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Heterogeneous *N*-deacetylation of chitin in alkaline solution

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Abstract

The influence of alkaline concentration, temperature and solution-to-chitin ratio on the *N*-deacetylation of shrimp chitin was investigated. Experimental conditions varied from 10 to 60% NaOH, 70 to 150 °C, and 5 to 45 mL of alkaline solution per gram of chitin. The degree of *N*-deacetylation increased mainly with increasing temperature or NaOH concentration. The effect of the solution-to-chitin ratio was insignificant. Response surface analysis indicated the maximal degree of deacetylation to occur at 60% NaOH, 107 °C and 26 mL alkaline solution per gram of chitin. The apparent rate constant ranged from 4.4×10^{-5} to 1.1×10^{-2} min^{-1}. In the temperature range of 70–150 °C, the activation energy for heterogeneous *N*-deacetylation is 56 kJ/mol. The rate constant also increased with increasing alkaline concentration. The influence of alkaline concentration suggests that *N*-deacetylation might be both reaction and diffusion controlled. © 1997 Elsevier Science Ltd.

Keywords: Chitin; Chitosan; Heterogeneous *N*-deacetylation; Rate constant

1. Introduction

Chitosan is a cationic biopolymer obtained from the *N*-deacetylation of chitin, a β-(1 → 4)-linked *N*-acetyl-D-glycan. The nontoxic, biodegradable and biocompatible properties of chitosan provide great potential for many applications [1–3]. The degree of *N*-acetylation influences not only the physicochemical characteristics [4–9], but also the biodegradability [10–12] and immunological activity [13] of chitosan.

Consequently, the accurate determination of the degree of *N*-deacetylation has been the goal of much research [14]. New analytical procedures based on enzymatic methods [15], HPLC [16], conductometric titration and NMR spectroscopy [17], IR spectroscopy [18] and other instrumentation are constantly being proposed. In addition, novel techniques of deacetylation such as intermittent water washing [19], the use of a water-miscible organic solvent [20], dispersing organic liquids [21], high temperature [21] or enzymatic *N*-deacetylation [22] have been reported. The kinetics of *N*-deacetylation is of prime importance in applying all of the new methods.

The kinetics of homogeneous alkaline *N*-deacetylation of chitin was reported to be a pseudo-first-order reaction [23]. Similar results were obtained for heterogeneous deacetylation at 150 °C [21]. However,

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the variation of alkaline concentration on the deacetylation rate has not been investigated. Furthermore, the consistency between repetitions and the effect of chitin source were never mentioned.

In this study, we apply the experimental design and response surface methodology to examine the heterogeneous alkaline deacetylation process. The kinetics of the process was also investigated.

2. Experimental

Materials.—Chitin was isolated from pink shrimp (*Solenocera melantho*) shell waste. After treated with 2.5 N NaOH (12.5 mL per gram of shrimp shell powder at 75 °C for 6 h) and 1.7 N HCl (9 mL per gram of shrimp shell powder at ambient temperature for 6 h) [24], the resulting chitin contained 5.2% protein, 1.4% ash and 0.0004% calcium. Commercial shrimp chitin (Ohka Enterprise Inc., Kaoshiung, Taiwan) was used in a comparison test. The protein, ash and calcium content of commercial shrimp chitin are 7.1%, 2.7% and 0.09%, respectively. Alkalis, acids and salts (NaOH, HCl, CH_3COONa, CH_3COOH, H_2SO_4) were analytical grade reagents from E. Merck (Darmstadt, Germany). Toluidine blue, PVSK (potassium polyvinyl sulfate) and cetylpyridium chloride monohydrate were supplied by WAKO Chemicals, Japan.

N-Deacetylation.—A central composite experimental design [25] was used to establish the conditions for deacetylating chitin (Table 1). The experiment covered the range of 5–45 mL of soln per gram of chitin, 10–60% NaOH concn, 70–150 °C in temperature. Samples were heated in glass flasks under reflux in a water bath or in silicon oil maintained at the desired temperature. A portion of the samples was removed for analysis after 5, 15, 30, 60, 120, 180, 240, 300, and 360 min of deacetylation.

Characterization of N-deacetylated chitin (chitosan).—After alkaline treatment, a colloid titration method [26] was used to determine the degree of *N*-deacetylation. Duplicate samples were titrated with 0.0025 N PVSK by the indicator method to a toluidine blue end-point. The procedure was modified slightly to be as follows: 0.5 g of chitosan was dissolved in 99.5 g of a 5% CH_3COOH soln. One gram of the chitosan–CH_3COOH soln was mixed with 30 mL of deionized water. Two to three drops of 0.1% toluidine blue were added to the soln. The soln was titrated with 0.0025 N PVSK, whose formality

Table 1
Apparent first-order reaction rate constants during *N*-deacetylation

N-deacetylation conditions			First-order rate constant $10^3 \times k'$ (min^{-1})	Correlation coefficient r^2
Solution/chitin (mL/g)	NaOH concn (%)	Temperature (°C)		
13.1	18.1	86.2	0.00980 [a]	0.3436
13.1	18.1	133.8	1.78	0.7551
13.1	51.9	86.2	1.22	0.8708
13.1	51.9	133.8	9.84 [b]	0.8051
36.9	18.1	86.2	0.0439	0.9706
36.9	18.1	133.8	2.47	0.9155
36.9	51.9	86.2	1.51	0.9051
36.9	51.9	133.8	11.3 [b,c]	0.6635
45.0	35.0	110.0	1.79	0.9615
5.0	35.0	110.0	1.19	0.9417
25.0	60.0	110.0	8.43 [b]	0.8314
25.0	10.0	110.0	1.77	0.9530
25.0	35.0	150.0	11.8 [b]	0.8957
25.0	35.0	70.0	0.401	0.9402
25.0 [d]	35.0	110.0	2.19 ± 0.23	0.9553–0.9862

[a] The change in the degree of *N*-deacetylation was too small to be accurately determined by the analytical procedure.
[b] Regression results were obtained from experimental data in the first hour before reaching the maximal degrees of *N*-deacetylation.
[c] A commercial shrimp chitin *N*-deacetylated under the same condition resulted in a rate constant of 15.1×10^{-3} min^{-1}. The correlation coefficient was 0.9991.
[d] Six replicates were conducted for these conditions.

(f) was calibrated by cetylpyridinum chloride monohydrate at the same concentration. The degree of deacetylation was calculated in a similar fashion as that reported by Sannan et al. [4].

$$A = 0.0025 \times 10^{-3} \times f \times V \quad (1)$$

$$X = A \times 161 \quad (2)$$

where A is the moles of D-glucosamine (2-amino-2-deoxy-D-glucose) residues, X is the weight of D-glucosamine residues, and V is the volume (mL) of PVSK consumed in the titration.

$$Y = 0.5 \times 0.001 - [(A - B) \times 42] - X \quad (3)$$

$$C = Y/203 \quad (4)$$

where Y is the weight of the *N*-acetyl-D-glucosamine residues in the *N*-deacetylated chitin, B is the apparent number of moles of the D-glucosamine residues in the starting chitin, and C is the number of moles of the *N*-acetyl-D-glucosamine residues in the *N*-deacetylated chitin. Thus the degree of deacetylation can be calculated by eq (5), wherein 161 and 203 are the molecular weights of D-glucosamine and *N*-acetyl-D-glucosamine (2-acetamido-2-deoxy-D-glucose), respectively.

$$\text{Degree of deacetylation} = \frac{X/161}{X/161 + Y/203} \times 100 = \frac{A}{A + C} \times 100 \quad (5)$$

When NaOH is present in abundance in the reacting medium, the rate of the deacetylation reaction can be written as:

$$-\frac{dC}{dt} = kC \cdot C_{NaOH} \approx k'C \quad (6)$$

Thus the slope of a semi-logarithmic plot of C versus time would be the apparent or pseudo-first-order rate constant k′.

3. Results and discussion

Alkaline deacetylation of chitin.—By using the indicator titration for the determination of the D-glucosamine content, the process of *N*-deacetylation can be monitored. Fig. 1 shows the change in the degree of deacetylation for several conditions. Apparently temperature and NaOH concentration dramatically affect the rate of deacetylation. In addition, different sources of shrimp chitin result in different rates of deacetylation. Commercial chitin was deacetylated at a faster rate than the chitin prepared in the laboratory. The difference in shrimp species or the chitin isolation procedure might cause a variation in morphology or composition between chitin samples from different sources. This in turn leads to different behavior during deacetylation. The higher amounts of impurity in commercial chitin might have increased the amorphous region and decreased the crystalline region within sample particles. This would lead to faster deacetylation rate and result in higher degree of *N*-deacetylation for chitosan prepared from commercial shrimp chitin.

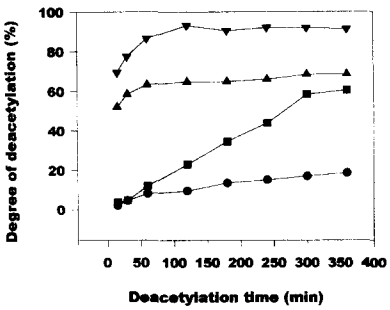

Fig. 1. Time-course of alkaline *N*-deacetylation of shrimp shell chitin under different conditions. ● 25 mL solution/g chitin, 35% NaOH, 70 °C; ■ 25 mL solution/g chitin, 35% NaOH, 110 °C; ▲ 36.89 mL solution/g chitin, 51.89% NaOH, 133.78 °C; ▼ 36.89 mL solution/g commercial chitin, 51.89% NaOH, 133.78 °C.

Optimal condition for N*-deacetylation.*—The degrees of deacetylation of chitin samples, after 6 h of alkaline treatment, were analyzed using the RSREG procedure of SAS [27]. The results are listed in Table 2. Reaction temperature plays the dominant role on influencing the degree of deacetylation. Other significant factors include the interaction of temperature and NaOH concentration, the quadratic term of temperature, and NaOH concentration, in the order of decreasing significance. The degree of deacetylation increases with increasing temperature or NaOH concentration. It decreases with the interaction term of temperature and NaOH concentration, or the quadratic term of temperature. The solution-to-chitin ratio shows minimal effect on the degree of *N*-deacetylation compared with other variables. Canonical analysis of the response surface (Fig. 2) indicates the stationary point to be a saddle point at 132 °C, 39% NaOH, and 30 mL of solution per gram of chitin. The maximum deacetylation within the experimental range was predicted to occur at 107 °C, 60% NaOH, and 26 mL of alkaline solution per gram of chitin.

Table 2
Response surface regression results between the degree of *N*-deacetylation after 6 h and the alkaline *N*-deacetylation variables

Parameter [a]	Parameter estimate Ci, Cii, or Cij	T for H0: parameter = 0	Probability > |T|
Intercept	−202.79	−5.998	0.0001
X1	0.94170	1.031	0.3267
X2	2.5396	3.635	0.0046
X3	3.0986	6.035	0.0001
X1 * X1	−0.017340	−1.531	0.1569
X2 * X2	0.004650	0.676	0.5143
X3 * X3	−0.008738	−3.831	0.0033
X2 * X1	−0.002073	−0.192	0.8513
X3 * X1	0.001327	0.236	0.8185
X3 * X2	−0.021549	−5.424	0.0003
R-square	0.9478		

[a] Notation of parameters: X1, solution-to-chitin ratio (mL/g); X2, NaOH concentration (%); X3, deacetylation temperature (°C).
[b] Degree of deacetylation = Intercept + C1 * X1 + C2 * X2 + C3 * X3 + C11 * X1 * X1 + C22 * X2 * X2 + C33 * X3 * X3 + C21 * X2 * X1 + C31 * X3 * X1 + C32 * X3 * X2.

Kinetics of N-*deacetylation.*—At the same alkaline concentration, the deacetylation process followed the pseudo-first-order kinetics (Table 1). For most conditions, semi-logarithmic plots between the amount of *N*-acetyl-D-glucosamine residues and the deacetylation time had correlation coefficients well above 0.80. Nevertheless, some irregular patterns were observed for the time-course data. First of all, the *N*-deacetylation rate at 13 mL of 18% NaOH per gram of chitin and 86 °C was the slowest among all the conditions. It appeared that more concentrated sodium hydroxide solution ($\geq$ 35%) was necessary to achieve substantial deacetylation within 6 h at 86 °C. When 18% NaOH solution was used to deacetylate chitin at or below 86 °C, the degrees of *N*-deacetylation (DD) remained relatively low (< 10%), even after 6 h of reaction. These low DD values were accompanied by a large variation in the titration results. As a consequence, it made the correlation coefficient for the semi-logarithmic regression of the *N*-acetyl-D-glucosamine amount versus time extraordinarily low. For those conditions that have high NaOH concentration (51.89 or 60%) and high temperature (133.78 or 150 °C), the deacetylation proceeded extremely fast. The degree of deacetylation leveled off within 2 h of reaction. The change in the amount of *N*-acetyl-D-glucosamine residues within the initial period did follow the pseudo-first-order kinetics. However, when the degree of deacetylation leveled off, the kinetic behavior changed. Roberts [14] mentioned that the maximum degree of *N*-deacetylation for a single alkaline treatment was about 75–85%. The maximum degree of *N*-deacetylation achieved in this study was about 70% for lab-prepared chitin, but it was 93% using a commercial chitin as the starting material. The difference might be due to the fact that chitin samples were isolated from different crustacean shells under different conditions. They therefore have different impurity and crystalline levels that influenced their *N*-deacetylation behavior. From six replicate tests at 25 mL of 35% NaOH per gram of chitin at 110 °C, the standard deviation for the rate constant determination was found to be ±11% of the average value (Table 1).

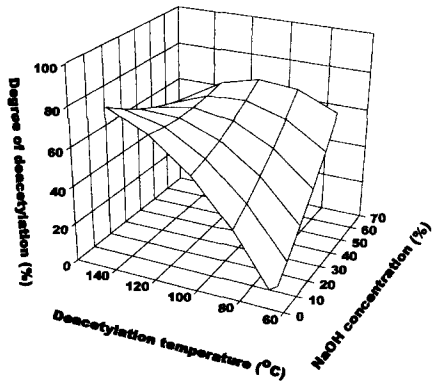

Fig. 2. Effects of NaOH concentration and temperature on the degree of *N*-deacetylation of shrimp chitin (after deacetylation with 25.0 mL alkaline solution per gram of chitin for 6 h).

From the apparent rate constants for 25 mL of 35% NaOH per gram of chitin at 70, 110 and 150 °C, the activation energy was found to be 13.4 kcal/mol

or 56.1 kJ/mol. This is comparable to the 8.5 to 13.8 kcal/mol range reported for heterogeneous *N*-deacetylation between 80 and 120 °C. [21]. Nevertheless, it is lower than the 22 kcal/mol for homogeneous *N*-deacetylation in the temperature range 25 to 40 °C [23], but higher than the 35.63 kJ/mol and 11 kcal/mol reported for heterogeneous *N*-deacetylation between 51.0 and 82.5 °C, and 80–100 °C respectively [28,29]. At 110 °C, a 6-fold increase in NaOH concentration resulted in nearly a 5-fold increase in the rate constant. The rate constant also increased about 5 times with a 2.9-fold increase in NaOH concentration at 134 °C. The concentration of alkali had more influence on the degree and rate of *N*-deacetylation at lower temperature. A 9-fold increase in the solution to chitin ratio at 110 °C increased 50% of the rate constant. At other temperatures, the rate constant increase was about 20% for a 3-fold increase in the solution to chitin ratio. These results suggest that the deacetylation rate constant depends not only on temperature, but also on the relative amount of alkalis to chitin.

It is well known that chitin degradation occurs during *N*-deacetylation. Under the most drastic conditions, the molecular weight of chitin decreased from above 2×10^6 to a viscosity average of 2.37×10^5 (a weight average of 3.56×10^5) for chitosan after 6 h of deacetylation. This represents a molecular weight decrease by a factor of about 5–10. Chitosan prepared under milder conditions had molecular weight of about 5×10^5 up to $1–2 \times 10^6$. The molecular degradation could be described by a pseudo-first-order kinetics (Fig. 3). In the first hour, the molecular weight decrease was relatively fast. The degradation rate constant was approximately 5.86×10^{-3} min^{-1} (data not shown). In the latter stage, the rate constant decreased to 5.40×10^{-4} min^{-1}.

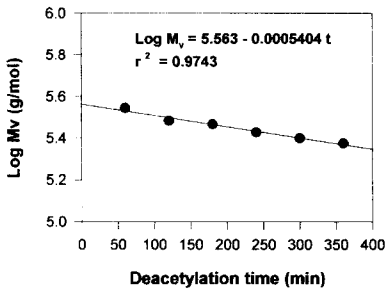

Fig. 3. Semi-logarithmic plot of viscosity-averaged molecular weight versus *N*-deacetylation time after 1 h at 36.9 mL alkaline solution per gram of chitin, 51.89% NaOH, and 133.78 °C.

Mechanism of N-*deacetylation.*—Under different concentrations of alkali, heterogeneous *N*-deacetylation appeared to be more complicated than a pseudo-first-order reaction. Concentration of alkali had significant influence on the degree of *N*-deacetylation and the *N*-deacetylation rate constant. This might be due to one or both of the following reasons. First, the amount of *N*-acetyl-D-glucosamine residues kept decreasing during deacetylation. In contrast, the amounts of lower molecular weight chitin (DD < 40%, $\sim 2 \times 10^6 > M_w > 1904$), chitosan (DD ≥ 40%, $1–2 \times 10^6 > M_w > 1610$), their oligomers ($1610–1904 > M_w \geq 322$), and D-glucosamine residues continued to increase. This would gradually increase the likelihood of the degradation reactions. The shift in the predominant reaction(s) could not only contribute to the leveling off in the degree of deacetylation, but also lead to the dependence of *N*-deacetylation on the concentration of alkali. Secondly, the morphological effect suggested in Roberts [14] could also give rise to the dependence of *N*-deacetylation on the concentration of alkali. Kurita et al. [30] suggested that heterogeneous *N*-deacetylation took place preferentially in the amorphous region of chitin, then proceeded from the edge to the inside of the crystalline region. As pointed out by Levenspiel [31], either the reaction on solid surface, or the diffusion of reactant from bulk fluid to the solid surface, may influence the rate of heterogeneous reactions. The diffusion rate of sodium hydroxide from alkaline solution to the surface or the inside of chitin particle would be related to the concentration of alkali. At the same solution-to-chitin ratio and temperature, the *N*-deacetylation rate constant increased with increasing sodium hydroxide concentration (Table 1). However, these data could neither be described merely by a higher-order reaction, nor by a diffusion-controlled reaction. Consequently, the heterogeneous *N*-deacetylation might be controlled by both reaction and diffusion. The exact mechanism and its quantitative description await future investigation.

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