Biospectra : ISSN: 0973-7057, Vol. 19(1), March, 2024, pp. 101-104 **Animal Science**
An International Biannual Refereed Journal of Life Sciences

ISSN : 0973-7057

Comparative studies on characterization of chitosan nanoparticles and crude chitosan extracted from carapace of freshwater crab *Sartoriana spinigera* (Wood-Mason,1871)

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Received : 19th January, 2024 ; Revised : 24th February, 2024
DOI:-https://doi.org/10.5281/zenodo.14257369

Abstract- Chitosan is deacetylated form of chitin extracted from exoskeleton of crustaceans such as crabs, prawn, and even cell wall of fungi. Chitosan being a hetero-polysaccharide and has proved to be biocompatible and also possesses hypo-cholesterolemic, anti-oxidative, drug delivery efficiencies. In the present study chitosan -in crude and nanoparticle form- has been extracted from carapace of freshwater crab *Sartoriana spinigera*, commonly found in Jharkhand, followed by their characterization to study their proper efficiency to perform various important functions. Average weight of crude chitosan extracted from carapace powder was 46.34%, and that of chitosan nanoparticle (by sTPP method) was 44.33%. Characterization by FTIR spectrum showed 7 absorption peaks confirming the structure of crude chitosan and chitosan nanoparticle. Degree of deacetylation of crude chitosan was 88.75% and that of chitosan nanoparticle was 92.25%. Average particle size of crude chitosan and nanoparticle was found to be 398±5 nm and 297.5±2.23 nm respectively. XRD of crude chitosan showed a peak at 2θ around 20° typifying its amorphous and pure structure. XRD of chitosan nanoparticle showed a peak at 2θ around 12°, 20°, 28°, 34° typifying its amorphous and complex structure after extraction by sTPP method. SEM results showed smooth, crystalline structure of both crude and chitosan nanoparticle. On the basis of characterization, it can be said that Chitosan in both crude and nanoparticle form extracted from *Sartoriana spinigera* can now be used as molecules to study their further efficiencies.

Key words: Chitosan, Nanoparticle, *Sartoriana spinigera*, Characterization

INTRODUCTION

Presently, as fatal diseases are rising day by day, society is moving towards cure that are of natural origin. Due to this, scientific community is also shifting its interest from strong harmful chemicals to finding natural bioactive substances as medicines. One such substance is Chitosan, which is a linear hetero-polysaccharide biopolymer, made up of β-1, -2-deoxy-2-amino-D- glucopyranose and β-1,4-2-deoxy-2-acetamido-D- glucopyranose residues.[1] Chitosan is a deacetylated form of chitin which is found in exoskeleton of crustaceans, insects and cell wall of fungi. Chitosan has the properties of being bioactive, biocompatible and non-toxic.[2] It is an important biopolymer that has proved to have medicinal efficacies.[3,4] However, these properties of chitosan depend on the process of extraction and characters of the molecule. Use of harsh chemicals during extraction process not only destroys its molecular structure, but also its basic characteristics such as degree of deacetylation, crystallinity, pH and more.

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Nanoparticle form of chitosan has proved to show better cationic effect, absorption enhancement effect[5] due to their smaller size. Most extractions of chitosan and its nanoparticle form have been done from marine animals.

In the present study, chitosan nanoparticle has been extracted from carapace of a freshwater crab *Sartoriana spinigera*, which is abundantly found in Jharkhand, India, during rainy season.[6] *Sartoriana spinigera* is of ethnobiological significance, as it is consumed by the tribals of Jharkhand to cure various ailments.

Aim of the present study was to extract crude chitosan from carapace of *S.spinigera*, convert it into nanoparticle form and then compare their physical and molecular characters.

MATERIALS & METHODS

Live specimen of *Sartoriana spinigera* were collected, their carapace was removed, washed and dried to obtain constant weight.

Extraction of crude chitosan from carapace of *Sartoriana spinigera*

Extraction of crude chitosan was done following the method of Takiguchi (1991)[7]. It involves following process of demineralization in 1N HCl, deproteinization in 4% NaOH and deacetylation in 40 % NaOH.

Conversion of crude chitosan into nanoparticle

Conversion of crude chitosan into nanoparticle form was done by sTPP method.[8]

Characterization of crude chitosan and nanoparticle

1. Physical characters such as pH, colour and % yield was measured.
2. FTIR spectrum: The FTIR- spectrum of standard chitosan[9] was compared with FTIR spectrum graph of crude chitosan and its nanoparticle form from carapace of *Sartoriana spinigera*.
3. Degree of deacetylation: DD% was calculated using the following Brugnerotto *et al.* (2010)[10]
4. Particle size: measurement of particle size of extracted samples of crude chitosan and chitosan nanoparticles was done by Zeta size.
5. Crystallinity: structure of samples and their purity was detected by X-ray diffraction at 2θ.
6. Scanning electron microscopy: by studying SEM images at different magnification, difference in surface of crude chitosan and nanoparticles was studied.

Samples were sent to Cytogene, Lucknow to study FTIR, Particle size, Crystallinity and SEM.

RESULTS & DISCUSSION

Table 1- Weight of respective components obtained after demineralization, deproteinization and deacetylation of dry carapace powder, and final weight of chitosan nanoparticle obtained

Batch	Weight of Dry carapace powder (g)	Weight of chitosan (g)	Weight of chitosan nanoparticle from crude chitosan (g)
1	20	9.62	8.85 (92%)
2	20	9.82	8.83 (90%)
3	20	9.14	8.23(90%)
Average	**20**	**9.52±0.49**	**8.63±0.43**

Results are presented in Table 1 and Figures 1-4. Table 1 showed an average of 9.52± 0.49 g of crude chitosan was obtained from 20 g of carapace powder of *Sartoriana spinigera*. After treatment with sTPP, an average of 8.63±0.43 g nanoparticle was obtained. Average chitosan nanoparticle yield was found to be 42.33% from carapace of *Sartoriana spinigera*.

pH of both crude chitosan and nanoparticle was found to be 7. Colour of crude chitosan was pale yellow and that of nanoparticle was creamy white. In the present study, conversion of crude chitosan into nanoparticle is done by using sTPP (sodium tripolyphosphate). The process is based on Ionic gelation method in which nanoparticles are formed as a result of electrostatic interaction between cationic chitosan and anionic tripolyphosphate. Other process of chitosan nanoparticle extraction includes Microemulsion micelles method, emulsification solvent diffusion method[11], but these involve harsh chemicals. Ionic gelation method is a simple extraction procedure with use of mild chemicals, and has been used by various researchers.

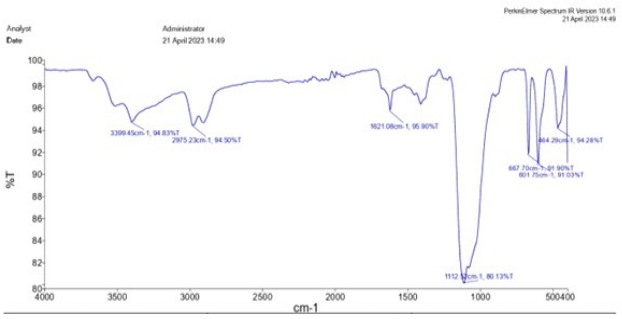

Fig 1: FTIR spectra of crude chitosan extracted from carapace of *Sartoriana spinigera* (% transmission/cm wave number)

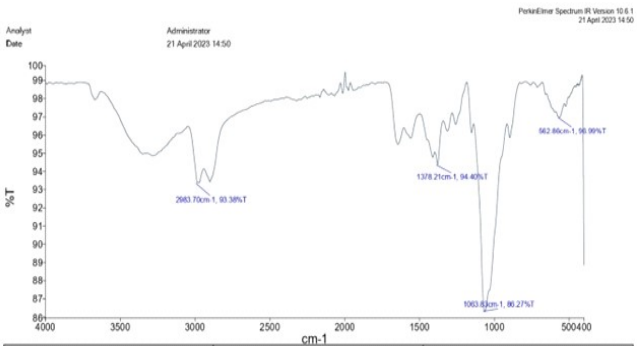

Fig 2: FTIR spectra of chitosan nanoparticle extracted from carapace of *Sartoriana spinigera* (% transmission/cm wave number)

Similarity of major peaks were observed between standard chitosan[9] and crude chitosan and nanoparticle extracted from carapace of *S.spinigera*. 7 out of 9 bands indicating ring stretching, CO stretching, CH2 bending and CH3 deformation, Amide II band, amide I band,CH stretching and NH stretching are found to be similar.

After calculation, degree of deacetylation of crude chitosan from carapace of *Sartoriana spinigera* was found to be 89.75%, and that of chitosan nanoparticle from carapace of *S.spinigera* was 92.25%. Higher degree of deacetylation indicates high quality of chitosan. DD% of chitosan extracted from cuttle bone *Sepia prashadi* was found to be 55.95%.[12] DD% of chitosan of shrimp waste was 89.79%.[9]

Particle size of crude chitosan was 398±5 nm and that of chitosan nanoparticle was 297.5±2.23 nm.

XRD patterns of crude chitosan and chitosan nanoparticles revealed the crystalline phase of the materials. A single characteristic peak at 2 θ ~ 20° was seen in case of crude chitosan extracted from *S.spinigera*, indicating its pure structure. However, chitosan nanoparticle showed more peaks at 12°, 20°, 28°, 34° typifying its amorphous and complex structure after extraction by sTPP method.

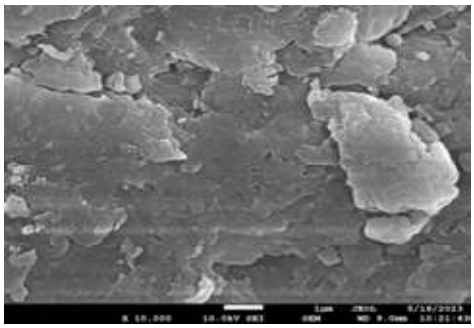

Fig 3: SEM image of crude chitosan extracted from carapace of *S.spinigera*

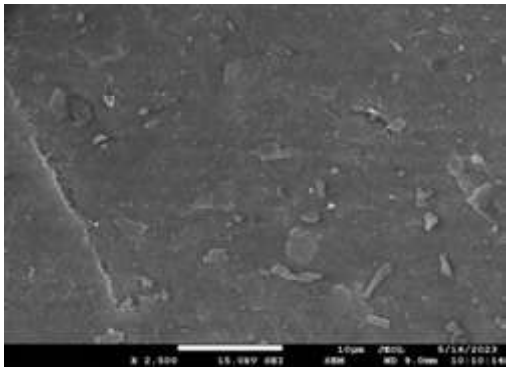

Fig 4: SEM image of chitosan nanoparticle extracted from carapace of *S.spinigera*

SEM studies were done to reveal the surface morphology. Crude chitosan showed rough surface of particles (fig. 3). However, fig. 4 showed smooth structure of chitosan nanoparticle.

The present study shows work on extraction of crude chitosan from carapace of freshwater crab *Sartoriana spinigera* and its conversion into nanoparticle form by sTPP method., which is a mild and simple process of conversion. Physical and chemical characterization of both crude and nanoparticle form of chitosan revealed their efficient capacity to be used in field of pharmacology. Chitosan nanoparticle of size 298 nm was obtained in the present study. High Degree of deacetylation, specific peaks in XRD pattern and smooth and nearly spherical structure of chitosan nanoparticle extracted from carapace of *S. spinigera* proves its efficiency to be used in many potential applications. Such biopolymers have a potent capacity to replace synthetic drugs with side effects.

ACKNOWLEDGEMENT

The researchers are thankful to ZSI, Kolkata for species identification of samples. They are also grateful to Cytogene laboratory, Lucknow for characterization of samples.

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