ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 7, Issue 4, 2014

ISSN - 0974-2441

Research Article

EXTRACTION AND PURIFICATION OF CHITOSAN FROM CHITIN ISOLATED FROM SEA PRAWN (*FENNEROPENAEUS INDICUS*)

SNEHA PAUL[1], AISWARYA JAYAN[2], CHANGAM SHEELA SASIKUMAR[1], SANJAY M CHERIAN[1]

[1]Department of Cellular & Molecular Biochemistry, Frontier Lifeline & Dr. K.M. Cherian Heart Foundation, Mogappair, Chennai - 600 101, India. [2]Department of Biotechnology, Karunya University, Coimbatore - 641 114, Tamil Nadu, India. Email: sheelsasic@yahoo.co.in

Received: 09 July 2014, Revised and Accepted: 31 July 2014

ABSTRACT

Aim: The main study focuses on the purification of chitosan from chitin isolated from *Fenneropenaeus indicus*, will be suitable for the pharmaceutical industry.

Objective: Chitosan is an amino polysaccharide prepared by processing prawn waste (shell) which involves the demineralization, deprotienization and deacetylation. Chitosan is a versatile natural polysaccharide, its second most abundant natural polymer. Researchers have found that chitosan as biocompatible, biodegradable and non-toxic, which have made wide applicability in the pharmaceutical field.

Result: The chitosan yield was found to be 57.69%, and it was analyzed for its physiochemical parameters, antibacterial and antifungal activity.

Keywords: Chitosan, Chitin, Natural polymer, Characterization, Purification, Physiochemical parameters, Antibacterial, Antifungal activity.

INTRODUCTION

Chitosan is a very simple substance. It is derived from chitin, a polysaccharide that is found in the cell wall of fungi and also in the exoskeletons of crustaceans. They are then being processed by removing the shells from shellfish such as shrimp, lobster, crabs etc. [1]. It is obtained by the partial deacetylation of chitin; natural polymer composed of β-(1-4)-linked D-Glucosamine, randomly distributed. It consists of two types of monomers; chitin-monomers and chitosan-monomers [2].

Commercially, chitosan is produced between 3800 and 20,000 Daltons, which is the structural element in the exoskeleton of crustaceans and cell wall of fungi [3]. The degree of deacetylation (%DD) can be determined by ultra violet-visible spectrophotometer, Fourier transform infrared (FTIR) and nuclear magnetic resonance spectroscopy. A common method for the synthesis of chitosan is from the N-deacetylation of chitin using sodium hydroxide in excess as a reagent and water as a solvent, this yield up to 98% product. Chitosan is biocompatible, non-antigenic, non-toxic, bio-functional and bio-degradable and enhances the transport of polar drugs across epithelial surfaces [4].

Chitosan has been used over a wide range of applications, such as wound healing agents, drug carriers, chelating agents, membrane filter for water treatment and bio-degradable coating or film for food packaging. It is also used as a potential biomaterial that can be used for nerve repair. Chitosan is used in water purification by spreading the powder over the surface where any toxic substances such as greases, oils, or dangerous heavy metals are immediately absorbed, and the scum is then easily removed. Chitosan is an antibacterial agent. Chitosan is used in food as a preservative agent to prolong the freshness of the product and makes it taste better. Purified chitosans are used for medicinal purposes where they have contact with skin undergo a strict inspection process, this promotes in healing wounds. Chitosan and its derivatives, such as trimethylchitosan (where the amino group has been trimethylated), have been used in non-viral gene delivery [5].

The novel characteristic features of chitosan not only relive its property in the pharmaceutical industry, but also in drug targeting and delivery. This research is mainly focused on the synthesis of purified chitosan, to determine the various qualities attributes of its physiochemical properties such as molecular weight, viscosity, DD, dry weight, ash value, solubility, pH etc. The confirmation of chitosan was done by FTIR and X-ray diffraction (XRD).

METHODOLOGY

Isolation and extraction of chitosan

The exoskeletons of the prawn waste (shell) were removed separately and was rinsed thrice with tap water and then twice with distilled water. Then they were dried in a hot air oven for about 24 hrs at 55°C. The sample obtained was soaked in boiling 4% sodium hydroxide using 1000 ml beaker for 1 hr. The sample was removed and then allowed to cool at room temperature for 30 minutes. They were then crushed further to small pieces of about 0.5-5.0 mm [6].

Demineralization

The sample obtained was demineralized using 1% hydrogen chloride with 4 times its quantity. They were then soaked for 24 hrs to remove minerals. The above samples were treated with 50 ml of 2% sodium hydroxide for 1 hr. The remains of the sample were washed with deionized water and then drained off [7].

Deacetylation

The process was then carried out by adding 50% sodium hydroxide to the obtained sample on a hot plate and boiling it for 2 hrs at 100°C. The sample was then allowed to cool at room temperature for 30 minutes. Then they were washed continuously with 50% sodium hydroxide. They sample obtained is filtered (chitosan is obtained). The sample was left uncovered, and oven-dried for 6 hrs at 110°C [8].

Purification of chitosan

The obtained chitosan has to be purified to make it suitable for use. The purification process was designed in three steps - removal of insoluble with filtration, reprecipitation of chitosan with 1 N sodium hydroxide, demetallization of retrieved chitosan [9].

Physiochemical parameters

Molecular weight: Intrinsic viscositybu Brook-field viscometer is used to determine the average molecular weight. The average molecular weight was obtained from Mark-Houwink equation:

$\eta = KM^{\alpha}$

Where [η] is the intrinsic viscosity, M is the average molecular weight of the solution, respectively and K and a are the Mark-Houwink constants specific for a given polymer [10].

Moisture content

Moisture content of the prepared chitosan was determined by the gravimetric method. The water mass was determined by drying the sample to constant weight and measuring the sample after and before drying. The water mass was the difference between the weights of the wet and oven dry samples [11].

Percentage of moisture content = (wet weight, g – dry weight, g) × 100 wet weight, g

Loss on drying

Loss on drying of the prepared chitosan was determined by the gravimetric method. The water mass loss was determined by drying the sample to constant weight and measuring the sample after and before drying. The water mass (or weight) obtained showed the difference between the weights of the wet and oven dry samples [12].

Percentage of loss on drying = (wet weight, g – dry weight, g) × 100 dry weight, g

Ash value

The ash value of chitosan was determined by taking the prepared chitosan sample which was previously ignited, cooled, and tarred crucible. The samples were heated in a muffle furnace preheated to 650°C for 4 hrs. The crucibles were then allowed to cool in the furnace to <200°C and then were placed into desiccators with a vented top [13].

Percentage of ash = (weight of residue, g) × 100 sample weight, g

pH and solubility

The pH measurement of the chitosan solutions was carried out using a microprocessor pH meter. Solubility: The solubility of chitosan was demonstrated in various solutions like distilled water, acetone, ethanol, acetic acid and lactic acid. The chitosan obtained here got dissolved completely in acetic acid.

DD

IR technique was used for determining the DD according to the previously reported methods [14,15]. The percentage of the acetylated amine group was determined by

N-acetyl % = 100-(A1652/A3446) × 100/1.33

Characterization of chitosan

The prepared biopolymer chitosan was analyzed by Shimadzu FTIR 8300 spectrometer in the wavelength between 400/cm and 4000/cm and in the solid state using potassium bromide pellets [16]. This polymer was also analyzed by XRD.

Antibacterial and antifungal by well diffusion method

The antibacterial assays were done on human pathogenic bacteria and fungi. The nutrient agar medium was poured into the petri plate. Allow the medium to solidify, 1 ml of inoculums was placed on the plates and spread it with cotton swab. Six wells around 10 mm diameter were cut out aseptically with the help of cork borer. Among six wells, one well was filled with 30 µl of diluted acetic acid solution and another five well with different concentration (10, 30, 60, 90, 120 µl) of chitosan solution. Incubate at 37°C for 24 hrs and note for zone of inhibition [17].

RESULTS AND DISCUSSION

An effort had been made to explore the physicochemical properties and antimicrobial activity as well as structural properties of prawn waste (shell) collected from Saidapet market, Chennai. The results of physicochemical and functional properties of the prepared chitosan are given in the (Table 1) (Fig. 1).

Table 1: Physiochemical parameters of chitosan

Physiochemical parameters	Chitosan
Yield	57.69%
Molecular weight	159653 g/mole
Moisture content	4%
Ash value	1.86%
Loss on drying	2%
pH	6.7
Solubility	Acetic acid
Degree of deacetylation	87%

Fig. 1: Crude chitosan isolated from *Fenneropenaeus indicus*

The prepared Chitosan from chitin was confirmed as reported data [18]. Chitosan [19] from shrimp shell contains moisture in the range 1.0-1.30% depending upon the season, relative humidity and intensity of sunlight. There is no significant difference in the percentage of moisture content between the reported data elsewhere 1-1.30%. The report generated by KFDA [20] the moisture content of chitosan powder should be <10%. The moisture content obtained was in the range of 4%. Chitosan has very low ash value, 1.86%; indicates the efficiency of demineralization step followed in the preparation of the chitosan sample by removing the minerals. Commercial chitosan [21] is reported to have ash value about 1.18%.

The molecular weight of the prepared chitosan was variable due to various factors such as high temperature, alkali concentration, time of reaction, chitin concentration, dissolved oxygen deliberation, shear stress, etc. and the determined molecular weight is 159653 g/mole [22,23].

The solubility of chitosan was checked with five different solvents such as water, ethanol, NaOH, acetic acid and lactic acid. It was not soluble in alkaline or neutral solution, but was soluble in acidic condition, whereas you compare with lactic acid, it was more soluble in acetic 90-95% solubility was seen. The pH value of chitosan also varies from the range 6.2 to 8.0.

The DD is an important parameter to be noted affecting solubility, chemical reactivity and biodegradability. DD may range from 30% to 95% [24] depending on the available source and procedure. It is calculated by using the equation and FTIR (infrared spectroscopic) analysis of the prepared chitosan [25]. This study (Table 1) revealed that; DD of the prepared chitosan is 87%, 100% DD is very rarely obtained. Commercial chitosan with various DD in the range of 75-85% is generally found.

Chitosan was extracted from chitin got from prawn shell and further purified and confirmed by FTIR - Fig. 2, Table 2 and XRD - Fig. 3. The FTIR studies of the Chitosan from *Fenneropenaeus indicus* species.

Table 2: Wavelength of the main bands obtained from chitosan

Vibration modes	Standard	Indian prawn
NH out-of-plane bending	752	712
Ring stretching	896	880.6
CO stretching	1026	1026
CH2 bending and CH3 deformation	1418	1432
Amide II band	1563	1576.2
Amide I band	1661	1652.8
Symmetric CH3 stretching	2930	2927.0
A symmetric CH3 stretching		
NH stretching	3268	3446.4

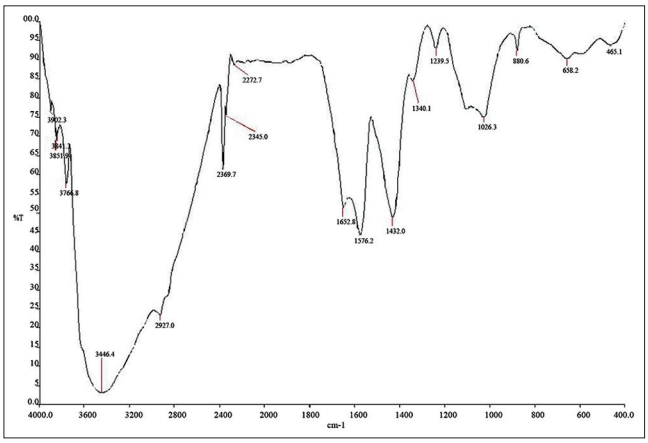

Fig. 2: Fourier transform infrared spectroscopy image of chitosan

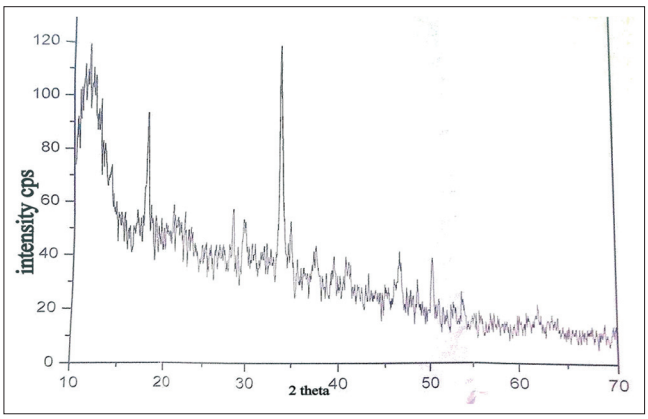

Fig. 3: X-ray diffraction pattern of chitosan

Characteristics Chitosan yield 67%.Then the major absorption band is observed between 1220/cm and 1020/cm which represents the free amino group (-NH2) at C2 position of glucosamine, a major group present in chitosan. Further the sample showed the absorption bands at the various peaks 712, 880.6, 1026, 1432, 1576.2, 1652.8, 2927.0, 3446.4, which is similar to standard chitosan. This shows the confirmation of chitosan [26].

XRD has also done to confirm the formation of chitosan and to determine the nature of chitosan powder. XRD patterns chitosan are illustrated in Fig. 3. The XRD pattern of chitosan exhibits broad diffraction peaks at 2θ = 10° and 21° which are typical fingerprints of semi-crystalline chitosan [27,28] found that fungal chitosan showed two crystalline reflections at 9.7° and 19.9°. Prashanth *et al.* in the year 2002 found that the wide-angle X-ray diffraction (WAXD) patterns of the shrimp chitosan showed two major characteristic peaks at 2θ = 9.9-10.7° and 19.8-20.7°. It is also reported that [29] the two characteristic crystalline peaks with slightly fluctuated diffraction

Table 3: Antimicrobial activity and zone of inhibition of chitosan (mm)

Test organism	Concentration of chitosan (µl/ml)					Concentration of acetic acid (µl/ml)
	10	30	60	90	120	30
	Zone of inhibition (mm)					
S. aureus	-	-	3	8	12	8
C. albicans	-	-	8	10	12	-

S. aureus: Staphylococcus aureus, C. albicans: Candida albicans

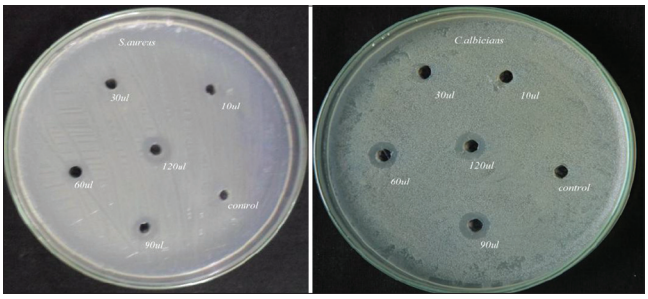

Fig. 4: The viability of chitosan against *Staphylococcus aureus* and *Candida albicans*

angles found in the WAXD patterns indicated that two types of α- and γ-chitosans exhibited a comparable degree of crystallinity and had two consistent peaks of 9-10° and 19-20°, where as chitosan extracted from *F. indicus* showed three consistent peaks at 11.3, 19.0, and 33.0.

The antimicrobial properties of chitosan were done by varying the concentration and showed an inhibition toward *Staphylococcus aureus* and *Candida albicans* types of tests microorganism, it has been shown in Table 3 and Fig. 4. More prominent zone was seen in higher concentration of chitosan. While, no antimicrobial activity was seen in control well [30].

CONCLUSION

This study shows the production of chitosan from sea prawn waste (shell), would successfully reduce the environmental pollution. The FTIR and XRD studies confirm the production of chitosan, and physicochemical properties of chitosan show that in can be used in various fields like pharmaceutical industry, food packaging, water treatment, drug delivery, etc. The source made from waste (chitosan) shows an excellent antimicrobial activity against human pathogens. Thus, it can be used as good potent source against the infectious pathogens.

REFERENCES

1. Shahidi F, Abuzaytoun R. Chitin, chitosan, and co-products: Chemistry, production, applications, and health effects. Adv Food Nutr Res 2005;49:93-135.
2. Dutta PK, Ravikumar MN, Dutta J. Chitin and chitosan for versatile application. J Macromol Sci Polym Rev 2002;42(3):307.
3. Sahoo S, Sahoo R, Nayak PL. Synthesis and characterization of gelatin-chitosan nanocomposite to explore the possible use as drug delivery vehicle. Eur Sci J 2013;9(18):135.
4. Hudson SM, Smith C. Polysaccharide: Chitin & chitosan: Chemistry and technology of their uses as structural materials. In: Kaplan DL, editor. Biopolymer from Renewable Resources. New York: Springer-Verlag; 1998. p. 96-118.
5. Rinaudo M. Chitin and chitosan: Properties and applications. Prog Polym Sci 2006;31:603-32.
6. Lamarque G, Lucas JM, Viton C, Domard A. Physicochemical behavior of homogeneous series of acetylated chitosans in aqueous solution: Role of various structural parameters. Biomacromolecules 2005;6:131-42.
7. Huang M, Khor E, Lim LY. Uptake and cytotoxicity of chitosan

molecules and nanoparticles: Effects of molecular weight and degree of deacetylation. Pharm Res 2004;21(2):344-53.
8. Muzzarelli RA, Rochetti R. Determination of the degree of deacetylation of chitosan by first derivative ultraviolet spectrophotometry. J Carbohydr Polym 1985;5:461-72.
9. Qian RQ, Glanville RW. Methods for purifying chitosan providence health system, 2005.
10. Delorino M, Salvacion P. Cresidio investigation of chitosan from squid pen as scar remover. World Appl Sci J 2009; 5 (special issue for environment):98-103.
11. Black CA, editor. Methods of Soil Analysis: Part I Physical and Mineralogical Properties. Madison, Wisconsin: American Society of Agronomy; 1965. p. 671-98.
12. Hu Y, Jiang X, Ding Y, Ge H, Yuan Y, Yang C. Synthesis and characterization of chitosan-poly (acrylic acid) nanoparticles. Biomaterials 2002;23(15):3193-201.
13. Jiang TD. Chitosan. Beijing, China: Chemical Industry Press; 2001. p. 91, 100, 108.
14. Domszy JG, Roberts GA. Evaluation of infrared spectro-scopic techniques for analysis chitosan. Macromol Chem 1985;186:1671-7.
15. Hiral A, Odani H, Nakajima A. Determination of degree of deacetylation of chitosan by 1H NMR spectroscopy. Polym Bull 1991;26:87-94.
16. Mohammad RK. A review of several reported procedures to determine the DD of N2acetylation for chitin and chitosan using infrared spectroscopy. Carbohydr Polym 2007;71:4972508.
17. Islam M, Masum S, Mahbub KR. *In vitro* antibacterial activity of shrimp chitosan against *Salmonella* paratyphi and *Staphylococcus aureus*. J Bangladesh Chem Soc 2011;24(2):185-90.
18. Islama M, Masumb S, Rahmana MM, Mollab AI, Shaikhc A, Roya SK. Preparation of chitosan from shrimp shell and investigation of its properties. Int J Basic Appl Sci 2011;11(01):77-80.
19. Tajik H, Moradi M, Rohani SM, Erfani AM, Jalali FS. Preparation of chitosan from brine shrimp (*Artemia urmiana*) cyst shells and effects of different chemical processing sequences on the physicochemical and functional properties of the product. Molecules 2008;13(6): 1263-74.
20. KFDA. Food Additives Code. Seoul: Korea Food and Drug Administration; 1995. p. 449-51.
21. Wang JC, Kinsella JE. Functional properties of novel protein: Alfalfa leaf protein. J Food Sci 1976;41:286-92.
22. Terbojevidh M, Cosani A. Molecular weight determination of chitin and chitosan. In: Muzzarelli RA, Peter MG, editors. Chitin Handbook. Italy: European Chitin Society; 1997. p. 87-101.
23. Laka M, Chernyavskaya S. Preparation of chitosan powder and investigation of its properties. Proc Estonian Acad Sci Chem 2006;55(2):78-84.
24. Di Martino A, Sittinger M, Risbud MV. Chitosan: A versatile biopolymer for orthopaedic tissue-engineering. Biomaterials 2005;26(30):5983-90.
25. Struszczyk H. Microcrystalline chitosan. I. J Appl Polym Sci 1987;33:177-89.
26. Puvvada YS, Vankayalapati S, Sukhavasi S. Extraction of chitin from chitosan from exoskeleton of shrimp for application in the pharmaceutical industry. Int Curr Pharm J 2012;1(9):258-63.
27. Bangyekan C, Aht-Ong D, Srikulkit K. Preparation and properties evaluation of chitosan-coated cassava starch films. Carbohydr Polym 2006;63(1):61-71.
28. Yen MT, Mau JL. Physico-chemical characterization of fungal chitosan from shiitake stipes. LWT Food Sci Technol 2007;40:472-9.
29. Yen MT, Yang JH, Mau JL. Physicochemical characterization of chitin and chitosan from crab shells. Carbohydr Polym 2009;75(1):15-21.
30. Toan NV, Hanh TT, Thien PV. Antibacterial activity of chitosan on some common food contaminating microbes. Open Biomater J 2013;4:1-5.