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Multistage deacetylation of chitin: Kinetics study

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ABSTRACT

In this work kinetic study of a high degree of deacetylated chitin, obtained from multistage deacetylation treatment is investigated. It is found that multistage alkali treatments are as effective as a single stage deacetylation treatment, and the product is more deacetylated. The influences of alkaline concentration, reaction temperature and time on the multistage deacetylation shrimp chitin are studied. The degree of deacetylation (DD) increased with increasing NaOH concentration, reaction temperature and time step of multistage treatment. The effect of NaOH concentration is significant; nevertheless; the effect of temperature is insignificant. Our results showed that the multistage deacetylation reaction followed the pseudo-first-order kinetics, and the apparent activation energy of single step treatment, compared with multistep deacetylation, is lower. The apparent rate constant of reaction ranged from 1.68×10^{-2} to 9.54×10^{-2} h^{-1} and the apparent activation energy is estimated 16.2 kJ/mol at the aqueous 50% NaOH solution, in temperature range of 70–90 °C.

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1. Introduction

Chitin is an ideally (1 → 4) linked aminopolysaccharide having acetamide groups at C2 and the second most abundant natural biopolymer after cellulose. Chitosan is an acid-soluble deacetylated and the main derivative of chitin (Shigemasa, Matsuura, Sashiva, & Saimoto, 1996).

Due to chitosan excellent biocompatibility, biodegrability and bioactivity, it is more and more considered as a very interesting substance for diverse applications as a biomaterial such as drug delivery system, surgical thread, and bone healing materials and wound dressing (Borchard, 2001; Mirzadeh et al., 2002; Singla & Chawla, 2001). The main sources for production of chitin are the shells of crab, shrimp and krill. Shrimp shells contain 40–45% chitin, it is obtained by extracting calcium carbonate and proteins from shrimp's shells (Mirzadeh et al., 2002; Pillai, Willi, & Sharma, 2009; Yaghobi, Mirzadeh, & Hormozi, 2002). Chitosan was characterized by its extent of N-acetylation, which influence not only in its physicochemical characteristics, chain morphology and molecules weight but also in its biomedical application, biodegradability and immunological activity. The extent of deacetylation is essential to study property relationship and possible industrial uses (Li, Revol, & Marchessault, 1998). Enhancement of DD and nitrogen content of chitin in N-acetylation reaction has been the interest to us for some time and we have already reported about possible increasing of DD through multistage process due to morphological effect. Our previous results showed that multistage procedure might be effective for increasing degree of deacetylation of chitin and subsequently improving biomedical application of the resultant chitosan as a blood hemostasis agent (Mirzadeh et al., 2002; Yaghobi & Mirzadeh, 2004). Significant differences observed between multistage and single alkali treatments on the nitrogen content and degree of deacetylation of the resulted chitosan (Mirzadeh et al., 2002). For this reason in this work we used multistage deacetylation and the N-acetylation reaction conditions are controlled. The experimental study is applied for multistage reaction behavior and the three step treatment on shrimp chitin to enhancement of DD is chosen.

The kinetics of homogeneous alkaline deacetylation of chitin was reported to be pseudo-first-order reaction (Ahmad Khan, Khiang Peh, & Ch'ng, 2002; Chebotok, Novikov, & Konovalova, 2007; Focher, Beltrame, Naggi, & Torri, 1990; Liu et al., 2009; Sannan, Kurita, & Iwakura, 1977). Similar results were obtained for heterogeneous deacetylation (Ahmad Khan et al., 2002; Methacacon, Prasitsilp, Pothsree, & Pattaraarchachai, 2003). In this study kinetics of multistage process is investigated and with the aim of improving DD of deacetylated chitin the results were compared with continuous deacetylation.

2. Experimental

2.1. Material and methods

Chitin used in these experiments was extracted from Persian Gulf shrimp's shell according to Kifune method (Kifune, Katsuhiro,

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Table 1
Characteristics of chitin.

Specification	Chitin
Viscosity-average molecular weight (Mv)	3.22×106
Degree of deacetylation (DD)	20%
X-ray diffraction peaks	10°, 19°4′
Ash at 900 °C	0.34

& Shinger, 1984) that was optimized in the reaction parameters (Mirzadeh et al., 2002; Yaghobi et al., 2002). The extraction parameters were optimized to mild acidic and alkaline concentrations. At first shrimp shells were washed with water, dried and cut to small pieces. The initial step of chitin extraction was carried out by acetic acid at room temperature for 2 h. This step was followed by filtration, neutralization and washing.

Deproteination was performed using alkaline treatment with 2N sodium hydroxide solution at 60–65 °C and then neutralization was carried out by process of washing. Demineralization was followed by 10% hydrochloride acid at room temperature for 3–5 h. In order to remove natural pigment in the chitin, acetone was added to the solid under reflux conditions for 2 h. Chitin extraction yield from shrimp shell was 28–30%. Table 1 shows the specification of chitin, which have been used in this study, the viscosity and molecular weight measurement were performed using an Ubbelohde viscometer at room temperature (Rhazi et al., 2000).

Deacetylation was carried out with 40–50 wt% NaOH at 90 °C in a nitrogen atmosphere. The FTIR spectra of prepared chitin and chitosan are shown in Fig. 1. The three stage deacetylation treatments were carried out for 1.5, 1.5, 2 h and these treatments were separated by washing and air drying and another case was a single continuous deacetylation treatment for 5 h. After each step, the obtained solid was filtered off and then washed with water and methanol. Table 2 shows the change of DD in several conditions.

Different methods have been proposed to determine the degree of deacetylation of chitin (Ahmad Khan et al., 2002; Takanori, Kurita, & Iwakura, 1978). In this work, degree of deacetylation was determined using Takanari method by FTIR spectroscopy (Takanori et al., 1978). This method gave data of degree of deacetylation by plots of the ratio of the absorbance of the amide II band at 1550 cm^{-1} to that of the band at 2878 cm^{-1} against the degree of deacetylation. The samples of chitosan were mechanically blended with KBr and the mixed powder was pelleted, and finally its FTIR spectrum was recorded with a Bruker IFS-48 spectrometer.

Table 2
Degree of multistage deacetylation of chitin obtained from various conditions.

No.	NaOH (wt%)	Temperature (°C)	DD (%)		
			1.5 h	1.5 h	2 h
1	50	90	87.4	88.6	90.9
2	45	90	56.7	63.4	78.6
3	40	90	30.7	33.2	34.5
4	50	70	75.6	79.4	81.3
5	50	80	82.5	86.8	89.6

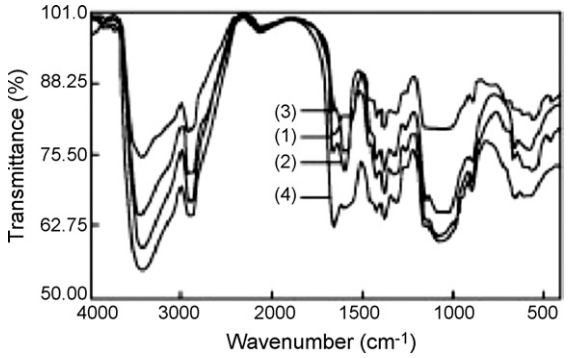

Fig. 2. FTIR spectra of CS-1:1.5 h (1), CS-1:3 h (2), CS-1:5 h (3), CS-2:5 h continuous (4).

3. Results and discussion

3.1. Multistage deacetylation

Fig. 2 shows the FTIR spectra of the different samples of chitosan, CS-1 and CS-2 denote deacetylation reaction for multistage and continuous treatment respectively. The number after CS-1 indicates the time of treatments. As shown in this figure a number of changes in FTIR spectrum of chitin during deacetylation have been attributed to the variations in the degree of deacetylation. In 1590 cm^{-1} region that is assigned to the amino groups of chitosan, a sharp peak appeared, showing that the extent of deacetylation was increased. Other changes were also observed at 1665 cm^{-1} bond, which is attributed to acetamide groups. As the degree of deacetylation was increased a new peak at 1590 cm^{-1} was appeared. In the meantime the peak at 1665 cm^{-1} was weakened. As the extent

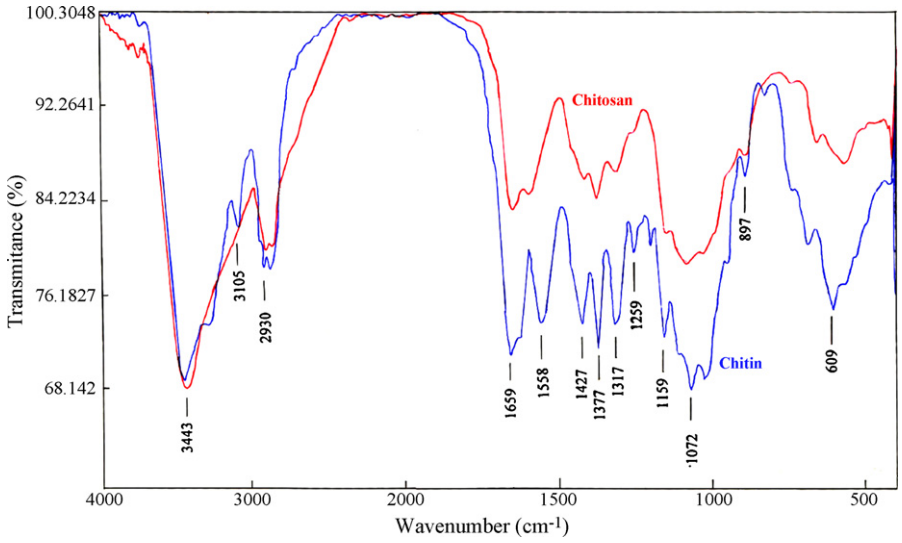

Fig. 1. FTIR spectra of prepared chitin and chitosan.

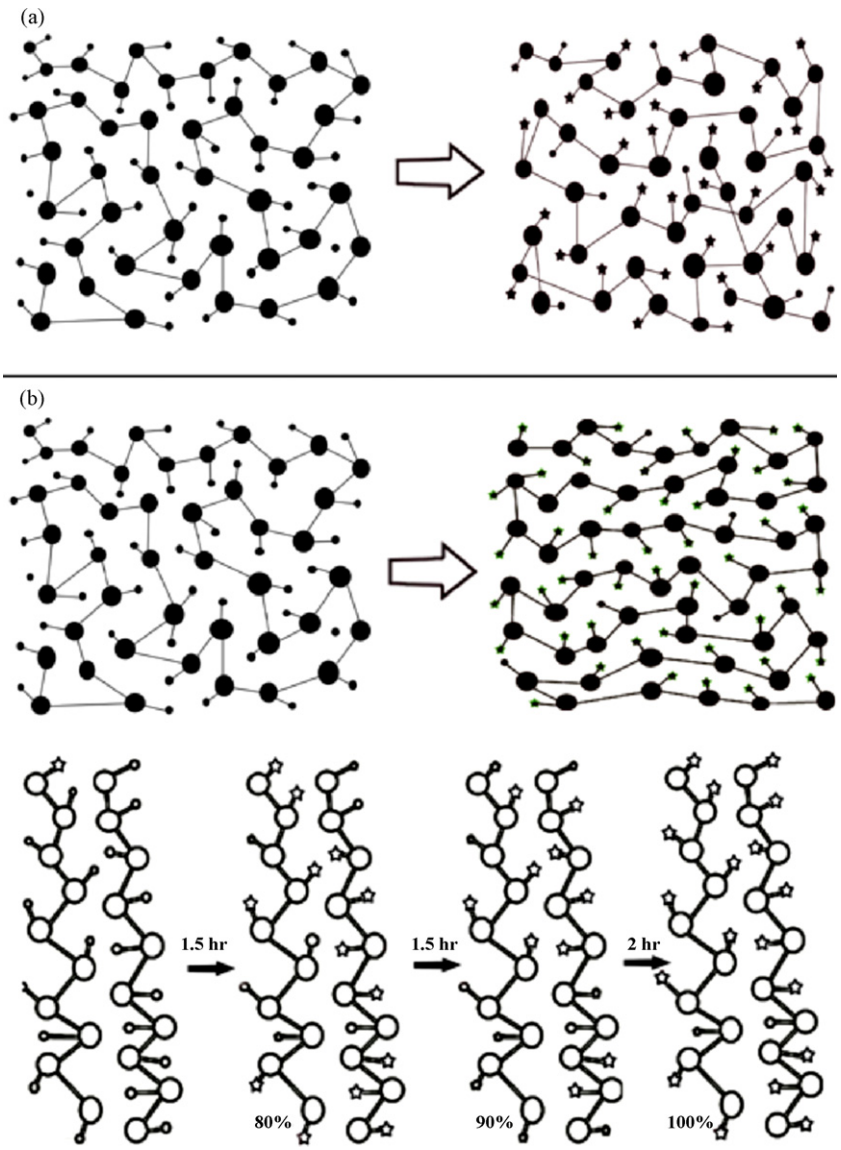

Fig. 3. Schematic representation of the morphological effect of chitin structure under (a) single stage and (b) multistage deacetylation treatment, (○): acetamide group, (*): NH_2.

of deacetylation reaches above 90%, this peak was removed completely.

This result was observed in the FTIR spectra of the multistage treatments by which with increasing of each alkali treatment the peak at 1590 cm^{-1} became sharper. As can be seen in this spectra with increasing the extent of deacetylation, variations in 1665 cm^{-1} and 1590 cm^{-1} bond are significant and among these samples CS-1:5 h (multistage) showing highest DD.

In general, in the single step alkaline treatment the deacetylation of chitin proceeds rapidly until the deacetylation reaches around 75–85%, after which further treatment has only a very limited effect on the extent of deacetylation. The most probable explanation for this condition is that the morphology of chitin chains is such that the remaining amide groups are inaccessible to the NaOH molecule for alkali treatment. It may be assumed that the variations of degree of deacetylation might be due to morphological effect. Therefore, it would be possible to show such chain configuration changes as shown in Fig. 3. In other words, in the multistage alkali treatment, the remaining acetamide groups are more accessible owing to the morphological changes induced. Based on this interpretation, a possible explanation for this effectiveness is the washing treatment that can affect the swelling of chitin with alkali. It seems likely that more washing after each stage of the alkali treatment caused swelling of a greater number of chitin chains and, therefore, more chains could be exposed to deacetylation process (Yaghobi & Mirzadeh, 2004).

To prove this phenomenon an acid–base titration of chitin–NaOH solution was carried out to determine the alkali concentration in the liquid phase at different time lengths of reaction. Acid used for titration was HCl 25% and titration agent was phenolphthalein. The alkali concentration (NaOH) was measured after 5 h. The results showed that the concentration of alkali is too low to facilitate deacetylation reaction to progress. As it is reported in the literature, when the alkali agent concentration is below 40% there is no deacetylation reaction (Robberts, 1989). For this work, after 5 h, the alkali concentration was 43%, which was very close to the critical non-reaction region. In other words, there is not enough driving force in order to conduct the reaction. To overcome this difficulty, after every 1.5 h the liquid phase was drained and the resultant chitosan washed with water and once again a solution of NaOH (50%) was added. The concentration of NaOH was also 43% after 53 h. It seems that the ability of NaOH

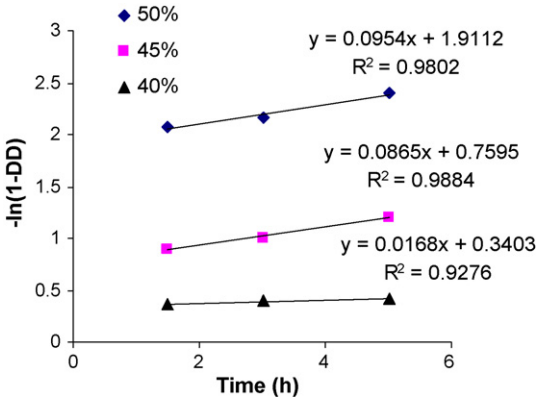

Fig. 4. Effect of NaOH concentration on the multistage deacetylation.

diffusion into chitin was highly diminished. It means that after initial 5 h of reaction, decreasing of NaOH concentration in the liquid phase stopped the deacetylation reaction.

3.2. Kinetics of multistage deacetylation

The rate of deacetylation reaction was assumed to be proportional to concentration of the acetamide group, the pseudo-first-order reaction. The rate constant k may therefore be expressed as follows (Sannan et al., 1977):

$$k = \frac{2.303}{t} \log \frac{a}{a-x} \quad (1)$$

where a is the original concentration of the acetamide group, x is the concentration of the resulting amino group at any time t.

Since the DD in this work was determined by FTIR spectroscopy method, $a/(a-x)$ is proportional to $(1-\mathrm{DD})$. The value of the $\ln(1-\mathrm{DD})$ were plotted against time step of multistage treatment. The plots gave straight lines as shown in Figs. 4 and 5. These linear relationships between $\ln(1-\mathrm{DD})$ and time appeared that multistage deacetylation reaction would be a pseudo-first-order reaction. A power law model may thus be considered for the rate of reaction and apparent activation energy was determined from slope of the linear plots $\ln(k_j)$ against reciprocal of absolute temperature (Fig. 6).

3.3. Effect of alkaline concentration on multistage deacetylation

The effect of alkaline concentration on multistage deacetylation is shown in Fig. 4. It seems that a law concentration such as 40% the multistage reaction followed the pseudo-first-order kinetics at the 5 h treatment, and apparent reaction rate constant ($1.68 \times 10^{-2}\ \mathrm{h}^{-1}$, R^2 value = 0.9802). Also with increasing of NaOH concentration multistage deacetylation process followed the first-order kinetics at the total treatment. The reaction rate constant decreased as a function of concentration rapidly. For example at 50% concentration, the reaction rate was 5.6 times more than that of the 40% concentration. The morphological effect could attribute to the dependence of multistage deacetylation on the concentration.

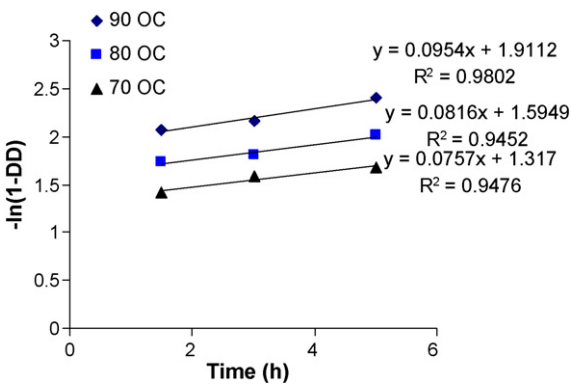

Fig. 5. Effect of reaction temperature on the multistage deacetylation.

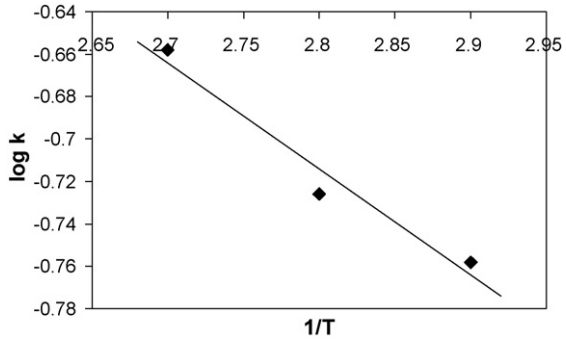

Fig. 6. The Arrhenius plot of $\log k$ vs. $1/T$.

3.4. Effect of reaction temperature on multistage deacetylation

Fig. 5 showed the effects of reaction temperature on N-deacetylation. At the same alkaline concentration, the multistage deacetylation process also followed the pseudo-first-order kinetics, and the rate constants declined as a function of temperature decreased. At 50% NaOH conditions, the apparent activation energy was estimated as about 16.2 kJ/mol from the slop of the straight line of the Arrhenius plot ($\log k - 1/T$) shown in Fig. 6. This is lower than the 22.56–49.80 kJ/mol reported for continuous N-acetylation between 40 and 100 °C (Liu et al., 2009; Sannan et al., 1977). So it is means that multistage method of deacetylation improves the process of deacetylation by morphological effects on chitin.

The rate constants k are computed by Eq. (1) and are found 2.19×10^{-1} at 90 °C, 1.87×10^{-1} at 80 °C and 1.74×10^{-1} at 70 °C.

4. Conclusion

Our results showed that multistage procedure might be effective for increasing the degree of deacetylation of chitin. This technique caused a greater degree of swelling thereby increasing the accessibility of chitins chains during the subsequent treatment and gave case for the multiple treatments became more effective than a single treatment of similar total time.

The time and concentration of alkaline have significant influence on degree and rate of multistage deacetylation; nevertheless; the effect of temperature is insignificant. Also under all conditions multistage deacetylation process followed the pseudo-first-order kinetics ($R^2 > 0.9$), and the apparent activation energy was estimated 16.2 kJ/mol, in the temperature range of 70–90 °C, which was lower than reported for single step treatments.

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