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Enhancement of Chitin's Degree of Deacetylation by Multistage Alkali Treatments

Nakisa Yaghobi and Hamid Mirzadeh*

Department of Polymeric Biomaterials, Iran Polymer and Petrochemical Institute
P.O. Box: 14965/115, Tehran, I.R. Iran

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ABSTRACT

In this study chitin was extracted from Persian Gulf shrimp's shell and deacetylation was carried out with 5 wt% NaOH at 90°C in a nitrogen atmosphere.Chitosan was prepared with high degree of deacetylation. The effect of deacetylation conditions in the multistage alkali treatments in comparison with continuous alkali treatment was studied. Chitosan's nitrogen content and the extent of degree of deacetylation were measured using FTIR spectroscopy and elemental analysis (CHNO analysis) respectively. Significant differences observed between multistage and single stage alkali treatments on the nitrogen content and degree of deacetylation of the resultant chitosan. In the multistage treatments deacetylation of chitin reached to 90.9% that is effective to improve biomedical application of the resultant chitosan. The morphological effect of the chitin macromolecules chains and the alkali agent concentration as a driving force for promotion of deacetylation reaction were studied. Our results showed that the ability of NaOH diffusion into chitin macromolecules was highly decreased in the continuous treatment while in the multistage treatments the degree of deacetylation was enhanced due to more diffusion of the alkali agent into chitin macromolecules and many acetamide sites on these chains were subjected to alkali treatments.

Key Words:
chitin; chitosan;
multistage treatments;
degree of deacetylation;
nitrogen content.

INTRODUCTION

Chitin is an aminopolysaccharide having acetamide groups at C-2 and it converts into chitosan having amino groups as shown in Scheme I [1].

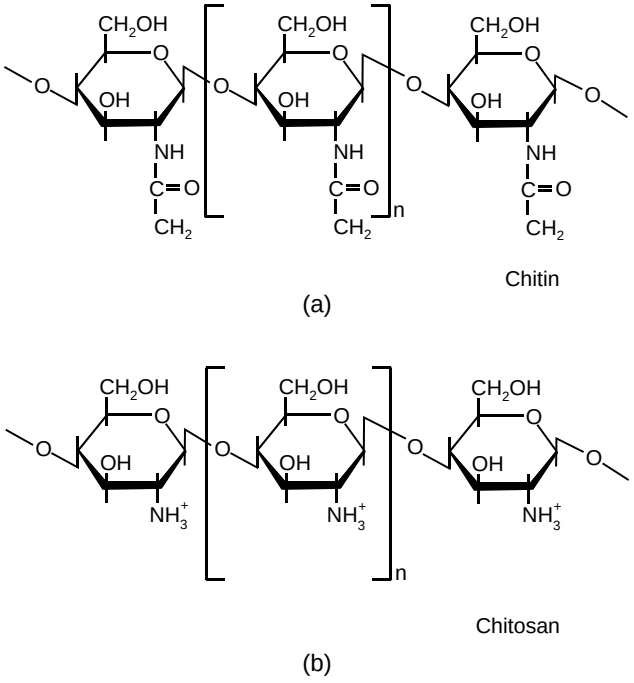
The main sources for the industrial production of chitin are the shells of crab, shrimp and krill. Shrimp's shells contain 40-45% chitin and it is obtained by extracting calcium carbonate and proteins from shrimp's shells [1]. Chitosan is an acid-soluble deacetylated and the main derivative of chitin and obtained from N-deacetylated of

(*) To whom correspondence should be addressed.
E-mail: h.mirzadeh@ippi.ac.ir

chitin. Deacetylation under acidic or basic conditions was applied for chitosan preparation. Although amides may in principle be hydrolyzed under either acidic or basic conditions, the use of acidic hydrolysis is precluded because of the susceptibility of the glycosidic links in chitin to acidic hydrolysis [2]. Deacetylation with aqueous alkali is the most commonly used method for the deacetylation of chitin but no standard conditions have been established [2]. The most frequently used alkali is NaOH [2,3]. The extent of deacetylation is governed by the alkali concentration, the temperature and the time of reaction [4].

An important parameter to examine is the degree of *N*-acetylation of chitin, the ratio of 2-acetamido-2-deoxy-D-glucopyranose to 2-amino-2-deoxy-D-glucopyranose structural unit. This ratio has a considerable effect on polymer solubility and solution properties [5].

Chitosan was characterized by its extent of *N*-acetylation, which affects not only its physicochemical characteristics, chain morphology and molecular weight [5,6] but also its biomedical application, biodegradability and immunological activity [7]. For this reason in this work we studied the effect of non-continuous time of alkali treatment in comparison with continuous alkali treatment to evaluate the extent of variation of degree of deacetylation on the resultant chitosan.



Scheme I. Schematic structures of chitin and chitosan [1].

EXPERIMENTAL

Materials and Methods

Chitin used in these experiments was extracted from Persian Gulf shrimp's shell according to a method that was optimized by reaction parameters, which were reported previously [7-8].

Deacetylation was carried out with 50 wt% NaOH at 90°C in a nitrogen atmosphere. A suspension of 1 g chitin in 50 mL of aqueous sodium hydroxide, in capacity of 5L minipilot, was mixed at 90°C under nitrogen purging in a 5L minipilot. This minipilot has a 5L steel reactor with a PID controller to control reaction temperature accurately (Figure 1).

The three 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.

Deacetylation reaction conditions are shown in Table 1. CS-1 and CS-2 denote deacetylation reaction for multistage and continuous treatment, respectively. The number after CS-1 indicates the number of treatments.

Degree of Acetylation and Nitrogen Content Determination

The quality and property of prepared chitosan vary with deacetylation reaction factors. The influence of

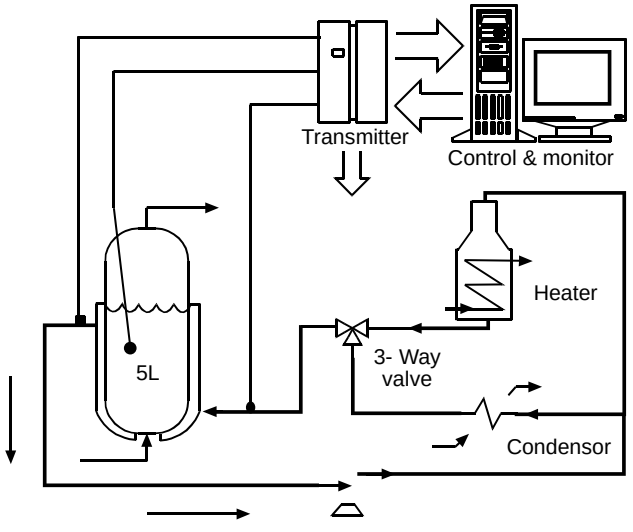


Figure 1. Schematic diagram of the pilot plant used for chitosan extraction.

Table 1. Operating conditions of the deacetylation reactions.

Treatment	Time of alkali treatment (h)	Reaction temperature, T (°C)	NaOH concentration (%)
Non-continuous CS-1-1	1.5	90	50
Non-continuous CS-1-2	1.5	90	50
Non-continuous CS-1-3	2	90	50
Continuous CS-c	5	90	50

the reaction parameters such as reaction duration, reaction temperature, nature of alkali agent and natural source of chitin was studied and optimized as reported elsewhere [8].

The extent of deacetylation is essential to study property relationships and possible industrial uses. Different methods have been proposed to determine the degree of deacetylation of chitin [9,10]. In this work, degree of deacetylation was determined using Takanari et al. [10] method by FTIR spectroscopy. 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. The elemental analysis (CHNO) was carried out by Elemetar-Analysensystems GmbH-Germany analyzer to evaluate the nitrogen content of the resultant chitosan.

RESULTS AND DISCUSSION

Table 2 shows the nitrogen (N%) content and the degree of deacetylation of the chitosan samples. Nitrogen content and degree of deacetylation in CS-1-3 were higher than CS-1-2 and CS-1-1. Figure 2 shows the FTIR spectra of the different samples of chitosan. 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 of deacetylation reached above 90%, this peak was disappeared 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.

Pure chitin and chitosan have nitrogen content of 6.86 % and 8.69 %, respectively [2]. Table 2 shows the nitrogen content of the chitosan derived from the multistage treatments in comparison with the other products. As shown in this table nitrogen content in the chitosan, derived from the single treatment (CS-2), is

Table 2. Nitrogen content and degree of deacetylation of chitosan samples.

Sample	Nitrogen content (W%)	Degree of deacetylation (DD)
CS-1-1	7.6	87.4
CS-1-2	7.7	88.6
CS-1-3	7.9	90.9
CS-2	7.0	80.5
Fluka	7.5	86.3
Sigma	7.5	86.3

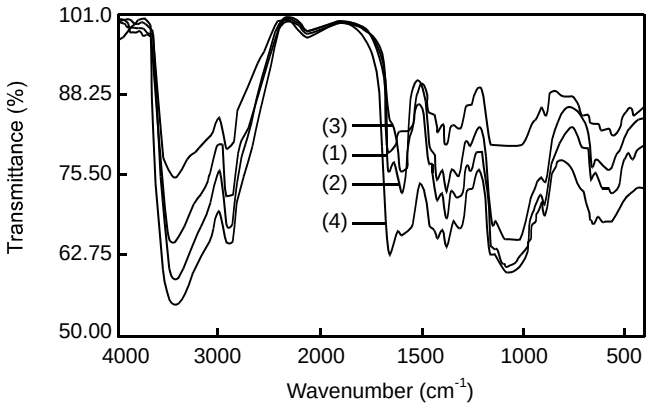


Figure 2. FTIR Spectrum of CS-1-1 (1), CS-1-2 (2), CS-1-3 (3), CS-2 (4).

lower than CS-1 samples. CHNO Elemental analysis results are also consistent with FTIR spectra.

As shown in Figure 2, a number of variations in the FTIR spectrum of chitin during deacetylation can be attributed to the changes in the degree of deacetylation. Figure 3 shows the FTIR spectra of the final product after three stages of deacetylation, i.e. CS-1-3, in comparison with samples made by Fluka and Sigma (Figure 4).

As it can be seen in this spectra, with increasing the extent of deacetylation, variations in 1665 cm^{-1} and 1590 cm^{-1} bonds are significant and among these samples CS-1-3 (multistage) showing the highest nitrogen content and degree of deacetylation.

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 are 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 Figure 5. 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

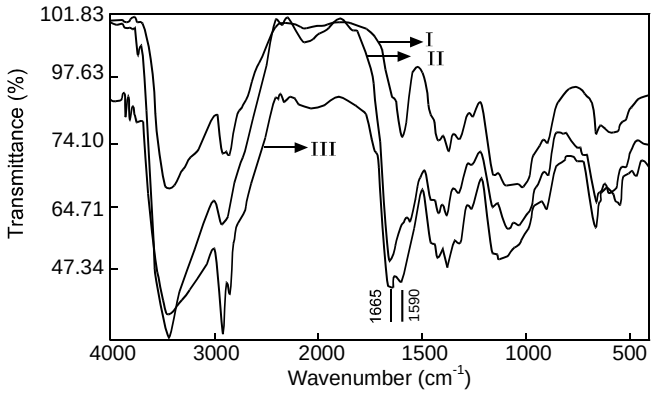


Figure 3. FTIR Spectra of CS-1-3 (I), chitosan from Fluka (II) and chitosan from Sigma (III).

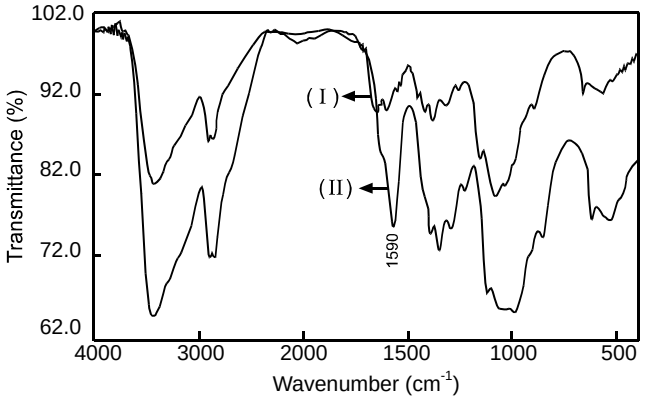


Figure 4. FTIR Spectrum of chitosan after continuous 53 h deacetylation treatment (I), in comparison with non-continuous 5 h deacetylation reaction (II).

of a greater number of chitin chains and, therefore, more chains could be exposed to deacetylation process [2].

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. Variations of NaOH concentration are shown in Table3. 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 [2]. 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

Table 3. NaOH Concentration in liquid phase versus reaction time.

Time (h)	NaOH Concentration (%)
0	50
5	43
53	43

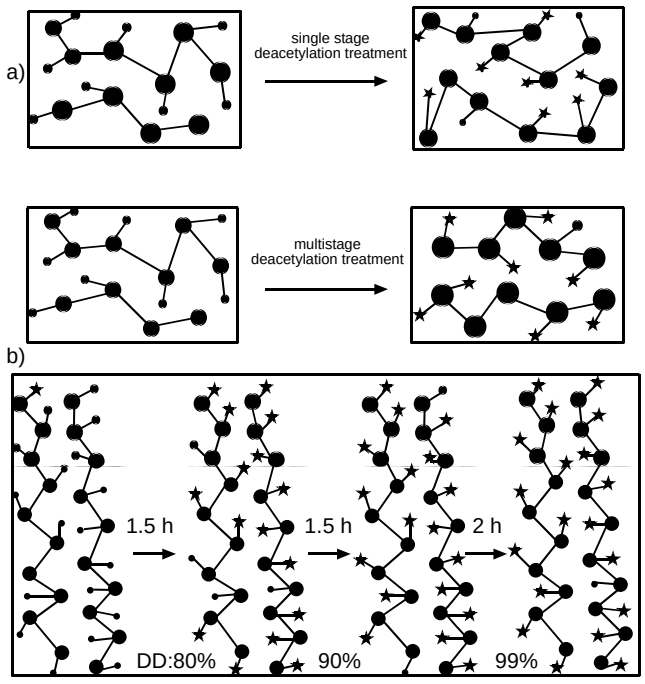


Figure 5. Schematic representation of the morphological effect of chitin structure under (a) single stage and (b) multistage deacetylation treatment, (•) : HN-C-CH_3, (★): NH_2, (∿∿) chitin's ring.

53 h. It seems that the ability of NaOH 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. Figure 4 shows the FTIR spectra of prepared chitosan after the continuous (single stage), 53 h, deacetylation reaction in comparison with non-continuous (multistage) reaction. As shown in this figure, in 1590 cm^{-1} and 1665 cm^{-1} regions a doublet peak appeared, showing a not fully deacetylation for single step alkali treatment. However, a singlet peak in 1590 cm-1 was appeared for CS-1-3 that was obtained from multistage reaction.

CONCLUSION

We found that multistage alkali treatments were as effective as a single stage deacetylation treatment, and the product was more deacetylated. This technique caused a greater degree of swelling thereby increasing the accessibility of chitins chains during the subsequent treatment and gave a case for the multiple treatments, which became more effective than a single treatment of similar total time.

Deacetylation reaction in initial 5 h was stopped because of decreasing of alkali agent concentration and, therefore, diffusion of deacetylation agent into chitin chains became very slow. Even when the reaction time was extended infinitly, full deacetylation did not take place.

Our results showed that this procedure might be effective for increasing the degree of deacetylation of chitin and subsequently improving biomedical application of the resultant chitosan.

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