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Characterisation and Analysis of Chitosan Extract from *Artemia franciscana* Using SEM, FT-IR and XRD Studies

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Introduction: *Artemia franciscana* is one of the best animal models which is used in various fields. After the hatching of Artemia the shell wastes are used for Chitosan extraction. The extraction process consumes less time when compare with other shells because of its shell size.
Materials and Methods: The Chitosan extract was prepared using Artemia shells. The Physico-chemical properties were analysed and characterization of Chitosan extract was done using SEM, XRD and FT-IR studies.The physicochemical properties of the extracted chitosan indicate that it has optimum values desirable for wound healing applications. Chitosan has high water and fat binding capacity that can help to maintain a moist environment on the wound surface and control inflammation, respectively. The degree of deacetylation (DD) of chitosan is critical in determining its

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biological activities. The DD value obtained in this study was 84.77%, indicating that the chitosan sample had a high degree of deacetylation. Outcomes: The morphology of the chitosan membrane is an important factor that influences its properties and potential applications, including wound healing.

Results: The SEM images obtained in this study revealed that pure chitosan exhibited a nonporous, smooth membranous phase with dome-shaped orifices, microfibrils, and crystallites. The XRD analysis results suggest that the chitosan used in this study is highly crystalline, with both crystalline and amorphous regions enhancing its bioactivity and biocompatibility, making it a promising material for wide range of application in medicine. The crystalline and amorphous structure of Chitosan is analysed by the XRD, SEM and FT-IR reveals its wide range of applications in medicine.

Keywords: Artemia franciscana; chitosan; extraction; XRD analysis.

1. INTRODUCTION

A modified natural carbohydrate polymer called chitosan is made from chitin, which is found in a variety of natural sources including crustaceans, fungus, insects, and some algae. The deacetylated version of chitin, chitosan, is a linear, polycationic, heteropolysaccharide mostly composed of glycosidic connections between 1,4-2-deoxy-2-amino-D-glucopyranose and 1,4-2-deoxy-2-acetamido-D-glucopyranose. Usually, demineralization, deproteinization, and deacetylation with the application of potent acids and bases comprise the extraction of chitosan from Artemia shell waste. Chitin undergoes partial deacetylation to produce chitosan. The protonated free amino groups on glucosamine increase the solubility of the molecule, making the crystalline form of chitosan more soluble in diluted acids (pH 6.0) than it is in aqueous solutions above pH 7.0. Chitosan and its oligomers have a variety of biological qualities that make them beneficial for animal health, including antioxidant, anti-inflammatory, cholesterol- lowering, immunity-boosting, anticancer, neuroprotective, antimicrobial, and antifungal effects [1-3].

Chitosan is a semicrystalline polymer in its solid state. Using low molecular weight, totally deacetylated chitin, single crystals of chitosan were produced [4-8]. The development of characterisation techniques has been carried out on a highly deacetylated polymer [9,10].

The distribution of the acetyl groups throughout the main chain when calculating the molecular weight affects a chitosan's solution characteristics in addition to its standard DA (Synowiecki et al., 2003). Due to the initial polymer's semicrystalline nature, the deacetylation, which is typically carried out in the solid state, results in an uneven structure. The degree of ionisation is related to the acid's pH and pK, according to analysis of the impact of chitosan protonation in the occurrence of acetic acid and hydrochloric acid on solubility [11,12]. As solubility also depends on ionic concentration and salting-out was observed at concentrations over 1 M HCl, it is likely that the chlorhydrate form of chitosan can be prepared. Chitosan's solubility in acetic acid is often investigated by dissolving it in 1% or 0.1 M acetic acid. Recently, chitosan was successfully converted to a water-soluble form at a pH of neutral in the presence of glycerol 2-phosphate. At pH 7–7.1 and room temperature, stable solutions were obtained, but when heated to roughly 40°C, a gel developed (.Abdou *et al*.,2008) The gelation temperature varied slightly depending on the experimental conditions, and the sol-gel transition was partially reversible [13-16].

The average DA of a chitosan sample must be determined in order to characterise it. In addition to potentiometric titration, several approaches like IR, elemental analysis, an enzymatic reaction, UV, 1 H liquid-state NMR, and solid-state 13C NMR have been projected. When calculating molecular weight from intrinsic viscosity using the Mark- Houwink relation, the solvent is also crucial. It was established that the collections affect both the measurement of viscosity and molecular weight using light scattering (Bajaj et al.,2011). As was already noted, chitosan is used to create hydrogels, films, fibres, or sponges, the majority of which are used in the biomedical industry, where biocompatibility is essential. We can only include a few of the most promising systems out of the many that are detailed in the literature. While being more simpler to work with than chitin, chitosan compounds often have inferior stability because they are more hydrophilic and, in particular, pH sensitive [17,18].

2. MATERIALS AND METHODS

2.1 Preparation of Artemia Shells

Cysts of brine shrimp (*Artemia franciscana*) were recently collected from the seashore (Thoothukudi district). Cysts were purified of debris, sand, and salt crystals, and then hatched in accordance with the guidelines outlined by Sorgeloos et al. [19]. The cyst shells were removed from the top of the hatching tank after the hatching procedure and processed as follows: I density separation in brine; (ii) washing several times in fresh water; (iii) density separation in fresh water; (iv) drying at 60°C overnight in a forced air oven; (v) grinding to a powder; and (vi) storage at -5 2°C for prolonged use [20].

2.2 Preparation of Chitosan

2.2.1 Demineralization

This process, which removes calcium carbonate and calcium chloride, the two main inorganic components of the exoskeleton of crustaceans, is carried out in diluted hydrochloric acid (HCl) solution.

2.2.2 Deproteinization

To remove proteins, deproteinization is done using an alkaline procedure using a diluted sodium hydroxide solution, or NaOH. The demineralized shells were deproteinized for 1–3 hours at 70–0.5°C with a constant agitation speed of 100 rpm and a solvent–solid ratio of 15:1 (w/v) using 1.5–3.5 M sodium hydroxide.

2.2.3 Deacetylation

Deacetylation was used to transform the recovered chitin to chitosan. Thechitin was incubated for 1.5–4.5 hours at (60–100)0.5°C with a consistent agitation speed of 100 rpm and a solvent to solid ratio of 10:1 (w/v) of sodium hydroxide solution at a concentration of 30–50% w/w. A hoover pump was used to separate the mixture into solid and liquid components, which were then successively washed with distilled water until the pH was neutral. The obtained solid material (chitosan) was next dried for three hours at 80°C in a moist oven, and the dry weight was recorded.

ARTEMIA CYST SHELL
↓
DEMINERALIZATION
↓
DEPROTEINIZATION
↓
DEACETYLATION
↓
CHITOSAN

2.3 Physio – Chemical Properties of Chitosan

2.3.1 Fat binding capacity

Fat-binding capacity of prepared chitosan was worked out using the equation proposed by Wang and Kinsella. Fat binding capacity was calculated using the pursuing relationship:

FBC(%) = fat bound (g)/ initial sample weight (g) × 10

2.3.2 Water binding capacity

Water binding capacity of chitosan was concluded by using the process reported by Wang and Kinsella [21]. Water binding capacity was worked out using the following relationship:

WBC (%) = water bound (g) /initial sample weight (g) × 100

2.3.3 Moisture content

The Moisture content was concluded by make use of the gravimetric method. The water mass is find out by drying the sample to stable weight and measuring the sample after and before drying. The water mass was the dissimilarity between the mass of the moist and oven dry samples. Moisture content was worked out using the pursuing relationship:

$$\%\ Moisturecontent = wetweight\ (g) - dryweight\ (g)/wetweight\ (g) \times 100$$

2.3.4 Degree of deacetylation

The acid-base titration process (Zhang et al. 2011) through some adaptations was applied to conclude the percent degree of deacetylation (DDA) of the derived chitosan experimentally.

The proportion degree of deacetylation (DDA) of chitosan was calculated by means of this Equation.

$$\% \text{ DDA} = (c1V1 - c2V2/M \times 0.016) \times 100$$

2.4 Characterization of Chitosan

2.4.1 The fourier transform infrared (FTIR) analysis

The Fourier Transform Infrared (FTIR) examinations showed the characteristic wavelengths and spectra of the separated chitin, raw chitosan and refined chitosan from Artemia cyst wastes.

The vibrational characteristics of amino acids and cofactors, which are sensitive to minute structural changes, are investigated using Fourier transform infrared (FTIR) spectroscopy.

2.4.2 X-RAY diffraction

X-ray diffraction (XRD) method was carried out on the samples to confirm, validate and evaluate the degree of crystallinity of the separated chitin and chitosan. The most well-known family of procedures for examining a material's structural characteristics is X-ray diffraction (XRD).

2.4.3 Surface morphology

A study of the surface morphology of the precursors and their particular separated bio sorbents was examined using the Jeol JSM-7600F Field Emission Scanning Electron Microscope. This study was done to analyse the grain structure and unevenness of the separated bio sorbents. The surface morphology of the artemia (brine shrimp) shell precursor particles was studied by Scanning Electron Microscopy (SEM).

3. RESULTS

3.1 Moisture Content

The moisture content of chitosan was found out using the equation below.

Loss on drying%= (wet weight - dry weight)/sample weight x100%

5g of chitosan was taken, soaked and weighed to get wet weight as 5.2g. And it was dried to find the dry weight which was found to be 5.01g.

Loss on drying% = (5.2 - 5.01) / 5 x 100% = 3.56%

The moisture content of chitosan sample= 3.56%

3.2 Water Binding Capacity & Fat Binding Capacity [21]

WBC%= Water bound g/ initial sample weight (g)% Initial sample weight = 3g
Water bound sample weight= 9.78g WBC%= (9.78/ 3)100= 326%
The water binding capacity of chitosan sample= 326%
FBC%= Fat bound g/ initial sample weight (g)% Initial sample weight = 3g
Fat bound sample weight= 6.69g FBC%= (6.69g/ 3g)100= 223%
The fat binding capacity of chitosan sample = 223%

Fig. 1. Artemia Shells for Chitosan extraction
The shells are separated and collected after hatching and collected from the culture tank. The separated shells are dried in oven for chitosan extraction process

Fig. 2.Chitin, After the process of Deproteinization
After the deproteinization (excessive removal of protein) from the Artemia shells followed by demineralization. The shells which is converted into Chitin polysaccharide

3.3 Degree of Deacetylation [22]

Degree of deacetylation was calculated using the equation below by substituting the values I got from titration.

$$DD(\%)=\{(CHCl\times VHCl-CNaOH\times VNaOH)\times 0.016\times 100\%\}/G\times(100-W)\times 9.94\%,$$

Where; CHCl is the aqueous HCl solution solution concentration (M), CNaOH is the aqueous NaOH solution concentration (M), VHCl is the volume of the aqueous HCl solution (M), VNaOH is the volume of the aqueous NaOH solution (M), G is the weight of the chitosan sample (g), and W is indeed the aqueous concentration of the chitosan. The theoretical equivalent mass of amino groups is 9.94%, and the weight of amino groups equivalent to 1 mL of 0.1 M HCl is equal to 0.016 (CHCL Concentration = 0.1 M VHCL volume = 10 ML CNAOH concentration = 0.1 M VNAOH volume = 10 ML

G weight = 1 g
W aqueous concentration of chitosan = 2%

DD% = {[(0.1 M) x (10 mL) - (0.1 M) x (10 mL)] x (0.016 g/mmol) x 100%} / (1 g x (100%- 2%) x 9.94%)

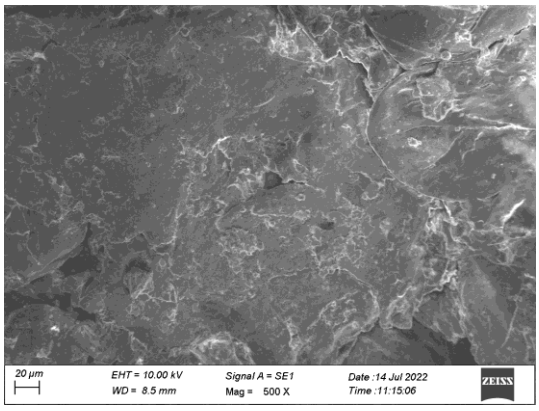
DD% = 84.77%

The degree of deacetylation of chitosan= 84.77%

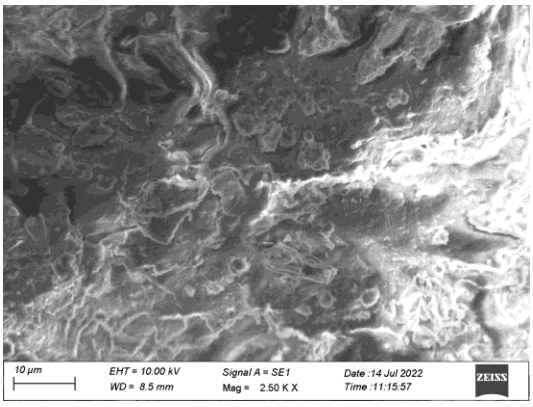
3.4 Characterization of Chitosan

Fig. 3. shows that (a,b,c,d,e,f and g) are Microscopic images (SEM) of chitosan extracted from *Artemia Franciscan* shells. The SEM image shows the presence of pores and fibers in the microstructure of the chitosan from Artemia shell wastes.

The SEM image of the chitosan shows a rougher and grainier surface with a significant increase in the number of pores of the chitosan sample.



a



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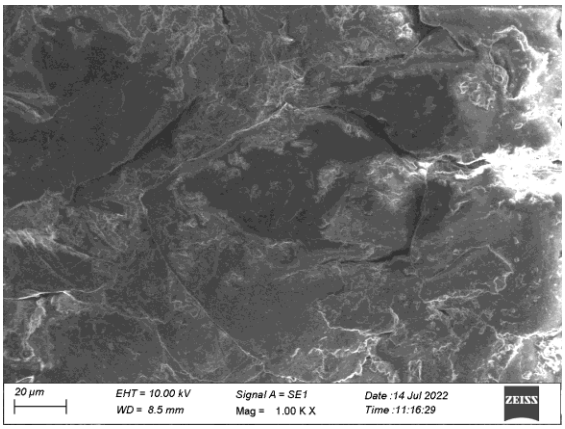
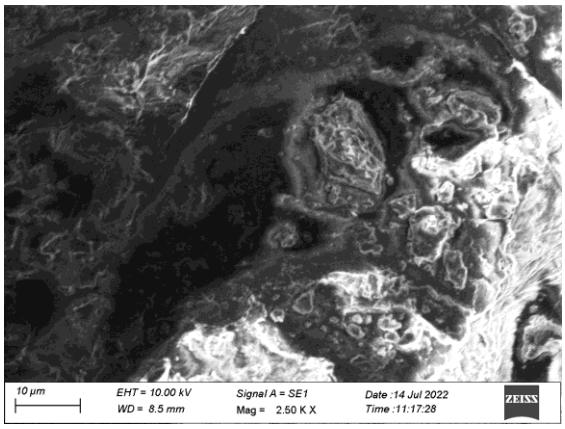
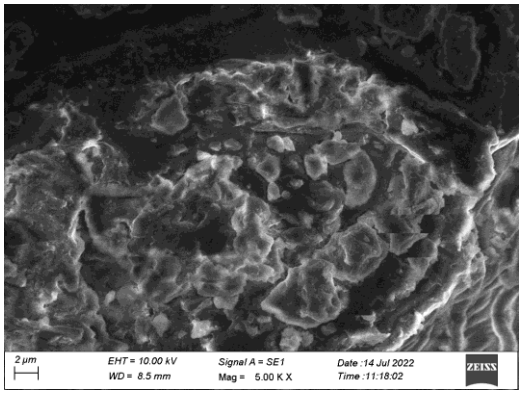
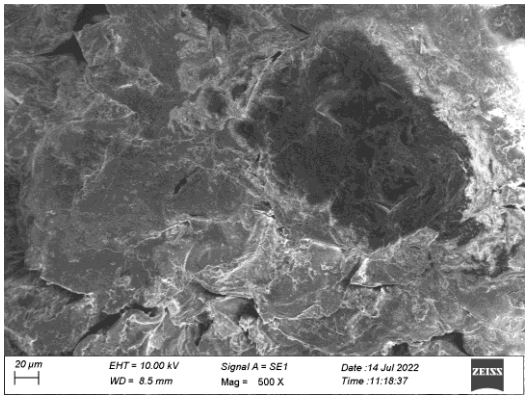


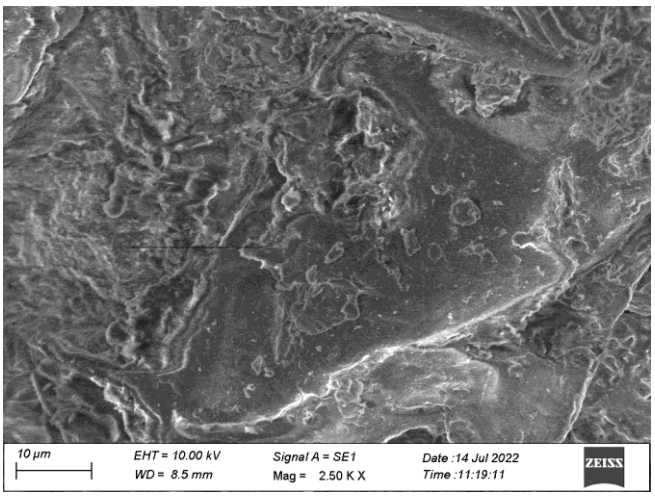


c **d**





e **F**



g

Fig. 3. SEM images of chitosan

Table 1 shows that experimental FTIR bands and the functional groups of chitosan isolated from Artemia shell wastes. The chitosan from the Artemia shells were exhibited the absorption bands are 3435.89, 2921.89, 2852, 2065, 1741, 1650, 1559, 1419, 1322, 1261, 1203, 1154, 1073, 713 and 576.

The O-H Stretching and OH Bending represents the Alcohol group. The C-H Stretching and C=C

Stretching represents Alkyne group. C-N Stretching represents Amine group and C=C Bending represents Alkene group.

3.5 XRD Diffraction Chitosan

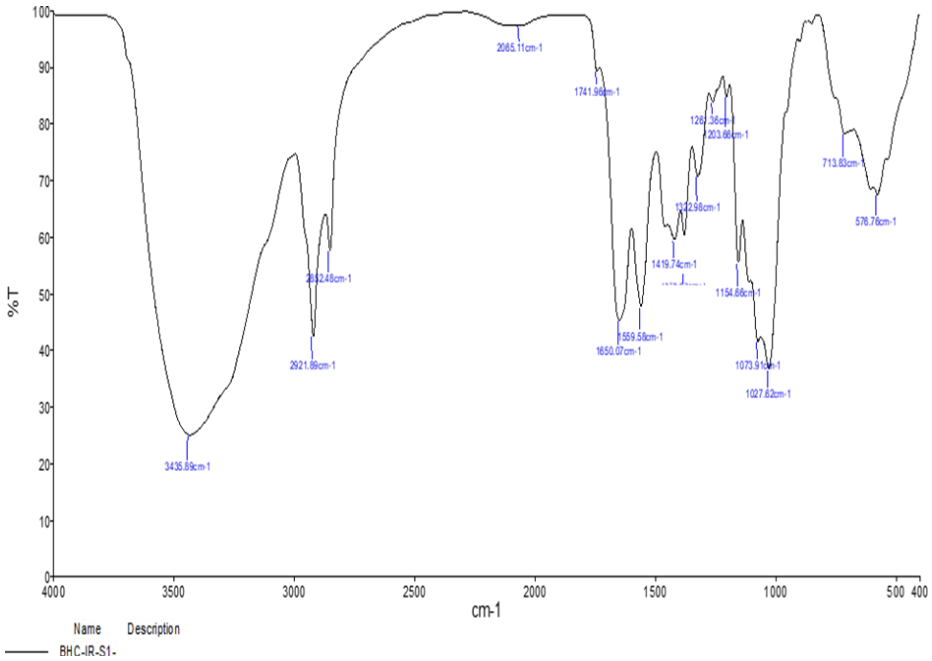
The peaks obtained from the XRD patterns of the chitosan sample in the 2θ position are (5.6°**,** 8.6°, 19.30°, 26.07°, 31.69°, 32.9°, 48°.

The crystalline size of the peaks is obtained from the chitosan sample are:

The XRD analysis of chitosan exhibits very broad peak at 2θ = 20° as per Figure. This result is similar to the previous studies conducted using pure chitosan. Kumar, et al. (2012), B. Aziz et al. (2017).

Crystalline size of chitosan was calculated using XRD crystalline size calculator (Scherrer equation) by InstaNANO. The values of each peak position and corresponding FWHM was added to the calculator and the results were obtained. The average of all crystalline sizes from the results was calculated.

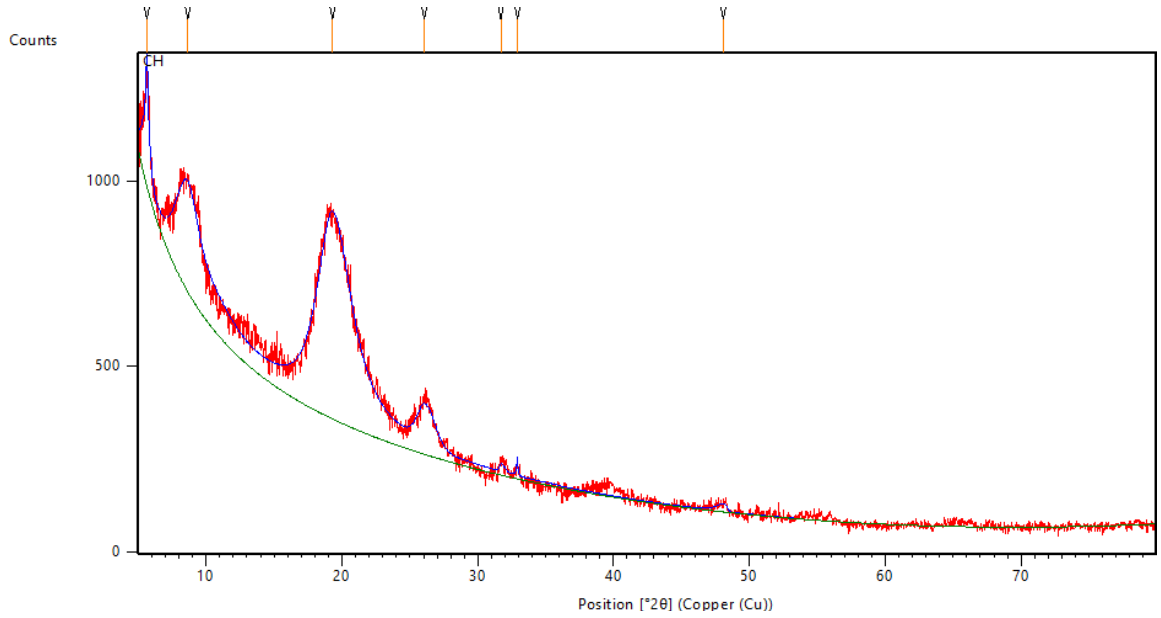
The crystalline (grain) size of chitosan= 21.65nm



Graph 1. FTIR chromatogram of chitosan

Table 1. FTIR fuctional groups of chitosan

Absorption	Appearance	Group
3435.89	O-H Stretching	Alcohol
2921.89	C-H Stretching	Alkene
2852.48	C-H Stretching	Alkene
2065	C=-C Stretching	Alkyne
1741	C-H Bending	Aromatic compound
1650	C=O Stretching	δ- Lactam
1559	C=C Stretching	αβ- Unsaturated ketone
1419	OH Bending	Alcohol
1322	O-H Bending	Phenol
1261	C-N Stretching	Amine
1203	C-N Stretching	Amine
1154	C-N Stretching	Amine
1073	C-O Stretching	Primary alcohol
713	C=C Bending	Alkene
576	C=C Bending	Alkene



Graph 2. Shows that XRD image of the chitosan extracted from *Artemia franciscana* shells

Table 2. Relative intensity of Chitosan extracted from *Artemia franciscana*

Pos. [°2θ]	Height [cts]	FWHM Left [°2θ]	d-spacing [Å]	Rel. Int. [%]
5.6465	240.96	0.3405	15.63911	64.49
8.6383	219.93	2.0696	10.22805	58.87
19.3071	373.62	2.7294	4.59359	100.00
26.0768	70.97	1.2927	3.41438	19.00
31.6969	17.57	0.2435	2.82064	4.70
32.9018	53.81	0.1212	2.72005	14.40
48.0822	20.82	1.3518	1.89081	5.57

Table 3. Variation in Crystalline size

Pos. [°2θ]	Crystalline size
19	3.08
26	6.59
31	35.41
32	71.36
48	6.72

4. DISCUSSION

Artemia (Brine shrimp) is very easy to hatch and cultivate. As Artemia shells comparing with other crustaceans, Artemia shells are very thin and too easy to extract the Chitosan. Though chitosan is considered as much more effective, the amount of chitosan yield from the Artemia shells is very low.

After Chitosan extraction, physicochemical and characterization work were analysed . When compare this physico-chemical values with previous studies (A.Sri Hari Kumar, A. Sahoo, P. et al.) using crab shells, shrimp shells, the physico chemical values of chitosan from Artemia fransciscana reveals average fat binding capacity, high water binding capacity and high moisture content.

Degree of deacetylation of chitosan is more important because, it impacts the physical, chemical and biological properties of chitosan, such as acid-base and electrostatic characteristics, biodegradability, self-aggregation, sorption properties, and the capability to chelate metal ions. The degree of deacetylation resolves mainly the content of free amino groups in the polysaccharide. (A.Sri Hari Kumar, A. Sahoo, P. et al.).

However, in this studies demonstrates, high degree of deacetylation (84.7%) occurs in chitosan sample extracted from *Artemia franciscana*.

The FTIR results shows some vital functional groups involved in the derived Chitosan. Mainly Alkene and Amine groups presents in the chitosan sample. The XRD analysis of extracted chitosan indicates some strong broad peaks at 2θ = 20º which means it has a crystalline structure. The crystalline Crystalline size of chitosan was calculated using XRD crystalline size calculator (Scherrer equation) by InstaNANO. The average of all crystalline sizes from the results was calculated. The crystalline (grain) size of chitosan= 21.65nm.

5. CONCLUSION

Chitosan have a variety of biological qualities that make them beneficial for animal health, including antioxidant, anti-inflammatory, cholesterol- lowering, immunity-boosting, anticancer, neuroprotective, antimicrobial, and antifungal effects. The crystalline size and degree of deacetylation of Chitosan enables us to identify the biological properties such as biodegradability, and it capacity to bind to chelate metal ions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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