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Synthesis of Nanochitosan membranes from Shrimp shells

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Abstract

Chitosan is the second most abundant natural polysaccharide after cellulose, which is the most widely used and distributed biological material. Chitosan is derived from chitin, which is a structural component of the external structures of crustaceans such as shrimp and crab, and it can be used to create nanoparticles in a number of ways. The formation of its excellent film properties can be used to create films, fiber and water gels containing water / nano particles min. The goal of this study is to easily and cheaply extract and characterize nanochitosan from marine crustaceans, as well as to develop nanochitosan membranes. Chemical-mechanical method, acid hydrolysis, sonication and filtration were used, where nano-chitosan membranes is obtained by casting and evaporation. Techniques used for characterization included Transmission electron microscopy (TEM), Fourier transform infrared (FT-IR), Scanning electron microscopy (FEI-SEM). Functional groups of chitosan characterized using Infra Red absorption showed several peaks. SEM micrographs showed roughly spherical nanochitosan particle diameters ranging from 22.1 to 51.6 nm. Image TEM showed spherical shaped chitosan nanoparticles with diameters ranging from 21 nm to 34 nm were obtained. Chitosan was obtained as a white powder and was highly soluble in 1% acetic acid using the procedure developed in this study. The findings point to the development of distinct films with high transparency, strength, and durability suitable for pharmaceutical and medical applications.

Keywords: Nanochitosan, shrimp shells, membrane

1. Introduction

Chitosan is derived from chitin, a structural component found in crustacean exoskeletons such as shrimp and crabs, and it can be used to create nanoparticles in a variety of ways [1]. Because of the proton amino groups, it interacts with polyanions to form multi-electrolyte complexes due to its faltering character. Chitosan is a biopolymer consisting of poly (1-4) -2-amino-2-deoxy-d-glucopyranose and it consists of an amino group in its composition [2], which is a larger natural polysaccharide after cellulose, the most widely used and distributed biological material. It has excellent film-forming properties and can be used to create films, fibers, and hydrogels containing water micro/nano-particles. Use the functions of OH and NH2 in the structure of chitosan to create a variety of products for specific applications with improved properties [3].

Chitosan a polymer with distinct and valuable properties, as it is a material with important technological and economic properties, as well as biological properties such as biocompatibility, bioactivity, and biodegradation, in addition to being non-toxic and derived from renewable natural resources. This is because some human enzymes, such as chitosan biodegradation lysozyme, are involved in the polymer metabolism process. This property is consistent with one of the biodegradation process's basic requirements, that it is susceptible to an enzymatic reaction Furthermore, chitosan can be produced in a number of different forms, including films, membranes, and porous structures [4]. Physical chitosan modification entails shrinking the particle size or chitosan granules so that it can be used more widely. Physical modification produces nanoparticle size [5], which involves de-acetylation of chitin molecules by removing the acetyl group and determining the single-group (-NH2) content in chitosan. Repeating the process can result in acetylation values of up to 98 percent, but this heterogeneous dea acetylation process can never achieve complete dea acetylation without modification[6] . This study aimed to extract and characterize nanochitosan from marine crustaceans

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and the development of nanochitosan membranes in an easy and inexpensive way

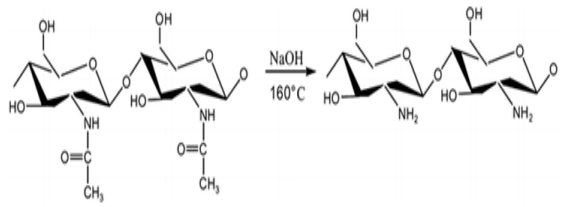


Fig 1. Preparation of chitosan from chitin

Chitosan has unique properties that make it useful in a variety of applications. However, due to its anti-bacterial erotic interesting, such as the strong influence, and bio-compatibility, and biodegradable, non-toxic, and the absorption of high humidity, chitin and chitosan are now receiving a lot of attention from researchers all over the world [6]. It has applications in a wide range of fields. The ability to immobilize microorganisms and speed wound healing [4]. Chitosan is also used for drug delivery [5].

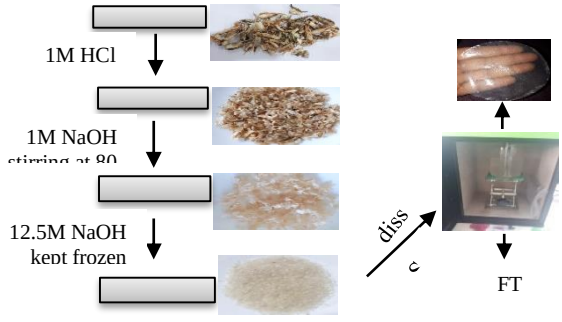
Tissue engineering is the science that combines engineering materials and biomedicine to create materials that can replace and/or stimulate the regeneration processes that occur in damaged tissues [7]. Tissue engineering is the process of repairing, replacing, maintaining, or improving the function of a tissue or organ [8]. Polymer scaffolds for tissue engineering must meet a few basic criteria that scientists have widely accepted. The scaffold should have a high porosity, a well-distributed pore size distribution, and a large surface area. Another condition is biodegradation, which occurs when the rate of lysis equals the rate of tissue formation. In addition, the scaffold must have the necessary structural integrity and mechanical properties to prevent the scaffold pores from collapsing during tissue neoformation. Finally, the scaffold should be non-cytotoxic and biocompatible, allowing cells to adhere, proliferate, migrate, and differentiate more effectively. [9]. Chitin or chitosan / nBGC hybrid scaffolds were developed using the lyophilization technique. Scaffolding revealed that a porous composite was sufficient when it was uniformly distributed nBGC (active glass ceramic particles biologically) on the pores' walls. Swelling and degradation properties, as well as the ability to become biologically active, were all demonstrated in the developed nanoscaffolds [6].

Due to the great mechanical strength of the chitosan, as well as the permeability of urea and creatinine [10]. Chitosan could be used as a substitute for a synthetic renal membrane. Many researchers studied the characteristics of the chitosan membrane's formation and proposed a variety of chitosan membranes for applications such as reverse osmosis, ion exchange, metal ion absorption, pigment spread, and the separation of mixed systems containing water and alcohol. [11].

Chitosan has several important properties for treating various types of wounds, including antifungal, bactericidal, and oxygen permeability. As a result, chitosan was modified into various forms for the treatment of wounds and burns; wound dressing applications typically require a biodegradable and biodegradable dressing [12-13]. Chitosan is a natural polymer with a wide range of properties, including biocompatibility, biodegradability, hemostatic activity, and antibacterial activity [14-15].

Chitosan can be used to treat the skin after burns. Chitosan is a solid, biocompatible substance with water absorption. Another benefit of this type of chitosan treatment is that it has a high oxygen permeability, which is necessary to avoid a lack of oxygen in the affected tissues, and the chitosan coating can absorb water and is destroyed by the body's enzymes automatically., this means that it does not need to remove chitosan from the burnt part from the affected tissue. Because in most injuries, especially burns, removing the wound covering can damage the affected site [5]. .

Chitosan has excellent physico-chemical properties that appear to make it a good candidate for DNA delivery by overcoming systemic barriers. When electrostatically interacting with nucleic acids, chitosan easily forms complex microscopes or nanoparticles. Chitosan's immunostimulatory properties have been known for more than two decades. However, its potential as a non-toxic and safe aid in the development of cancer vaccines was only recently realized Surprisingly, chitosan appears to have no toxicity when used as a gene delivery vehicle [16-18]. It was studied the ability of chitosan to promote the growth and deposition of material rich in minerals by osteoblastic widely in bone tissue engineering [19-21]. Several studies have focused on calcium phosphate and chitosan (CP) compounds for this purpose in bone tissue engineering [22-23].



2. Materials and methods

Materials

Sodium Hydroxide from BDH Chemicals, Hydrochloric acid from Central drug House- India,

acetic acid glacial from Alpha Chemika – India, Ethanol from Hayman kimia.

Methods of the study

1.Synthesis of Chitosan

-Extraction of Chitin

A- Demineralization

500 ml of 50% HCl was added to 50 g of shrimp shells to demineralize them. For two hours, the reaction was started with stirring at 35 °C. The shells were then filtered and washed several times with distilled water to achieve a pH of 7. It has been bleached in ethanol immerse for 30 min, dried in 70 ° C.

B- Deproteinization.

The dried, demineralized shells were deproteinized with sodium hydroxide at a concentration 1M of 1:10 (g/ml), solid:liquid ratio for 3 hours, the reaction was stirred at 80 °C. Steel was filtered and washed with distilled water until the PH =7. It was then dipped in ethanol for 30 min to further bleach it, the resulting chitin was dried in a 70 °C oven.

C. Manufacturing of Chitosan.

It has been achieved chitin by chitin interaction with 12.5 Molar sodium hydroxide by a solid / liquid of 1:15 (g / ml). For 24 hours, mixture cools and freezes. The mixture was then heated up to 115 °C, and the reaction was stirred for 4 h. The resulting chitosan filtration and washed with distilled water to achieve a pH 7, and dried in an oven 70 ° C. [3]. Suit the degree acetylated disarmament (DA) with the degree of chitosan shift from chitin, which is dependent on the sodium hydroxide concentration as well as the reaction temperature and time, depending on the manufacturing method [6].

2-*Extraction of nanochitosan.*

An emulsion was created by dissolving chitosan 1% (w/v) in glacial acetic acid 1% (v/v) and stirring. Using (0.1 M) NaOH, the pH of the solution was to 6. The product was ultrasonicated for 30 minutes to reduce the size of the chitosan particles [24]. At an interval of one minute every 10 min.

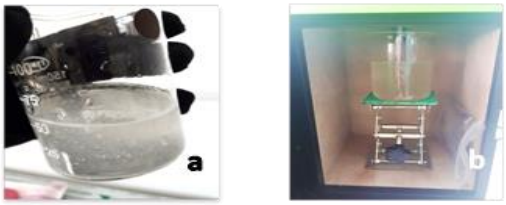


Fig 2. (a) Chitosan is in gel form after being dissolved with glacial acetic acid, (b) Ultrasound dispersion of chitosan gel

- Identification of Nanochitosan

1. Fourier Transform Infrared (FTIR).

To investigate the change of functional groups, the FTIR spectra of nanochitosan were measured with FTIR (Thermo Fisher Scientific) and a Hyperion Scanning Microscope. The samples were ground and combined with KBr. The powder that resulted was ground and pressed into fine granules, and its absorbance was measured.

2. Environmental Scanning Electron Microscopy (FEI-SEM).

The electron microscopy (FEI-SEM (ZEISS SIGMA VP)) model EM10C was used to characterize NCS prepared from shrimp shells at various magnification levels.

3. Transmission Electron Microscope (TEM).

The diameters of the synthesized prepared from shrimp shells were examined using an electron microscope (ZEISS LEO 912) model AB-100 KV of FEI technology.

-Preparation of nanochitosan membranes.

After sonication, 30 ml of nanochitosan was taken and poured into Petri dishes until evaporated and then left to dry completely. It has been carefully separated from the plate.

3. Results and discussion

3.1 Description Nanochitosan prepared from shrimp shells.

Chitosan samples were prepared for the various reaction conditions that were chosen. Chitosan was obtained as a white transparent powder, indicating the effectiveness of the bleaching treatments carried out after the purification and protein removal steps. Chitosan is dissolved with 1% acetic acid and a gel-like solution is formed. After treatment it becomes transparent liquid form Fig 3.

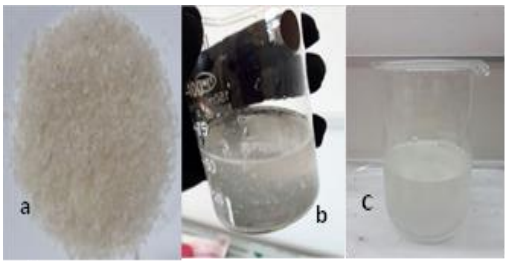


Fig 3. (a) Chitosan powder, (b) Chitosan after being dissolved in acetic acid, (c) Nanochitosan

-Nanochitosan identification.

1-FT-IR determination of nanochitosan.

The FTIR of the chitosan samples in (Fig 4) show the characteristic absorption bands at 3450.97 cm1, which are attributed to an overlap of the groups the NH and OH stretching vibrations caused by the polymer's hydrogen bonds. The region 2909.41−2357.14 cm−1 representing the C−H (symmetric) bond, the absorption region at 1625.649 cm-1 indicates (-C = O stretching) of amide I, as a result. The band 1384.090 cm-1 indicates NH2, 1059,740 cm-1 refer to the chemical group C - O - C (glucose - β-1 -4), the saccharide structure is characterized by skeletal vibrations involving C-O stretching. By analyzing the infrared spectrum of chitosan, this highlights the similarity of chitosan with its precursor in previous works which matches with [2- 3]. The FTIR for both forms of chitosan and nanochitosan showed very close uptake bands, but differed in intensity. It was found that the spectrum of nanochitosan has less intense absorption bands than chitosan, and it can be said that the main functional groups of chitosan have not

changed after hydrolysis with acetic acid and subjected to sonication. The FT-IR of the nanochitosan was given in the (Fig 5). The FTIR showed nanochitosan strong absorption bands at 3443.18182 cm-1 indicating the presence of OH hydroxyl group and NH stretching vibrations and the peak at 2925.97 cm-1 belongs to the CH stretching vibration and that at 1619.80591 cm-1 is ascribed to the NH2 bending. The peaks at 1043.18 cm-1 are assigned to C-O stretching vibrations.

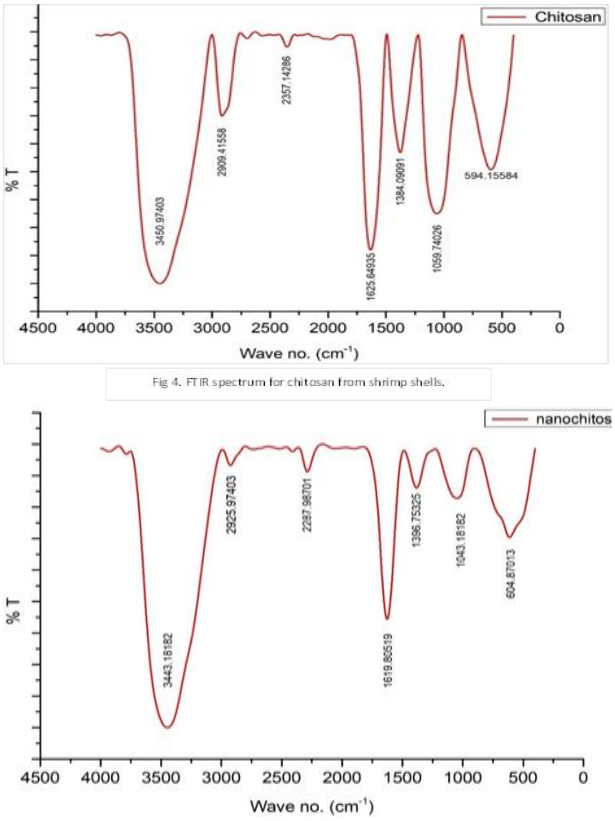


Fig 4. FTIR spectrum for chitosan from shrimp shells.

Fig 5. FTIR spectrum for nanochitosan from shrimp shells.

-Scanning electron microscopy technique (FE-SEM).

Analysis Calcium was found to be absent in the demineralized shells using EDS. It also depicts the elements that comprise chitosan (Fig 6). The Au signal peaks correspond to the sample holder. which carry the sample to be measured.

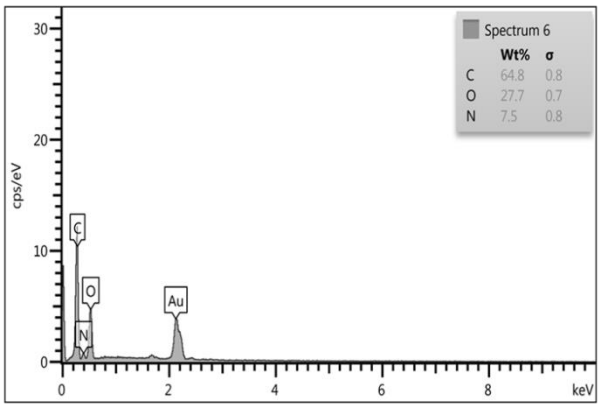


Fig 6. EDS for chitosan from shrimp shells

The diameter morphology of NCS was studied using SEM and energy dispersive spectroscopy (SEM-EDS), and the resulting microscopic images are shown in Figure 1. (Fig 7). The microstructure of nanochitosan was studied by SEM (Fig 7) and it showed nearly spherical NCS nanoparticles with nanochitosan particle diameters ranging from 22.1 to 516nm.

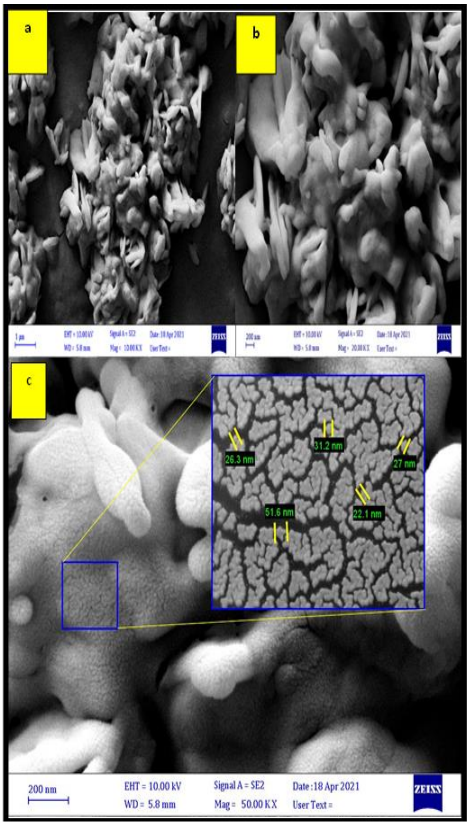


Fig 7. (a) SEM micrograph of Nanochitosan magnified to 10.0 KX. (b)SEM micrograph of NCS magnified to 20 KX. (c) SEM micrograph of NCS magnified to 50 KX

Fig 7. (a) SEM micrograph of Nanochitosan magnified to 10.0 KX. (b)SEM micrograph of NCS magnified to 20 KX. (c) SEM micrograph of NCS magnified to 50 KX.ganization of the manuscript.

TEM identification.

Image TEM (8) showed spherical shaped chitosan nanoparticles. Nanochitosan particles with diameters ranging from 21 nm to 34 nm were obtained. From the frequency graph in (Fig 8.c) it is clear that the breakdown of the amorphous parts in nanochitosan reaches a nanocrystalline scale of 20-50 nm.3.5.3 TEM identification.

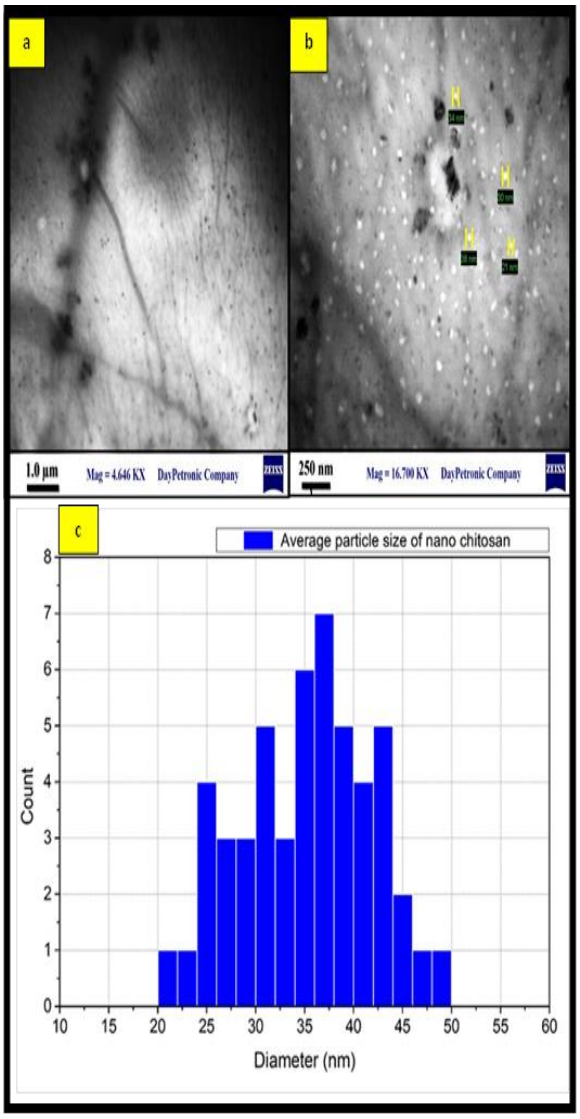


Fig 8. (a)TEM micrograph of nanochitosan at a magnified to 4.646 KX. (b) TEM micrograph of NCS magnified to 16.700 KX. (c) Diameter count distribution of NCS.

Characterization of nanochitosan membranes

Tensile strength is considered very important in determining the suitability of material for applications that have a drying temperature and methods of forming a large influence. The solvent evaporates faster with high temperature, causing the quick re-arrangement of strings winding chitosan molecules. The intermolecular gap closes, the bonding tightens, and the mechanical strength improves. Chitosan molecules in suspension have a high evaporation rate. During the drying process, it causes the formation of molecular hydrogen bonds to improve the mechanical properties of the product. In the case of films made using the solution casting method, the chitosan molecule is smaller and the repetition rate is lower than the molecular surface diffusion rate. Secondary drying of chitosan in natural solution causes severe deformation including shrinkage and wrinkling, as well as having great mechanical strength [25-26]. (Fig 9) also clearly shows that the film formed from the suspension is transparent and highly elastic.

Fig 9. Nanochitosan membrane

4. Conclusions

Chitosan, was obtained, is a white powder high solubility in acetic acid by 1%. Functional groups of chitosan characterized using Infra Red absorption showed several peaks. SEM micrographs showed several peaks showing the OH-functional groups of chitosan and showing the presence of amine groups. SEM and TEM image showing that chitosan nanoparticles are spherical in shape. The breakdown of the amorphous parts of the nanochitosan reaches the nanocrystalline scale of 20 to 50 nm. The findings point to the development of distinct films with high transparency, strength, and durability suitable for pharmaceutical and medical applications.

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