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Preparation of Chitosan Nanoparticles from Crab Shell and Their Characterization

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Abstract

Preparation and characterization of Chitosan nanoparticles (CHNP) is presented with the aim of determining the particle size and morphology, surface chemistry, thermal stability through a simple and cost effective process of production preparation. CHNP were prepared through drop wise addition of sodium tripolyphosphate solution to chitosan solutions in the ratio of 1: 1 under stirring. Chitosan gave a yield of 61% from the crab shell and a degree of deacetylation (DOD) of 75%. The low DOD could be responsible for the high yield of chitosan which is due to the incompletely removed acetyl groups in chitin. The final weight of the chitosan was observed to have increased after the conversion to nanoparticles, and this could be as a result of the crosslinking of the chitosan and the TPP. The nanoparticle size was determined with UV-Visible spectrometer showing a characteristics maximum adsorption of 0.056 at 230 nm and the XRD characterization showed two characteristics peaks at 2θ = 16° and 23°. The ionotropic gelation method used in this study was an effective and simple method in achieving reproducible nanoparticles with the desired safety and physicochemical characteristics. Due to their nano particle size, the produced CHNP could find application in pharmaceutical industries for drug delivery and repair of tissue. Also, in wastewater treatment, CHNP could find application because of its tendency to adsorb toxic dyes and metals as well as a filler for non-degradable polymers.

Keywords

Chitosan, Nanoparticles, Nanotechnology, Deacetylation, Ionic Gelation, Pharmaceutical

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1. Introduction

Nanotechnology is the emerging science concerned with nanometer scale and nanoparticles are one of the building blocks in nanotechnology. In recent time, polymers and nanotechnology have been of interest in several areas such as therapeutic innovation and pharmaceutical industry among others. Nanoparticles refers to solid colloidal particles in the nanometer range of 10 to 1000 nm. Due to their nano particle sizes, they are known to have unique physical and chemical properties as well as their large surface areas. The preparation of nanoparticles be done both from synthetic polymer like polyethylene or natural polymers like polysaccharide, protein. The preparation of nanoparticles from synthetic polymers comes with some disadvantages because they can only be achieved through heat, high shear force or organic solvent which affect the stability of drug. But the preparation of nanoparticles from natural polymers are achieved through simple and mild processes which doesn't involve the use of high shear force and organic solvent.

Over the last few years, Chitosan nanoparticles (CHNP) have gained considerable attention due to their inherent biological properties. Chitosan is obtained from Chitin (Poly β-(1-4) -N-acetyl-D-glucosamine) found in the structures of molluscs, crustaceans, insect outer shells, squid and cartilage [2]. This natural polymer is also seen in the cell walls of mushrooms and fungi [3-4]. Crab (*Callinectes amnicola*) a crustacean

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found in in riverine and mud areas in West African countries [5-6]. Though nutrients and minerals can be sourced from crabs for humans, but the shells have no basic use they are rather disposed to the environment as pollutant. However, processing these shells into biopolymers like chitin, chitosan, as well as nano-derivatives will not only result in available raw materials for biopolymer but also reduce environmental pollution.

Chitosan and its derivatives are biological based materials having excellent bioactivities as well as unique chemical and physical properties. They find application in more than 200 industrial products [7], such as in membranes as tissue engineering materials and for dialysis, in textile industries [8]. In the pharmaceutical industries, CHNP are essential for controlled release of drugs, and this improves efficacy and stability of drugs. Due to their very small size, they can easily pass through deliver drugs and biological barriers into the tissues targeted in a system [9]. They also used for treatment of waste water to remove toxic metals and dyes [10].

Recent works done on CHNP has been on expanding the understanding of its properties as well as methods of modifying its physical or chemical properties, which are mostly applied in optimizing nanoparticles drug delivery as well as release features. Hence this current research is aim at providing a possible cheaper source of synthesizing this biopolymer of interest from naturally readily available crab shells. This paper will also investigate the degree of deacetylation, morphology, thermal property, crystallinity, so as to further provide a clearer understanding of the properties of chitosan and its nanoparticles.

2. Material and Method

2.1. Chemicals and Materials

Sodium hydroxide (NaOH), hydrochloric acid (HCL), sodium tripolyphosphate (TPP), acetic acid and other chemicals used as well as all apparatus and materials used such as beaker, conical flask, pipette are of analytical grade purchased from Sigma-Aldrich. Crab shells were sourced from (Ikotun market in Lagos, Nigeria) and materials such as retort stand, sieve were. The crab shell was washed with distilled water so that unwanted materials and dirt could be removed, sun-dried for 48hrs, pulverized into fine powder using mechanical grinder.

2.2. Extraction of Chitin

Chitin was obtained out of the powdered crab shell through demineralization, and deproteination process. Demineralization of chitin was done by the addition of 2 g of the powdered shell to 20 mL 0.7 M of HCl followed by heating the mixture at 65°C for three hours in a bath. Then the pH of the resulting mixture was adjusted by washing with distilled water and then dried to constant weight 65°C. Then the deproteination was carried out by treating the resulting product with 1.2 M of NaOH followed by heating at 65°C for 30 min. The product that resulted was washed thoroughly with distilled water to a neutrality and dried to constant weight at 60°C in an oven to produce chitin.

2.3. Chitin Deacetylation

The chitin was deacetylated by treatment it with NaOH (50%) and then heating the mixture for 3 hours at 100 in a bath. Then the suspension was then filtered and the cake was thoroughly washed with distilled water to a neutral pH before been dried at 65°C in an oven to produce Chitosan (CS). To determine the degree of deacetylation (DOD), Equation 1 was employed foolowing a method described by Domsy and Roberts [11].

$$\text{DOD}\ (\%) = 100 - \frac{A1653}{A3448} \times \frac{100}{1.33} \quad (1)$$

Where A_{1655} and A_{3450} represent amide absorbance (1653 cm^{-1}) and hydroxyl absorbance at (3448 cm^{-1}) respectively.

2.4. Ionic Gelation of Chitosan

CHNP was then synthesized from the Chitin using ionic gelation method [12]. To prepare a solution of chitosan, 32 g Chitosan powder was dissolved in 2 L of 2% acetic acid then stirred for 24h at 60°C; and TPP solution was prepared by dissolving of 73.4 g of sodium Tri-poly-phosphate (TPP) in 2 L distilled water. Chitosan solution was then added to sodium TPP solution in drops to give a final ratio of 1: 1 v/v (CS: TPP). Then the CHNP were filtered and washed severally with distilled water, which was made to stand for a day before been filtered with a sintered glass. The samples when were the taken for analysis.

2.5. Characterization of CHNP

2.5.1. Morphological

The size and morphology of CHNP were studied in Quanta (FEI) 400 ESEM/EDAX A little amount of CHNP which has been vacuum dried were kept on the stub of scanning electron microscopy (SEM) with the use of a double-sided adhesive tape through a sputter at 50 mA for a period of 6 min. Then the stub holding the sample was put into the SEM Chamber. Afterwards the photomicrograph of the sample was done at acceleration voltage of 20 KV.

2.5.2. Fourier Transform-infrared (FTIR) Spectroscopy

The FTIR analysis of chitosan and CHNP were carried out with the aid of A2-technologies portable attenuated total

reflectance FTIR spectroscopy (ATRS-FTIR, UK). The spectra of the sample were taken in the range of 4000 cm^{-1} to 400 cm^{-1} (middle infrared) with a 4 cm resolution at room temperature in the mode for absorbance for 10 scans [13]. FTIR spectra of CHNP were gotten by placing 1 mg of the sample on the sensor and the spectrum was then compared with the spectrum of chitosan and TPP standard.

2.5.3. UV-Visible Spectrometry of CHNP

In order to verify the formation of nanoparticles the solution was scanned in 200 – 600 nm range in a spectrophotometer (752 N) using a quartz cuvette with diluted acetic acid as the reference [1]

Thermal Properties

The thermal degradation properties of CHNP was done with the aid of a thermo gravimetric analyzer TGA-Q500 (TA Instruments, New Castle, USA). The temperature was varied from 0 – 900°C in this experiment and the rate of heating was set at 10°C min^{-1}. A 60 cm^3 min^{-1} flow rate was set for the flow of Nitrogen from the beginning to the end of the experiment.

2.5.4. Determination of Crystallinity

Wide-angle X-ray diffraction (XRD) of CHNP was obtained using a LabX XRD-6000 Shimadzu X-ray diffractometer having a high speed and high precision vertical goniometer. The diffraction spectra were analyzed over a 2θ range, using (λ = 0.154 nm) CuKα Cu Kα radiation, with a rate = 20 /min with a sampling pitch of 0.05°, diffraction angle from 10 and 80. The d-spacing was calculated with the Bragg's equation, λ = 2d sin θ.

2.6. Data Analysis

All experiments were done in triplicate and analyzed with Analysis of Variance (ANOVA). and Duncan's Multiple Range Test (DMRT).

3. Results and Discussions

3.1. Yield of Chitosan from Crab Shells

Chitosan gave a yield of 61% from the crab shell and a degree of deacetylation (DOD) of 75% was btained.

According to Deleanu *et al.* [14], practical grade chitosan flakes obtained from crab shells have a minimum DOD of 85%. Sadeghi *et al.* [15] also reported a 98% DD of chitosan procured from Primex, Iceland. The low DOD in this study compared to those from other research works must have contributed to the high 61% chitosan yield which is due to the incompletely removed acetyl groups in chitin. The final weight of the chitosan was observed to have increased after the conversion to nanoparticles, and this could be as a result of the crosslinking of the chitosan and the TPP.

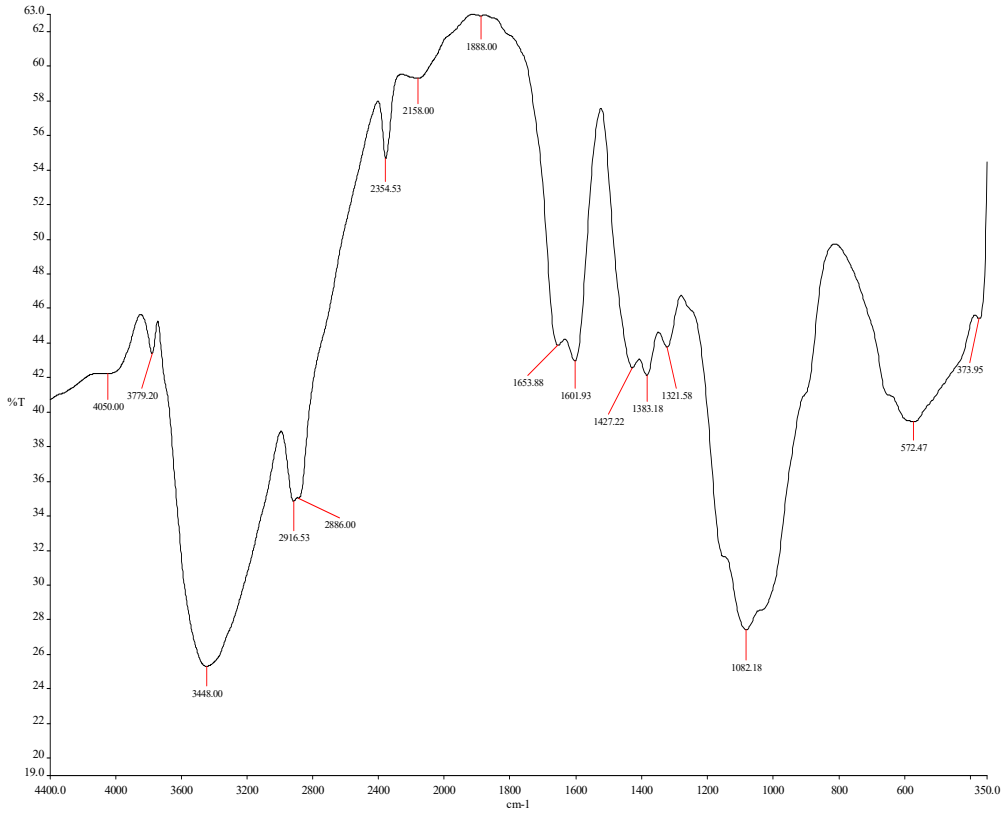

Figure 1. FTIR spectrum of chitosan.

3.2. Characterization of CHNP

3.2.1. FTIR Analysis of Chitosan and CHNP

The FTIR spectrum of chitosan is shown in Figure 1. The peaks of the spectrum are as follows (Table 1): 1653.88, 3448.00, 1082.18, 1383.18, 1653.88 and 1383.18 cm^{-1} respectively representing the N-H bending vibrations of primary and secondary amine; O-H stretching of phenolic and alcoholic groups; C-N stretching; C-H bending in a ring; C-O stretching of carboxylic acids and alcohols and C-C stretching (in ring) respectively. These were the expected functional groups as they represent the major components of chitosan. The FTIR spectrum of CHNP is presented in Figure 2. The peaks of the spectrum are as follows (Table 2): 3470.00, 1635.00, 1383.16, 1635.00, 1383.18 and 889.00 cm-1 respectively representing the N-H bending vibrations of primary and secondary amine; O-H stretching of phenolic and alcoholic groups; C-H bending in a ring; C-O stretching of carboxylic acids and alcohols; and C-C stretching (in ring) and PO_4^{-2} group respectively. The PO_4^{-2} group observed in the spectrum of the CHNP was also observed in the findings reported by [1]. Hence the peak seen at 889 representing PO_4^{2-} group is marked difference seen in the spectrum of CHNP from that of chitosan which gives an indication along other characterization done to confirm the formation of CHNP.

Table 1. FTIR of chitosan.

Functional group	Wavelenght (cm^{-1})
OH stretch	3448.00
C-H bend	1383.18
N-H bend	1653.88
C-C stretch (in ring)	1383.18
C-O stretch (alcohol)	1653.88
C-N stretch	1082.88

Table 2. FTIR of CHNP.

Functional group	Wavelenght (cm^{-1})
OH stretch	3470.00
C-H bend	1388.16
N-H bend	1635.00
C-C stretch (in ring)	1388.16
C-O stretch (alcohol)	1635.00
PO_4^{2-}	889.00

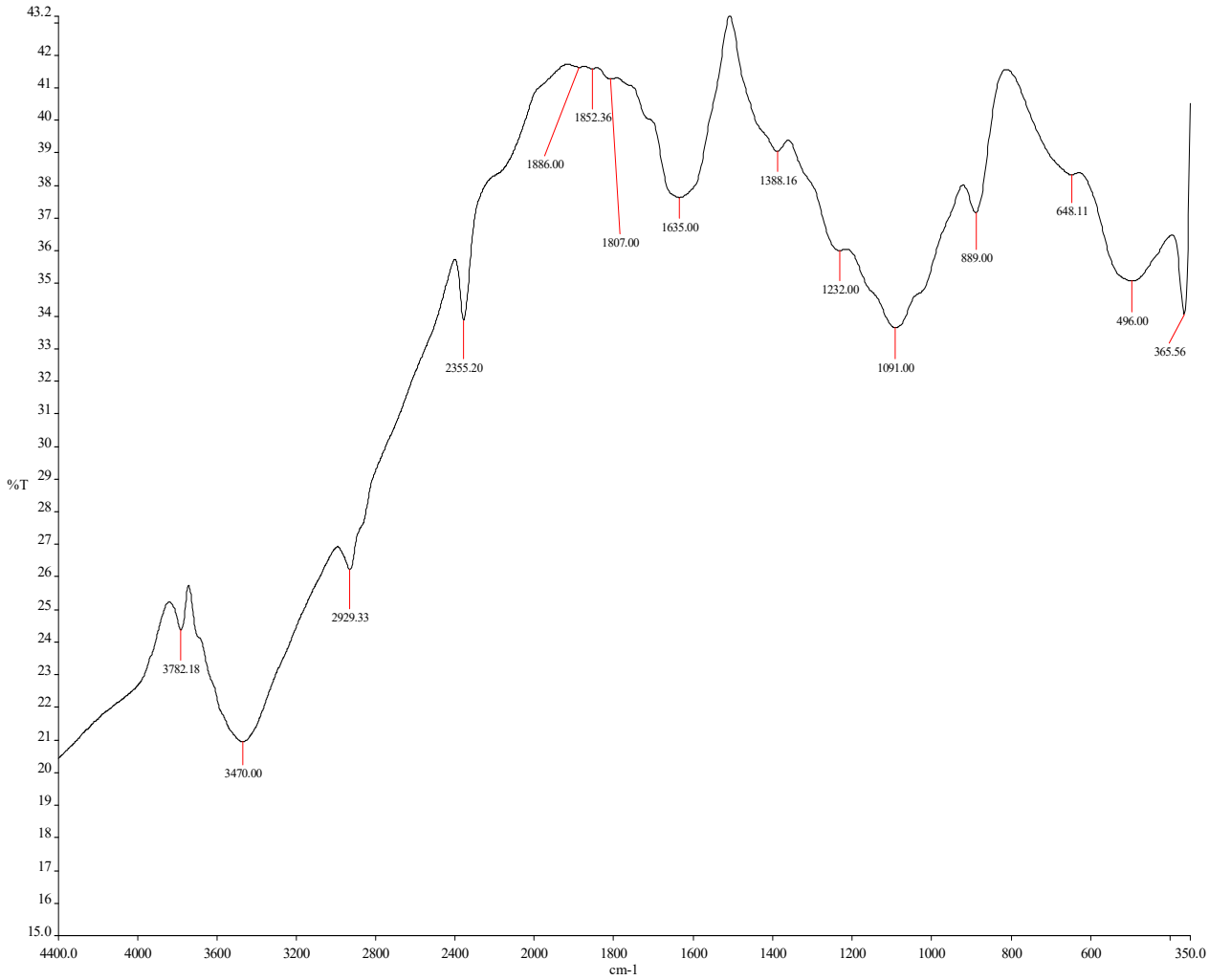

Figure 2. FTIR spectrum of CHNP.

3.2.2. UV-Visible Spectrometry of CHNP

The Characterization of prepared CHNP by U. V. spectrophotometer showed the peak at 230 nm (Figure 3). This could be as a result of the amido group present in chitosan [1]. Krishnaveni and Priya [16] observed a peak at 310 nm for CHNP in their research work on the green synthesis of silver nanoparticles obtained from *Catharanthus roseus*, *Calotropis gigantea,* Chitosan and Chitin. Similarly, a peak was observed at 201 by Liu *et al*. [17] where they prepared and characterize nanoparticles from trypsin based hydrophobically modified chitosan. [1] also saw a peak for CHNP at 223 nm in their work on preparation of CHNP and their in-vitro characterization. The result of the UV-Visible spectrophotometer in this study is in agreement with literature that nanoparticles were formed, as the peak was observed within 200 nm to 400 nm. This observation from the UV-Visible spectrophotometer further corroborate the result of the FTIR spectrum of the CHNP prepared.

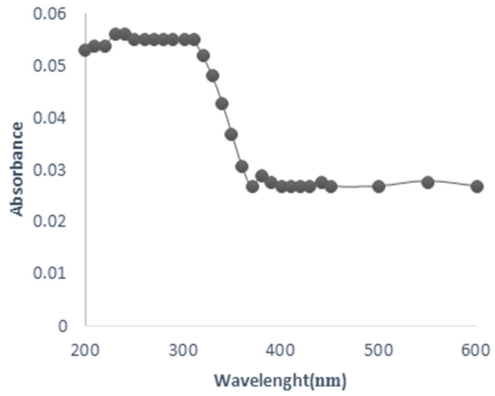

Figure 3. UV-Visible Absorption spectra of CHNP.

3.2.3. Morphology of CHNP

The surface morphology of CHNP was studied using Scanning electron micro-graphs (SEM). SEM micrographs of CHNP is shown in Figure 4. It was observed that CHNP aggregated to form a solid lump, having a coarse surface. This coarseness of the surface is dependent on the DOD [18]. The DOD of chitosan in this study was seen to be 75% which is due to the incompletely removed acetyl groups present in the chitin and is known to have strongly influenced the coarse nature of the CHNP as seen in the SEM micrograph. The particles shapes were observed to be broad and spherical. Anand *et al.* [19] reported similar shapes and micrographs were reported by where CHNP were synthesized from the shell of shrimps. Olajide *et al*., [5] reported similar shapes and micrographs in his work where crab shell was used to synthesize CHNP, and similar observation was given by Maram *et al.,* [12]. The spherical polymer particles customarily make a large amount of active sites available.

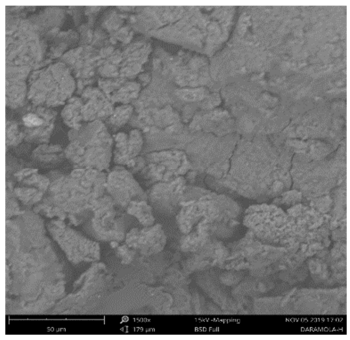

Figure 4. SEM analysis of CHNP.

3.2.4. Thermogravimetric Analysis of CHNP

The result of the thermogravimetric analysis of CHNP is shown in Figure 5. The curve showed a downward trend as the temperature was raised from 100 to 900°C, which indicate loss of weight. At 100°C, the weight of the sample reduced to 90%, and this could be associated with loss of water content with temperature increase. As the temperature was futher increased to 300°C, a further loss in weight was observed. Remarkably, a drastic weight loss was observed between 300°C to 650°C. CHNP was observed to lost about 55% of its total mass at the temperature of 650°C. A possible reason for the observed drastic weight loss might probably be from the depolymerisation, loss of CH_2OH and NH_2 moieties. Similar observation was reported in the findings of Olajide *et al*. [5] in their work with observed weight loss with temperature reduction and this weight loss was as well said to be possibly associated with the depolymerisation, loss of CH_2OH and NH_2 moieties. The final stage of the weight loss was observed between 650°C to 900°C, where about 5% of the weight was lost, thereby bringing the total weight loss to 70%. With this observation in the weight loss in CHNP with temperature it suggests its possible application in wastewater treatment at temperatures lesser than 200°C without significant effect on the CHNP structure.

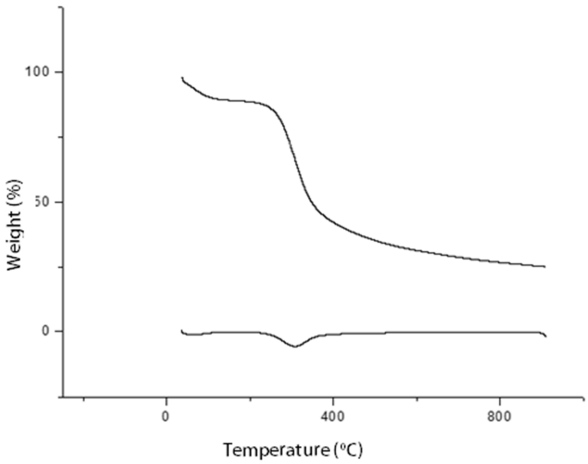

Figure 5. Thermogravimetric analysis of CHNP.

3.2.5. XRD Analysis of Chitosan Nanoparticle

The crystallinity of chitosan is an important parameter which affects the ability of a materials to access water or metal ions to be adsorbed into its internal sites [20]. X-ray diffractogram of the CHNP (as shown in Figure 6) reveals that the first two major peaks appeared at 2θ = 16°C and 26°. This shows a shift in the normal chitosan peaks at 2θ = 10° and 20° [12]. However, the spectrum also shows a broad band between 2θ = 55° and 90°, depicting the amorphous regions of the CHNP. This observation is corroborated by the findings by Olajide *et al.* [5] where the XRD of CHNP was reported to give two peaks at 2θ = 17° and 24°, and amorphous region between a broad band between 2θ = 55° and 90°. Therefore, the observations of the XRD analysis of CHNP in this study further corroborate other characterization already done to show that nanoparticles of chitosan have been prepared from chitosan through ionic gelation method.

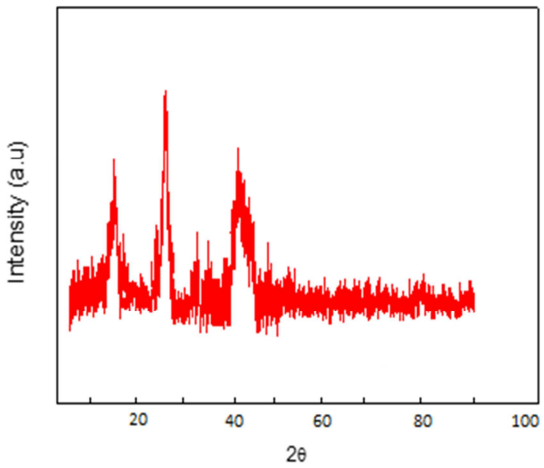

Figure 6. XRD analysis of CHNP.

4. Conclusion

CHNP were prepared from crab shells through ionic gelation method. The prepared CHNP were characterized using scanning electron microscope, UV-Visible spectrometer and X-ray diffraction. Thermogravimetric analysis revealed their thermally stability, and could be used even at elevated temperatures below 200°C. Due to their nanoparticle size, the produced CHNP could find pharmaceutical industries for drug delivery and repair of tissue. Also, in wastewater treatment, CHNP could find application because of its tendency to adsorb toxic dyes and metals as well as a filler for non-degradable polymers. Finally, recent works done on CHNP has been on expanding the understanding of its properties as well as methods of modifying its physical or chemical properties, which are mostly applied in optimizing nanoparticles drug delivery as well as release features. As a possible cheaper source of synthesizing this biopolymer of interest from naturally readily available crab shells, this paper has further given a clearer understanding of the properties of chitosan and its nanoparticles produced from crab shells.

Declarations

Ethics Approval and Consent to Participate

Not applicable

Consent for Publication

Not applicable

Availability of Data and Materials

Not applicable

Conflict of Interest

The authors declare that they have no competing interests

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Authors' Contributions

Oluwatosin Emmanuel Daramola: Investigation, Methodology, Resources, Software, Data curation, Writing-Original draft preparation, Formal analysis.

Oluwaseun Adekoya Adelaja: Conceptualization, Validation, Supervision, Writing-. Reviewing and Editing.

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