

# The Effect of Reaction Time and Temperature During Heterogenous Alkali Deacetylation on Degree of Deacetylation and Molecular Weight of Resulting Chitosan

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**ABSTRACT:** The objective of the study is to elucidate the effect of reaction time and temperature during heterogenous alkali reaction on degree of deacetylation (DD) and molecular weight (MW) of the resulting chitosans, and to establish the reaction conditions to obtain desired DD and MW chitosan products. Chitin was extracted from red shrimp process waste. DDs and MWs were determined by infrared spectroscopy (IR) and static light scattering, respectively. The results are as follow: The DD and MW of chitin obtained were 31.9% and 5637 kDa, respectively. The DD of the resulting chitosan increased along with reaction time and/or reaction temperature. The DDs of the resulting chitosan that were obtained from 140°C were higher than those reacted at 99°C. The highest DD of the resulting chitosans after alkali deacetylation at 99 and 140°C were 92.2 and 95.1%, respec-

tively. The DDs of chitosans increased fast at the beginning of reaction process then slowed over time. The reaction rate and rate constant of the deacetylation reaction decreased with increasing DD of the reactant. The MWs of chitosans decreased along with the deacetylation time. MW of those chitosans reacted at 140°C are smaller than those at 99°C. The rate of chitosan degradation was above 43.6%/h in the initial stage, then decreased to about 20%/h. The degradation rate constants raised substantially in the late stage. © 2003 Wiley Periodicals, Inc. *J Appl Polym Sci* 88: 2917–2923, 2003

**Key words:** chitosan; heterogenous alkali deacetylation; reaction rate; reaction order; rate constant; molecular weight; degree of deacetylation

## INTRODUCTION

Chitinous materials including chitin and chitosan are considered to be versatile, environmentally friendly biomaterials.<sup>1</sup> Chitinous materials can be applied in food processing, agriculture, biomedicine, biochemistry, wastewater treatment, membranes, and microcapsules applications.<sup>1–12</sup>

The molecular weight (MW) and its distribution affect physical and chemical properties of polysaccharides,<sup>13</sup> such as the rheological properties of chitosan,<sup>12,14</sup> effect axial ratio,<sup>3,4</sup> chain flexibility,<sup>15,16</sup> and mechanical properties and pore size of membranes and microcapsules of chitosan,<sup>3,6,7,17</sup> water holding capacity of cosmetics,<sup>18–20</sup> antimicrobial activity,<sup>21,22</sup> lower LDL, cholesterol and triglycerol capacity,<sup>23</sup> blood coagulation activity, and wound-healing activity<sup>24</sup>; gel forming, osmotic pressure modification, viscosity enhancing, or fiber formation also depend on molecular weight and its distribution.<sup>13</sup> The degree of

deacetylation (DD) effects release of core material,<sup>3,4</sup> chain flexibility,<sup>15,16</sup> immunoadjuvant,<sup>25,26</sup> mechanical properties and pore size of membranes,<sup>27</sup> bactericide effect,<sup>28–31</sup> enzyme-binding ability,<sup>32</sup> metal binding,<sup>33</sup> and biodegradation of chitosan film.<sup>34</sup>

In principle, either the acids or the alkalis can be used to deacetylate chitin to chitosan. However, glycosidic bond are very susceptible to acid; therefore, alkali deacetylation are used more frequently.<sup>35</sup> There are two methods of alkali treatment. One is the homogeneous alkaline treatment, where chitins are mixed with alkali to prepare alkali chitin. Usually it is performed at lower temperature and longer reaction time. Chitosans with a DD of ca. 50% are water soluble.<sup>36,37</sup> The heterogeneous alkaline treatment is conventional and more often used to deacetylate chitin. It was patented in the United States in 1930. Solid chitins are suspended in alkali solution and the reaction is at a higher temperature in a shorter time. Chitosan are soluble in acidic aqueous solution at a DD higher than 60%.<sup>35</sup>

DDs and MWs of resulting chitosans by the heterogenous alkaline treatment were affected by alkali concentration, solution to solid ratio, reaction time and temperature, and reaction environment during alkaline treatment. Higher alkali concentration as well as higher solution to solid ratio facilitate deacetylation

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reaction.<sup>38,39</sup> However, solution to solid ratio of 20 times are frequently used to conserve the alkali and reduce cost. The longer the alkali reaction times, the higher the degrees of deacetylation. However, deacetylation reactions occurred quickly during the first hour then slowed down over time during reaction. Wu and Bough<sup>40</sup> reported DD of chitin increased from 13 to 72% during the first hour and increased to 75% at the second hour. Increase rates were about 1.3% per hour between the second and fifth hours when reacted with 50% NaOH, at 100°C. Lin,<sup>38</sup> and Chang and colleagues,<sup>39</sup> reported similar results. On the other hand, the longer the alkali reaction time, the lower the MW of chitosan.<sup>39,40</sup> The higher the reaction temperature, the higher the degree of deacetylation of chitosan,<sup>27,41</sup> and the lower MW.<sup>27</sup> Bought and colleagues<sup>42</sup> reported that MW and viscosity of chitosan obtained by deacetylation under nitrogen gas are higher than those under air. This may be due to chitosan being degraded by oxygen. Therefore, reaction processes under vacuum or nitrogen environment have been used to avoid oxygen degradation.

As chitosan is deacetylated from chitin, not only does the deacetylation reaction go on but the degradation reaction also occurs,<sup>27,35,39</sup> because substituted groups on C(2)—C(3) of chitin molecules are in *trans* arrangement. This will retard the elimination of the acetyl group on the C(2) position but not the degradation of  $\beta$ -1,4 linkage.<sup>35</sup> Deacetylation reactions are very effective during the early stage but become much ineffective during the late stage. However, degradation of the  $\beta$ -1,4 linkage still continues.

Functional properties depended on the MW and DD of chitinous materials used. However, MW and DD of chitinous materials depended on the conditions of preparation. Alkali treatment at elevated temperatures is a very common practice method to produce various MW and DD chitinous materials. However, the alkali process creates environmental problems and chemical modification of glucose ring. Most of the literature has been focused on changes of DD and much less on the change of MW of the resulting chitosan during alkali reaction. Therefore, the objectives of the study are to explore the effect of reaction time and temperature on DD and MW of the resulting chitosans during alkali deacetylation, and to establish the conditions to obtain desired MW and DD chitosan products.

## MATERIALS AND METHODS

### Preparation of chitin

Chitin was prepared from shrimp (*Solenocera prominentis*) waste by a modified method of Stanley and colleagues.<sup>43</sup> Ground shrimp waste was treated with 0.5N NaOH at ambient temperatures to hydrolyze the

surface flesh. The alkali-treated waste was washed then dried and disintegrated to obtain powder. The powder passed through sieves of 40–60 mesh. The flake free powder was soaked in 2N HCl for 2 h to remove the minerals until no CO<sub>2</sub> evolved. The demineralized powder was soaked in 2N NaOH at 80°C to hydrolyze the protein, then washed with water until neutral. The alkali-treated powder was soaked in 1% KMnO<sub>4</sub> at room temperature for 1 h to oxidize the astaxanthin, then soaked in 1% oxalic acid at 60°C for 1 h to neutralize the KMnO<sub>4</sub>. This was then washed and dried to get a white chitin powder.

### Preparation of chitosan

Chitin powder obtained were suspended in 50% NaOH solution at a chitin to solution ratio of 20 and reacted at 99 or 140°C for 1 to 9 h. A fresh NaOH solution was used to replace old ones after each hour of reaction. After the reaction, the slurries were washed with water and dried to get 60–100% DD chitosan. The reaction was performed under nitrogen to avoid oxidation.

### Degree of deacetylation measurement

Infrared spectrometry was used to determine the degree of deacetylation of the chitosans.<sup>44</sup> Chitosan powder was sieved through a 200 mesh then mixed with KBr (1:100) and pressed into a pellet. The absorbencies of amide I (1655 cm<sup>-1</sup>) and of the hydroxyl band (3450 cm<sup>-1</sup>) were measured using a Bio-Rad FTS-155 infrared spectrophotometer (Cambridge, MA). The band of the hydroxyl group at 3450 cm<sup>-1</sup> was used as an internal standard to correct for disc thickness and for differences in chitosan concentration in making the KBr disk. The percentage of the amine group's acetylation in a sample is given by  $(A_{1655} / A_{3450}) \times 115$ . Here,  $A_{1655}$ ,  $A_{3450}$  are the absorbencies at 1650 and 3450 cm<sup>-1</sup>, respectively.

### Molecular weight determination

The static light scattering method was used to measure the average MW of the chitinous materials.<sup>45,46</sup> Chitin was dissolved in *N,N*-dimethylacetamide (DMAc) + 5% LiCl, whereas chitosan was dissolved in 0.01M HCL + 0.09M NaCl. Different concentrations (0.001 ~ 0.10 g/L) of chitin and chitosan solutions were prepared. The solvents and solutions were filtered through 0.02  $\mu$ m (Whatman, Anotop 25, Clifton, NJ) and 0.45  $\mu$ m (Lida, Kenosha, WI) filters, respectively. The scattered light intensity of the solutions between 30° and 140° was measured by a Malvern light scattering photometer (Malvern 4700, Worcester, UK) at 632.8 nm and 30  $\pm$  0.1°C. The MW was calculated from the Zimm plot processed by Malvern soft-

ware (version 1.26 for Windows). Each sample measurement was repeated 5 times. Refractive index increments ( $dn/dc$ ) of chitin solution of 0.10 mL/g reported by Terbojevich et al.<sup>45</sup> was used, whereas 0.189 mL/g of chitosan solutions was determined by an interferometric refractometer (Wyatt/Optilab 903, Santa Barbara, CA).

### Deacetylation rate ( $R_{DD}$ ) and degradation rate ( $R_{MW}$ ) calculation

During alkali treatment both deacetylation as well as degradation reactions were processed simultaneously. Deacetylation rate was defined as percentage increase in DD per hour. It can be expressed by the following:

$$R_{DD} = (DD_t - DD_0)/t \quad (1)$$

Here,  $R_{DD}$  is rate of deacetylation reaction.  $DD_0$  is DD of chitin at time zero.  $DD_t$  is DD of the resulting chitosan after reaction for  $t$  h.

Degradation rate was defined as percentage decrease in MW per hour during alkali reaction. It can be expressed by

$$R_{MW} = [1 - (MW_0/MW_t)] \times 100\%/t \quad (2)$$

Here,  $R_{MW}$  is degradation rate during alkali reaction.  $MW_0$  is the MW of chitosan at time zero.

$MW_t$  is the MW of the resulting chitosan after reaction for  $t$  h.

### Reaction order and rate constant

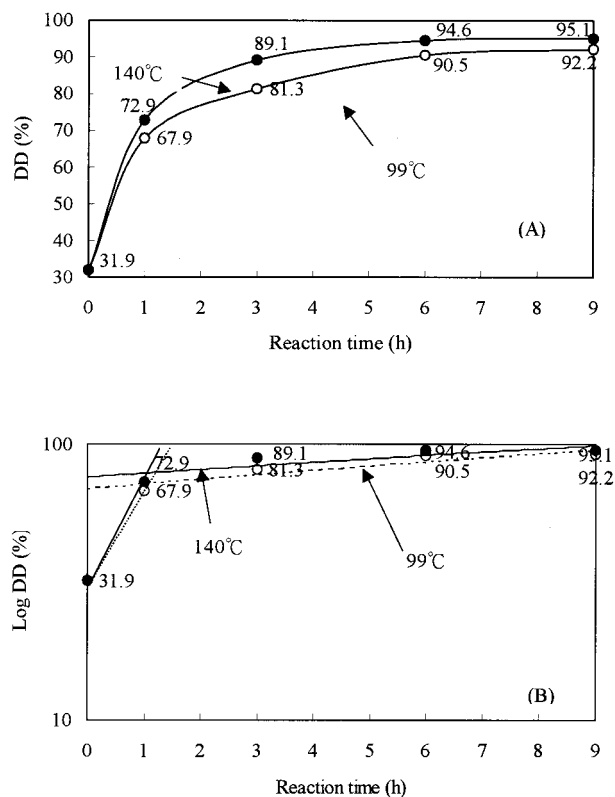
When plotting the concentration of reactant or product against time, it is a zero-order reaction if the relationship is linear. If not, then plotting the logarithmic concentration against time and when the relationship is linear, then it is a first-order reaction. Figure 1 shows that the relationship of logarithmic DD against time was linear. Therefore, the reaction was a first-order reaction. The rate constant of deacetylation can be expressed as in the following:

$$k_{DD} = 2.303 \log (DD_t/DD_0)/t \quad (3)$$

Here  $k_{DD}$  is rate constant of alkali reaction.  $DD_0$  is DD of reactant at time zero.  $DD_t$  is DD of chitosan after reaction  $t$  h.

From Figure 2, the relationship of logarithmic MW against time was linear. So the reaction was also a first-order reaction. The rate constant ( $k_{MW}$ ) of alkali degradation can be calculated by<sup>47,48</sup>

$$(1/MW_t) - (1/MW_0) = (k_{MW}/m)t \quad (4)$$



**Figure 1** The degrees of deacetylation (DD) of the resulting chitosans during heterogenous alkaline deacetylation reactions at 99 and 140°C. (A) The  $y$  axis is in normal coordinates; (B) the  $y$  axis is in logarithmic coordinates.

Here,  $k_{MW}$  is rate constant during alkali degradation.  $MW_0$  is the MW of reactant at time zero.  $MW_t$  is the MW of product after reaction for  $t$  h. The  $m$  is the average MW of chitosan monomer;  $m = 161 + 42(1 - DD)$ .

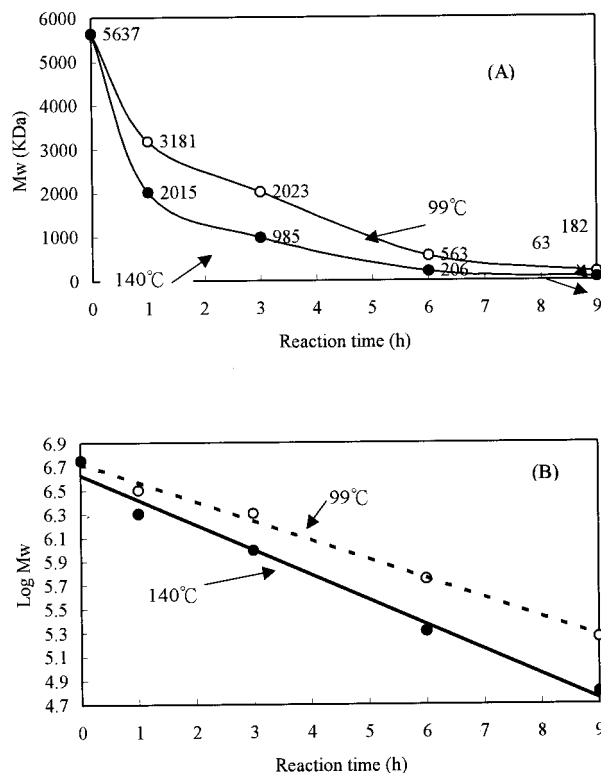
## RESULTS AND DISCUSSION

### Characterisitic of chitin

DD and MW of chitin prepared from red shrimp waste were 31.9% and 5637 kDa respectively.

### Effect of reaction time on DD of chitosan

Results in Figure 1(A) show that DD of chitosan increased with reaction time whether the reaction was performed at 99 or at 140°C with 50% alkali. The longer the reaction time, the smaller the increase in the degree of deacetylation. Changing the ordinate into a logarithmic scale, we got Figure 1(B). After the first hour, the results were in linear. Therefore, the reaction was a first-order reaction. Equations (1) and (3) were used to calculate the reaction rate ( $R_{DD}$ ) and rate constant ( $k_{DD}$ ), and the results are listed in Table I. The data show that at the beginning of deacetylation DD increased very fast. For those reacted at 99°C, the



**Figure 2** The weight average molecular weight (MW) of the resulting chitosan during heterogeneous alkali deacetylation reactions at 99 and 140°C. (A) The y axis is in normal coordinates; (B) the y axis is in logarithmic coordinates.

reaction rate at the first hour ( $R_{DD0,1}$ ) was 36.0%/h. However, the reaction rate between the first and second hour,  $R_{DD1,2}$ , and second and third hour,  $R_{DD3,6}$ , were 6.7 and 3.1%/h, respectively. The value of  $R_{DD6,9}$  was only 0.6%/h. The rate constant for those reacted at 140°C showed similar trends but higher than those reacted at lower temperature. The rate constant for those deacetylated at 99°C also decreased with reaction time. The rate constant decreased from 0.755 to 0.090  $\text{h}^{-1}$  during the first hour ( $k_{DD0,1}$ ), and between the first and third hours ( $k_{DD1,3}$ ). The rate constant between the third and sixth hours ( $k_{DD3,6}$ ) decreased further to 0.036  $\text{h}^{-1}$  and further down to 0.006  $\text{h}^{-1}$  between the sixth and ninth hours ( $k_{DD6,9}$ ). For those reacted at 140°C, changes in rate constant were similar to those reacted at 99°C. The data above show that reaction rate as well as rate constant decreases over time during alkali deacetylation. This is the reason why it is very difficult to raise the degree of deacetylation to a very high level. Similar results have been reported in the literature. The reaction rate and rate constant are listed in Table II.

Wu and Bough<sup>40</sup> reported that the reaction rate during the first hour ( $R_{DD0,1}$ ) was 59.0%/h, and decreased to 3.0 and 1.3 %/h for those reacted between the first to second hour and second to fifth hour. The reaction rate constant during the first hour ( $k_{DD0,1}$ )

was 1.712  $\text{h}^{-1}$ , then decreased to 0.041 and 0.017  $\text{h}^{-1}$  for those reacted between the first to second hours and second to fifth hours, respectively. Mima and colleagues<sup>27</sup> reported the DD of the resulting chitosan was 78% after the first hour of reaction using 47% alkali at 110°C. The  $R_{DD1,4}$  was 1.3%/h. Among the data reported in the literature, the lowest reaction rate during the first hour ( $R_{DD0,1}$ ) was about 20%/h. However, the decreasing trends was similar.<sup>49</sup>

Lin<sup>49</sup> speculated that the higher reaction rate or rate constant at the beginning may be due to the higher concentration of alkali used to facilitate the deacetylation reaction. However, in this report, alkali solution was changed every hour, but the results were similar. This might be mainly due to number of acetyl groups decreasing over time during the alkali reaction. Therefore, there are less chances for NaOH molecules to react with acetyl groups, resulting in a decrease of the reaction rate and rate constant. Plotting data of this study and data reported in the literature, we got Figures 3(A) and 3(B). Data in Figure 3(A) show that reaction rate was higher than 12%/h as long as the DD of chitin was less than 40%. The reaction rate decreased linearly with increasing DD of the resulting chitosan. The reaction rate was  $R_{DD} = -1.606DD + 80.627$ ,  $R^2 = 0.857$  for those chitin with DD less than 40%. As the DD of chitin was over 40%, the reaction rate was between 0.2 and 8.1 %/h. It was significantly less than those of chitin with DD less than 40%. The equation indicated that the sluggish increase in DD and limited extent increases in DDs as DD of reactant chitin were higher than 40%. The regression coefficient was bad when all the data in the literature were put together. However, the regression coefficient was good and linear when only data of this report was used. The equation of  $R_{DD} = -0.294DD + 27.716$ ,  $R^2$

**TABLE I**  
The Rate of Deacetylation Reaction ( $R_{DD}$ , %/h), Rate of Degradation Reaction ( $R_{MW}$ , %/h), Rate Constant of Deacetylation Reaction ( $k_{DD}$ ,  $\text{h}^{-1}$ ), and Rate Constant of Degradation Reaction ( $k_{MW}$ ,  $\text{h}^{-1}$ ) of Chitosans from Different Heterogenous Alkali Reaction Temperatures

	99°C	140°C	99°C	140°C	
$R_{DD0,1}$ <sup>a</sup>	36.0	41.0	$k_{DD0,1}$ <sup>a</sup>	0.755	0.827
$R_{DD1,3}$	6.7	8.1	$k_{DD1,3}$	0.090	0.100
$R_{DD3,6}$	3.1	1.8	$k_{DD3,6}$	0.036	0.020
$R_{DD6,9}$	0.6	0.2	$k_{DD6,9}$	0.006	0.002
$R_{MW0,1}$	43.6	64.3	$k_{MW0,1}$	0.026	0.061
$R_{MW1,3}$	18.2	25.6	$k_{MW1,3}$	0.016	0.045
$R_{MW3,6}$	24.1	26.4	$k_{MW3,6}$	0.072	0.212
$R_{MW6,9}$	22.6	23.1	$k_{MW6,9}$	0.205	0.600

<sup>a</sup>  $R_{DD0,1}$ ,  $R_{MW0,1}$ ,  $k_{DD0,1}$ ,  $k_{MW0,1}$  represent reaction rate for deacetylation, for molecular weight degradation, reaction rate constant for deacetylation and for molecular weight degradation, respectively, during alkali reaction during the first hour.

TABLE II  
The Rate of Deacetylation Reaction ( $R_{DD}$ , %/h) of Chitosans Reported in the Literature

Reference	Deacetylation condition	$R_{DD}$ (%/h)* <sup>a</sup>	$k_{DD}$ (1/h)
Wu and Bough, 1978 <sup>40</sup>	DD of chitin: 13%	$R_{DD0,1} = 59.0$	$k_{DD0,1} = 1.712$
	Temperature: 100°C	$R_{DD1,2} = 3.0$	$k_{DD1,2} = 0.041$
	NaOH concentration: 50%	$R_{DD2,5} = 1.3$	$k_{DD2,5} = 0.017$
Lee, 1988 <sup>50</sup>	DD of chitin: 8%	$R_{DD0,1} = 65.9$	$k_{DD0,1} = 2.223$
	Temperature: 100°C	$R_{DD1,2,5} = 4.3$	$k_{DD1,2,5} = 0.055$
	NaOH concentration: 50%	$R_{DD2,5,4} = 2.0$	$k_{DD2,5,4} = 0.025$
	Volume of alkali solution/weight of chitin: 20	$R_{DD4,6} = 0.8$	$k_{DD4,6} = 0.010$
		$R_{DD6,8} = 0.4$	$k_{DD6,8} = 0.004$
Lin, 1991 <sup>49</sup>	DD of chitin: 37%	$R_{DD0,1} = 17.0$	$k_{DD0,1} = 0.378$
	Temperature: 95°C	$R_{DD1,7} = 1.3$	$k_{DD1,7} = 0.023$
	NaOH concentration: 50%		
	Volume of alkali solution/weight of chitin: 15		
Lin, 1992 <sup>38</sup>	DD of chitin: 35.2%	$R_{DD0,3} = 12.7$	$k_{DD0,3} = 0.245$
	Temperature: 99°C	$R_{DD3,6} = 2.2$	$k_{DD3,6} = 0.029$
	NaOH concentration: 50%	$R_{DD6,9} = 1.0$	$k_{DD6,9} = 0.012$
	Volume of alkali solution/weight of chitin: 20		

\* : Calculated by these authors.

= 0.901. The results indicate that reaction rate for those chitosans with a DD less than 40% decreased with the increasing DD. However, absolute values of those slopes are smaller than those with DD less than 40%. This implies that the effect of DDs of chitosan were not as significant as those of lower DDs.

Results in Figure 3(B) showed that the DD of chitin affected the rate constant of alkali deacetylation. The rate constant was larger than  $0.245 \text{ h}^{-1}$  as the DD of

chitin was less than 40% and decreased with increasing DD of reactant. The regression equation is  $k_{DD} = -0.062DD + 2.643$ ,  $R^2 = 0.966$ . However, as DD of the reactant was larger than 40%, rate constants were between  $0.002$  and  $0.100 \text{ h}^{-1}$ , and they were significantly smaller than those reactants with a DD less than 40%. The regression coefficient was low when all data were included. However, the regression was linear when a coefficient larger than 0.9 was obtained when data of this report was used. The equation obtained was  $k_{DD} = -0.004DD + 0.362$ ,  $R^2 = 0.923$ . The equation shows that rate constant decreased with increasing the DD of reactant as those reactants with DD less than 40%. The slope was also smaller than that of the DD less than 40%, indicating that rate constant was less affected by DD of the reactant.

#### Effect of reaction temperature on DD of reactant

Under same alkali deacetylation condition, the higher the reaction temperature the higher the DD of the chitosan obtained. Results in Figure 1 show that the DD of chitosan reacted at 140°C for 1–9 h was higher than those reacted at 99°C. DD of 67.9 vs 72.9% was obtained after reaction for 1 h at 99 vs 140°C. Mima and colleagues<sup>27</sup> reported that a DD of 57 and 91% were obtained after reaction for 2 h at 60 and 110°, respectively. Similar results of DD of 66.23–83.57, 74.8–83.74, and 76.77–87.66% were obtained after reactions for 3–5 h at 70, 80, and 90°C respectively.<sup>41</sup> The results of Mima and colleagues,<sup>27</sup> Chinadit and colleagues,<sup>41</sup> and this report indicated higher reaction temperatures are required to prepare chitosans with higher DDs.

Conditions to obtain higher a DD product is not equal to having a higher reaction rate or higher rate constant. Results in Table I show the reaction temper-

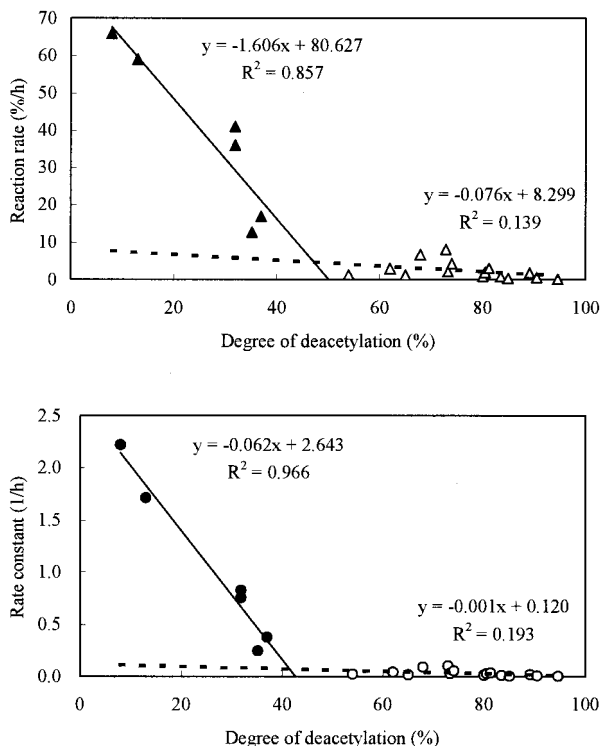


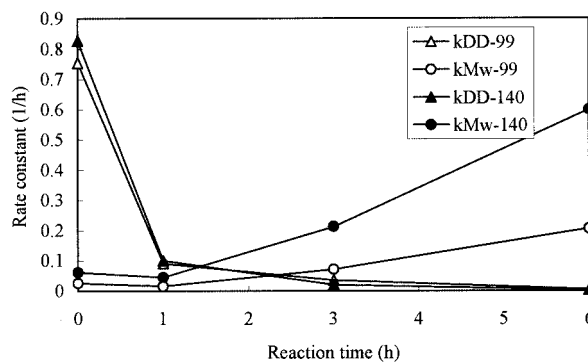
Figure 3 The plots of the degree of deacetylation of reactants vs (A) reaction rate of deacetylation ( $R_{DD}$ , %/h) and (B) rate constant of deacetylation ( $k_{DD}$ ,  $\text{h}^{-1}$ ).

atures at a 140°C reaction rate during the first hour ( $R_{DD0,1}$ ), and first to third hours ( $R_{DD1,3}$ ) are 41.0 and 8.1%/h, respectively, significantly higher than that of 36.0 and 6.7 %/h, respectively, of the same reaction period under a reaction temperature of 99°C. However,  $R_{DD3,6}$  and  $R_{DD6,9}$  reacted at 140°C were 1.8 and 0.2%/h, respectively, smaller than that of 3.1 and 0.2%/h, respectively, of their counterparts at a reaction temperature of 99°C. Results show that the reaction rate of those reactions at 140°C were larger than those reacted at 99°C during the first third hour. However, after the third hour, the reaction rates for those reacted at 99°C were larger than those reacted at 140°C. From the relationship of reaction rate or rate constant vs DD of product in Figure 3, it was shown that reaction rate and rate constant decreased with increasing DD of the reactant. In order to prepare higher DD chitosans and to conserve the energy, the reaction temperature higher than 140°C can be used at the beginning. Lee<sup>50</sup> reported a higher reaction rate of  $R_{DD0,1} = 82\%/h$ , and a high DD product can be obtained with a reaction temperature at 157°C. For a late process, a lower reaction temperature of 99°C will do the job. In this way, the higher DD product can be prepared with less energy consumption.

#### Effect of reaction time on MW of chitosan

Figure 2(A) show the changes of MW of chitosans during alkali reaction at 99 and 140°C between 1 and 9 h. MW of chitosan decreased along with deacetylation time. Decreases in MW during the early stages are significantly rapid until the sixth and ninth hours; then the decrease in MW becomes significantly slow. Wu and Bough<sup>40</sup> reported similar results when changing the ordinate scale to logarithmic scale; then data shows good linear relationship between MW and reaction time [Fig. 2(B)]. This indicates that degradation reactions were first-order reactions during the heterogenous deacetylation reaction. Using eqs. (2) and (4), the degradation reaction rate ( $R_{MW}$ ) and rate constant ( $k_{MW}$ ) were calculated, and then listed in Table I. Data showed that values of reaction rate of 1 h were the largest regardless of the reaction temperature. MW decreased continuously along with degradation time; however, reaction rates were only half of the first hour.

The results of the rate constant showed that the trends of changing the rate constant differed from that of the reaction rate. The rate constant of the first hour ( $k_{MW0,1}$ ) was larger than the rate constant of the third hour ( $k_{MW1,3}$ ); however, the rate constants of the late reaction were larger than the previous one and increased significantly (Fig. 4). Data in Figure 4 seem to contradict the common impression that the MW of the reactants did not change significantly during the late stage of hot alkali reaction [Fig. 2(A)]. Results of the



**Figure 4** The rate constant of deacetylation ( $k_{DD}$ ,  $h^{-1}$ ) and rate constant of degradation ( $k_{MW}$ ,  $h^{-1}$ ) of chitosan during heterogenous alkali deacetylation at 99 and 140°C.

reaction rate showed that the reaction rate between 3 and 9 h were 18.2–24.1 and 23.1–26.4 for reactions at 99 and 140°C, respectively (Table I). The reaction rates during the first hour ( $R_{MW0,1}$ ) were 43.6 and 64.3%/h for those reactions at 99 and 140°C. In other words, around 40–60% decreases in MW during the first hour hot alkali degradation reaction and around 20% decreases during the late stage reaction.

Results in Figure 4 showed that the value of  $k_{DD}$  was very high and decreased rapidly along with the reaction. However, value of  $k_{MW}$  increased rapidly after 1 h. This may be that during the 1 h of heterogenous alkali deacetylation reaction, the majority of NaOH molecules react with the acetyl group and only a minor number of NaOH molecules react with glycosidic bonds. Therefore, the DD of chitosan increased significantly. Although there are only small amounts of NaOH molecules involved in the reaction with glycosidic bonds, the degradation reactions were random and just one degradation reaction in the middle of chitosan chain would reduce MW significantly. This is the reason why the MW of chitosan decreased rapidly at the beginning. As the alkali reaction passed on, the remaining acetyl groups became scarce, e.g., the concentration of reactant decreases; furthermore, the substituted group on C(2)—C(3) are in the *cis* form, which will retard the deacetylation reaction.<sup>35</sup> Fewer NaOH molecule would reacts with the acetyl group; therefore, more NaOH molecules attack glycosic bonds, thus increasing the reaction rate and rate constant of the degradation reaction, and decreasing the reaction rate and rate constant of the deacetylation reaction. The reaction rate of the degradation reaction remained 20%/h and the rate constant increased significantly during the late stage.

#### Effect of reaction temperature on the MW of chitosan

Results in Figure 2 showed that the MW of chitosan is smaller for those resulting from a higher reaction tem-

perature than that of the lower temperature at the same reaction time. Table I also showed that reaction rate ( $R_{MW}$ ) and rate constant ( $k_{MW}$ ) of the degradation reaction at a higher temperature were larger than that of lower temperatures. This indicated that degradation occurred faster at higher temperature. Mima and colleagues<sup>27</sup> reported MW of chitosan was 790 and 500 kDa after deacetylation at 60 and 110°C, respectively. Degradation in chitosan was more severe during higher temperature deacetylation than that reacted at lower temperature. High molecular weight chitosan product can be obtained by using low reaction temperature. Higher deacetylated and higher molecular weight chitosan can be obtained by reacting at a higher temperature during 1 h, then reacted at a lower temperature during the rest of the reaction to avoid degradation and conserve energy. Or chitosans prepared with DD 75–85% with a higher alkali reaction than the methods of Mima and colleagues<sup>27</sup> can be used, and the methods of Domard and Rinaudo<sup>51</sup> can be used to proceed to the second stage reaction.

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