to account for all the HONO added to the chitosan solution, the amount which decomposes, oxidizes, or is lost to the gaseous phase must be considered. Ideally the depolymerization procedure should be conducted so that the rate of depolymerization is much greater than the rates of loss by these mechanisms (i.e., $n_c \gg n_d$). If this cannot be achieved, then n_d must be determined. Let f_d be defined as the fraction of the initial HONO that decomposes, so that

$$f_d = \frac{n_d}{n}. \tag{9}$$

Substitution of Eqs (8) and (9) into the mass balance, Eq. (3), yields

$$n_c = n - n_d - n_{eg} = n(1 - f_d)\left(1 - \frac{1}{\mathrm{dp_1}}\right). \tag{10}$$

By combining the above relationship with Eqs (5) and (6) the change in $\mathrm{dp_n}$ or M_n may be determined for a given initial HONO-to-chitosan ratio (n/m_1).

$$\frac{1}{M_2} - \frac{1}{M_1} = \frac{n}{m_1}(1 - f_d)\left(1 - \frac{1}{\mathrm{dp_1}}\right) \tag{11}$$

and

$$\frac{1}{\mathrm{dp_2}} - \frac{1}{\mathrm{dp_1}} = \frac{n}{m_1}(1 - f_d)\left(1 - \frac{1}{\mathrm{dp_1}}\right)M_0. \tag{12}$$

These simple relationships for prediction of the extent of depolymerization are analogous to time-dependent kinetic models derived for random chain scission and homolytic depolymerization processes [10]. This derivation makes no presumption about the MW distribution, only that HONO side reactions with chitosan are insignificant, and that all amine groups are equally reactive. The final MW distribution should be determined by the initial distribution of amine groups [6], since the homogeneous deamination reaction is expected to occur randomly.

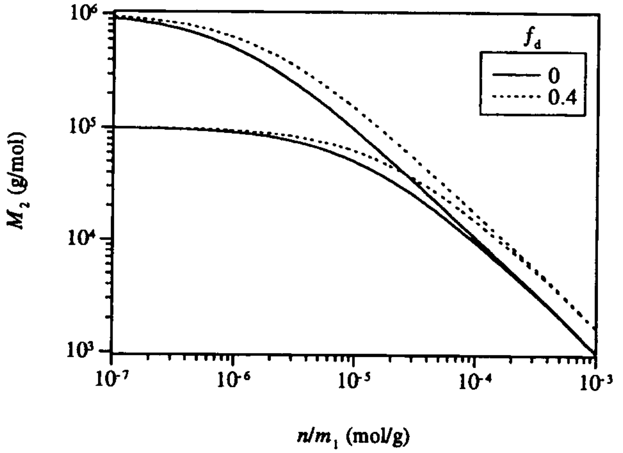

Fig. 2. Effect of the nitrous acid-to-chitosan ratio (n/m_1) on the predicted M_n after depolymerization, for two values of M_1 (10^5 and 10^6 g/mol) and f_d (0 and 0.4).

Prediction of the extent of chitosan depolymerization based on the degradation conditions provides flexibility in producing chitosan samples of tailored MW. The effect of the HONO-to-chitosan ratio on the final MW achieved is illustrated in Fig. 2; this demonstrates the wide range of molecular sizes that may be produced. Clearly, the significance of competing HONO decomposition mechanisms must be quantified or minimized in order to accurately apply Eqs (11) and (12).

4. Application of the extent of depolymerization relationships

The theoretical expression for prediction of the extent of depolymerization provides the opportunity to (*i*) estimate the molecular size of high MW chitosans, and (*ii*) produce chitosan of a chosen MW from a higher MW sample. The first application involves depolymerization under controlled reaction conditions, measurement of the MW of the depolymerized material, and computation of the initial MW. Consider an experiment in which a known amount of HONO (n) is used to depolymerize a known mass of chitosan (m_1), and the value of M_2 is then measured experimentally by techniques such as osmometry or reducing end group methods, which are more accurate when applied to smaller polymers. The initial MW, M_1, could then be calculated with Eq. (11). Controlled depolymerization provides a simple means of estimating the size of high MW chitosans, provided that the extent of decomposition, f_d, is known or the reaction is conducted so that f_d is negligibly small.

The second use of the expression for the prediction extent of depolymerization is essentially the reverse of the above; i.e., when chitosan of a known M_1 is depolymerized, then the final MW can be predicted based on the n/m_1 ratio. When relatively large chitosan is converted to low or moderately lower MW material, Eqs (11) and (12) can be simplified to

$$M_2 = \frac{m_1}{n(1 - f_d)} \tag{13}$$

and

$$dp_2 = \frac{m_1}{nM_0(1 - f_d)}. \tag{14}$$

Thus, smaller chitosans of gravimetrically predictable size can be created from larger starting material of unknown MW.

Obviously, the quantitative application of Eqs (11–14) is facilitated if the depolymerization reaction is conducted so that $f_d \cong 0$. If that condition cannot be achieved, then the value of f_d must be known. Unfortunately, the instability of HONO solutions [11–13], together with the volatility of HONO from dilute aqueous solutions [14], contribute significantly to the disappearance of HONO. The results of the accompanying chitosan depolymerization study [7] demonstrate that, although the rate of HONO disappearance by chitosan depolymerization is generally much greater than the combined rate of disappearance by decomposition, oxidation, and volatilization, the observed values of f_d may be significant under certain experimental conditions. In order to compare the depolymerization and apparent decomposition rates, additional experiments have been performed to establish the combined kinetics of HONO decomposition, oxidation, and vaporization under conditions similar to those used in the depolymerization experiments.

The fraction of HONO that does not participate in the deamination reaction is simply the ratio of the instantaneous apparent rate of HONO decomposition, R_N, to the total rate of disappearance of HONO from solution due to depolymerization and decomposition. Both the apparent decomposition characteristics of HONO and the depolymerization kinetics of chitosan are first order with respect to HONO concentration, [HONO] [7]. Since the rate of chitosan depolymerization, R_C, is also proportional to the protonated amine concentration, [GNH$_3^+$], it follows that

$$f_d = \frac{R_N}{R_N + R_C} = \frac{k_N[\text{HONO}]}{k_N[\text{HONO}] + k_0[\text{HONO}][\text{GNH}_3^+]} = \frac{k_N}{k_N + k_0[\text{GNH}_3^+]}. \tag{15}$$

In this expression, k_N is the apparent overall decomposition rate constant encompassing decomposition, oxidation and vaporization, and k_0 is the observed chitosan depolymerization rate constant. From Eq. (15) it is clear that f_d is independent of [HONO] and decreases with increasing chitosan concentration. Moreover, f_d is not only a function of reaction temperature, but also of the copolymer composition because the inherent reactivity of the amine decreases with increasing degrees of deacetylation [7].

It is therefore important to establish optimal conditions that minimize the effects of various HONO loss mechanisms that compete with the depolymerization reaction. Among the strategies available, increasing the mass transfer resistance between the aqueous solution and gas phase [15–19] and altering the solvent composition [20] have been demonstrated to reduce the disappearance of HONO from aqueous solution. Since

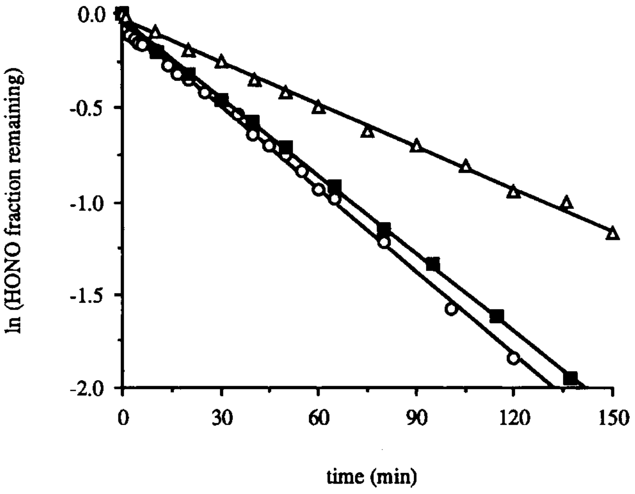

Fig. 3. Comparison of the rate of nitrous acid decomposition/volatilization in 'capped' 50 mM HCl solution (△), 'open' 50 mM HCl solution (○) and 'open' 12.5 mM HNO_3 + 37.5 mM HCl solution (■), all at 35°C.

Eguchi et al. [20] suggested that substitution of HNO_3 for HCl reduced the decomposition rate of HONO, the effectiveness of this approach under conditions appropriate for chitosan depolymerization (i.e., pH 1–2) was evaluated. The rates of HONO disappearance from an HCl-based acidic solvent (50 mM HCl), as used in the depolymerization study, and a HNO_3-containing solvent (12.5 mM HNO_3 and 37.5 mM HCl) were compared (Fig. 3), but this substitution decreased the apparent rate constant only by about 5% (Table 1).

A far more effective method of reducing the apparent decomposition of HONO is to increase the gas-phase mass-transfer resistance. Instead of allowing free convection and

Table 1
Apparent rate constants for the disappearance of nitrous acid

Temp. (°C)	Solvent composition	S/V ratio (cm^{-1}) [a]	Solution pH	Apparent rate constant k_N (min^{-1}) [b]
35	50 mM HCl	0.28	1.47	$1.47 \pm 0.04 \times 10^{-2}$
35	12.5 mM HNO_3, 37.5 mM HCl	0.28	1.47	$1.39 \pm 0.03 \times 10^{-2}$
35	50 mM HCl	'capped'	1.46	$7.71 \pm 0.43 \times 10^{-3}$
55	50 mM HCl	0.28	1.59	$4.41 \pm 0.21 \times 10^{-2}$
55	50 mM HCl	0.20	1.45	$2.77 \pm 0.14 \times 10^{-2}$
65	50 mM HCl	0.28	1.70	$6.91 \pm 0.29 \times 10^{-2}$
65	50 mM HCl	0.20	1.54	$3.85 \pm 0.23 \times 10^{-2}$

[a] Reactor surface-area-to-solution-volume ratio.
[b] 95% confidence interval.

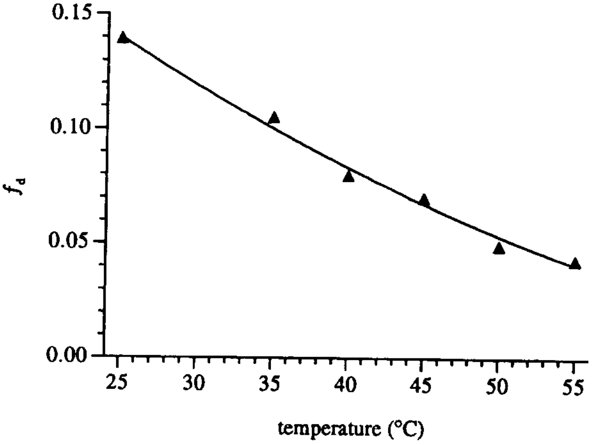

Fig. 4. Effect of reaction temperature on the fraction of nitrous acid that does not participate in the depolymerization reaction, based on experimental results with 1% chitosan (69% dd) solution in an 'open' reactor.

vaporization to occur within the 'open' reactor, the reaction vessel was fitted with a cap having only a 1 mm diameter hole for the escape of gaseous products. The results, shown in Fig. 3, clearly demonstrate the reduction in apparent decomposition rate due to 'capping' the reactor; at 35°C the apparent decomposition rate was reduced by 48% (Table 1). Alternatively, the loss of HONO can be diminished by decreasing the surface area of the solution exposed to the atmosphere per unit of solution volume through reduction of the diameter of the reactor. As shown in Table 1, a significant reduction in the apparent rate constant was observed at two temperatures when the surface area of solution exposed to the gas phase per unit volume of solution was reduced from 0.28 to 0.20 cm^{-1}.

In addition to mass-transfer considerations, the relative rates of chitosan depolymerization and apparent HONO decomposition are determined by reaction temperature and chitosan concentration and composition. For a 1.0% (w/w) chitosan (69% dd) solution reacting with HONO in an 'open' reactor (S/V ratio 0.28 cm^{-1}), the value of f_d can be computed as a function of reaction temperature based on the chitosan depolymerization and apparent HONO decomposition rate constants previously presented [7]. The resulting temperature dependence is shown in Fig. 4, where the value of f_d drops from 14% of the initial HONO added at 25°C to about 4% at 55°C. Thus, a significant reduction in f_d can be achieved by increasing the reaction temperature.

The effectiveness of an increase in the chitosan concentration for a reduction in the value of f_d is demonstrated in Fig. 5, for solutions open to the atmosphere and for 'capped' reactors. At 35°C and with a chitosan of 69% dd, a marked decrease in the value of f_d is observed with an increase in chitosan concentration; this effect is especially pronounced with the 'capped' system.

Thus, in summary, the need for a convenient, practical procedure for manipulation of the molecular size of chitosan, in a fashion predictable by polymer theory, is clearly met

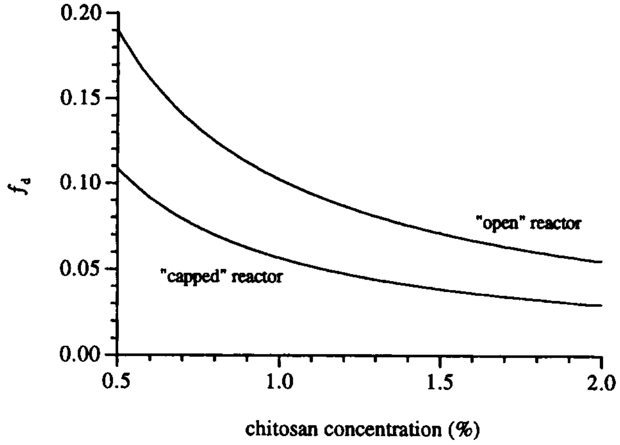

Fig. 5. Effect of chitosan (69% dd) concentration on the fraction of nitrous acid that does not participate in the depolymerization reaction at 35°C.

by the treatment of acidic solutions of the aminopolysaccharide with HONO. The fraction of HONO that does not participate in the depolymerization reaction with chitosan can be minimized by impeding the transfer of decomposition products from the liquid into the gas phase, in addition to increasing the depolymerization reaction temperature and chitosan concentration. More specifically, by performing reactions in 'capped' vessels, using low S/V ratio conditions at temperatures above 50°C and employing chitosan concentrations as high as is compatible with proper mixing of the solution, f_d can be minimized or neglected altogether. Thus, the expressions derived for prediction of the extent of depolymerization can be confidently applied for manipulation of the MW of chitosan.

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