International Scholars Journals

International Journal of Urology and Nephrology ISSN 2756-3855 Vol. 12 (1), pp. 001-006 January, 2024. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

From waste to value: Investigating a potential multi-functional pharmaceutical excipient from crab shells

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Received 25 June, 2023; Accepted 30 September, 2023

An efficient and beneficial waste disposal mechanism is highly desired. This study was carried out to extract and characterize a potential multifunctional pharmaceutical excipient from crab shell wastes. Shells of *Pachygrapsus mamoratus* were obtained from Oron, a coastal town in Akwa Ibom State of Nigeria. Chitin was extracted from the powdered shell by deproteination and demineralization; and chitosan was derived by alkaline deacetylation of the chitin. The polymer was subjected to Fourier transform infrared (FTIR) spectroscopy and differential scanning calorimetry (DSC). It was also evaluated for flow properties, pH and hydration and swelling characteristics. The shells gave a yield of 17% chitosan. FTIR analysis of the polymer showed C-H bond of substituted cyclic hydrocarbon, cyclic C-N bond, C-O bond of glucose molecules, C-H bond of side chain –CH_2OH, presence of β-ester linkage, N-H of amides and bonded and free O-H groups. The last transition in the thermogram of chitosan was a polymer degradation exotherm with a peak at 337.9°C. The chitosan had higher bulk density, higher flow rate, lower Carr's index and lower Hausner's ratio compared to sodium carboxymethylcellulose. It also had lower hydration and swelling capacities. Therefore, the crab shell-derived chitosan has better thermal stability, better flow properties but poorer swelling properties compared to sodium carboxymethylcelluose.

Key words: Crab shell, chitosan, physicochemical characteristics, pharmaceutical excipient.

INTRODUCTION

The different approaches to domestic solid waste management can be arranged in decreasing order of preference and efficiency as: waste prevention, re-use, recycling, composting, land filling, dumping and burning (USEPA, 2014). Extraction of chitosan from crab shells for pharmaceutical use is as efficient as waste recycling. Chitosan is obtained by deacetylation of chitin, a component of shells of crustaceans such as shrimps and

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Abbreviations: HR, Hausner's ratio; CI, Carr's index; FTIR, fourier transform infrared; DSC, differential scanning calorimetry; SCMC, sodium carboxymethylcellulose; BD, bulk density; TD, tapped density; MSC, moisture sorption capacity; HC, hydration capacity; SC, swelling capacity.

crab (Wang et al., 2006). Chitin is similar to cellulose, the difference being that the C-2 hydroxyl groups are replaced by acetamido residue. It consists mainly of unbranched chains of β-(1,4)-acetamido-2-deoxy-D-glucan (also known as β-(1,4)-N-acetyl-D-glucosamine). Thus, chitin is a polymer of N-acetyl-D-glucosamine with β-1,4 linkages. It is practically insoluble in water. Chitosan, also known as soluble chitin, is chemically known as 2-amino-2-deoxy-β-D-glucopyranose. It is composed randomly of β-(1,4)- linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine. The amino group in chitosan has a pKa of 6.5 which leads to a protonation of acidic to neutral solution. This makes chitosan water-soluble and a bioadhesive which readily bonds to negatively charged surfaces such as mucosal membrane (Sonia and Sharma, 2011). Chitosan has found a wide application as a drug delivery agent (Hu et al., 2013). It is used as a binder in wet granulation (Kepsutlu et al., 1999), as a drug-carrier in microparticle system, in preparation of hydrogels, as a bioadhesive polymer, as a site-specific drug delivery agent such as to the stomach and colon, as a biodegradable polymer for implants, for hormone delivery and for slow release of drugs from tablets and granules (Yin et al., 2009). It is also used as diluents in direct compression and as a viscosifier. It is used as permeation or absorption enhancer for poorly permeable drugs such as neomycin and cimetidine and for transport of polar drugs across epithelial surfaces (Yin et al., 2009). It is therefore, a multifunctional pharmaceutical excipient. Moreover, a high variety of properties are achieved when chitosan is admixed to amphiphilic compounds (Chiappisi and Gradzielski, 2015).

Crabs are important seafood in coastal areas and they constitute important source of protein in these areas. Disposal of the shell wastes is however, a continuous environmental burden (Burrow et al., 2007). The fact that these wastes can be utilized as sources of chitin and chitosan which are widely utilized in pharmaceutical, medical and environmental fields has provided a more interesting solution to seafood waste accumulation.

The objectives of this study were: to provide a means of disposing the seafood wastes in the coastal towns of Nigeria, to extract chitin and convert it to chitosan; and to carry-out physicochemical characterization of the chitosan. The physicochemical characteristics of the chitosan will be compared with those of sodium carboxymethylcellulose (SCMC), the two substances being cellulose-related. Sodium carboxymethylcellulose is a cellulose derivative with many applications as a pharma-excipient.

MATERIALS AND METHODS

Materials

Sodium hydroxide pellets (BDH Chemicals, England), 36% v/v hydrochloric acid (BDH Chemicals, England) and distilled water which was prepared in the Process Laboratory of Department of Pharmaceutics and Pharmaceutical Technology, University of Uyo, Uyo, Nigeria.

Crab shell collection and blending

Shells of *Pachygrapsus mamoratus* were obtained from Oron, a coastal town in Akwa Ibom State, Nigeria. They were sun-dried for five days to remove moisture from the shells. The dried shells were crushed using mortar and pestle and then powdered using laboratory blender (Christison, United Kingdom).

Extraction of chitin

Chitin extraction was carried-out using a modified form of the method described by Burrow et al. (2007). The process involved two steps namely: deproteination and demineralization.

Deproteination

A 75 g sample of the powdered exoskeleton was weighed and then divided into three equal parts and placed in three different 250 ml beakers labeled A B, C so as to obtain results in triplicates. Each of the samples was treated with 100 ml of 4% w/v NaOH solution and boiling for 1 h in order to dissolve the proteins and sugar. The system was allowed to cool and the supernatant was decanted off. The sediment was dried on cardboard paper for 30 min at room temperature. The powder was further crushed to pieces in a mortar and then screened through a 0.5 mm sieve.

Demineralization

The resulting powder was demineralized with 135 ml of 1% w/v HCl and was left overnight. This was done to remove the calcium carbonate content of the sample. The demineralized crab shell was then treated for 1 h with 50 ml of 2% w/v NaOH to decompose the albumen into water soluble amino acid which was drained off. The sample was washed with deionized water and then air-dried.

Conversion of chitin to chitosan

The chitin was converted to chitosan by deacetylation process using the method described by Burrow et al. (2007). It was carried out by adding 100 ml of 50% w/v NaOH to each sample and then heating at 100°C for 2 h in a water bath. The samples were then removed and cooled for 30 min at room temperature. Afterward, each sample was washed continuously with 50% w/v NaOH and filtered to retain the solid matter which is chitosan. Each sample of the chitosan was placed in a 250 ml beaker and air-dried for 3 h and then oven-dried at 120°C for 24 h to obtain dry chitosan.

Physicochemical characterization

The Fourier transform infrared spectrum and differential scanning thermogram of the chitosan were obtained while the other physicochemical properties were determined and compared with those of sodium carboxymethylcellulose.

Fourier transform infrared (FTIR) spectroscopy

A sample of the chitosan was prepared in a potassium bromide disk

in a hydrostatic press at 6 to 8 tons pressure. The FTIR spectrum was recorded at scanning range of 350 to 5,000 cm^{-1} using a spectrophotometer (model 8400S, Shimadzu Corporation, Kyoto - Japan).

Differential scanning calorimetry (DSC)

DSC analysis of chitosan was carried out on a 1 mg sample in an A1 40 µL crucible using a DSC – 204Fl machine (NETZSCH Co., Germany). The scanning was done at 20°C/min heating rate over a temperature range of 0 to 500°C under nitrogen environment.

Angle of repose

For the angle of repose, 20 g powder of each polymer was poured inside a funnel of orifice diameter 0.75 cm clamped at a height of 10 cm from the table surface. The powder was allowed to flow freely and the angle of repose, Θ, was calculated using the equation

$$\Theta = Tan^{-1} (2h/D) \quad (1)$$

Where, h = height of heap and D is the diameter.

Flow rate

A 20 g sample was placed in a flow rate machine (Erweka, GMBH, Germany). The time of flow was determined and the flow rate was calculated.

Bulk and tapped densities

A 20 g sample was placed in a 50 ml measuring cylinder and the bulk volume was taken. The system was tapped 100 times after which the volume was retaken. The bulk density (BD) and tapped density (TD) were calculated as the ratio of mass to the corresponding volume. The Carr's index (CI) and Hausner's ratio (HR) were calculated using Wells and Aulton (2007) equations:-

$$CI = \frac{TD-BD}{TD} X 100\% \quad (2)$$

$$HR = \frac{TD}{BD} \quad (3)$$

pH

The pH of 2% w/v dispersion of each polymer was determined 24 h after preparation using a pH meter.

Moisture content

A 2 g sample of polymer was weighed and transferred into an electronic moisture analyzer (Type MB 35, OHAUS, Switzerland) and the percent moisture content was determined.

Moisture sorption capacity (MSC)

The moisture sorption capacity was determined by gravimetric method. A 2 g sample of polymer was placed in a desiccator containing distilled water (relative humidity 100%) for 5 days after which it was reweighed. The percentage moisture uptake was determined using equation 4 (Beristain et al., 2006).

$$MSC = M_m / M_d \times 100\% \quad (4)$$

Where, M_m is the mass of moisture absorbed and M_d is the mass of the dry polymer.

Hydration and swelling capacities

Compacts (500 mg) weight (W_d) of the polymers were prepared by compressing the powder using a tableting machine (Shanghai Tiaxiang and Chenta Pharmaceutical Machinery Co., China) fitted with a 12 mm flat faced punch and die. The thickness 'h' and diameter '2r' of the compacts were measured with the aid of micrometer screw gauge. The compacts were placed on glass plates and transferred into a beaker containing 60 ml of distilled water. At 10 min intervals, the glass plates with the hydrated compacts were removed, dried by blotting with tissue paper and then weighed using the method of Builders et al. (2009) with slight modification. The process was continued until a constant weight (W_e) of the hydrated mass was obtained. The hydration capacity (HC) was determined using equation 5.

$$HC = W_e / W_d \quad (5)$$

The thickness 'h' and diameter '2r' of the hydrated mass at constant weight were also measured. Volumes of compacts were calculated using Equation 6.

$$V = \pi r^2 h \quad (6)$$

The swelling capacity (SC) was calculated as the ratio of the final volume of swollen mass to the initial volume of the compact.

Statistical analysis

Data, obtained in triplicates from evaluations were expressed as mean values ± standard error of the mean. Statistical analysis was done using Student's t-test. Significance of difference was taken at *p - values* less than 0.05.

RESULTS

The crab shells gave a yield of 17 ± 0.5% chitosan. The FTIR spectrum of the polymer (chitosan) is shown in Figure 1. The absorption peaks were observed at 399.28, 447.50, 675.11, 858.35, 1035.81, 1471.74, 1643.41, 2287.65, 3362.04, 3834.61 and 4488.50 cm^{-1}. The DSC thermogram is shown in Figure 2. It showed a diffuse endotherm over the temperature range of 30 and 85°C (with peak at 54.8°C), followed by a sharp endotherm which peaked at 107.2°C. This was followed by another endotherm which peaked at 182.6°C and subsequently an exotherm which peaked at 337.9°C. The areas of the four transitions were 632.5, 771.5, 1647.0 and 1849.0 J/g, respectively. The flow properties of sodium carboxymethylcellulose and those of chitosan are presented in Table 1. There were significant differences

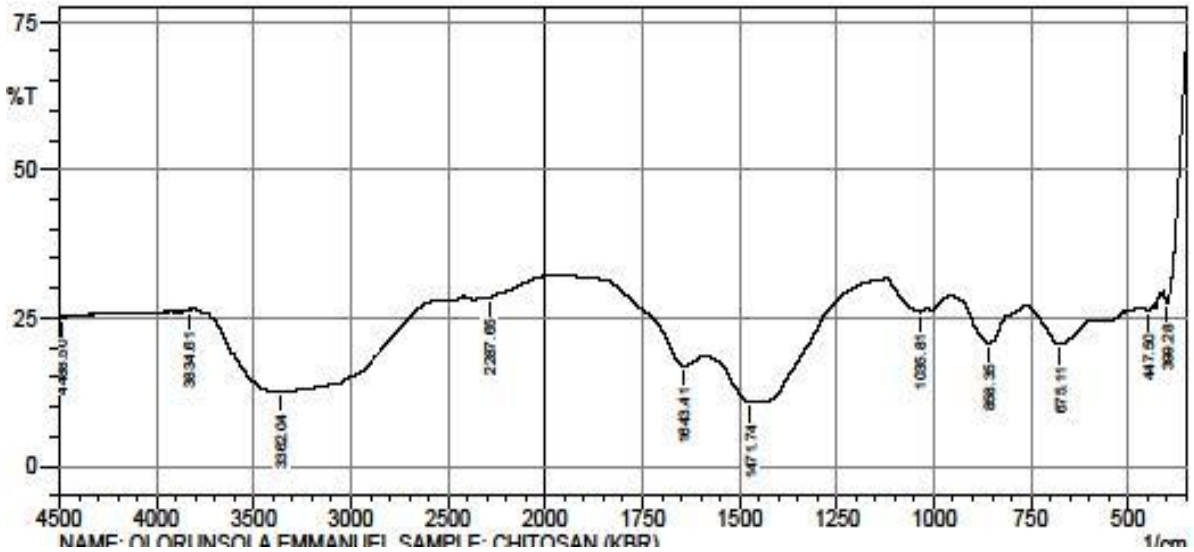

Figure 1. Fourier transform infrared spectrum of chitosan.

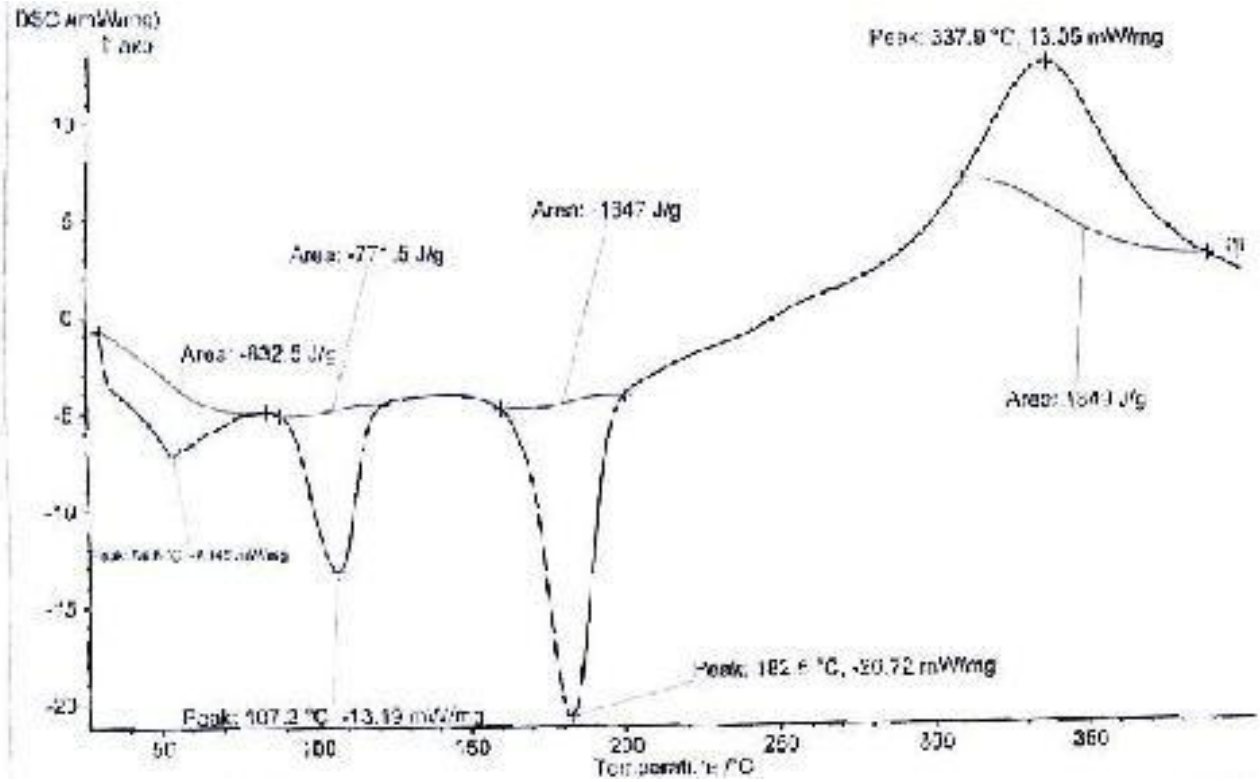

Figure 2. Differential scanning thermogram of chitosan.

in all the flow parameters of the two polymers. Chitosan had lower angle of repose, higher flow rate, higher bulk density, higher tapped density, lower Carr's index and lower Hausner's ratio. The pH, hydration and swelling characteristics of the two polymers are presented in Table 2. There were significant differences in these parameters determined for the two polymers. Chitosan had higher pH, lower moisture content, lower moisture sorption capacity, lower hydration capacity and lower swelling capacity.

Table 1. Flow properties of the polymers.

Parameter	Sodium carboxymethylcellulose	Chitosan	p-value
Angle of repose (°)	50.12 ± 0.36	35.32 ± 0.56	< 0.05
Flow rate (g/min)	9.15 ± 0.06	19.23 ± 1.12	< 0.005
Bulk density (g/ml)	0.20 ± 0.01	0.59 ± 0.02	< 0.005
Tapped density (g/ml)	0.31 ± 0.01	0.75 ± 0.03	< 0.005
Carr's Index (%)	35.48 ± 0.40	21.33 ± 0.15	< 0.025
Hausner's Ratio	1.55 ± 0.00	1.27 ± 0.02	< 0.05

Table 2. pH, hydration and swelling characteristics of the polymers.

Parameter	Sodium carboxymethylcellulose	Chitosan	p-value
pH of 2 % w/v dispersion	5.27 ± 0.03	12.30 ± 0.88	< 0.005
Moisture content (%)	10.31 ± 0.33	6.64 ± 0.45	< 0.025
Moisture sorption capacity	96.42 ± 2.66	33.65 ± 1.77	< 0.005
Hydration capacity	4.98 ± 0.05	1.15 ± 0.06	< 0.0005
Swelling capacity	4.22 ± 0.03	1.40 ± 0.05	< 0.005

DISCUSSION

Absorption peaks of FTIR below 600 cm^{-1} are not used for characterization (Coutt, 2008). Therefore, the peaks at 399.28 and 447.50 cm^{-1} cannot be assigned to specific functional groups. The peak at 675.11 cm^{-1} can be attributed to C-H bending vibration of substituted cyclic hydrocarbon while that at 858.35cm^{-1} can be assigned to cyclic C-N stretching. The absorption peak at 1035.81 cm^{-1} can be assigned to C-O bending of glucose molecule. Those at 1471.74 and 1643.41 cm^{-1} can be assigned to C-H bending of side chain $–CH_2OH$ and presence of β-esters. The absorption peak at 3362.04 cm^{-1} fell within the range of 3300 and 3500 cm^{-1} which is characteristic of N-H of amides while those at 3834.61 and 4488.50 cm^{-1} correspond to stretching vibration of bonded and free O-H groups (Coutts, 2008). All these absorption peaks are typical of chitosan molecule. The various absorption peaks of sodium carboxymethylcellulose were differently assigned to C-H bending of substituted cyclic hydrocarbon, C-H stretching of cyclic hydrocarbon, O-H bending and C-O stretching of alcohol and O-H stretching of bonded and free hydroxyl groups (Olorunsola et al., 2014). Hence, the major difference in the functional groups of chitosan and sodium carboxymethylcellulose is the acetamido residue of chitosan.

The diffuse endotherm of chitosan which peaked at 54.8°C can be ascribed to loss of the absorbed water (Horvat et al., 2005); and 632.5 J of heat per gram sample of the polymer was absorbed in the process. The second transition (with peak at 107.2°C) is a relaxation endotherm (Chung et al., 2002) with enthalpy of 771.5 J/g. Endothermic relaxation is a second order reaction just like glass transition (Horvat et al., 2005). The third endotherm can be ascribed to polymer melting. The area under the curve (164.7 J/g) represents the latent heat of melting while the peak (182.6°C) represents the melting point of the polymer (Builders et al., 2009). The last transition, a diffuse exotherm can be ascribed to the polymer degradation (Iqbal et al., 2013). The exothermic transition temperature of chitosan is higher than that of SCMC. Hence, it has a better thermal stability. The significantly higher bulk density of chitosan is an indication that it has better packing and/or higher density. Chitosan has a significantly higher flow rate ($P< 0.005$). The angle of repose of SCMC which is greater than 50° suggests a very poor flow while that of chitosan which fell between 30 and 40° suggests a fair flow (Wells and Aulton, 2007). The Carr's index of SCMC which is greater than 35% and the Hausner's ratio which is greater than 1.5 suggest that the polymer has a poor flow. On the other hand, the Carr's index of chitosan which was 21.33% and the Hausner's ratio of 1.27 suggest that the polymer possesses a fair flow (Wells and Aulton, 2007). Therefore, chitosan possesses a better flow and may be a better diluent in direct compression.

The pH of sodium carboxymethylcellulose is in the acidic region while that of chitosan is in the basic region. Sodium carboxymethylcellulose has a pKa of 4.3 (Tian et al., 2006). Being a weak acid, it will undergo full ionization and exhibit maximum solubility at high pH (Aulton, 2007). Hence, it will form better hydrogel at low pH. Conversely, chitosan has a pKa of 6.5 (Sonia and Sharma, 2011) and being a basic polymer, it will undergo full ionization and exhibit maximum solubility at low pH (Aulton, 2007). This could be responsible for the better activity of chitosan as a permeation enhancer at low pH

(Yin et al., 2009). The protonation constant and solubility of chitosan has been shown to vary with the degree of deacetylation (Wang et al., 2006).

Sodium carboxymethylcellulose with higher moisture content is also characterized by a higher moisture sorption capacity. Hence, even though it contains more water, it has the ability to absorb more if exposed to a humid condition. However, it had been shown that the level of hygroscopy of the polymer does not mean poor stability (Tian et al., 2006). Therefore, it might be difficult to compare the stability of the two polymers based on hygroscopy alone. The lower hydration capacity ($P < 0.0005$) and lower swelling capacity ($P < 0.005$) of chitosan are indication that the polymer is not a strong hydrogel as SCMC. It is however, important to note that while SCMC will improve drug delivery by acting mainly as a bioadhesive agent, chitosan will improve drug delivery both as a bioadhesive agent and by temporarily increasing intestinal permeability (Kos et al., 2008).

Conclusion

The extraction of chitosan from crab shell wastes has a dual benefit. It serves as a means of disposing seafood wastes and as a means of generating a multifunctional pharmaceutical excipient. The chitosan possesses better thermal stability, higher bulk density, better flow properties but weaker swellability compared to sodium carboxymethylcellulose.

Conflict of interests

The authors did not declare any conflict of interest.

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