

Extraction and Characterization of Chitosan and Preparation of Nano-composites with Resorcinol Formaldehyde Resin

Ganesh Bhandari, Rajesh Pandit and Netra Lal Bhandari*

*Chemistry Department, Tri-Chandra Multiple Campus, Tribhuvan University, Kathmandu, Nepal
E-mail: netra.tu.edu@gmail.com*

Abstract

Chitosan is a functionalized form of N-deacetylation of chitin that has a linear chain of β -(1, 4)-glucosamine ($C_6H_{11}O_4N$)_n extracted from edible crustaceans such as crabs by demineralization, deproteinization and N-deacetylation respectively. It was characterized by Fourier transform infrared (FTIR) spectroscopy and X-Ray diffraction (XRD) analysis that revealed the size of Chitosan nanoparticles and confirm the presence of major functional groups as -OH, -NH₂, C=O, C-O-C, -CH₃ and -CH. Different composition composites of resorcinol formaldehyde resin of chitosan was prepared with solution casting and are characterized by compressive strength test. The result shows that relay about the decrease in the compressive strength with addition of chitosan in composites.

Keywords: *Chitosan is a functionalized, Nano-composites, Resorcinol Formaldehyde Resin*

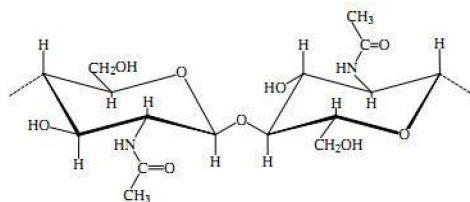
Introduction

Biopolymers such as chitin, collagen, hemicellulose are natural polymers that are abundantly available and extractable from natural sources which are replenished through plantations or growth of animal providing renewability compared to synthetic polymer. The problem associated with synthetic polymers such as toxicity and non-biodegradability is suppressed by the use of biopolymers with their immunogenic behavior^{1,2}. They are biodegradable and thus create minimum wastes. They are derived from biological compositions, including polysaccharides, protein and lipids³.

Polysaccharide are the polymers of carbohydrate molecules bound together by glycosidic linkages found in starch and glycogen, cellulose and chitin and when hydrolyzed gives different types of oligosaccharides and polysaccharides⁴.

Chitin is a nitrogenous long chain polymer of an N-acetyl glucose having a molecular formula (C₈H₁₃O₅N)_n. Chitin has used for several medicinal, industrial and biotechnological purposes⁵. It is synthesized from the *N-acetyl* -D-glucosamine. These units form covalent β -(1→4) - linkage (which is similar to the linkages between the glucose units of cellulose).

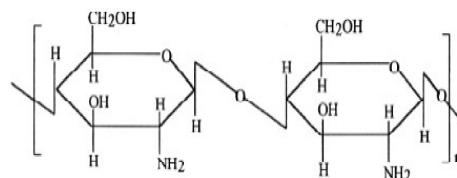
* *Corresponding author*



Scheme 1: Structure of Chitin

Chitosan is chemically defined as a copolymer of β -(1, 4) glucosamine ($C_6H_{11}O_4N$) n , with a varying content of N-acetyl groups produced by deacetylation of chitin. It contains the hydroxyl group (-OH) and mainly shows the chelating ability for many transition metal containing organometallic compounds.

Chitosan shows the properties like biocompatibility, biodegradability, hydrophilicity, biological activity and low-toxicity. The biological properties shown by chitosan are spermicidal, Immuno adjuvant, fungistatic and anticholesteremic behavior. The characteristics of chitosan in solutions depend on the degree of deacetylation. It is a colorless, crystalline or amorphous powder, which is insoluble in water, organic solvents and diluted acid and alkali. It contains the amino group which is highly reactive in nature. The presence of free $-NH_2$ groups, primary $-OH$ groups and secondary $-OH$ groups in chitosan makes it as a useful chromatographic support ⁶.



Scheme 2: Structure of chitosan

The primary source of chitin is the marine crustacean shell waste from shrimp, prawns and crabs. Shrimps are widely used as a sea food but due to insolubility nature of its shell it is causing pollution ⁷. However, the chemical conversion of shrimp into chitosan is taken largely under consideration. Biological and chemical methods can be employed for chitosan extraction which contain both demineralization and deproteinization stage. The chemical extraction method yields into higher percentage purity of chitin ⁵.

Both chitin and the chitin-derived chitosan have wide ranging applications in the field of biomedicine, microbiology, tissue engineering, food technology, agriculture, electrochemical technology, textile, energy and bio-nanotechnology ⁸. Chitin's purity plays an important role for biomedical application in order to avoid contamination. Chitosan membranes have been proposed as an artificial kidney membrane because of their suitable permeability and high tensile strength ^{9,10}.

Even having large number of advantages, thermal stability, hardness, gas barrier properties and bacteriostatic activity are acting as a barrier to meet its applications. Thus, modification (chemical modification, blending and graft copolymerization) of chitosan has gained importance to meet the desired properties.

Although having many advantages, it can biologically degrade under environmental conditions such as moisture, nutrient, microorganisms, temperature and others. It can be taken as advantageous when compared to synthetic polymer as they take millions of years to be degraded³. Furthermore, chitosan can be extracted from various sources such as crustacean, algae that differ in physiochemical properties such as molecular weight, polymer structure, stereochemistry, primary sequence and chemical reactivity, etc.

Resorcinol crystallizes from benzene as colorless needles that are readily soluble in water, alcohol, and ether, but insoluble in chloroform and carbon disulfide. Resorcinol reacts with formaldehyde to produce partially condensed reaction products. Chitosan composite is formed from resorcinol formaldehyde. The reaction is exothermic so that the heat is maintained during the reaction interval. Resorcinol is also used as a chemical intermediate for the synthesis of pharmaceuticals and other organic compounds and also in endodontic therapy treatment for human being¹¹.

The main aim of present work is to isolate chitosan from chitin containing crabs and prawn shells. The chitosan thus extracted and prepared in resorcinol formaldehyde resin to prepare the nanocomposites that shows better mechanical properties and environmentally more compatible composites than neat composites.

Experimental Methods

Raw materials

Shrimp shell and crab shells were scraped free of loose tissue, washed, dried and powdered. It was sieved through a 0.215-0.525 mm mesh. Then they were subjected to demineralization, deproteinization and deacetylation¹².

Chemical demineralization

Powdered form of crab shells was then treated with 7% (v/v %) HCl in the ratio of (1:10) that is (g/mL) for 24 hrs. The mixture was kept in ambient temperature and was washed with distilled water till neutral. The demineralized samples were dried and weighed and emission of CO₂ gas was observed.

Deproteinization

After demineralization, deproteinization of chitin was carried out by using 10% (w/v %) NaOH (Fisher Scientific) at 60 °C for 24 hours. The resulting solution was washed with distilled water and ethanol (10mL/gram, Bengal Chemicals, 97%) for neutralization and purification of chitin. It was then dried at 50 °C till a constant weight was obtained.

Deacetylation of chitin

Chitin was treated with 50% NaOH (w/v-%) solution at 60 °C for 8 hours and filtered. The residue was washed with hot distilled water and crude chitosan was obtained by drying in hot air oven at 50 °C overnight. The detail of the deacetylation process to prepare chitosan was presented in scheme 3.



Scheme 3: Deacetylation process of chitin to prepare chitosan

Preparation of resorcinol-formaldehyde/chitosan nano-composites

Resorcinol 16g (SDFCL, 99.0%) was mixed with 16g of water and then about 0.32g of oxalic acid (Fisher Scientific, 95%) was added which was then heated in a reflux condenser at about 95 °C -100 °C. After that, about 8g of formaldehyde (SDFCL, 41%) was carefully added in resorcinol solution to prepare the resin. Various composition composites of resin with chitosan were prepared by solution casting method¹³.

After the addition of chitosan, the mixture was refluxed to ensure complete combustion of the formaldehyde with resorcinol. As the mixture content start to light freight, it was transferred into an ice cube carefully. In order to remove the water of condensation, the liquid was evaporated for about 24 hrs, at 60°C in hot oven. After that, the temperature was raised to about 120°C for 12 hours in oven. At this stage, the reaction mass was a hot viscous liquid and upon cooling, the mass solidified to a clear amber-like resinous product which was brittle and grind able¹¹⁻¹⁵.

Characterization techniques

Fourier transform infrared (FTIR) spectroscopy

The functionalization of chitin to chitosan and the nano composites was confirmed by Fourier transform infrared (FTIR) spectroscopy^[16, 17]. The FTIR analysis was carried out in the Nepal Academy of Science and Technology (NAST), Nepal by using IR Prestige-21 FTIR Spectrometer (SHIMAZDU) to the identify species, functional groups, and vibration modes associated with each peak. Spectra were collected in the spectral range of 4000–400 cm⁻¹ using KBr pellet method.

X-ray diffraction (XRD) analysis

X-ray diffraction (XRD) analysis was used for the crystal size measurement of the nano particles of chitosan in powdered form or in composites. It is also known as x-ray crystallography is defined as an analytical technique used to identify phase of a material. The crystal phase and structure of the samples were determined by X- ray diffractometer (Bruker D2 Phaser) with a monochromatic CuK α radiation

source ($\lambda = 0.15418$ nm) at angle 2θ ranging from 20° C to 80° C at Nepal Academy of Science and Technology (NAST), Nepal. The accelerating voltage of 30 kV and emission current of 10 mA was used.

Mechanical characterization

The mechanical properties of the composites were measured by the compression test. It was measured by crushing cubic composite specimens in compression testing machine. The test was carried out by compressive testing machine (CTM, Capacity 500KN) shown in figure 1, manufactured by HARRIS & TARRIS company at Central Material Testing Laboratory, Pulchowk Campus, Tribhuvan University, Lalitpur, Nepal). The cuboidal composites having dimension (10.3 mm, 10.8 mm, 9.6 mm) for each of different composition were used for the compressive strength test.



Figure 1: Photographs of compressive strength testing machine at Central Material Testing Laboratory, Pulchowk Campus, Tribhuvan University, Lalitpur, Nepal).

Results and Discussion

Fourier transform infrared spectroscopy (FTIR)

The structure of chitosan obtained by deacetylation of chitin from prawn shells was studied under Fourier transform infrared (FTIR) spectroscopy. The wave number of FTIR spectra of chitosan was measured in between $4000\text{-}500\text{ cm}^{-1}$.

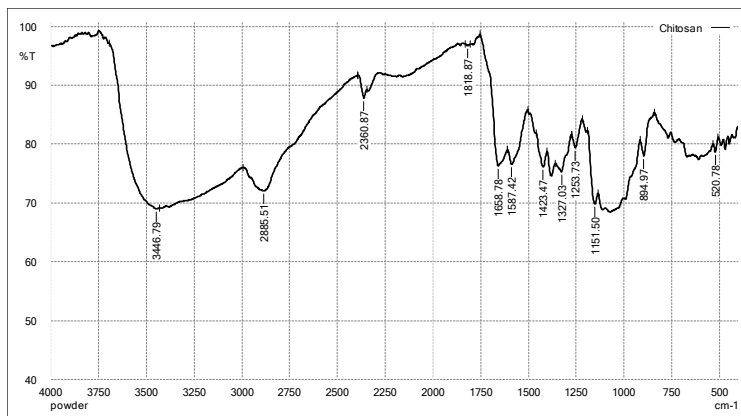


Figure 2: FTIR spectra of chitosan

Chitosan exhibited characteristic peaks at (1658 cm^{-1}) C=O stretching, (1423 cm^{-1}) C-H bending vibrations ¹⁶. The O–H stretching (3446 cm^{-1}), C–H stretching (2885 cm^{-1}), C–O–H vibration (1253 cm^{-1}), C–O–C stretching peak (1151 cm^{-1}), C–O/C–N stretching peak (2360 cm^{-1}), ring stretching (894 cm^{-1}), CH₂ bending and CH₃ deformation (1423 cm^{-1}) and the peak (520 cm^{-1}) indicated the presence of saccharide unit and (1587 cm^{-1}) NH₂ group were the characteristic peaks of chitosan ^{7,17}.

X-Ray diffraction (XRD) analysis

The XRD patterns of chitosan samples extracted from prawn shells exhibited strong reflections at 2θ value of 22 corresponding to the plane 200. The crystalline size $thkl$ of the synthesized chitosan nano powder was calculated from the XRD pattern using the Debye-Scherrer equation ¹⁸.

$$thkl = K\lambda / B \cos\theta$$

where k is the shape factor ($k = 0.9$ for most of the spherical crystal), λ is wavelength of monochromatic X-ray beam ($\lambda = 1.54060\text{ \AA}$ for CuK α radiation), B is full width at half maximum (FWHM) of the peak at the maximum intensity, θ is the peak diffraction angle that satisfies Bragg’s law for (hkl) plane and $thkl$ is crystallite size and the symbol h , k and l represent the miller indices and the respective plan is hkl . The (200) peak from the XRD pattern was used to calculate the chitosan nano size in this study from Debye-Scherer equation.

2θ corresponding to the (200) miller plane = 22

FWHM = 0.005652

(λ) = 1.54060

thus, $thkl = 0.9 \times 1.54060 / 0.005652 \times \cos 11$

= $1.38654 / 0.005481568$

= 249.91 \AA (24.991 nm)

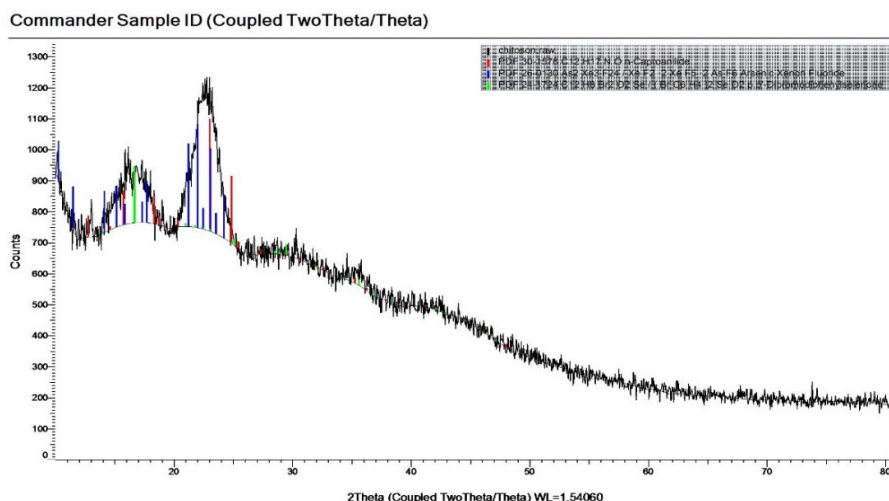


Figure 3: XRD pattern of chitosan from prawn shells

Hence, the size of chitosan was obtained to be 24.991 nm indicate that the synthesis chitosan was crystalline in size (F. A. Al Sagheer et. all). The broad peaks suggest that their particles could be of a nano metric size. Different phases were identified by comparing the experimental XRD pattern to standard complied by the International Centre for Diffraction Data (ICDD) using chitosan. Each pattern showed chitosan as the only phase.

Mechanical characterization of resin

Compressive strength is a mechanical test used to check the capacity of material to withstand loads tending to reduce its size¹⁹. It can be measured by plotting applied force against the deformation in a testing machine.

The compressive strength was calculated by using following formula.

$$\text{Compressive strength (CS)} = \text{Compressive force (F)} / \text{Cross section area of specimen (A)}$$

For Cross section area, Length of cube (resin) = 10mm

$$\begin{aligned} \text{Cross section area} &= 10^2 \text{ mm}^2 \\ &= 100 \times 10^{-6} \text{ m}^2 \\ &= 10^{-4} \text{ m}^2 \end{aligned}$$

Table 1: Calculation of compressive strength of different composition composites of resorcinol-formaldehyde resin with chitosan nanoparticles

S.N.	Chitosan (wt. %)	Compressive force (F)	Compressive strength (MPa)
1	0	14	0.525
2	1%	13	0.4872
3	1.5 %	12	0.450
4	3%	9	0.3375

The variation of compressive strength with chitosan content of composite maybe represented in bar diagram which are as follows

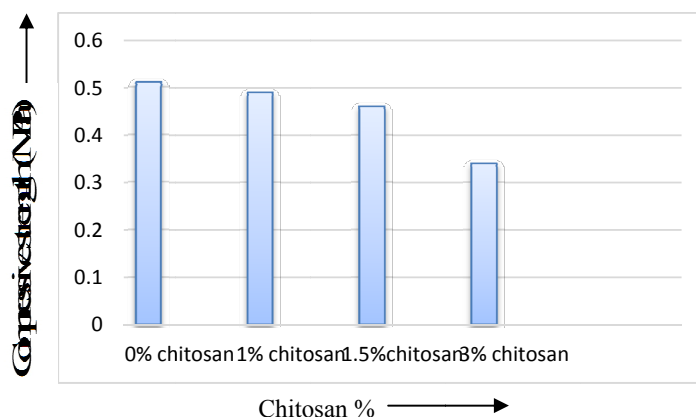


Figure 4: Variation of compressive strength with chitosan content chitosan R/F resin

When the weight % of chitosan was (0%, 1%, 1.5%, 3%) the compressive strength was found to be (0.525, 0.4872, 0.450, 0.335) Mpa, respectively. The bar diagram shows decrease in compressive strength with increase in % of chitosan by weight. This is due to the poor chemical bonding of chitosan crystal with R-F resin^{19,20}.

Conclusions

Chitosan was extracted from Chitin through a deacetylation process and the powdered phase of chitosan was revealed by FTIR technique. The Nano composite of chitosan was thus prepared by resorcinol formaldehyde resin which was characterized by compressive strength test. The compressive strength of chitosan was found to decrease with addition of chitosan due weak chemical bonding.

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