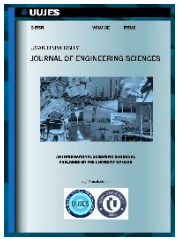

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Research article

CHARACTERIZATION AND PROPERTIES COMPARISON OF NIGERIAN CRAB-SHELL EXTRACTS

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Abstract

Efforts aimed at large scale isolation of chitin and chitosan from marine exoskeletons are being intensified due to their emerging usage in several applications. In this study, extraction of chitin and chitosan was carried out on crab shell of Nigerian origin. Chemical treatments involving demineralization with 1.2M HCl and deproteinization using 1M NaOH at 100°Cwere employed. This was followed by deacetylation of chitin to produce chitosan. The extracts chitin and chitosan were characterized using SEM, FTIR, DTA and XRD analytical tools. Results of these characterizations compared well with established spectral bands and degradation temperatures of both chitin and chitosan which are 388°Cand 342°Crespectively. The presence of $CaCO_3$ in a standard crystalline form as revealed by XRD analysis coupled with the exhibition of peculiar microstructural features through SEM suggest that the biopolymer could be spurn into fibrous mat for wound dressing, scaffold for drug delivery as well find application in suture material development. Contributions from this study have the potential of extending the frontiers of knowledge in tissue engineering. Results of Fourier Transform Infrared (FTIR) analysis on crab shells, chitin and chitosan showed the absorption band of 1798, 3460-3268, 1628-1558 cm^{-1} respectively.

Keywords: *Marine exoskeletons; spectral bands; degradation temperature; biopolymer; chitin; chitosan.*

1. Introduction

Chitin is the second most abundant natural polysaccharide cellulose known to be non-elastic and nitrogenous. It exhibits a linear chain composed of- β - (1-4) - linked by the 2-acetamido-2- deoxy-β-D-glucopyranose monomers [1,2]. Naturally, chitin occurs in the form of α-, β-, and γ- and are usually extractable from the tissue of crustacean's exoskeleton, squid pens, and fungi where it exists as protein-chitin matrix that yield hard shells [3,4]. According to Kjartansson et al. [5], aside the matrix, other contents include

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lipids from the muscle residues and carotenoids, mainly astaxanthin and its esters. In most instances, the crustacean shell consists of 30-40% protein, 30-50% calcium carbonate and calcium phosphate, and 20-30% chitin. These are however functions of the species and season in which the marine animal is harvested. Thus, to obtain chitin of high purity, thorough removal of the associated constituents must be carried out. At this instance, chitin may be obtained as cellulose with the OH^- at position C-2 replaced by an acetamido group thereby enabling it serves as a structural polysaccharide [6].

In contrast to chitin, chitosan a cationic polysaccharide usually is obtained by the process of chitin deacetylation. It exhibits linear chain consisting of β-(1, 4)-linked 2-acetamino-2-deoxy-β-Dglucopyranose and 2-amino-2- deoxy-β- D-glucopyranose. Chitosan is insoluble in water, organic solvents and aqueous bases; however, after stirring, it often becomes soluble in acids like nitric, perchloric, phosphoric, hydrochloric, formic, acetic and citric. The major difference between chitin and chitosan is the acetyl group content of the biopolymer. Hence, the degree of deacetylation (DD) is usually employed to differentiate chitosan from chitin. According to Muslih et al. [7], chitosan is obtained when the DD> 50%. The intrinsically positively charged chitosan often naturally chemically binds negatively charged fats like lipids and bile acids. This has made chitosan very useful in applications such as drug delivery, tissue engineering, food preservation, biocatalyst immobilization, waste water treatment, molecular imprinting, suture thread, burn and wound dressing.

The three common extraction method of chitin include fermentation, enzymes and chemical actions [8]. While the extraction by fermentation is very expensive, enzymatic extraction does not denature the chitin. According to Jung et al. [9], harsh acid treatments may cause chitin to hydrolyse, giving rise to inconsistent physical properties and the resulting solution becoming source of pollution to the environment. Furthermore, the quality of chitin produced is usually a major concern. According to Kishore et al. [10] chitin quality is a function of the molecular mass (average and polydispersity) and the degree of acetylation.

The main aim of this work is to extract and compare the properties of chitin and chitosan extracted from crab shells of Nigerian origin with a view of determining their quality and potential area of application.

2. Materials and Method

Waste crab shells were collected from a dump site in Mushin area of Lagos state, Nigeria. The shells were scraped free of loose tissue, washed with water and dried under the sun at an average daily temperature of 35°Cfor 5 days. The dried shells were ball milled and sieved to 300 μm using mechanical sieve vibrator. Demineralization was carried out at room temperature (32°C), using dilute hydrochloric acid solution. The solution was prepared by dissolving 200ml of HCl in $2dm^3$ of distilled water to produce1.2M HCl. Then, 1.34kg crab shell powder was soaked in 1 liter of the 1.2M HCl solution. A brown-like coloured solid solution was obtained, tested using a litmus paper and found to be acidic; the pH being 5. This washing process was continued with distilled water until the pH read 7. The demineralised sample was filtered and dried in the oven at 70°Cfor 5 h to constant weight of 490g. Deproteinization was also carried out on the sample by heating in a beaker of 1M sodium hydroxide (NaOH) solution prepared by dissolving 40g of NaOH in $1dm^3$ of distilled water at 100°Cfor 1h. This treatment was repeated several times until a colourless solution was obtained indicating the absence of protein. The sample was further treated for effective protein removal by soaking in fresh set of alkali solution for 21 h then washed with distilled water until it became neutral (pH 7), after which the samples were filtered, and oven dried at 70°C

Depigmentization was carried out by soaking the extracted chitin in 1M of Hydrogen peroxide (H_2O_2), for 32 h at 26°C room temperature, filtered and dried to a constant weight of 258g.

In order to produce chitosan, deacetylation of chitin obtained was carried out by adding 26% NaOH solution and boiled in water bath at 95°C for 7 h. The sample was then soaked with fresh set of alkali for 35 h, washed with distilled water until neutral, filtered and oven dry at 70°C to a constant weight of 245g.

2.1 Chitin yield calculation

The quantity of chitin obtained from processed crab shells is calculated thus:

$$Yield = \frac{Mass\ of\ extracted\ chitin}{Mass\ of\ crab\ shell\ powder} x\ 100 \quad (1)$$

$$Yield = \frac{258}{1341} x\ 100 = 19.2$$

The chitin yield from crab powder is 19.2%

2.1.1 Chitosan yield calculation

$$Yield = \frac{245}{1341} x\ 100 = 18.3\%$$

2.2 FTIR Spectroscopy

Fourier Transform Infrared (FTIR) spectrometer, model A Nicolet 6700 M at Redeemers University, Nigeria was used in carrying out FTIR analysis of samples. Ten milligrams of fine samples were dispersed in a matrix of KBr (500 mg), followed by compression at 22–30 MPa to form pellets. The transmittance measurements were carried out in the range of 500–4000 cm^{-1} at a resolution of 4 cm^{-1}.

2.3 XRD analysis

The x-ray diffractometry measurements were performed on an EMPYREAN XRD-6000 diffractometer using Cu Ka radiation (l=1.540598nm, Ni-filter) at 40 kV, 30 mA. The samples without preferred orientations were scanned in steps of 0.026261 in the 2Theta range 4.99 to 75 using a count time of 29.7s per step. The crystallinity index (Crl) for crab powder, chitin and chitosan was calculated using equation (2) [11].

$$Crl(\%) = \frac{lc}{lc+la} x\ 100 \quad (2)$$

Where lc and la represent the intensities of the crystalline and the amorphous region respectively. Crystalline size normal to hkl plane (D_{hkl}) was calculated from the full width at half height of the source curve using equation (3) [12].

$$Dhkl = \frac{k\lambda}{\beta \cos\theta} \quad (3)$$

Where k is a constant (indicative of crystallite perfection and is assumed to be 1); λ (Å) is the wave length of incident radiation (1.5406 Å); β (rad) is the width of the crystalline peak at half height and θ (deg) is the diffraction angle corresponding to the crystalline peak.

2.4 DTA analysis

The Diffraction Thermal Analysis measurements were carried out on DTA analyser model; NETZSCH DTA 404 PC at the Centre for Energy Research and Development, Obafemi Awolowo University Ile-Ife, Nigeria. Sample of mass 5mg was combusted in DTA/TG crucible Al_2O_3, within a temperature range of 0-1000^oC.

2.5 Scanning Electron Microscopy *(SEM)*

The samples micrographs were produced via a scanning electron microscopy model; Phenom Eindhoven, Netherlands. It works with an electron intensity beam of 15 kV, while the samples to be observed were usually mounted on a conductive carbon imprint left by the adhesive tape. This is usually prepared by placing the samples on the circular holder and coated for 5 min to enable it conduct electricity.

3. Results and discussion

3.1 Degree of Acetylation (DA) Measurement

The DA was calculated using Equation 4 [13]:

$$DA = [(A1660/A3450)X100]/1.33 \quad (4)$$

Where A1650 is the absorbance of amide I vibration; A3450 is the absorbance of OH vibration; 1.33 is a factor that represents the ratio of A1650/A3450 for fully N-acetylated chitin. The computation yields 66% as chitin degree of acetylation.

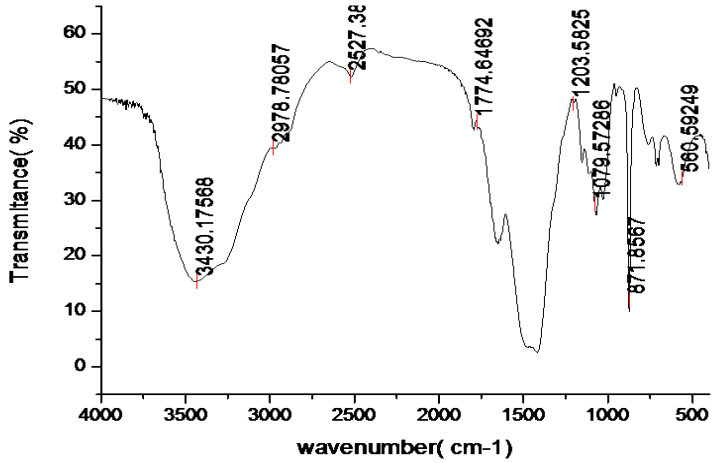


Fig. 1 FTIR spectrum of Crab powder

Fig. 1 displayed different functional groups exhibited by crab shell powder sequel to FTIR analysis results presented in Table 1. A characteristic broad peak was detected in the region of 3500cm-1 and 3200 cm-1, which could be attributed to O-H of the water molecule and N-H bending. The peak at 2891cm-1 is assigned to -CH stretching of the aliphatic compound, while the peak at 1653 cm-1 is the secondary amide stretch (amide I band) C=O. A small absorption band occurring at 2521cm-1 represents carboxylic acid. As reported by Musarrat et al., [14], the bands around 1798, 1420-1430 and 876 cm-1 represent bending and stretching of $CaCO_3$. The presence of absorption band at 141 cm-1 confirms the presence of -CH_3 bending and -CH_2 deformation.

Table 1 FTIR vibration modes of crab shell powder samples

Vibration modes	α-chitin standard	Crab chitin (cm-1)	Chitosan (cm-1)	Crab shell (cm-1)
OH, out of plane bending	690	700	696	
NH out of plane Bending	752	748	750	759
Ring stretching	896	895	895	
CH_3 wagging along chain	952	952	950	950
CO stretching	1026	1028	1026	1030
C-O-C	1073	1074	1074	1068
Asymmetric in phase ring stretching mode	1116	1116	1116	1112
CH_2 bending and CH_3 deformation	1418	1417	1417	1417
Amide II band (NH) stretching	1563	1558	1558	
Amide I (C=O) secondary amide stretching	1661	1660 and 1626	1662 and 1624	1653
CH stretching	2878	2891	2893	2893
Symmetric CH_3 stretching and asymmetric CH_2 stretching	2930	2960	2931	2970
NH stretching	3268	3279	3267	3275
OH stretching	3439	3460	3460	3446
CH_2 wagging amide III		1315	1315	
CH_2 bending and CH_3 symmetric deformation	1379	1379	1379	
$CaCO_3$				873
Asymmetric bridge oxygen stretching		1157	1157	1153

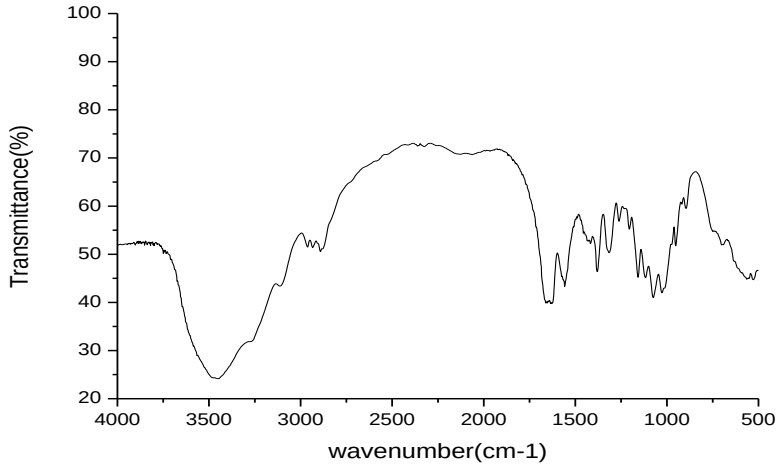


Fig. 2 FTIR of chitin from crab shell

As shown in Fig. 2, the FTIR spectrums of chemically extracted chitin have narrow absorption bands, typical of crystalline polysaccharide [15]. The chitin spectrum peaks at 3460 cm-1and 3268 cm-1, which is attributed to the -OH of the aliphatic compound and -NH stretching respectively. The band at 2930 cm-1 represents symmetric -CH_3 stretching and asymmetric stretching of -CH_2.Stretching vibration of amide I, II and III peaks at 1660, 1558 and 1315 respectively. As reported by Abdou et al.,[16], the presence of two amides I (C=O) band around 1660 and 1626, confirms that it is alpha (α-) chitin. Amide I band is known to be responsible for the splitting of wave numbers between 1600 to 1500cm-1 corresponding to the inter-sheet hydrogen bonding. This is due to the hydroxyl methyl group that can be linked to the band peak at 1630 cm-1 and the intra-sheet hydrogen bonding at the C=O stretching region with wave number 1660 cm^{-1}.

This inter- and intra-sheet hydrogen bonding is characteristic of chitin, which gives chitin its highly insoluble property [17]. The vibration of Amide III was observed at peak 1315 cm-1 due to the presence of protein content in the chitin complexes (Table 1). The spectrum also features peaks at, 1157, 1116, 1074, and 1028 cm-1, relating to the asymmetric bridge oxygen in C-O-C.

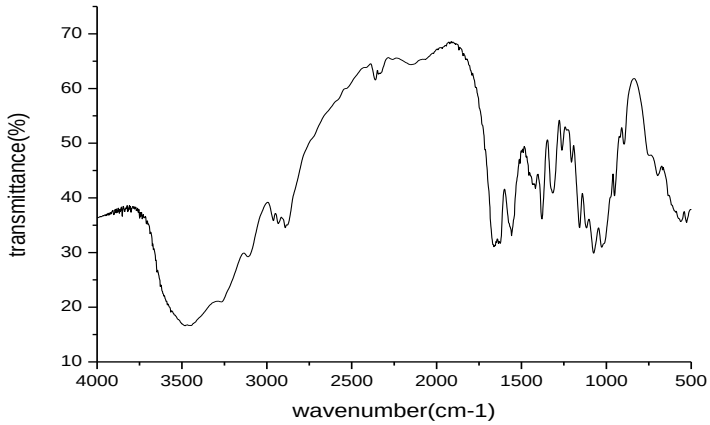


Fig. 3 FTIR of chitosan from crab shell

The absorption bands of chitosan obtained from crab shell (Fig. 3) were relatively similar to those of chitin except the disappearance of band 2532cm-1, which could be attributed to deacetylation of -$NHCOCH_3$ group. The band at 1558 cm-1 has a larger intensity than at 1662 cm-1, which suggests effective deacetylation. As reported by Suneta and Pradip [18], when chitin deacetylation occurs, the band at 1656 cm-1 decrease paving the way for a growth at 1597cm-1 indicating the prevalence of -NH_2 group. The absorption bands at 3460, 3267,3111,2931,1662,1628,1558,1447,750 and 696cm-1compared well with the standard chitosan spectral.

3.2 Morphological characteristics of chitin and chitosan

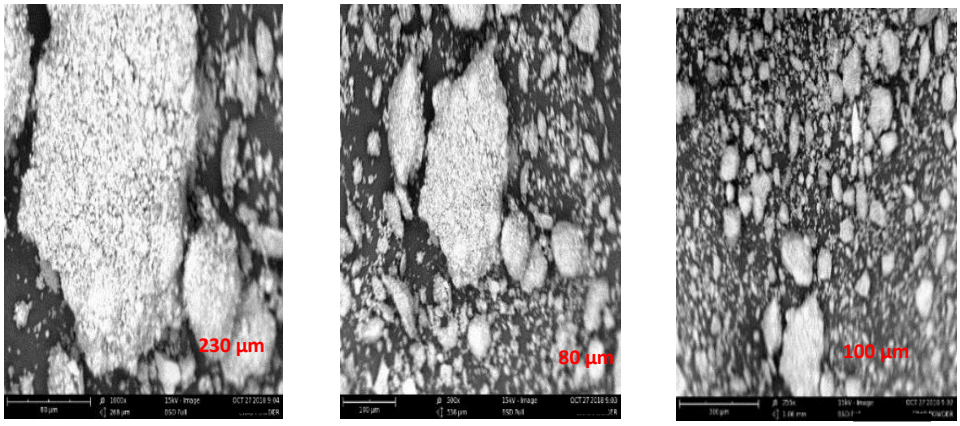
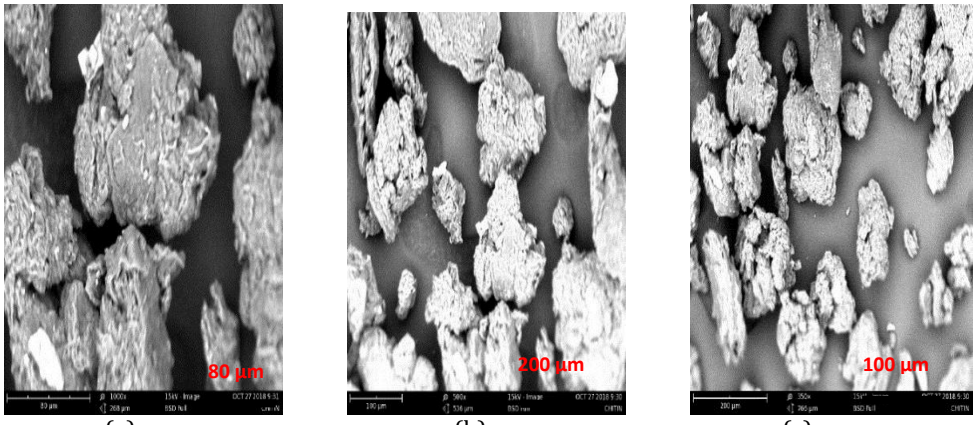


Plate 1 SEM micrographs of crab shell powder at varied magnifications

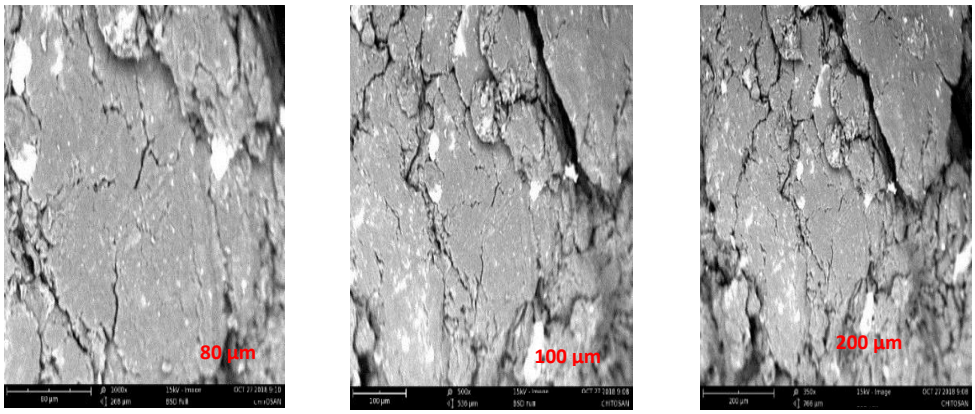
The SEM analysis results of crab shell powder are presented in Plate 1(a – c). The micrographs showed whitish and crystalline particles dispersed within the dark matrix. These crystal features could be attributed to the presence of calcium carbonate and other trace elements. However, after exposure to acid and alkali treatments, there were noticeable changes in the crystal morphologies.



(a) (b) (c)

Plate 2 SEM micrographs of chitin at varied magnifications showing globular fibrils

Plate 2a-c showed globular fibrils of chitin.



(a) (b) (c)

Plate 3 SEM micrographs of chitosan at varied magnifications showing thin and long thread-like fibrils

Chitosan SEM micrographs displayed thin and thread-like fibrils (Plate 3a-c). These microstructural features suggest that both chin and chitosan could be electro-spun into fibres that could be suitable for suture and wound dressing.

3.3 XRD Characterisation of extracted chitin and chitosan samples

The XRD of crab shell in Fig. 4 reveals the existence of crystalline form of $CaCO_3$ and chitin. The calcite peak is found to be at 2θ= 23.5,44,48, 49, while the remaining peaks represent chitin. Fig. 5 shows the full XRD spectrum of chitin having one strong peak at 19.3, corresponding to plane 110, with two other weak peaks at 12.7, and 26.4. Chitosan XRD spectrum shows a strong peak at 20.0 with two weak peaks 10 and 27 (Fig. 6). The crystallinity of crab shell, chitin and chitosan are compared in Table 2.

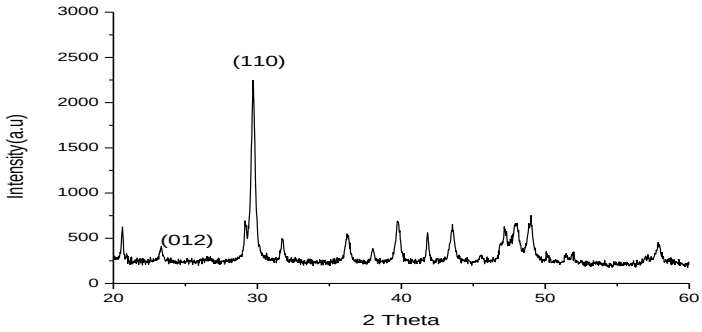


Fig. 4 XRD of crab shell

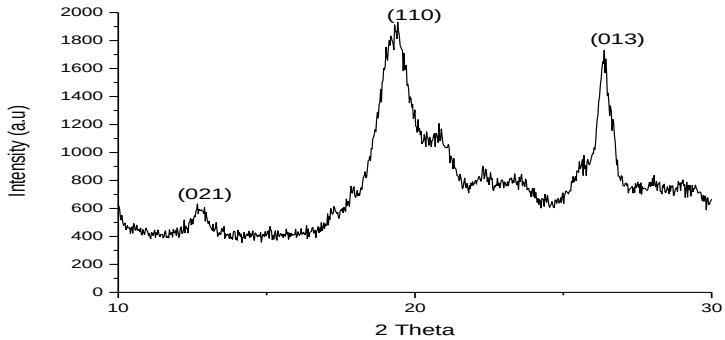


Fig. 5 XRD of chitin from crab shell

Table 2 Crystalline index and size of crab shell powder, chitin and chitosan

Sample	Crl (%)	D_{110}(Å)	Title
Crab powder	77	0.0830	100
Chitin from crab shell	80	0.0159	Chitin from crab shell
Chitosan from crab shell	79	0.0141	Chitosan from crab shell

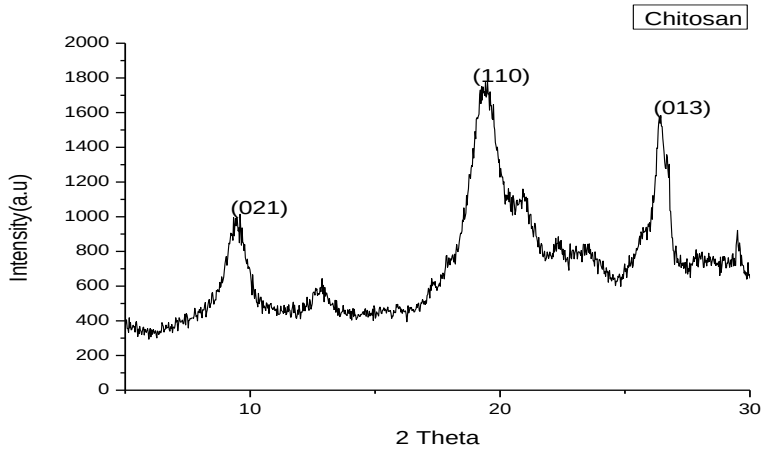


Fig. 6 XRD of chitosan

3.4 Thermal behaviour of extracted chitin and chitosan samples

Fig. 7 shows the DTA plot of crab shell displaying two major peaks at 705.3°Cand 870°C respectively for the beginning and completion of decomposition. These peaks correspond to 21.78mw/mg and 4.544 mw/mg weight losses respectively. The DTA of chitin shown in Fig. 8 suffered initial weight loss of -1.722 mw/mg at 235.6^{0}C attributed to vaporization of water from the sample. At this instance, chitin degradation starts from 388°Cresulting in weight loss of 12.23 mw/mg. This could be due to the degradation of saccharide molecule. However, decomposition continues at 484°Cand was completed at 912.6°Cwith a weight loss of 13.6 mw/mg. The DTA plot of chitosan is presented in Fig. 9 showing initial weight

loss of -1.722mw/mg at 135℃ Degradation actually started at 342℃and completed at 912.6℃resulting in weight loss of 12.23mw/mg.

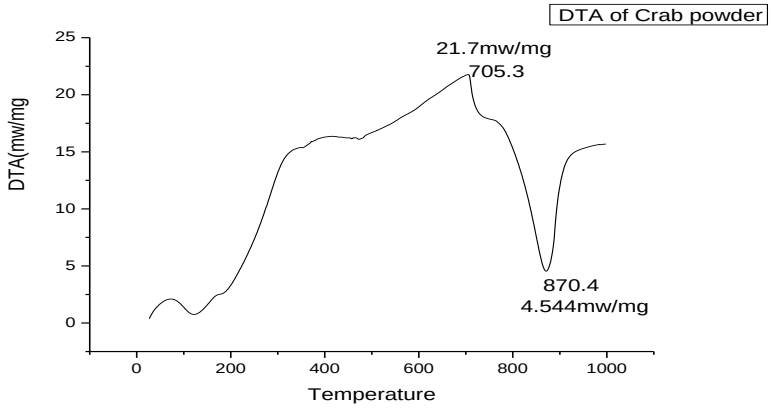


Fig. 7 DTA of crab shell powder

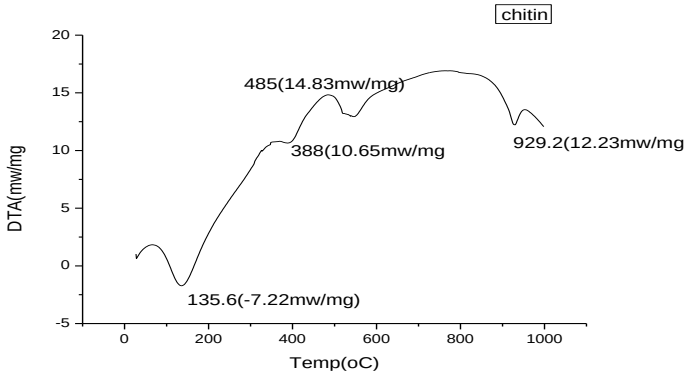


Fig. 8 DTA of chitin

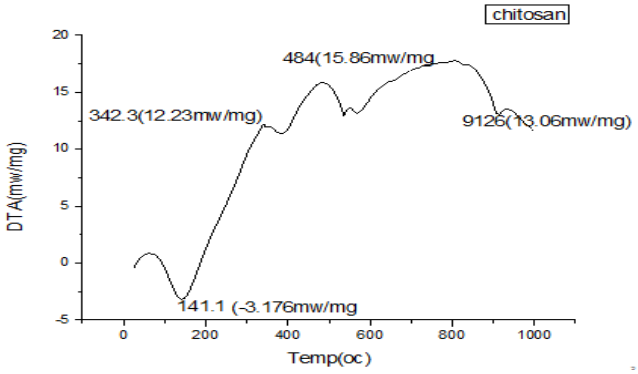


Fig. 9 DTA of chitosan

4. Conclusion

Chitin and chitosan have been successfully extracted crab shell, characterized and comparisons of their properties were also made. The yield of chitin from crab shell was found to be 19.2%, while that of chitosan is 18.3%. The crystalline index and crystal size of chitosan was 79% and 0.0140Å respectively which is lower, when compare to that of chitin that has crystallinity index of 80% and crystal size of 0.0159Å. This may be attributed to deacetylation of the acetyl group with higher concentration of NaOH and increase in the duration of treatment time. The Degradation temperatures of chitin and chitosan were observed to be 388℃ and 342℃ respectively, which is quite higher than the melting temperature of polymers such as PLA which has its melting temperatures between 159℃ and 178℃ Thus, it is concluded that the biopolymer could be incorporated into polymer matrix for melt extrusion without degrading the property. Furthermore, chitosan can be incorporated into the matrix of composites used in suture production.

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