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PREPARATION OF CHITIN AND CHITOSAN FROM SHRIMP SHELL OF PERSIAN GOLF AND THE DEGREE OF DEACETYLATION DETERMINATION

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ABSTRACT

Chitin, a natural polysaccharide, widely exists in the outer skeleton of arthropods such as shrimp. Chitin and its deacetylated derivative chitosan have been reported to be useful for biomedical applications like wound healing ointments and dressings, drug delivery system, and also shows several biological activities. It is predicted that chitosan will be one of the most demanding material in 2005 and about 75% of chitosan will be used in biomedical areas as a key material.

In this study chitin and chitosan were prepared from shrimp shells in Iran for the first time and the degree of deacetylation was estimated by analytical methods such as FT- IR and ^{1}H- NMR.

Dilute HCl and then NaOH were mixed with shrimp shell powder in a proper condition in order to obtain chitin. Chitin was refluxed in 30, 45 and 60% NaOH solutions for 1-3 h. The mixture was filtered and the residue washed with water and dried.

Chitin (1.5 g) obtained from the above procedure, and the chitosan yield was 60- 90%. One of the chitosan samples obtained in this study was soluble that FT- IR and ^{1}H- NMR determined the degree of deacetylation of this sample as 76 and 76.9% respectively.

We could reach to an easy and optimized method by changing the reaction time, reagents concentration and elimination of decolorizing agent (decolorizing was done during the repetition of purification process) in process.

A relationship between the degree of deacetylation and solubility properties of chitosan was confirmed as other chitosan samples that we obtained in this work could not be solved well and it needed to use other analytical devices such as solid NMR.

Keywords: Shrimp shell, Persian Golf, Chitin, Chitosan preparation, Degree of deacetylation, IR, NMR

INTRODUCTION

Chitin, a polysaccharide, found in the outer skeleton of arthropods in particular, for example, in epidermis of crustaceans such as crabs and shrimp, and in hyphae and spores of molds. Braconnot first described it in 1811(Braconnot, 1811). Rouget discovered chitosan ([α (1 $\rightarrow$ 4) 2- amino 2- deoxy β- D glucan] (Fig. **1**), the main derivative of chitin in 1859 (Rouget, 1859). Briefly, to prepare chitin, crab and shrimp shells are treated in boiling aqueous sodium hydroxide solution after decalcification in dilute hydrochloric acid and deproteination in a dilute sodium hydroxide solution. Chitin is then deacetylated to become chitosan in a concentrated sodium hydroxide solution at boiling point. Other methods also have been reported in the papers and patents. In fact they tried to change the procedure condition in regard to the reaction temperature and time, acid and base concentrations and the frequency of repeating the deacetylation process, to make an optimal method (Horowitz *et al*., 1957; Teng *et al*., 2001).

Chitosan a drug carrier for the 21st century is used in pharmaceutical preparations due to its desirable characteristics such as biocompatibility, digestibility, non- toxicity, and good bioavailability and absorption properties. Chitosan showed several other properties like antimicrobial and lipid and glucose- lowering effects. The properties of chitin and chitosan such as the origin of the material; the degree of N- acetylation, molecular weight and solvent and solution properties are important in the researches (Paul and Sharma, 2000; Majeti and Kumar, 2000).

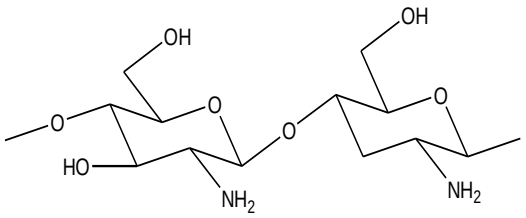

Fig **1**: Chitosan structure

The ratio of 2- acetamido- 2- deoxy- D- glucopyranose to 2- amino- 2- deoxy- D- glucopyranose structural units is one of the important parameter in chitin, namely the degree of deacetylation (DD). Chitin deacetylated to such an extent that it becomes soluble in dilute aqueous acetic acid and formic acids. Chitosan is the fully or partially N-deacetylated derivative of chitin and to define the above ratio, attempts have been made with many analytical tools (Majeti and Kumar, 2000) which include IR spectroscopy, pyrolysis, gas chromatography, gel permeation chromatography and UV spectroscopy, first derivative of UV spectroscopy, H- NMR, ^{13} C solid state NMR, thermal analysis, various titration scheme, acid hydrolysis and HPLC, separation spectrometry methods and near- infrared spectroscopy (Majeti and Kumar, 2000; Wu, 1988; Khan *et al*., 2002). The purpose of this work was to prepare chitin from shrimp shell that is abundant in south of Iran and in the second step, chitosan was prepared and the degree of deacetylation determined.

MATERIALS AND METHODS

All the necessary compounds such as sodium hydroxide, hydrochloric acid, chitosan were purchased from Merck and Fluka. IR spectrum of chitosan film was recorded on an Infrared spectrophotometer (Perkin Elmer Pergamon 781). In order to compare the electrophoresis pattern of the prepared chitosan samples with standard, Electrophoresis device (HELENA) was used. ^{1}H NMR spectra of chitosan/ CH_3 COOH were obtained by using ^{1}H NMR (Bruker FT- 400). Shrimp shell powder was prepared from the fresh shells obtained from the south of Iran (Persian Golf).

Chitin preparation:

Method 1:

Shrimp shell powder (10 g) was stirred for 16 h with 100 ml of 6% hydrochloric acid. The reaction mixture was filtered and the residue was washed with enough water until neutralization. The precipitate was refluxed in 500 ml of 10% sodium hydroxide for 120 min. the result material was filtered and washed with water until the washings were neutral. The mixture was stirred in 4% HCl solution for 12 h. After filtration and washing, it was refluxed in 8% NaOH for 90 min. filtration and washing the residue with water gave acetyl chitin with a pink color. Acetyl chitin was refluxed in 4% NaOH solution for 60 min. 2.2 (22%) chitin powder was obtained after filtrating, washing with water and drying.

Method 2:

Shrimp powder (10 g) was stirred for 1 h with 100 ml of 10% NaOH. The reaction mixture was filtered and the residue washed with water until neutralization. In order to decalcification, it was stirred for 3h in a 15% HCl solution and heated to 80°C. After filtering and washing, in order to defatting, the residue was heated to 70°C in 15% NaOH solution for 5 h and filtering, washing and drying procedures were performed and chitin was obtained in 5. 8 g (58%). FT- IR and 1H- NMR confirmed the structure.

Preparation of chitosan:

Chitin (1.5 g) obtained from the above procedure, was refluxed in 30, 45 and 60% NaOH solutions for 1-3 h the mixture was filtered and the residue washed with water. After drying chitosan was obtained in (60- 90%) yield. Electrophoresis, FT- IR and 1H- NMR confirmed the structure.

Preparation of chitosan film:

A 0.8% chitosan solution in 2% acetic acid was prepared. The solution was filtered and placed into a proper surface and air- dried for 24 h and was washed with methanolic ammonia followed by distilled water. After complete drying the films were used to obtain the FT-IR spectra (Khan *et al*., 2002).

RESULTS AND DISCUSSION

Chitosan a key material of 20th century has been predicted as the most consuming material in 2005. It can be prepared from chitin found in the outer skeleton of arthropods. Chitosan with several useful activities without side effects has found a special place in biomedical sciences (Paul and Sharma, 2000). There have been some problems in regard to pure chitin production, as it shows in the studies the usual method has been done by mixing shells with hot acid and base and decolorizing by $KMnO_4$ in room temperature. Concentrated formic acid could remove colored impurities completely but it causes chitin deacetylation. Then chitosan is prepared by concentrated potassium

hydroxide in Ni containers under the nitrogen atmosphere in 80°C. In order to prepare the HCl salt of chitosan, HCl in 50°C is used in the next step. Repeating the previous steps and washing with ether and ethanol purify it. Also chitin has been decalcified by dilute HCl solution and deproteinated in dilute NaOH, and then decolorized by 5% $KMnO_4$ and oxalic acid solution or by sun exposure. In fact every method has attempted to optimize the procedure (Horowitz *et al*., 1957).

In this work besides chitosan preparation, in order to reach to self-production in our country, we have tried to reach to an easy and optimized method by changing the reaction time and reagent concentration in process. First of all we prepared chitin from shrimp shell by stirring into the dilute HCl and refluxed in dilute NaOH. In method 1 chitin was obtained in 22% yield after purification and 58% in the second method. In our work decolorizing agent was not used because of decolorizing during the repetition of purification process. By changing the reaction time and the base strength, we obtained different chitosans. One of the chitosans that we obtained had an optimum solubility and it was chosen for preparing chitosan film and NMR spectroscopy.

Using 30% NaOH and refluxing for 1 h caused to obtain chitosan in 78.5%. The soluble chitosan was prepared by using concentrated NaOH (60%). Chitin is insoluble in acidic solutions and organic solvents. Chitosan (deacetylated chitin) is soluble in dilute aqueous acetic acid, formic acid. Several analytical methods include IR, ^{1}H- NMR and ^{13}C- NMR have been attempted to define deacetylation ratio as an important parameter effects on chitin solubility and solution properties (Majeti and Kumar, 2000; Wu, 1988; Khan *et al*., 2002).

Electrophoresis pattern of chitosan in 2% acetic acid solution (the one was prepared from chitin hydrolysis in 60% NaOH in this study) was compared with the standard chitosans, confirmed the existence of chitosan primarily. Also IR and 1H- NMR spectra confirmed it. FT- IR is more sensitive method for the chitosans with lower degree of acetylation (Khan *et al*., 2002). Chitin spectrum showed the absorbance at 1651 Cm^{-1} assigned to the carbonyl functional group of acetyl component. Acetyl is eliminated after hydrolysis and chitosan is produced so that the carbonyl band goes to be eliminated. The degree of deacetylation (DD) of the chitosan samples was calculated using the equation was proposed as $DD= 100- [(A_{CO}/ A_{OH}) \times 100 /1. 33]$ (Dormard and Rinaudo, 1983; Khan *et al*., 2002; Miya *et al*., 1980).

A_{CO} and A_{OH} were the absorbance of amide band as a measure of N- acetyl group content and hydroxyl band as an internal standard. 1. 33 denoted the value of the ratio of A_{1657}/ A_{3468} for fully N- acetylated compound.
So DD for standard medium and low molecular weight chitosans was calculated 57 and 54%. The degree of deacetylation for the soluble chitosan sample in this work was calculated in the similar way and obtained 76%.
^{1}H- NMR chitosan spectrum was recorded in D_2O, and the ring protons (H_1- H_6) resonances between 3 and 4 ppm. The protons related to hydroxyl and amine groups can be exchanged by deuterium and do not show any peaks in spectrum. So the rest of the hydrogen's peaks in chitosan structure unite could be observed in the ^{1}H- NMR (Ottoy *et al*., 1996; Kubota *et al*., 2000).

^{1}H- NMR was also recorded in presence of CF_3COOD. The peaks existed between 4.5 and 5 are hidden under the HOD peak, therefore the resonance between 3 and 4 ppm corresponds to five other protons that were chosen as an internal standard. The degree of N- acetylation can be calculated from the ratio of integral intensity of the N- acetyl protons to the sum of integral intensities of the above five protons. The spectrum of the standard chitosan with medium molecular weight showed the five protons between 3 and 4 ppm and the peak at 1.9 ppm corresponded to CH_3 protons signal. The integral intensity of the five protons obtained about 70 and for each proton the intensity was 13.8. So the integral intensity of methyl protons had to be thrice of each proton (41. 5). The DD of the soluble chitosan sample obtained in our study was calculated as 76. 9%.

There is a relationship between the degree of deacetylation and solubility properties of chitosan as other chitosan samples that we obtained in this work could not be solved well and it needed to use other analytical devices such as solid NMR that has not been available yet.

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