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ORIGINAL ARTICLE

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SYNTHESIS AND CHARACTERIZATION OF CHITOSAN FROM RAW SHRIMP SHELL WASTE

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Abstract

Chitosan is a polysaccharide that is completely natural, non-toxic, biocompatible and biodegradable. Various commercial and biomedical applications exist for chitosan. The objective of this research was to synthesis chitosan nanopowders and then characterizes them. Waste shrimp shell is employed in this study as the starting point for the production of chitosan. Through the use of X-ray diffraction (XRD), Scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FT-IR), the structural and chemical characteristics of the nanopowders have been explored. The examination of the thermal stability of synthesized chitosan performed using thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). The present study revealed that chitosan nanopowders were successfully synthesized by chemical method with minor modification. The XRD study showed two characteristics crystalline peaks approximately at $2\theta = 9.63^\circ$ and 19.93° which is similar to the previous papers. Therefore, it can be said that chitosan nanopowders were successfully synthesized.

Keywords: Chitosan, Shrimp Shell, Chemical method, Chitosan nanopowders, Structural and Chemical properties, Thermal stability.

Introduction

Chitosan is a natural polysaccharide that is harmless, adaptable and biodegradable. A homopolymer of N-acetyl-D-glucosamine connected by β- (1→4) bonds that was extracted from chitin (Austin & Brine, 1981). Chitin is the most frequently occurring natural polymer after cellulose. Last few years, chitosan has drawn a considerable amount of interest because of its biological characteristics and affordable manufacture (Srivastav et al., 2018). It has a number of commercial and biomedical uses like pharmaceutical, tissue engineering, drug delivery and waste-water treatment (No et al., 1989). In this current work, chitosan can be prepared from shrimp shells using chemical method that only takes minor alterations such as demineralization, deproteinization and deacetylation (Taher et al., 2017). For better washing ethanol is used. The aim of the study is to synthesis chitosan nanopowders and then characterizes them for further study. The prepared chitosan used in biomedical field as like teeth implantation will be our next target. Titanium and Zirconia are

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used for dental implants but we choose chitosan because of its low cost, availability and biocompatible. Chitosan can be characterized using a variety of methods: The structural characteristics of synthesized chitosan are probed using X-ray diffraction (XRD). The morphological and chemical properties of obtained chitosan are studied through Scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FT-IR) respectively. For the purpose of evaluating the thermal stability of synthetic chitosan, Thermogravimetric analysis (TGA) and Differential scanning calorimetry (DSC) are used.

Materials and Methods

Materials and instruments

In the neighborhood market, GOLLAMARI, KHULNA, raw shrimp shells were purchased. Sigma-Aldrich Laborchemikalien GMbH (Seelze, Germany) supplied purified sodium hydroxide pellets. Merck KGaA (Darmstadt, Germany) supplied us with hydrochloric acid (HCl, 37%). Ethanol (99%) was purchased from VWR International S.A.S (France). Then, distilled water was used to dilute them to the proper concentration for the subsequent procedures. Without additional purification, all of the compounds were employed. Rigaku SmartLab XRD (Japan) was used for XRD. 'Spectrum Two FT-IR Spectrometers', PerkinElmer was used for FT-IR analysis.

Preparation of chitosan

Chitosan must first be transformed into chitin in order to be extracted from shrimp shells. Demineralization and deproteinization are often the first two processes in the process of extracting chitin from raw shrimp shells (Agarwal et al., 2018). Deacetylation can then transform chitin into chitosan (Nessa et al., 1970). To eliminate the attached muscle residue from the raw shrimp shells, deionized water was used. After that, the shrimp shells were dried in the sun for at least 48 hours until they were crispy.

(a) Raw shrimp shells (b) Washed shrimp shells (c) Dried shrimp shells

Figure 1. Pretreatment of shrimp shells.

Step 1: Demineralization

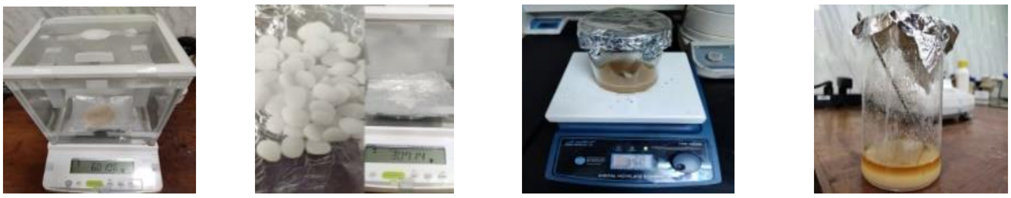
In this step, at first the dried shell was ground into powder by the process of hand milling (mortar and pestle). The fresh shrimp shell powder (20 g) was then dealt with 120 ml of 5% hydrochloric acid (HCl) in an acid-resistant jar with a solid-to-solvent ratio of 1:6, stirring for 6 hours at ambient temperature to remove the organic content, notably calcium carbonate ($CaCO_3$). After that, deionized water was used to wash the solution until it reached a "neutral pH". The finished demineralized shells were washed, bleached by submerging for 15 minutes in ethanol and then dried in a 60°C oven. After drying, the demineralized shells were ground by mortar and pestle.

(a) Dry shrimp shells (b) Grinding by mortar and pestle (c) Grinding and weighed (d) Treated with 5% HCl and stirred for 6 hours at room temperature

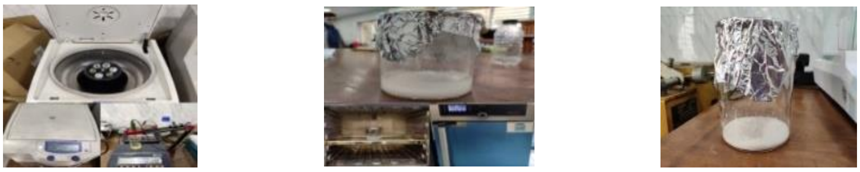
(e) Washing with deionized water (f) Drying at 60°C (g) Final product

Figure 2. Steps of demineralization process.

Step 2: Deproteinization



(a) Weighed demineralized powder (b) Sodium hydroxide (NaOH) (c) Demineralized powder treated with 5% NaOH and stirred for 3 hours at 60°C (d) Solution after stirring



(e) Washing with deionized water (f) Drying at 60°C (g) Final product

Figure 3. Steps of Deproteinization process.

In order to remove the protein content of shrimp shells, demineralized powder (6.0106 g) was treated with 5% sodium hydroxide (NaOH, 60.106 ml), at a solid-to-solvent ratio of 1:10 (weight/volume). This procedure was carried out for three hours at a temperature of 60°C. The resultant solution was then rinsed in deionized water until it reached a "neutral pH". Then, it was immersed in ethanol for 15 minutes for further bleaching and dried in an oven at 60°C. After drying, the resulting product was ground by mortar and pestle and weighed. The resultant product was chitin.

Step 3: Deacetylation

(a) Weighed deproteinization powder

(b) Sodium hydroxide (NaOH)

(c) Chitin treated with 60% NaOH and stirred for 4 hours at 100°C

(d) Washing with deionized water

(e) Drying at 60°C

(f) Final product

(g) Weighed final product (Chitosan)

Figure 4. Steps of Deacetylation process.

In this step, chitin (4.4700 g) was treated with 60% sodium hydroxide (NaOH, 44.7 ml) with a solid-to-solvent ratio of 1:10 (weight/volume). At a temperature of 100°C, the mixture was stirred for 4 hours. In this technique, acetyl groups are only partially eliminated from the structure of chitin in order to transform it into chitosan. The resultant chitosan was then baked in an oven at 60°C after being rinsed with deionized water until it reached a "neutral pH". After drying, the resulting chitosan were ground by mortar and pestle and weighed.

Results

X-ray Diffraction (XRD) characterization

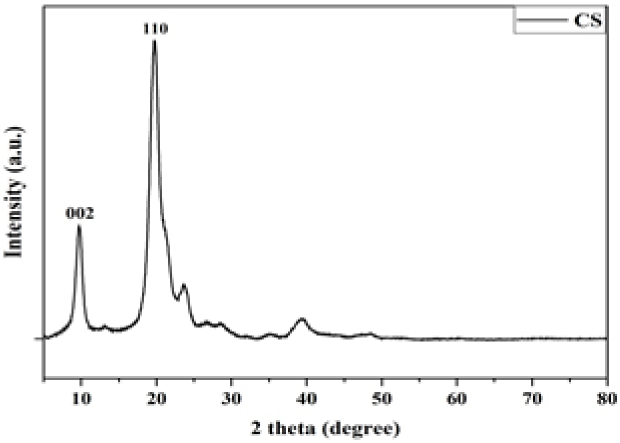


Figure 5. X-ray diffraction pattern of prepared Chitosan (CS).

Table 1. Table for Diffraction angle and Plane

Sample	Diffraction angle (2θ)	Plane
Chitosan (CS)	9.63° and 19.93°	(002) and (110)

Table 2. Table for Crystallite size

Sample	Crystallite size (D)
Chitosan (CS)	7.26 nm

Scanning Electron Microscopy (SEM) characterization

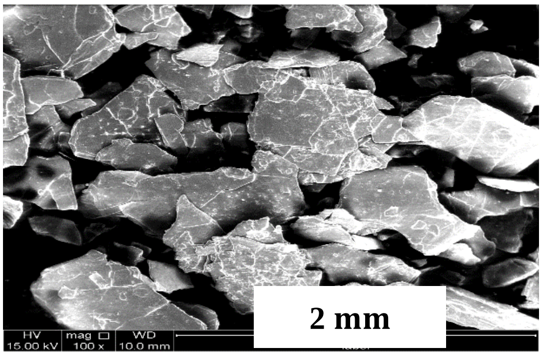


Figure 6. SEM image of prepared Chitosan (CS).

FT-IR characterization

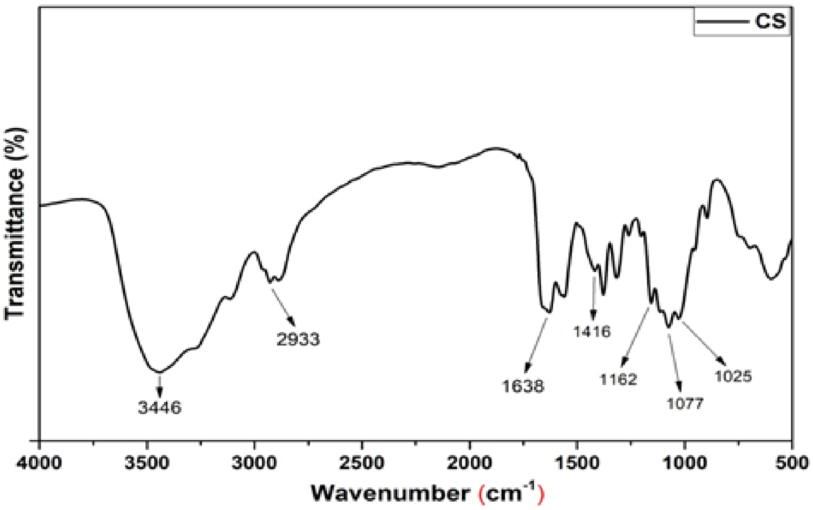


Figure 7. FT-IR spectrum of prepared Chitosan (CS).

Table 3. Functional groups and their corresponding bands of prepared chitosan

Wavenumber (cm^{-1})	Functional group
3446	N-H stretching
2933	C-H stretching
1638	C=O stretching
1416	N-H deformation
1162	C-H stretching
1077	C-O-C stretching
1025	C-O stretching

Thermogravimetric (TG) characterization

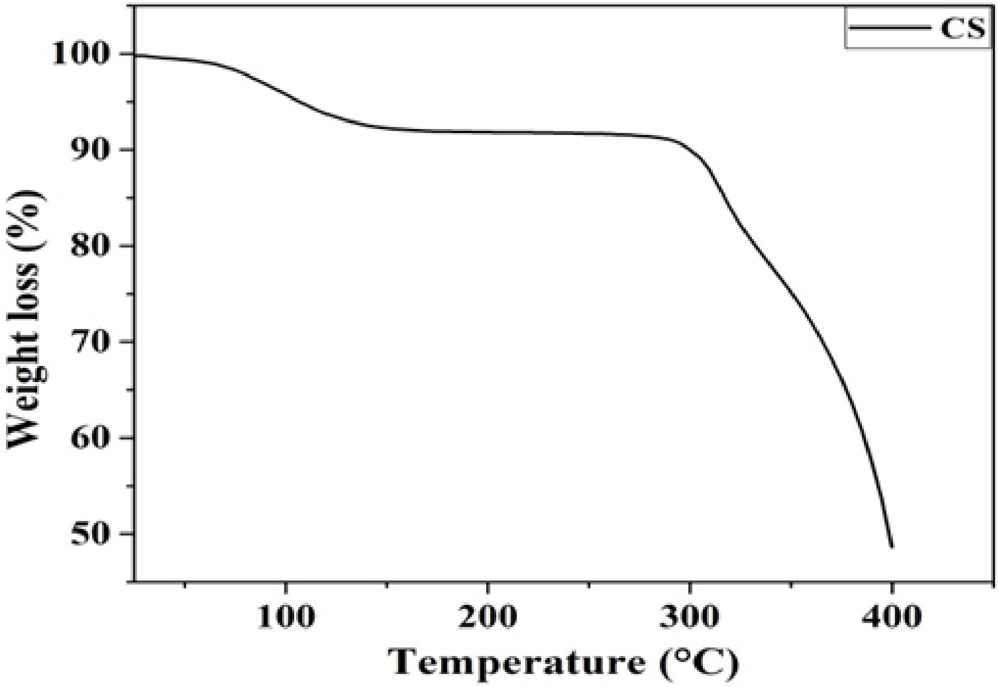


Figure 8. TG curve of prepared Chitosan (CS).

Differential scanning calorimetry (DSC) characterization

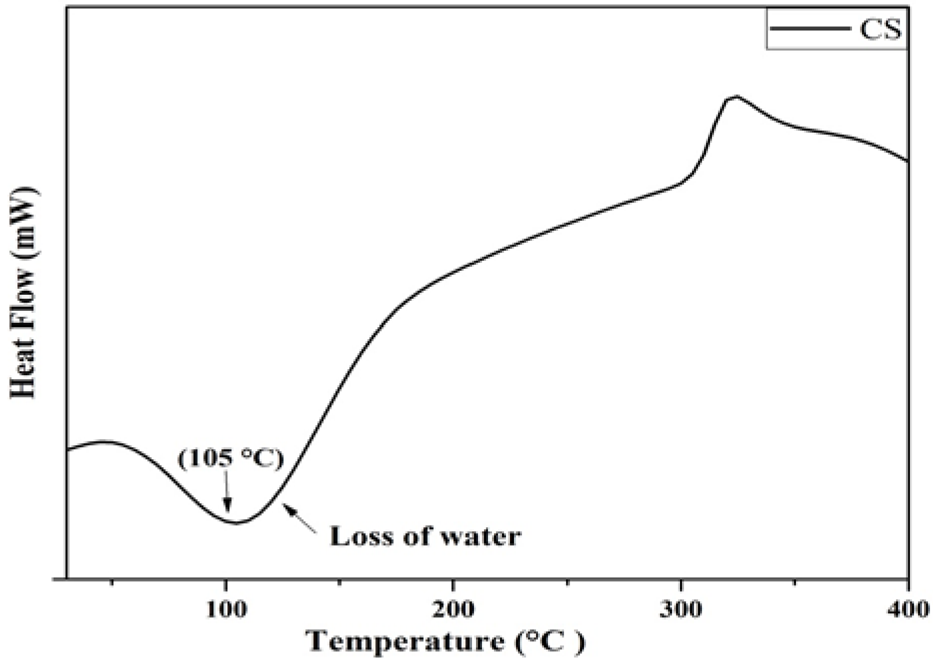


Figure 9. DSC curve of prepared Chitosan (CS).

Discussion

X-ray Diffraction (XRD) analysis

Standard chitosan from Sigma Aldrich has peaks in the XRD pattern at 2θ = 10° and 20°, which are associated with crystals I and II in the structural formation of chitosan (Isa et al., 2012). Figure 1 shows two distinctive diffraction peaks at 2θ = 9.63° and 19.93° that linked to the (002) and (110) planes, which are typical fingerprints of crystal chitosan. The crystal-I and crystal-II in the structure of chitosan are related to the peaks at 9.63° and 19.93°. Crystal-I corresponds to the hydrated crystalline structure, whereas crystal-II corresponds to the relatively crystalline lattice (110) of chitosan (Pokhrel et al., 2016). Both peaks in this investigation showed a significant level of crystallinity. Chitosan was effectively synthesized and appears to be partly crystalline polysaccharide, according to the XRD pattern (Günister et al., 2017).

The crystallite size (D) of chitosan (CS) has been calculated by using the Debye – Scherrer formula,

$$\text{Crystallite size, } D = \frac{K\lambda}{\beta\cos\theta}$$

where, λ is the wavelength of X-ray (Cu Kα = 0.154 nm), D is the crystallite size (nm), β is the Full Width at Half Maximum (FWHM) of the X-ray diffraction peak at the maximum intensity calculated from the origin software, peak angle θ is expressed in radians and K is crystallite shape factor constant (K = 0.9). The crystallite size of prepared chitosan (CS) is 7.26 nm.

Scanning Electron Microscopy (SEM) analysis

A scanning electron microscope is used to examine the morphology of the produced chitosan sample. Figure 2 depicts a non-smooth and non-homogeneous surface with shrinkage and straps from a SEM study of produced chitosan (CS) (Islam et al., 2011).

FT-IR analysis

Figure 3 depicts the prepared CS's FT-IR spectrum. A broad peak at about 3446 cm^{-1} is due to the amino group's N-H stretching vibration. C-H stretching is the cause of the peak at 2933 cm^{-1}. The peak at 1638 cm^{-1} is attributed to carbonyl (C=O) stretching vibration. N-H deformation is what causes the peak at 1416 cm^{-1}. The C-H stretching vibration is attributed with producing the peak at 1162 cm^{-1}. The C-O-C stretching vibration is responsible for the peak at about 1077 cm^{-1}. Stretching vibration of the C-O is what induces the peak at 1025 cm^{-1}. The characteristics of the prepared chitosan sample have approximately matched with the characteristics of chitosan nanopowder extracted by chemical method and FT-IR analysis reveals that chitosan is successfully synthesized (Cardenas & Miranda, 2004).

Thermogravimetric (TG) analysis

The weight loss of the produced chitosan from ambient temperature to 450°C at a heating temperature rate of 10°C min^{-1} in a nitrogen atmosphere is represented in figure 4 by thermogravimetric (TG) analysis. The initial weight loss of prepared chitosan is obtained between 74^{o}C and 140^{o}C corresponding to the loss of moisture. Chitosan experiences thermal deterioration between 295 to 334°C, which is indicative of deacetylation, vaporization and reduced volatile product levels. This observation indicates that chitosan is effectively synthesized (Cardenas & Miranda, 2004).

Differential scanning calorimetry (DSC) analysis

Figure 5 illustrates the differential scanning calorimetry (DSC) investigation to examine the thermal stability of a produced chitosan sample. The first endothermic peak of chitosan obtained at 105^{o}C that can be ascribed to the loss of water. The second chitosan peak is obtained between 305^{o}C and 334^{o}C with maximum value at 322^{o}C which indicates a decomposition of amine groups, is similar to the previous studies. Therefore, differential scanning calorimetry (DSC) analysis reveals that chitosan is successfully synthesized (Sreenivasan, 1996).

Conclusion

Chitosan was prepared from deacetylation of chitin by chemical method with minor modification. For better washing ethanol was used. The prepared chitosan sample was characterized by XRD, SEM, FTIR, TGA and DSC. The diffraction peaks at $2\theta = 9.63°$ and 19.93° that were revealed by the XRD results are related to earlier investigations. The synthesized chitosan's crystallite size was determined to be 7.26 nm. Chitosan can be plays a vital role for absorption due to its amide group. All of these characterizations of prepared chitosan sample give results as like as previous papers. For this reason, it can be said that chitosan is successfully synthesized from shrimp shell for further study. The prepared chitosan can be further used in biomedical field, drug delivery, food industry etc. because prepared chitosan sample are of nano level now.

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