Preparation and Characterization of Chitosan from Shrimp shell waste

Arafat A., Sabrin A Samad, Shah Md. Masum, Mohammad Moniruzzaman

Abstract— Chitosan was prepared from shrimp processing waste (shell) using the same chemical process as described for the other crustacean species with minor modification in the treatment condition. The physicochemical properties, molecular weight (165394 g/mole), degree of deacetylation (87%) as well as yield (19%) of prepared chitosan indicated that shrimp processing waste (shell) are a good source of chitosan. FT-IR spectra gave characteristics bands of $-NH_2$ at 3450cm^{-1} and carbonyl at 1629cm^{-1}. X-ray diffraction (XRD) patterns also indicated two characteristics crystalline peaks approximately at 10° and 20° (2θ).The surface morphology was examined using scanning electron microscopy (SEM).

Index Terms— Chitosan, Degree of Deacetylation, FT-IR, Scanning electron microscope.

1 INTRODUCTION

Chitosan is a linear polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). It is made by treating shrimp and other crustacean shells with the alkali sodium hydroxide. The biopolymer is characterized as either chitin or chitosan according to the degree of deacetylation (DD) which is determined by the proportion of D- glucosamine and N-acetyl-D-glucosamine. Structurally, chitosan is a straight-chain copolymer composed of D-glucosamine and N-acetyl-D-glucosamine being obtained by the partial deacetylation of chitin. Chitosdan is the most abundant basic biopolymer and is structurally similar to cellulose, which is composed of only one monomer of glucose.

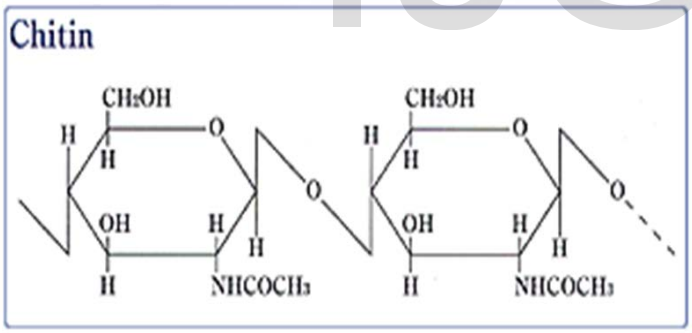

Figure 1: Structure of Chitin

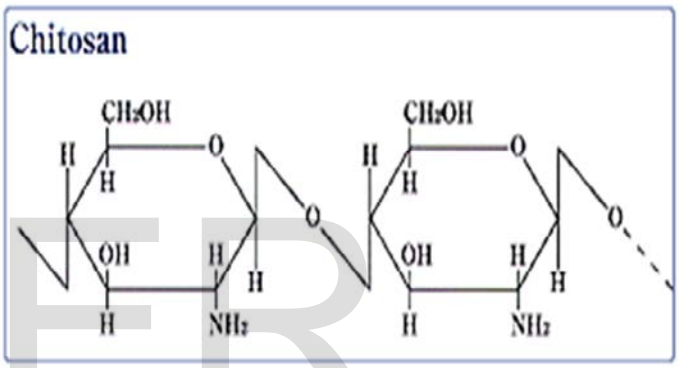

Figure 2: Structure of Chitosan

Chitosan solubility, biodegradability, reactivity, and adsorption of many substrates depend on the amount of protonated amino groups in the polymeric chain, therefore on the proportion of acetylated and non-acetylated D-glucosamine units. The amino groups (pKa from 6.2 to 7.0) are completely protonated in acids with pKa smaller than 6.2 making chitosan soluble. Chitosan is insoluble in water, organic solvents and aqueous bases and it is soluble after stirring in acids such as acetic, nitric, hydrochloric, perchloric and phosphoric [1]. The typical production of chitosan from crustacean shell generally consists of three basic steps: demineralization, deproteinization and deacetylation [2]. Due to its simplicity, relative instrument availability, and independence of sample solubility, IR spectroscopy is one of the most studied methods for characteristics of chitin and chitosan [3]. Shrimps are in general sold headless and often peeled of the outer shells and tail. About 30-40% by weight, shrimp raw material is discarded as waste when processed shrimp is headless, shell on products [4]. The main aim of this present work was to prepare chitosan from fishery waste materials which are hazard and toxic for environment.

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2 MATERIALS AND METHODS:

2.1 Chitosan production

2.1.1 Raw materials:

Detailed submission guidelines can be found on the author resources Web pages. Indigenous shrimp shells were collected from Khulna, Bangladesh. Chitosan is easily obtained from crab especially Dungeness crab (*Cancer magister*), shrimp particularly the Pacific shrimp (*Pandalus borealis*), lobster, or crawfish shells. These are the richest source of chitin and the major sources of crustaceans that are processed into chitin and chitosan.

Figure 3: Shrimp shell

Figure 4: Shrimp shell powder

2.1.2 Chemicals and Reagents: All chemicals (NaOH, HCl) were used industrial grade and were purchased from local market.

2.1.3 Preparation of Chitosan: Chitosan preparation is divided into three consecutive steps. Demineralization of Shrimp shells, Chitin processing (Deproteinization) and Chitosan processing (Deacetylation).Shrimp shells were dried in sun 2 days. After sun drying, the shrimp shells were crispy. Then the shells were grounded into powder. Dried Powder shrimp shells were placed in opaque plastic bottles and stored at ambient temperature.

2.1.3.1 Demineralization of Shrimp shells: In this step, finely powdered shrimp shell is demineralized with HCl. At room temperature the shrimp shells were demineralized with 5% HCl for 24 hours with a solid to ratio of 1:6. After 24 hours, the shells were quite squashy and were rinsed with water to remove acid and calcium chloride and dried in an oven to 60°C. Small amount of treated shell was reacted with again 10% HCl solution, which showed no bubble generation. This test ensures complete demineralization of shells.
Yield: Weight of demineralized Shell 35.2 gm.

2.1.3.2 Deproteinization: Demineralized shells was deproteinized with 5%NaOH solution for 48 hours at 60-70°C at a solid to solvent ratio of 1:10 (w/v). After processing, the residue was washed with distill water to remove NaOH. Then it is dried for 2 days and the product found is called chitin.
Yield: Weight of Chitin 24.15 gm.

2.1.3.3 Chitosan processing (Deacetylation): Deacetylation is the process to convert chitin to chitosan by removal of acetyl group. After rinsing with distilled water, the decalcified chitin was transferred to a 60% sodium hydroxide solution. The solution was heated in a domestic microwave oven for 2 hour for deacetylation. After rinsing with distilled water and drying at 60°C, the deacetylated chitin (now known as chitosan) was ready for use.
Yield: Weight of Chitosan 18.97 gm.

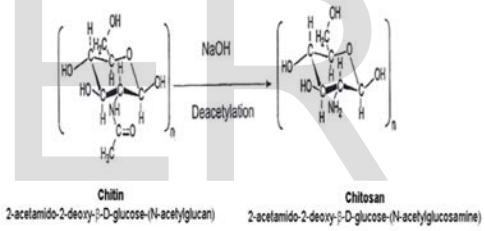

Figure 5: Conversion of chitin to chitosan by Deacetylation.

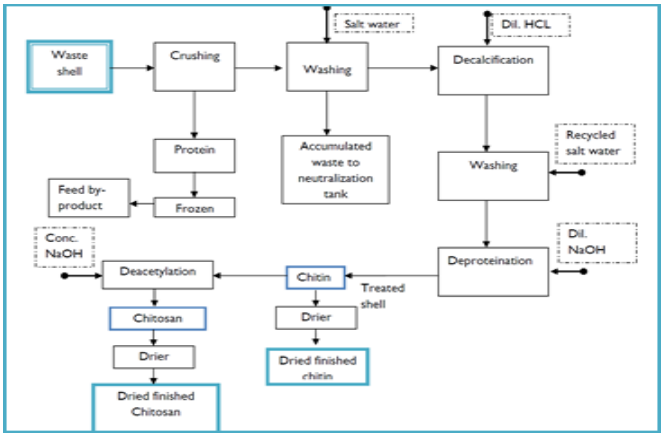

Figure 6: Manufacturing process of Chitosan.

2.2 Degree of Deacetylation of Chitosan:
Titration methods:

Dried chitosan (0.2 g) was dissolved in 20 cm^3 0.1M hydrochloric acid and 25cm^3deionized water. After 30 minutes continuous stirring, next portion of deionized water (25 cm^3) was added and stirring continued for 30 minutes [5]. When chitosan was completely dissolved, solution was titrated with a 0.1 mol·dm^{-3} sodium hydroxide solution using automatic burette (0.01 cm^3accuracy). Degree of deacetylation (DA) of chitosan was calculated using formula:

$$DA[\%] = 2.03 \cdot \frac{V_2 - V_1}{m + 0{,}0042 \cdot (V_2 - V_1)}$$

where: m – weight of sample, V_1, V_2– volumes of 0.1 mol·dm^{-3}sodium hydroxide solution corresponding to the deflection points, 2.03 – coefficient resulting from the molecular weight of chitin monomer unit, 0.0042 – coefficient resulting from the difference between molecular weights of chitin and chitosan monomer units.

For titration volume of 0.1 mol·dm^{-3}sodium hydroxide solution required, V_2–V_1 =10.5 ml.

Degree of Deacetylation of Chitosan calculated= 87.32%.

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3 Results and Discussions

3.1 Characterization of Chitosan:

Chitosan was prepared from shrimp shell discussed in the material and method section. The main difference between Chitin and Chitosan is that Chitosan is soluble in 1% acetic acid. The characterization of Chitosan was confirmed by FT-IR analysis and XRD analysis.

3.1.1 FT-IR Analysis of Chitosan:

Figure 7 shows that The IR spectra of chitosan showed a strong absorption band at 3454 cm^{-1} due to OH and amine N-H symmetrical stretching vibrations. A peak at 2926 $cm^{-1}$was due to symmetric -CH_2 stretching vibration attributed to pyranose ring [6]. The sharp peak at 1384 cm^{-1} was assigned to CH_3 in amide group [7].

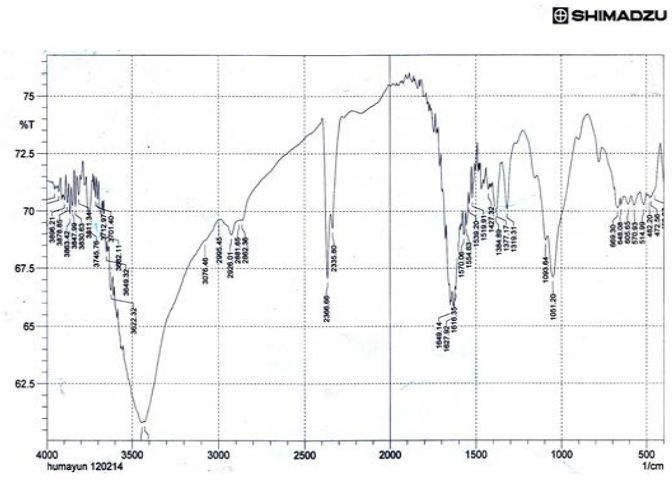

Figure 7: FTIR spectra of prepared Chitosan.

The broad peak at 1021 and 1093 cm^{-1} indicated the C-O stretching vibration in chitosan and peaks at 1627 and 1554 cm^{-1} were due to -C=O stretching (amide I) and NH stretching (amide II). The absorption bands at 1203 cm^{-1} was assigned to the anti-symmetric stretching of C-O-C Bridge and 1098 and 1021 cm^{-1} were assigned to the skeletal vibrations involving the C-O stretching [8]. Fourier Transform Infrared spectrum shows characteristic peaks of carbonyl at 1,629.85 cm^{-1} and amide at 3,450.65 cm^{-1} which ensures that this is the FT-IR of Chitosan.

3.1.2 XRD Analysis of Chitosan:

Figure 8 shows the used Chitosan biopolymers in this study. The XRD pattern of Chitosan exhibits Broad diffraction peaks at 2 theta= 10° and 20° which are typical diffraction peaks of Chitosan to amorphous Character [9] respectively. It is reported that, the characteristics crystalline peaks with slightly fluctuated diffraction angles found in WAXD patterns indicated that two types of alpha-Chitosan and gamma-Chitosan exhibited comparable degree of crystallinity and had a consistent peak between 19-20° [10].

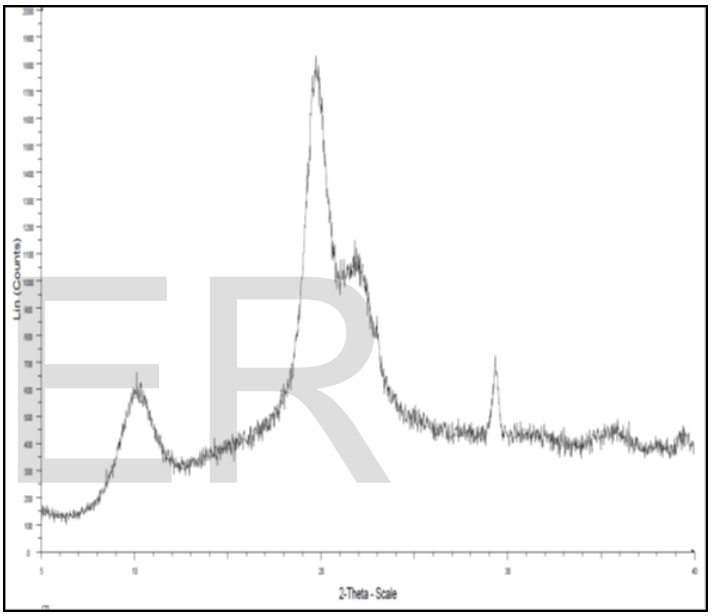

Figure 8: XRD spectrum of Chitosan

3.1.3 Scanning electron microscope (SEM) Analysis:

Chitosan prepared from shrimp shell waste was examined by scanning electron microscopy (SEM) having a magnification range of 5,000 and accelerating voltage 20 kV. The SEM micrograph illustrates the morphology of the prepared chitosan from shrimp shells.

The micrographs showed non-homogenous and non-smooth surface as shown in Fig. 9.

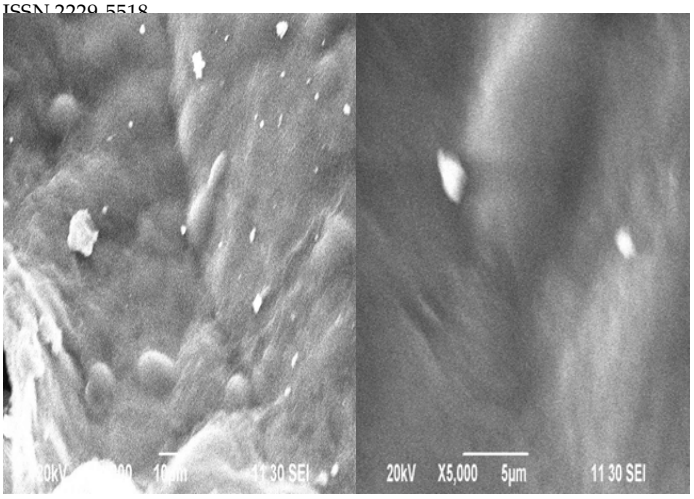

Figure 9: SEM image of prepared Chitosan.

4 Conclusion

The present observations indicate that the prepared chitosan in this study is soluble in 1% acetic acid solution. The FTIR, XRD, SEM of the prepared chitosan confirmed that the prepared material is Chitosan. The preparation of chitosan from shrimp processing waste (shells) would successfully minimize the environmental pollutants. Chitosan Can be plays a vital role for absorption due to its amide group.

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