Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Original article https://doi.org/10.12980/jclm.5.2017J7-2

Material properties of chitosan from shells of *Egeria radiata*: Drug delivery considerations

Stephen Olaribigbe Majekodunmi[1*], Emmanuel Oluwatoyin Olorunsola[1], Uche Collins Ofiwe[2], Aniefiok Sunday Udobre[3], Ekefre Akpan[1]

[1]*Department of Pharmaceutics and Pharmaceutical Technology, University of Uyo, P.M.B. 1017, Uyo, Akwa Ibom State, Nigeria*

[2]*National Agency for Science and Engineering Infrastructure, Centre of Excellence, Nanotechnology and Advanced Materials, Akure, Ondo State, Nigeria*

[3]*Department of Pharmaceutical and Medicinal Chemistry, University of Uyo, P.M.B. 1017, Uyo, Akwa Ibom State, Nigeria*

ARTICLE INFO

Article history:
Received 6 Jan 2017
Received in revised form 14 Apr, 2nd revised form 27 Apr 2017
Accepted 18 Jun 2017
Available online 22 Jul 2017

Keywords:
Egeria radiata shell
Chitosan
Material property
Drug delivery

ABSTRACT

Objective: To determine the material properties of chitosan derived from shells of *Egeria radiata* in the perspective of drug delivery.

Methods: Chitosan was extracted from the shells of *Egeria radiata* by deproteination, demineralization, decolourization and deacetylation processes. The obtained chitosan was characterized by Fourier transform infra-red spectroscopy, scanning electron microscopy, X-ray diffraction spectrometry, X-ray fluorescence and degree of deacetylation.

Results: The yield of chitosan from the shells was found to be 48.6%. The Fourier transform infra-red spectroscopy spectrum of obtained chitosan showed 12 major peaks between 660.62 and 3 642.57 cm^{-1} and specifically a band of large intensity at 1 485 cm^{-1} suggestive of effective deacetylation of chitin to chitosan. The scanning electron microscopy showed a non-homogeneous particles distribution which became rougher as the magnification increased. The X-ray diffraction spectrometry revealed the presence of aragonite and coesite while the X-ray fluorescence showed heavy presence of calcium with intensity of 0.7369 equivalent to 72% of total elemental composition. Titanium, lead, arsenic and other toxic elements were absent. The degree of deacetylation was found to be 67.8%.

Conclusions: The derived polymer is a chitosan characterized by high calcium content showing that it could be more suitable for formulation of basic and neutral drugs. There is an indication of its suitability for bioadhesive drug delivery and permeation enhancement over a relatively wide pH range and also relatively limited use for sustained drug delivery.

1. Introduction

Seafood is one of the most highly consumed worldwide especially around the coastal areas. The shells of *Egeria radiata* (Family: Donacidae) (*E. radiata*) are found littering the ground or treated as biowaste or sold to animal feed manufacturers[1,2]. In some environments, these shells constitute a nuisance derogating the environment. In some instances, they are burnt thus further constituting environmental pollution. Moreover, approximately 45%–55% of the shrimp biowastes are generated during processing and canning of sea foods[3]. The best option is to extract valuable substances like chitin and chitosan from them.

Chitosan, a fibre-like substance, non-toxic, biodegradable polymer of high molecular weight is a modified natural carbohydrate polymer derived from chitin through deacetylation[4]. Chitin is a homopolymer of β-(1→4)-linked N-acetyl-D-glucosamine. Chitin and chitosan are similar to cellulose. The difference is in the amine group in the position C-2 of chitin and chitosan instead of the hydroxyl group found in cellulose[5]. Chitosan possesses positive ionic charge which gives it the ability to chemically bind with negatively charged fats, lipids, cholesterol, metal ions, proteins and macromolecules[6].

Chitosan is very useful in a wide variety of applications which include pharmaceuticals, food industries, textile industries, biotechnology, cosmetics, biochemistry, biomedical and agricultural engineering. They are useful due to their biocompatibility, biodegradability, adsorption and their unique ability to chelate metal ions[7].

E. radiata, a mollusc in the class of Bivalvia, order Veneroida, is found in large fresh rivers of Western Africa including the Volta (Ghana), Cross River and Akwa-Ibom States of Nigeria (local name: Nkop), Sanaga (Cameroon)[8]. The picture of the shells of the mollusc is shown in Figure 1.

Chitin has been found in a wide range of natural sources. These sources include crustaceans, fungi, insects, annelids, molluscs, and coelenterates. However, chitosan is mainly derived from crustaceans primarily because a large amount of the crustacean exoskeleton is available as a by-product of food processing[9]. Since *E. radiata* is abundantly available in the coastal region of Nigeria[8], it could be economical to derive chitosan from the shells. The objectives of this research are to extract chitosan with optimal condition from the shells of the mollusc and to determine its material properties in perspective of drug delivery.

*Corresponding author: Stephen Olaribigbe Majekodunmi, Department of Pharmaceutics and Pharmaceutical Technology, University of Uyo, P.M.B. 1017, Uyo, Akwa Ibom State, Nigeria.
Tel: +2348090621133
E-mail: steo_majek@yahoo.com
The journal implements double-blind peer review practiced by specially invited international editorial board members.

Figure 1. Photograph of shells of *E. radiata.*

2. Materials and methods

2.1. Materials

Shells of *E. radiata* were obtained from Itam Market, Itam, Itu Local Government Area of Akwa-Ibom State, Nigeria. Other materials used were sodium hydroxide pellets, potassium permanganate crystals and 36% v/v hydrochloric acid (BDH Chemicals, England); and distilled water prepared in the Process Laboratory of Department of Pharmaceutics and Pharmaceutical Technology, University of Uyo, Uyo, Nigeria.

2.2. Methods

2.2.1. Extraction of chitosan

The shells were washed and oven-dried for three consecutive days at 50 ˚C. Size was reduced into powder by an industrial blender and later made to pass through Endecott sieves to obtain a 250 micron size. Extraction of chitosan from *E. radiata* was carried out via three procedures, namely, deproteination, demineralization and deacetylation processes. Powdered shells (500 g) were treated with 4% w/v NaOH at room temperature for 24 h. The alkali was drained from the shell and washed with distilled water repeatedly until the pH dropped to neutral. After this process of deproteination, demineralization was carried out by treatment with 4% v/v HCl at room temperature for 12 h to yield chitin. The acid was drained off from and the chitin was washed with distilled water and dried at room temperature on a white tile. This process was repeated with 2% w/v NaOH and then with 1% v/v HCl. Further decolourization was achieved by soaking chitin in 1% w/v potassium permanganate for 30 min followed by 1% w/v oxalic acid for 30 min. The decolourized chitin was deacetylated to form chitosan by treating with 65% w/v NaOH for 3 days at room temperature. The alkali was drained and the chitosan was washed repeatedly with distilled water until the pH was lowered. The resulting chitosan was dried at room temperature and then stored in a bottle until needed.

2.2.2. Determination of yield of chitosan

The mass of chitosan obtained was recorded and the yield was calculated using the equation:

$$\% \text{ Yield} = \frac{\text{Mass of chitosan}}{\text{Mass of shell}} \times 100$$

2.2.3. Characterization of chitosan

2.2.3.1. Fourier transform infrared (FTIR) spectroscopy

The sample of chitosan was characterized using infrared spectrophotometer (Shimadzu IR Prestige 21) in the range of 400 to 4000 cm^{-1}.

2.2.3.2. Scanning electron microscopy (SEM)

The structure of chitosan was examined using electron microscope (Model SEM PROX, Phenomworld, Eindhoven, Netherlands).

2.2.3.3. X-ray diffraction (XRD)

XRD analysis of chitosan was carried out to detect the degree of crystalinity. The XRD pattern of the fine powder samples were measured at room temperature. An enhanced mini-materials analyzer diffractometer (Model: EMMA, GBC Scientific Equipment Pty Ltd., Australia) equipped with Cu target X-ray tube with wavelength of 1.540 59 Å and step size of 0.05 was used for this purpose. X-ray diffraction was taken in the 2θ range of 5° to 70°. The mineral search was carried out using the GBC X-ray analysis TRACES Software (Version 6) which operates on the full ICDD PDF-2 database file.

2.2.3.4. Energy dispersive X-ray fluorescence (XRF)

The quantitative and qualitative elemental analysis of chitosan isolated was determined using an XRF spectrometer (Model: EDX3600B, Skyray instrument Inc., USA). This system detects elements between sodium (Na, Z = 11) and Uranium (U, Z = 92) with high resolution and fast analysis.

2.2.3.5. Determination of degree of deacetylation

The degree of deacetylation was carried out using potentiometric acid-base titration method[10] with modifications. Chitosan homogenous solution was prepared using dilute HCl containing 0.4 mol/L and titrated against 0.1 mol/L NaOH at room temperature. The end point was detected by inflection of pH values. The two inflections were mainly noted. The first one corresponded to HCl neutralization and the second to neutralization of ammonium ions of chitosan. The difference between the two points was noted to give the amount of amino groups in chitosan chain.

% Degree of deacetylation (DD %) = 100 – DA%

The DA% was calculated using the following equation:

DA % = Difference in inflection in pH value × 100%/Initial pH value

3. Results

The yield of chitosan from the shell was 48.6%. The FTIR spectrum of the chitosan as shown in Figure 2 contained 12 major peaks lying between 660.62 and 3642.57 cm^{-1}. The observed peaks were located at position 660.62, 702.55, 862.53, 1076.68, 1484.06, 1789.11, 2352.32, 2519.46, 2971.42, 3080.00, 3421.00 and 3642.57 cm^{-1}.

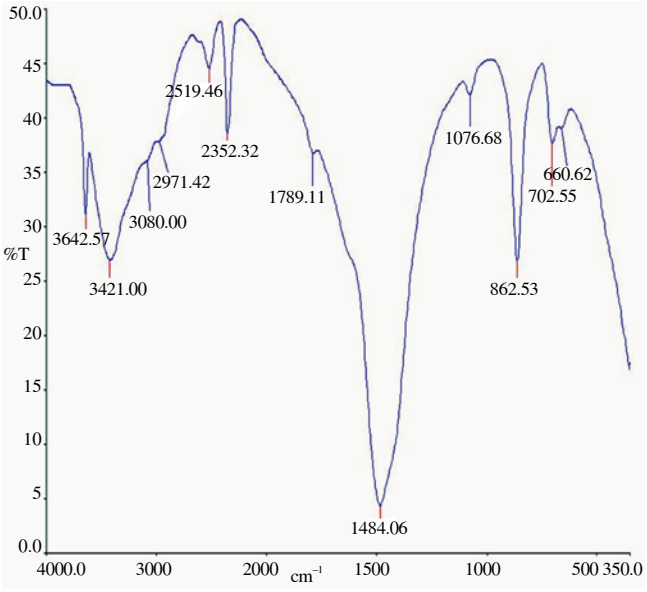

Figure 2. FTIR spectrum of *E. radiata* chitosan.

The surface morphology of the chitosan is shown in Figure 3. The particle surface became rougher as the magnification was increased. Figure 4 shows the XRD pattern of the chitosan. The pattern shows strong reflections at 2θ angles of 18.1°, 26.2°, 27.0°, 34.1°, 45.9°, 47.2° and 50.8°. The highest peak for both SiO_2 and $CaCO_3$ was observed at position 27° 2θ.

The results of XRF analysis are presented in two forms, namely qualitative elemental content (Figure 5) and quantitative elemental content (Table 1) while the degree of deacetylation (DD) was 67.8%.

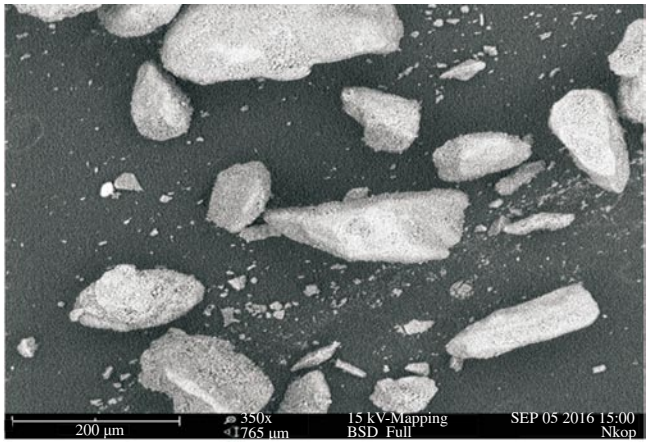

Figure 3. SEM of *E. radiata* chitosan.

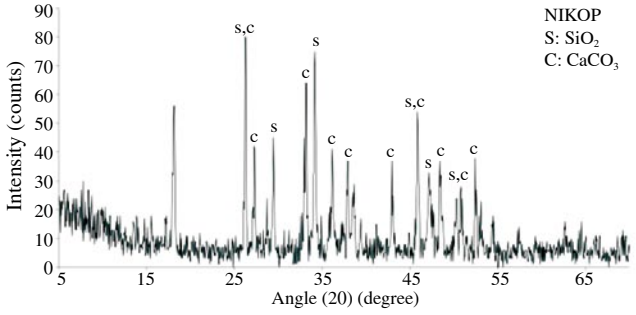

Figure 4. XRD pattern of *E. radiata* chitosan.

Table 1

Elemental composition of chitosan obtained from shells of *E. radiata.*

Element	Intensity	Content (%)
Mg	0.0001	0.0609
Al	0.0008	0.2211
Si	0.0014	0.0398
P	0.0059	0.2783
S	0.0051	0.3478
K	0.0000	0.0000
Ca	0.7369	72.3685
Ti	0.0000	0.0000
V	0.0000	0.0010
Cr	0.0001	0.0030
Mn	0.0003	0.0113
Co	0.0001	0.0007
Fe	0.0029	0.3565
Ni	0.0003	0.0153
Cu	0.0007	0.0151
Zn	0.0010	0.0322
As	0.0001	0.0000
Pb	0.0000	0.0000
W	0.0001	0.0000
Au	0.0002	0.0042
Ag	0.0002	0.0042

4. Discussion

Seafood shells usually contain protein, minerals and chitin[11]. Protein was removed during the first stage of extraction while minerals were removed during the second stage to obtain chitin. Chitosan was derived from the chitin by the deacetylation. The yield of chitosan from the shell is higher than that obtained from crab shell that was reported by Olorunsola *et al*.[12].

The FTIR band at 1484.06 cm^{-1} has a larger intensity which suggests effective deacetylation of chitin to form chitosan with a prevalence of NH_2 groups[13].

The scanning electron micrograph shows an inhomogeneous particles distribution. It also shows that the particle size and shape

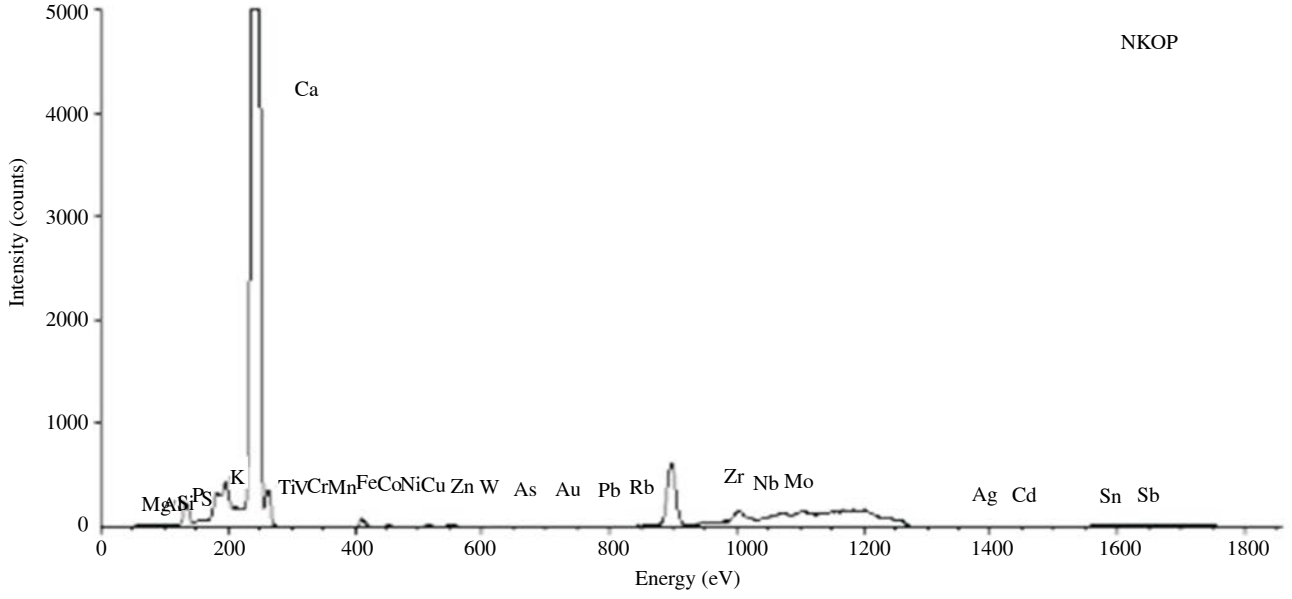

Figure 5. XRF pattern of *E. radiata* chitosan.

are even at low magnification.

The mineral search in X-ray diffraction pattern showed the presence of aragonite and coesite. Aragonite is one of the two common naturally occurring crystal forms of calcium carbonate (the other form being the mineral calcite). It occurs naturally in almost all mollusk shells. Coesite is chemically known as SiO_2.

The qualitative elemental feature of XRF gives a qualitative picture of the elemental composition using a graphical representation, with each peak labeled after the element. The quantitative elemental analysis computed by the XRF software provides information on the percentage of individual element in the sample relative to the weight of the sample analyzed. Prominent elements in the sample are represented with high percentage content. Physical examination of the spectrum shows the prominent elements as the most towering peaks. Impurities or low content elements are shown as low and almost grounded peaks and Table 1 shows the heavy presence of calcium with an intensity of 0.7369 and 72% of the constituents. Titanium, arsenic, lead and gold are non-existing demonstrating the safe use of chitosan in pharmaceutical preparations.

Protonation constant and hence solubility of chitosan has been shown to vary with the degree of deacetylation[14]. The amino group in chitosan has a pKa of 6.2 to 7.0 which leads to protonation of acidic to neutral solution[15]. This makes chitosan to be water-soluble at this pH range and forms a bioadhesive which readily bonds to negatively charged surfaces such as mucosal membrane. At higher pH, it forms a hydrogel[16].

Chitosan is one of the most valuable polymers in drug delivery due to its biodegradability and biocompatibility[17]. It is used in the formulation of nanoparticles, microspheres, hydrogels and films for different pharmaceutical applications. Such applications include oral, nasal, ocular, parenteral and transdermal delivery[4]. It is also used as a biodegradable polymer for implants, for hormone delivery and for slow release of drugs from solid formulations[18]. In the dissolved state, it acts as permeation or absorption enhancer for poorly permeable drugs and for transport of polar drugs across epithelial surfaces[18]. Hence, solubility and biodegradability are vital to applicability of chitosan.

The degree of deacetylation is important in establishing the structure-property relationship of chitosan and its possible application[15]. The work of Wang *et al*.[14] showed that the pKa increased from 6.17 to 6.51 with decrease in degree of deacetylation from 94.6% to 73.3%. Therefore, chitosan from the shells of *E. radiata* with degree of deacetylation of 67.8% will be characterized by higher pKa value and better solubility. Since water-solubility is a requirement for bioadhesion[16] and permeation-enhancing activity[18] of chitosan, the polymer from this mollusc could be effective for permeation enhancement over a relatively wide pH range and for formulation of bioadhesive delivery system. However, its use as a sustained-release matrix former may be limited by its low degree of deacetylation since hydrogel formation is required for sustained delivery[4].

The polymer derived from the shells of *E. radiata* is a chitosan. It is characterized by high calcium content and could be more suitable for formulation of basic and neutral drugs. It is characterized by degree of deacetylation of 67.8%. There is an indication that this polymer will be effective for bioadhesive drug delivery and for permeation enhancement over a relatively wide pH range. Its suitability for sustained drug delivery might be limited by the relatively low degree of deacetylation. Further work is therefore needed to validate the suitability of this polymer obtained from the shells *E. radiata* for drug permeation enhancement, bioadhesive drug delivery and sustained drug release.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Suchiva K, Chandrkrachang S, Methacanon P, Peter MG. Proceedings of the 5th Asia Pacific Chitin and Chitosan Symposium and Exhibition, Bangkok, Thailand 2011; 458-62.

[2] Hossain MS, Igbal A. Production and characterization of chitosan from shrimp waste. *J Bangladesh Agric Univ* 2014; **12**(1): 153-60.

[3] Lertsutthiwong P, Kow NC, Chandrkrachang S, Stevens WF. Effect of chemical treatment on the characteristics of shrimp chitosan. *J Met Mater Miner* 2002; **12**(1): 11-8.

[4] Kumar MNVR. A review of chitin and chitosan applications. *React Funct Polym* 2000; **46**: 1-27.

[5] No HK, Meyers SP. Utilization of crawfish processing wastes as carotenoids, chitin, and chitosan sources. *J Korean Soc Food Nutr* 1992; **21**(3): 319-26.

[6] Li Q, Dunn ET, Grandmaison EW, Goosen MFA. Applications and properties of chitosan. *J Bioact Compat Polym* 1992; **7**: 370-97.

[7] Rout SK. Physicochemical, functional and spectroscopic analysis of crawfish chitin and chitosan as affected by process modification [dissertation]. Baton Rouge: Lousiana State University; 2011.

[8] Etim L, Brey T. Growth, productivity, and significance for fishery of the bivalve *Egeria radiata* (Donacidae) in the Cross River, Nigeria. *Arch Fish Mar Res* 1994; **42**: 63-75.

[9] Shepherd R, Reader S, Falshaw A. Chitosan functional properties. *Glycoconj J* 1997; **14**(4): 535-42.

[10] Yuan Y, Chesnult BM, Haggard WO, Bumgardner JD. Deacetylation of chitosan: material characterization and *in-vitro* evaluation via albumin and pre-osteoblastic cell cultures. *Materials* 2011; **4**: 1399-416.

[11] Burrow F, Louim C, Abazinge M, Onokpise O. Extraction and evaluation of chitosan from crab exoskeleton as a seed fungicide and plant growth enhancer. *Am-Eur J Agric Environ Sci* 2007; **2**(2): 103-11.

[12] Olorunsola EO, Olayemi OJ, Majekodunmi SO, Etukudo UB. Extraction and physicochemical characterization of a potential multifunctional pharma-excipient from crab shell wastes. *Afr J Biotechnol* 2015; **14**(40): 2856-61.

[13] Limam Z, Selmi S, Sadok S, El-Abed A. Extraction and characterization of chitin and chitosan from crustacean by-products: biological and physicochemical properties. *Afr J Biotechnol* 2011; **10**(4): 640-7.

[14] Wang QZ, Chen XG, Liu N, Wang SX, Liu CS, Meng XH, et al. Protonation constant of chitosan with different molecular weight and degree of deacetylation. *Carbohyd Polym* 2006; **65**: 194-201.

[15] De Alvarenga ES. Characterization and properties of chitosan. In: Elnashar M, editor. *Biotechnology of biopolymers*. Rijeka: Intech; 2011, p. 91-109.

[16] Sonia TA, Sharma CP. Chitosan and its derivatives for drug delivery. *Adv Polym Sci* 2011; **243**: 23-54.

[17] Hu L, Sun Y, Wu Y. Advances in chitosan-based drug delivery vehicles. *Nanoscale* 2013; **5**: 3103-11.

[18] Yin L, Ding J, He C, Cui L, Tang C, Yin C. Drug permeability and mucoadhesive properties of thiolated chitosan nanoparticles in oral insulin delivery. *Biomaterial* 2009; **30**: 5691-700.