

Identification and Isolation of Chitin and Chitosan from Cuttle Bone of *Sepia prashadi* Winckworth, 1936

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Abstract: The potential of chitin and its derivatives mainly chitosan extends to various existing and proposed applications in an unusually large number of fields. In the present study, the prospect for using the exoskeleton of cephalopods as a raw material for chitosan manufacture was tried out. This includes the extraction of chitin from the cuttlebone (by deproteinization and demineralization) and chitosan by deacetylating the chitin. The chitin yield was 24% in the cuttlebone of *S. prashadi* and the chitosan yield was 62.6% from the chitin. The FTIR spectrum of chitin and chitosan was also confirming the presence of chitin and chitosan in the resultant sample analyzed. The degree of acetylation of chitosan was calculated as 55.95% using the FTIR stretching bands. The yield was higher than that the crab shells and other sources. So the results of the present study throw more light on the study expanding the tentacles to find out other potential sources for the extraction of chitin and chitosan.

Key words: Chitin • Chitosan • *S. prashadi*

INTRODUCTION

Chitin is a naturally occurring nitrogen containing polysaccharide related chemically to cellulose poly- β - (1>4)-N-acetyl-D - glucosamine [1], which forms a semitransparent horny substance and is principal constituent of the exoskeleton or the outer covering of insects, crustacean and arachnids. It is generally insoluble in alkaline solution and organic solvent [2]. Chitin and its derivatives find applications in various field mainly paper making, textiles printing and sizing, flocculation, ion exchange chromatography, removal of metal ions from industrial effluents, manufacture of pharmaceuticals and cosmetics and as an additive in food industry [3].

Chitosan (poly- β - (1>4)-glucosamine), a derivative of chitin, has attracted the interest of many researchers and industries owing to its antimicrobial activity, biocompatibility and biodegradability. They were also found to interact strongly with pesticides and metal ions in aqueous solutions [4-8]. Chitin based materials are also used for the treatment of industrial pollutants and adsorbs silver thiosulfate complexes and actinides. They are also used in wastewater treatment and will trap pollutants such as insecticides (DDT) and PCBS [9].

Chitosan can be used in the thin layer chromatography for the separation of the nucleic acid [2]. Diabetic ulcers were also showed immediate response to chitosan [3]. The ability of Chitosan to remove tannin and to deacidify coffee extract are utilized in the coffee industry and for clarification of such beverages as wine, beer and fruit juices and pressed or extracted fermented or unfermented vegetables. Chitosan which selectively aggregates leukemia cells *in vitro* is suitable for the development of novel antitumour agents. Chitosan has also been found to be effective in the removal of petroleum and petroleum products from waste water. Chitosan and other derivates have a wide application to ion exchange chromatography, chelation chromatography, high pressure liquid chromatography, gel chromatography and thin layer chromatography [10].

The marine environment is an exceptional reservoir of bioactive natural products, many of which exhibit structural/ chemical features not found in terrestrial natural products. Now the attention of the researchers has been turned towards the marine biota. Since most of the marine animals are found to be having a wide variety of bioactive substances. Many classes of bioactive compounds exhibiting antitumour, antileukemia,

antibacterial and antiviral activities have been reported worldwide to be accumulated in marine invertebrates [11-15].

Though wide variety cephalopods are available, only limited studies have been carried out on these animals. Out of 2,292 tones of cephalopod landings on all-India basis, the cuttlefishes rank first with nearly 60% of the total catch; whereas the squids contributed less than 40% [16]. Cuttlebones are widely used as a homemade remedy for ear ache and skin diseases in India and China [17]. Further the cuttlebone also reported antibacterial and antifungal activity against some of the human pathogenic microorganisms [18].

Hence the present attempt has been made to identify and isolate natural polymer chitin and its derivative chitosan from the cuttlebone of *S. prashadi* Winckworth, 1936 (Phylum: Mollusca; Class: Cephalopoda).

MATERIALS AND METHODS

Sampling: Cuttlefish *S. prashadi* specimen (Fig. 1a) were collected from Cuddalore landing centre (Latitude 10°42' N; Longitude 79°46' E), south east coast of India. Cuttlebones (Fig. 1b) were removed from the animal, washed, dried and pulverized with pestle and mortar into fine powder and used for further studies.

Chitin was extracted from the squid pen following the method of Takiguchi [19] by demineralising and deproteinising the cuttlebone powder. Chitosan was extracted from the chitin through deacetylation process following the method of Takiguchi [20].

Fourier Transform Infra Red (FTIR) Spectrum: FT-IR spectroscopy of solid samples of *S. prashadi* and standard chitin and chitosan from crab shell relied on a Bio-Rad FTIS - 40 model, USA. Sample (10µg) was mixed with 100µg of dried Potassium Bromide (KBr) and compressed to prepare a salt disc (10mm diameter) for reading the spectrum further. Degree of acetylation (DA) of chitosan was calculated following the method of Baxter *et al.* (1992) [21] using the stretching bands of FTIR spectrum.

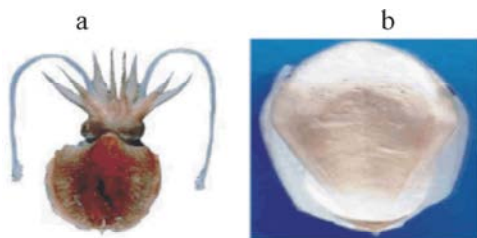


Fig. 1: Isolation of chitin and chitosan
a. *S. prashadi*; b. Cuttle bone.

RESULTS AND DISCUSSION

In recent years, great attention has been paid to the bioactivity of natural products obtained from plant, animal and in addition of marine origin. Chitin was extracted from squid pens (*Loligo vulgaris*) using two different methods. The physico chemical characteristic (degree of acetylation and molecular weight) of the chitosan was tested using HNMR and CNMR studies [22]. Muzzarelli *et al.* [23] depolymerised the chitosan by a wheat germ lipase preparation showing the logarithmic concentration of its lipase substance and its involvement in dietary. Das *et al.* [24] extracted chitin from the shell of two coastal portunid crabs of Bangladesh. Shigamassa and Al - Khateeb [25] investigated various acetylated chitosan derivatives and mixture of chitin and chitosan. The range of the degree of deacetylation (DDA) was analyzed by H-NMR spectroscopy and infrared spectroscopy using the different absorption bands such as amide I bands at 1655 cm⁻¹ and 1630cm⁻¹ or amide II band at 1560 cm⁻¹. Brugnerotto *et al.* [26] studied the use of infrared spectroscopy for characterization of the composition of chitin and chitosan covering the entire range of degree of acetylation (DA). The IR spectra of the structural units of these polymers validated the choice of base lines and characteristic bands.

Vikhoreva *et al.* [27] reported the preparation and anticoagulant activity of low-molecular weight sulfated chitosan shown by FTIR, NMR-methods and elemental analysis also demonstrated the anticoagulant activity of chitosan sulphates and antiXa activity like heparins. Lee *et al.* [28] observed that DNA/ Chitosan self aggregate complexes could be critical in the design and tailoring of novel gene delivery systems using non-viral vectors.

β - Chitin extracted from squid pens of *L. plei* and *C. sanpaulensis* was tested by applying different treatment, acid and alkali attacks, to evaluate its effects on the polymers purity and structure [29]. The anti - oxidant potency of chitin derivative-glucosamine HCl was investigated by employing various established in-vitro system, such as super oxide (O₂⁻)/ hydroxyl (ox) - radical scavenging, reducing power and ferrous ion chelating potency [30].

Chitin and chitosan are used in the preparation of the materials used in wound healing, antibacterial, antifungal agents, dialysis membrane, biomedical beads, fabrics and gauzes [31]. Nair and Madavan [32] and Das *et al.* [24] demonstrated that the crab, *Scylla serrata* and *Portunus pelagicus* contains 16.07% and 20.19% of chitin respectively from the body shell waste. The cuttlebone of *S. officinalis* was found to possess 20% of chitin [22, 33], whereas, in general, the squid/ octopus reported 3-20% of

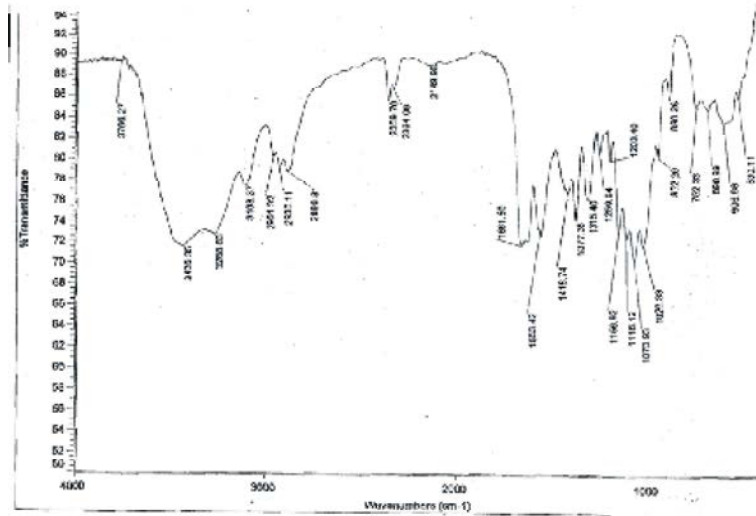


Fig. 2: FT-IR spectrum of standard chitin.

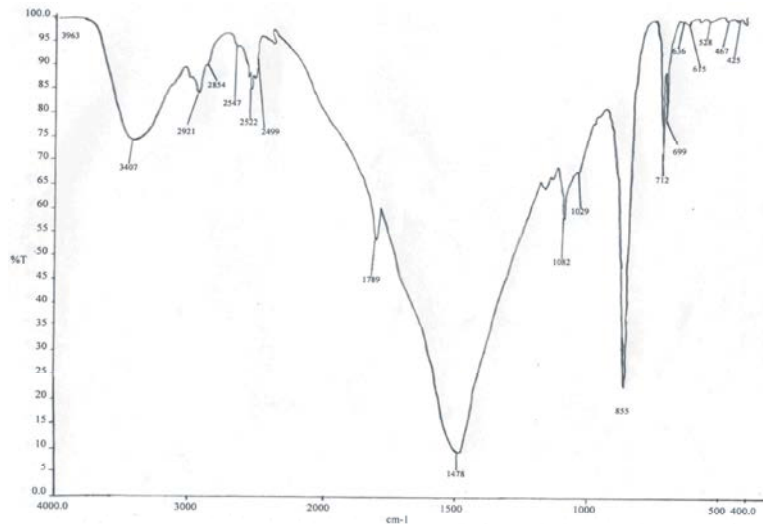


Fig. 3: FT-IR spectrum of chitin from *S. prashadi*.

chitin [2]. But in the present study, the yield of chitin from the cuttlebone of *S. prashadi* was found to be higher (24%) than the previous reports.

The percentage yield of chitin and chitosan were found as 24% and 62.6% respectively. The FTIR spectrum of the chitin and chitosan was obtained and the effective peaks were compared with that of the standard chitin and chitosan. The FTIR spectrum of the standard chitin contains eleven major peaks at the range of 690.99, 752.33, 896.28, 1026.33, 1073.93, 1418.74, 1661.50, 2886.81, 2961.32, 3268.63 and 3436.35 cm^{-1} (Fig. 2); whereas the FTIR spectrum of the *S. prashadi* sample also recorded the same number of peaks lying between 699 and 3407 cm^{-1} (Fig. 3).

The FTIR spectrum of the standard chitosan showed twelve major peaks at the range of 897.41, 1026.63, 1077.93, 1154.64, 1259.54, 1422.73, 1587.94, 1660.55, 2361.41, 2922.85, 2922.85 and 3377.95 cm^{-1} (Fig.4); whereas the chitosan sample from *S. prashadi* recorded the peaks in the range of 904 to 3377 cm^{-1} (Fig. 5). The usefulness of chitosan as a wound healing accelerator and its effectiveness in protecting wound from bacterial invasion by suppressing bacterial proliferation has been well documented [34]. Varghese (2002) [35] reported a chitosan yield of 4.4% to 8.3% in different size groups of mantis shrimp *H. nelanoura*. The properties of chitosan depend on various intrinsic parameters such as percentage of degree of deacetylation (DDA) and molecular weight

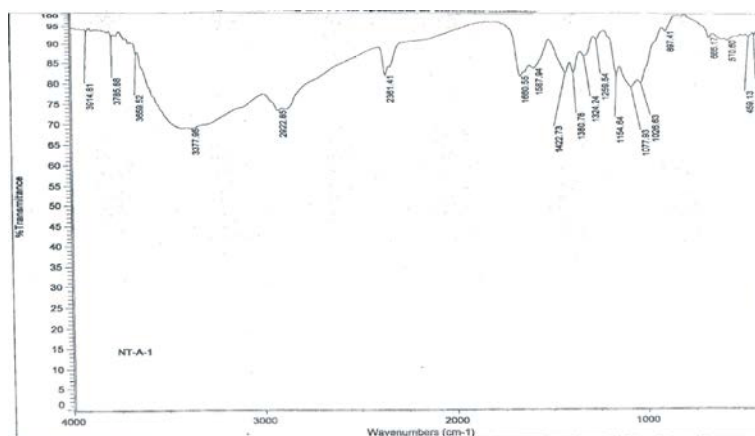


Fig. 4: FT-IR spectrum of standard chitosan.

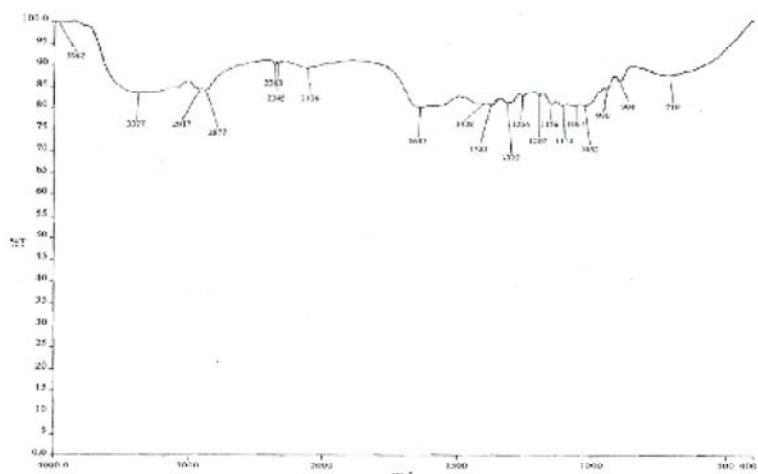


Fig. 5: FT-IR spectrum of chitosan from *S. prashadi*.

[36, 37]. The amide I band shows a well defined peak at 1650 cm^{-1} with a minor shoulder at 1625 cm^{-1} 3450 cm^{-1} (corresponding to that located at 3350 cm^{-1} for the structural units) as the reference one and the band at 1320 cm^{-1} characteristics of -OH, -NH₂, -CO groups was chosen to measure the extent of N-acetylation [26]. The characterization of a chitosan sample requires the determination of its average DA. The degree of deacetylation of chitin about 50% (depending on the origin of the polymer), it becomes soluble in aqueous acidic media and is called chitosan. The solubilization occurs by protonation of the -NH₂ function on the C-2 position of the D-glucosamine repeat unit, whereby the polysaccharide is converted to a polyelectrolyte in acidic media [38]. In our investigation, the degree of acetylation was found to be 55.95%.

In the study of IR spectra of N-acetyl-D-glucosamine and D-glucosamine, it has been reported [26] that the repeating units in these polymers; comparing both

spectra, it could be appreciated that a specific band appears at 1320 cm^{-1} for N-acetylglucosamine. The band located at 2900 cm^{-1} , often used in the literature as reference band to analyze chitin and chitosan, must be excluded as, for glucosamine, it may not be distinguished from the background. As reference peak it has been [26] either the large band centered at 3350 cm^{-1} (very near to that at 3450 cm^{-1} chosen for polymers) or the 1420 cm^{-1} band, which also seems to be suitable from the comparison of the two monomers. Parasakthi (2004) [39] observed the FTIR peaks at 534.61 cm^{-1} , 1024.16 cm^{-1} , 1321.88 cm^{-1} , 1380.81 cm^{-1} and 1640.40 cm^{-1} in chitin from the shell of *S. aculeata* which resembling the peaks of crab carapace, legs and the claw. Whereas in the chitin sample of *Loligo duvauceli* the peaks at 533.44 cm^{-1} , 896.73 cm^{-1} , 1032.11 cm^{-1} , 1315.60 cm^{-1} , 1377.88 cm^{-1} , 1561.36 cm^{-1} and 1657.29 cm^{-1} were also coincided with that of the cuttlebone samples confirming the presence of chitin.

Saraswathy *et al.* (2001) [40] have observed the major absorption band between 1220 and 1020 cm^{-1} represents the free amino group ($-\text{NH}_2$) at C_2 position of glucosamine, a major present in chitosan. Further the sample showed the absorption based for the free amino group between 1023.15 cm^{-1} and 1259.99 cm^{-1} (carapace), 1022.58 cm^{-1} and 1260.30 cm^{-1} (claw) and 1022.41 cm^{-1} and 1260.20 cm^{-1} (leg). When the peak at 1384 cm^{-1} represents the C-O stretching of primary alcoholic group ($-\text{CH}_2\text{-OH}$). The primary alcoholic group represented by a band in 1378.54 cm^{-1} (carapace), 1380.14 cm^{-1} (claw) and 1379.92 cm^{-1} (legs) respectively. The absorbance bands of 3450 cm^{-1} , 2878 cm^{-1} , 1420 cm^{-1} , 1655 cm^{-1} and 1320 cm^{-1} indicated the hydroxyl stretching, C-H stretching, C-H deformations, C = O stretching in secondary amide (amide I) and C-N stretching in secondary amide (amide III) respectively [41]. In the present study observe the same absorbance band in the level of 3377 cm^{-1} , 2877 cm^{-1} , 1440 cm^{-1} , 1643 cm^{-1} and 1320 cm^{-1} . A more detected structure in the 3500 - 3000 cm^{-1} region was observed for α chitin compared to β chitin [42]. In this investigation also the structural stretching band was observed in the range of 3470 cm^{-1} .

Chitosan is the only pseudo natural cationic polymer and thus, it finds many applications that follow from its unique character (flocculants for protein recovery, depollution, etc.). Being soluble in aqueous solutions, it is largely used in different applications as solutions, gels, or films and fibers. The slow growth of the industry has been attributed by some to the negative role of patents, which have tended to show down market development. It is also believed that instead of examining ways of using chitin/ chitosan as a substitute for ingredients already in usage in agriculture and industry. More research on identifying specific uses and advantages would help expand the spectrum.

CONCLUSIONS

The natural biological properties of chitin and chitosan are valuable for both plant and animal applications and such developments can be considered as valuable extensions of the use of the same. Nowadays chitin and chitosan were commercially prepared from crab shells. In the present investigation chitin and chitosan were isolated from cuttlebone of *S. prashadi*. The yield was higher than that of the crab shells and other sources. So the results of the present study throw more light on the study expanding the tentacles to find out other potential sources for the extraction of chitin and chitosan.

The result of the present study also records the utilization of not only chitin and chitosan but also the derivatives of raw materials and products to be used in a wide spectrum of fields and applications.

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