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Original Article

Utilization of blue crab shells for the synthesis of chitosan nanoparticles and their characterization

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Abstract

Chitosan, a useful biopolymer, was obtained from pulverized shells of blue crabs via demineralization, deproteination and de-acetylation. The chitosan was converted to nanoparticles using sodium tripolyphosphate (TPP). The FTIR spectrum of chitosan nanoparticles showed various functional groups, such as hydroxyl, carbonyl, and amine among others, as expected in chitosan. The SEM analysis revealed clusters of crystals at the surfaces, while TEM showed a very smooth and fine surface morphology. The particle size was estimated at < 200nm. The XRD spectrum showed shifting of the 2Θ peaks angles 10 and 20° for normal chitosan to 17 and 25° for the chitosan nanoparticles. The TGA and its derivative (DTGA) showed that the bio-polymer was thermally stable.

Keywords: tripolyphoshate, crystallinity, biomaterials, nanoparticles, spectroscopy, spectrum

1. Introduction

Chitin, also known as poly β-(1-4) -N-acetyl-D-glucosamine, is an important high-molecular-weight natural polysaccharide, which can be used in an eco-friendly manner to produce chitosan (Hsu *et al.*, 2004; Rinaudo, 2006).

Chitin constitutes a large fraction in structures of crustaceans, molluscs, squid cartilage, and insect outer shells (Fernandez-Kim, 2004). The cell walls of mushrooms and other fungi also consist of this natural polymer (Shahidi & Abuzaytoun, 2005; Tharanathan & Kittur, 2003).

The conversion yield of chitin to chitosan depends on the method used for its extraction and the degree of deacetylation (Martino *et al.*, 2005).

Chitosan is heterogeneous, cationic, and hydrophobic by its nature, and is influenced by pH (Guibal, 2004). Deacetylation of chitin using enzymatic or alkaline approach breaks some of the N-acetyl bridges (Figure 1) and forms D-glucosamine units, which possess free amine groups that invariably aid the solubility of chitosan (Kalut, 2008). It is soluble in lactic acid, formic acid and acetic acid (Guibal, 2004; Okuyama *et al.*, 2000; Varma *et al.*, 2004).

A lot of modifications have been proposed and tested to impact changes to both physical and chemical properties of chitosan (Coelho *et al.*, 2007; Ngah *et al.*, 2002, 2005). Nanorization of chitosan is currently being explored because of the unique properties exhibited by nanomaterials. Chitosan nanoparticles are biobased materials with excellent physical, chemical and bioactive properties. The reduction of chitosan particle size to nanoscale can be achieved by dissolving chitosan in 0.1 M acetic acid, and contacting the resulting solution with sodium tripolyphosphate (TPP) in drop-wise fashion (Maram *et al.*, 2013).

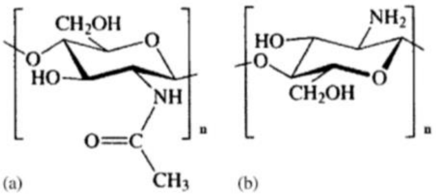

Figure 1. Structures of chitin (a) and chitosan (b)

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Chitosan nanoparticles are being utilised in many industries for their numerous advantages. In the pharmaceutical industries, chitosan nanoparticles are of great importance in the controlled release of drugs, which improves drug stability and efficacy. Their tiny size enables passing through biological barriers to deliver drugs to targeted tissues in the body (Shi & Fan, 2002). Chitosan nanoparticles are also used in membranes for dialysis, and as organ/tissue re-engineering materials (Martino *et al.*, 2005). In the textile industries, chitosan nanoparticles have been employed to strengthen fabrics, and to enhance the washability of textiles. The nanoparticles also impart antimicrobial activity on textile fabrics (Ting & Shen, 2005).

Chitosan nanoparticles find applications in wastewater treatment to remove toxic metals. The biopolymers are also utilized in areas where reinforcement and strengthening of materials are necessary (Rinaudo, 2006). Aranaz *et al.* (2009) reported that chitosan and its derivatives are used in more than 200 industrial applications.

The purpose of this research was to synthesise chitosan nanoparticles from the shells of blue crab (*Callinectes amnicola*) and to assess their properties using various characterization techniques.

Callinectes amnicola (blue crab) is a crab species commonly found in West African countries, especially in Nigeria. The blue crabs reside in mud and in riverine areas (Defelice *et al.*, 2001; Lawal-Are & Kusmiju, 2000). Blue crabs are important sources of minerals and nutrients to humans, but the shells are mostly disposed and to litter the marine environment, hence constituting an environmental nuisance. It is worthy of note, however, that these underutilized shells could be processed into natural biopolymers such as chitin, chitosan, and their nano-derivatives.

2. Experimental

2.1. Materials

Blue crab shells were obtained from a local market (Makoko market) in Lagos, Nigeria. The shells were thoroughly washed with water to remove dirt and other unwanted materials, and were sun-dried for 48 h. All the chemicals used, such as HCl, TPP etc., were of analytical grade, and were purchased from Sigma Aldrich.

2.2. Preparation and deacetylation of chitin

The blue crab shells were pulverized into fine powder. Chitin was extracted from the crab shell powder by demineralisation and deproteination. Demineralization was carried out by adding 1 g of the powder to 10 mL 0.7 M hydrochloric acid and subsequent heating at 65 ºC in a water bath for three hours. The resulting product was then washed with distilled water to pH 7 and dried in an oven at 65 ºC until constant weight. The powder was then deproteinated by treating with 1.2 M NaOH and heating the mixture at 65 ºC for 30 min. The material was washed with distilled water to neutrality, and oven-dried at 60 ºC until constant weight, to obtain chitin. Deacetylation of chitin was done by treating the powder with 50 % NaOH, and the resulting suspension was heated in a water bath at 100 ºC for 3 h. After that, the suspension was filtered and the filter cake washed thoroughly with distilled water to neutral pH and oven-dried at 65 ºC to give powdered chitosan.

The degree of Deacetylation (DD) was calculated according to Domsy and Roberts (1985) as shown in Equation 1:

$$DD\ (\%) = 100 - \frac{A_{1655}}{A_{3450}} \times \frac{100}{1.33} \qquad (1)$$

Here A_{1655} represents amide absorbance at 1655 cm^{-1} and A_{3450} represents hydroxyl absorbance at 3450 cm^{-1}.

2.3 Preparation of chitosan nanoparticles

Although various methods (De Moura *et al.*, 2008; Chattopadhyay & Milind, 2010, etc.) have been used to synthesise chitosan nanoparticle, in this study the chitosan nanoparticles were synthesised according to the method described by Maram *et al.* (2013). Chitosan (16 g) was dissolved in 1 L of 2 % acetic acid by stirring overnight at 60 ºC to give 0.1 M chitosan solution. 36.7 g of sodium tripolyphosphate (TPP) was dissolved in 1 L of distilled water to make an 0.1 M solution. The chitosan solution was added in a dropwise manner to the TPP solution to final ratio 1:1 (CS: TPP v/v). The formed chitosan nanoparticles were filtered and washed several times with distilled water.

The resulting chitosan nanoparticles were then allowed to stand for 24 h and were then filtered through sintered glass of porosity G-3.

2.4 Characterization of chitosan nanoparticles

The prepared chitosan nanoparticles were characterized using scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-Ray diffraction, thermogravimetry (TGA), and FTIR (Shimadzu (8400S) Spectrometry).

3. Results and Discussion

3.1 Yield and degree of deacetylation of chitosan

The yield of chitosan from the crab shell powder was 18.92% . The degree of deacetylation (DD) of the obtained chitosan (Equation 1) was 94.98%. Sadeghi *et al.* (2008) reported 98% deacetylated chitosan, purchased from Primex, Iceland. Deleanu *et al.* (2014) reported that chitosan flakes (practical grade chitosan from crab shells) purchased from Sigma Aldrich Chemie GmbH has at minimum 85% deacetylation.

In this study, the high degree of deacetylation must have contributed to the low 18.92% chitosan yield by removal of acetyl groups present in the chitin. There was an increase in the total weight after the chitosan sample had been converted to nanoparticles, which could stem from crosslinking of chitosan and TPP.

3.2 FTIR analysis of chitosan nanoparticles

The FTIR spectrum of chitosan nanoparticles is presented in Figure 2. The spectrum has the following peaks

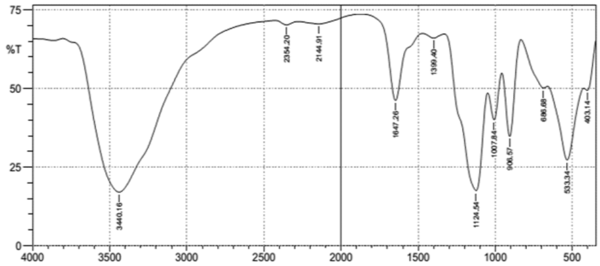


Figure 2. FTIR spectrum of chitosan nanoparticles

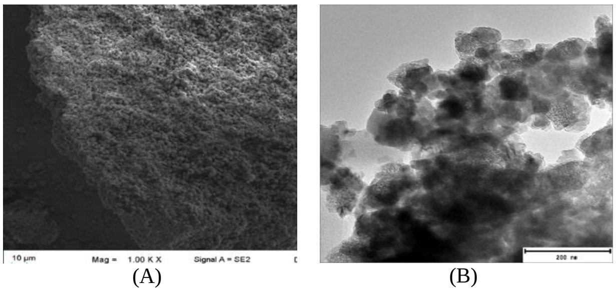
(Table 1): 3440.16, 1647.26, 1399.4, 1124.54, 1399.40, 906.57 and 1007.24 cm^{-1} respectively representing O-H stretching of alcoholic and phenolic groups; N-H bending vibrations; C-H bending in a ring, C-N stretching, C-C stretching (in ring), N-H wagging of primary and secondary amines; and C-O stretching of alcohols and carboxylic acids. These functional groups were expected as they are the major components of chitosan.

Table 1. Functional groups seen in the FTIR spectrum of chitosan nanoparticles

Functional group	Wavelength (cm^{-1})
OH stretch	3440.16
C-H bend	1399.40
N-H bend	1647.26
C-N stretch	1124.54
C-C stretch (in ring)	1399.40
C-O stretch (alcohols,)	1007.24
N-H wag (1 and 2 amines)	906.57

3.3 Morphology of chitosan nanoparticles

Scanning electron and transmission electron micrographs of chitosan nanoparticles are shown in Figure 3 (A and B). The SEM image presents chitosan nanoparticles aggregated to form a solid lump with a coarse surface. The coarse nature of chitosan surface depends on the degree of deacetylation (Abdel-Fattah *et al.,* 2007).



(A) (B)

Figure 3. SEM (A) and TEM (B) images of chitosan nanoparticles

The transmission electron micrograph (TEM) of chitosan nanoparticles was obtained using a Transmission Electron Microscope (Joel 100S FEI, 120 kV) at the University of Witwatersrand, South Africa. The micrograph depicts very smooth and fine surfaces, as observed in Figure 3 B. The fine surfaces could be as a result of the addition of tripolyphosphate (TPP) to chitosan solution, which converted the chitosan to nano-scale particles. It is evident from the TEM micrograph that nanorising chitosan gives a finer surface, which could contribute to its efficiency when applied industrially in a drug delivery system, in wastewater purification, etc. The particles shapes are spherical and broad. Similar micrographs and shapes were reported by (Maram *et al.,* 2013). The spherical polymer particles invariably offer large numbers of active sites (Maram *et al.,* 2013).

3.4 XRD analysis of chitosan nanoparticles

X-ray diffractogram of the chitosan nanoparticles is shown in Figure 4. The first two major peaks appeared at $2\Theta = 17^{o}$ and 24^{o}. This presents a shift of the peaks for normal chitosan ($2\Theta = 10^{o}$ and 20^{o}), as reported by Maram *et al.* (2013). Sivakamia *et al.* (2013) also reported that XRD of chitosan nanoparticles showed two peaks at $2\Theta = 17^{o}$ and 25^{o}. The crystallinity of chitosan is a key parameter affecting accessibility to internal sites for water or metal ions (Jaworska *et al.*, 2003). There is however a broad band between $2\Theta = 50^{o}$ and 90^{o} in the spectrum, connoting amorphous parts of the chitosan nanoparticles.

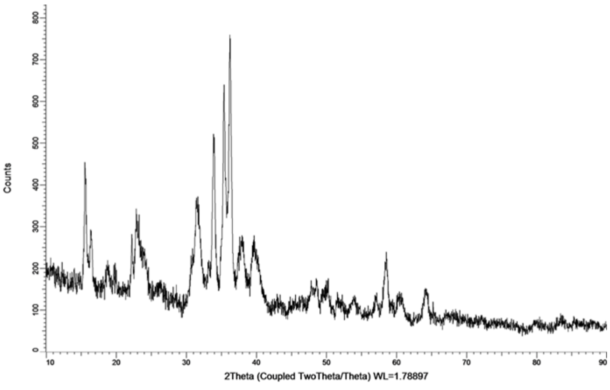


Figure 4. XRD of chitosan nanoparticles

3.5 Thermogravimetric analysis of chitosan nanoparticles

The thermogravimetric spectrum of chitosan nanoparticles is presented in Figure 5. The TGA curve showed a downward trend (loss of sample mass) from 100% to 97% at 100 ^{o}C. The could be a result of moisture loss with temperature increase. Further increase in the temperature to 300 ^{o}C reduced the mass of the sample by another 4%. A drastic reduction in weight was observed at temperatures from 300 ^{o}C to 640 ^{o}C. Practically chitosan loses close to 50% of its mass at these temperatures. The drastic reduction in weight might be from depolymerisation and loss of NH_2 and CH_2OH moieties. The last stage of weight loss occurred between 640°C and 900 °C, by about 11%, bringing the total weight loss to 60%. The implication of this analysis is that chitosan nanoparticles could be applied in waste water treatment at temperatures not exceeding 200 °C, without much effect on its structure.

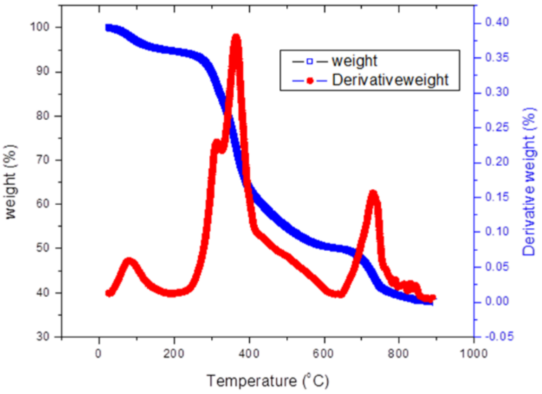


Figure 5. TGA and DTGA of chitosan nanoparticles

4. Conclusions

Chitosan nanoparticles were prepared from blue crab shells and characterized. The nanomaterials obtained were characterized with the aid of transmission electron microscopy. Thermogravimetric analysis showed that the biomaterials were thermally stable, and could be used even at elevated temperatures. As a result of the reduction in the particle size, the final product could be employed in pharmaceutical industries for body tissue repairs due to its non-toxicity; it could also be used in wastewater treatment as it has the tendency to adsorb toxic metals. Its anti-microbial ability makes it useful in textile industries as it inhibits the growth of microorganisms on textile fabrics.

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