

A Simple and Effective Method for Preparation of Chitosan from Chitin

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ABSTRACT- Background- Chitosan is the most abundant natural amino polysaccharide. Researchers have found, chitosan is biocompatible, biodegradable and nontoxic, which have made wide applicability in the pharmaceutical field.

Methods- Chitosan was prepared by deacetylation of chitin and characterized by U.V Spectrophotometry, FTIR (Fourier transform infrared spectroscopy), DLS (Dynamic Light Scattering), & Scanning electron microscopy (SEM).

Results- The present study showed that by SEM shown a long thin crystal structure on a smooth surface and prepared chitosan was soluble in 1% acetic acid solution.

Conclusion- Chitosan was prepared from deacetylation of crustacean chitin and the morphology studied by SEM that showed that chitosan had a long thin crystal structure on a smooth surface. We were observed the prepared chitosan was soluble in 1% acetic acid solution.

Key-words- Chitin, Chitosan, Deacetylation, DLS, FTIR, SEM

INTRODUCTION

Chitosan (CS) has emerged as alternative synthetic polymer due to its abundance, low production cost, biodegradable, biocompatible, renewable and non toxic nature. It is the second most abundant natural polymer next to cellulose, but most abundant natural amino polysaccharide and the estimation of its annual production is almost same as cellulose [1]. The elemental composition of the chitosan polymer is carbon (44.11%), hydrogen (6.84%) and nitrogen (7.97%). Due to their high percentage of nitrogen compared to synthetically substituted cellulose (1.25%), they are of commercial interest. Over the last several years CS has received increased attention as one of the promising renewable polymeric materials having a wide scope of applications that are both fascinating and as yet uncharted. Possible and usual applications of chitin, chitosan, and their derivatives are estimated to be more than 200 [1].

Chitosan contains an amino group having pKa value ~6. Thus it is positively charged and is readily soluble in aqueous acidic solution. It is a unique linear polycation with a high charge density, reactive hydroxyl and amino groups as well as excessive hydrogen bonding.

It can be isolated naturally from the cell wall of fungi, but commercially it is prepared from chitin. Chitin is white, hard, inelastic, high molecular weight crystalline polysaccharide extracted from shrimp and crab shells. At least 10 giga tons (1013kg) of chitin are synthesized and degraded each year in the biosphere. Chitin is deacetylated by using sodium hydroxide in excess as a reagent and water as a solvent to form chitosan [2]. The commercially available CS is 66% to 95% deacetylated and it has an average molecular weight ranging between 3800–20,000 daltons. The degree of deacetylation is determined by the content of free amino groups in the polysaccharides and used to differentiate between chitin and CS. Chitin with a degree of deacetylation (DD) of 75% or above is generally known as CS. Chitosan can be characterized physico-chemically by determining the degree of deacetylation, molecular weight, solubility, viscosity, crystallinity, and physical forms [3]. The properties of CS may vary on the basis of its source and other factors during the manufacturing process can influence the physicochemical characteristics of the final chitosan product. CS is poorly soluble in water, therefore, chemical modification is needed to improve its solubility and widening their applications. The presence of reactive functional groups in CS offers a wide range of derivatives such as quaternized, N, N, N-trimethyl, carboxyalkyl, thiolated, sugar-bearing, bile acid-modified and cyclodextrin-linked chitosan.

Chitosan is a heteropolymer consisting of β -(1 \rightarrow 4)-2-acetamido-D-glucose and β -(1 \rightarrow 4)-2-amino-D-glucose units, with the latter mostly exceeding 80%. The

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distribution of the two monosaccharide units in chitosan relies upon the alkaline treatment. Chitosan is chemically analogous to cellulose, which is a plant fiber. Cellulose, chitin, and chitosan share similar backbone structures, as shown in (Fig. 1). The difference among these three molecules is the functional group at the C-2 position. In molecular chain of chitin, it consists of linear structures of 2-acetamido-2-deoxy- β -D-glucose through β (1 \rightarrow 4) linkage, by replacing hydroxyl group at C-2 position in cellulose molecular chain and in chitosan acetamido group of chitin is replaced by amino group. Chitosan is a versatile biopolymer which is a glucosamine glycan. It contains three types of reactive functional groups, an amino/acetamido group as well as both primary and secondary hydroxyl groups at the C-2, C-3 and C-6 positions, for each monomer with a unit formula of C₆H₁₁O₄. The amino contents are the important factors contributing to differences in their structures and physicochemical properties, and its distribution is random, which make it easy to generate intra- and inter-molecular hydrogen bonds. Chitosan can be easily modified by various methods due to the presence of hydroxyl and amino groups on its backbones. Modifications of chitosan are generally done to improve physicochemical properties of chitosan and expansion of its applications in various fields.

MATERIALS AND METHODS

Preparation of chitosan from chitin- For chitosan production, one gram of crustacean chitin was treated with 30-70% NaOH solution in 1:50 w/v ratio [4]. Then the sample was deacetylated at 15 psi pressure at 121°C for 30min using an autoclave. After this the sample was harvested, the resulting chitosan was separated from the reaction medium containing sodium hydroxide solution by filtration using sieve. The separated chitosan was washed several times with distilled water until its pH becomes neutral. The product so obtained washed with acetone twice for the removal of pigments and any other impurities. This washed and neutralized chitosan was oven-dried for attainment of finished product.

Estimation of chitosan yield- The weight of chitosan produced was measured and yield was calculated using the following formula;

$$\text{Yield (\%)} = \frac{\text{Practical yield} \times 100}{\text{Theoretical yield}}$$

Characterization of prepared chitosan- The prepared chitosan was characterized by following method.

Moisture content- Moisture content of chitosan samples were determined by thermogravimetric analysis (TGA) using portable bench top AD MS 70 moisture analyzer. The water mass was determined by drying the sample to constant weight and measuring the sample weight after and before drying. The water mass was the difference between the weights of the wet and dry the samples [5]. Approximately 250 mg chitosan sample harvested at different time interval was placed on sample pan and moisture content was monitored by the constant heating pattern at 180°C. At the end of the cycle, total moisture content (% moisture content) and total dry weight of the sample was determined.

Moisture content (%)=

$$\frac{[\text{Wet weight (g)} - \text{Dry weight (g)}] \times 100}{\text{Wet weight (g)}}$$

Degree of deacetylation- Infrared spectroscopy (IR) technique was used for THE determination of DD. The degree of deacetylation (DDA) of chitosan was calculated using the baseline proposed by Domszy and Roberts [6]. The computation equation for the baseline is given below:

$$\text{DD} = \frac{100 - [A_{1655} \times 100 / 1.33]}{A_{3450}}$$

Where A₁₆₆₅ and A₃₄₅₀ are the absorbances at 1655 cm⁻¹ of the amide-I band as a measure of N-acetyl group content and 3450 cm⁻¹ of the hydroxyl band as an internal standard to correct for film thickness or for differences in chitosan concentration powder form. The factor '1.33' denoted the value of the ratio of A₁₆₆₅/A₃₄₅₀ for fully

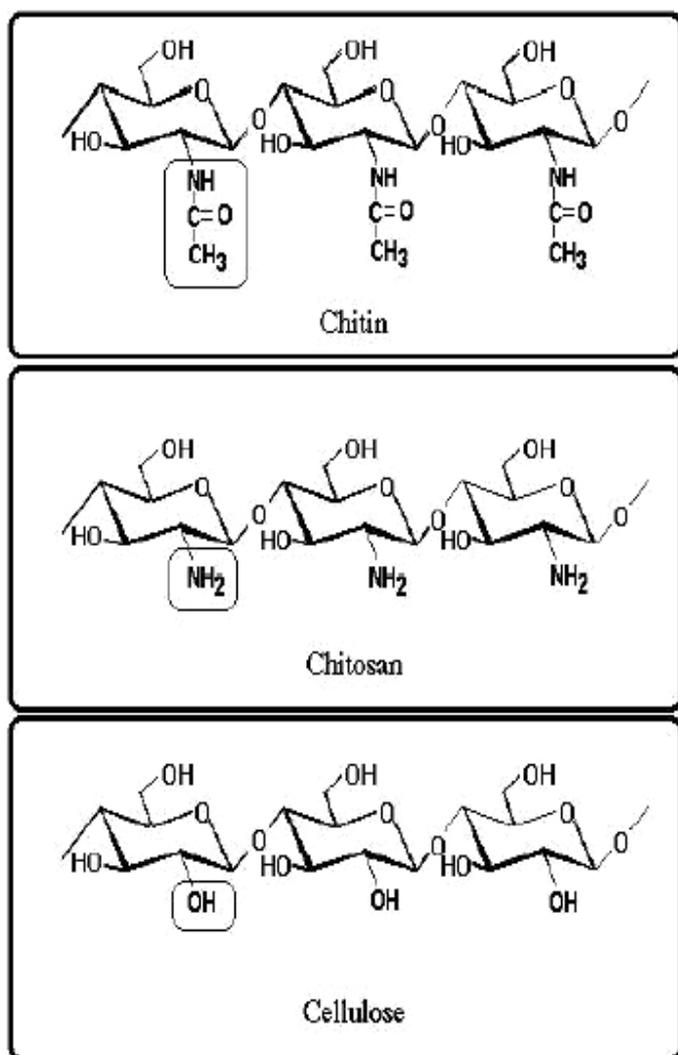


Fig. 1: Structure of Chitin, Chitosan, and Cellulose

N-acetylated chitosan. It was assumed that the value of this ratio was zero for fully deacetylated chitosan.

Crystallinity- The crystallinity of chitosan samples was also evaluated by FTIR analysis. The formula for calculation of crystallinity given below;

$$\text{Crystallinity} = A_{1379}/A_{2929}$$

pH and Solubility- The pH measurement of chitosan solution was carried out using pH meter cyber scan 510 (Germany). Solubility: Chitosan powder (0.2 gm) harvested at different time interval were dissolved in 20 ml 1% acetic acid solution in a beaker for 30min on a magnetic stirrer at 250 rpm at 25°C. After 30 min of agitation solution was filtered by Millipore 20 μ nylon membrane filtration assembly. The retardant (insoluble fraction) collected on membrane filter was washed with distilled water. Total dry weight content of chitosan insoluble fraction was analyzed by moisture analysis Total dry weight content of chitosan insoluble fraction was analyzed by moisture analyzer at above mentioned parameters. Chitosan solubility in percentage was calculated by following equations.

Solubility% =

$$100 - \frac{[\text{Weight of insoluble fraction} \times 100]}{\text{Initial weight of sample}}$$

Viscosity- Viscosity of chitosan was determined with a cone and plate Brookfield viscometer (CAP 2000 + Brookfield engineering laboratories, inc.). Chitosan solution was prepared in 1% acetic acid at a 1% concentration on a dry weight basis. Measurement was made by placing 150 μl of 1% chitosan solution on temperature control sample plate in duplicate using No. 01 spindle at 750 rpm at 25°C with values reported in centipoises (cPs) units.

Dynamic Light Scattering (DLS) analysis- The average particle size and zeta potential of chitosan was analyzed through DLS method done by Zetasizer Nano S (Malvern, UK). The DLS measurements were done with a wavelength of 532 nm at 25°C and angle of detection of 90°. Approx. 1 mg of sample was dissolved in 1 ml Milli Q water and 100 μl solution was further diluted for the measurement of particle size and zeta potential. All measurements performed in duplicate.

Scanning electron microscopy- The structure of chitosan was examined using Quanta 400 ESEM/ EDAX from FEI. Vacuum dried small amount of prepared chitosan samples were kept on an SEM stub using double-sided adhesive tape at 50 mA for 6 min through a sputter. Afterward, the stub containing the sample was placed in the scanning electron microscopy (SEM) Chamber. The photomicrograph was taken at acceleration voltage of 20KV.

FTIR analysis- FTIR analysis of different chitosan sample was performed with a2 technologies portable attenuated total reflectance (ATR) Fourier transform infrared spectroscopy (ATR-FTIR). Sample spectra were recorded in the middle infrared range from 4000cm⁻¹ to 400 cm⁻¹ with a resolution of 4cm in the absorbance mode for 10 scans at room temperature. Chitosan samples prepared by the alkaline deacetylation studied for the determination of degree of deacetylation. FTIR spectra of prepared chitosan samples were obtained by placing 1mg of sample of sample on the sensor of the instrument.

RESULTS

The chitosan was prepared by deacetylation of chitin using different % of NaOH. The best quality chitosan was obtained when chitin was deacetylated with 50% NaOH.

Estimation of chitosan yield- The yield of chitosan ranged between 37–50% (Table 1).

Characterization of physiochemical property of prepared chitosan- The prepared chitosan was characterized by the following method.

Moisture content- The moisture content of prepared chitosan was found to be in the range of 5–12% (Table 1).

Degree of deacetylation (DD)- The DD % was found to be in range of 65–80% (Table 1).

Crystallinity- The crystallinity of prepared chitosan samples was varied from 0.95 to 1.07 (Table 1).

pH and Solubility- The pH percentage of prepared chitosan samples was varied from 6.8–8 while the solubility of prepared chitosan samples ranges from 55% to 90% (Table 1).

Viscosity- The viscosity of prepared chitosan samples were observed between 82 to 123 cPs (Table 1).

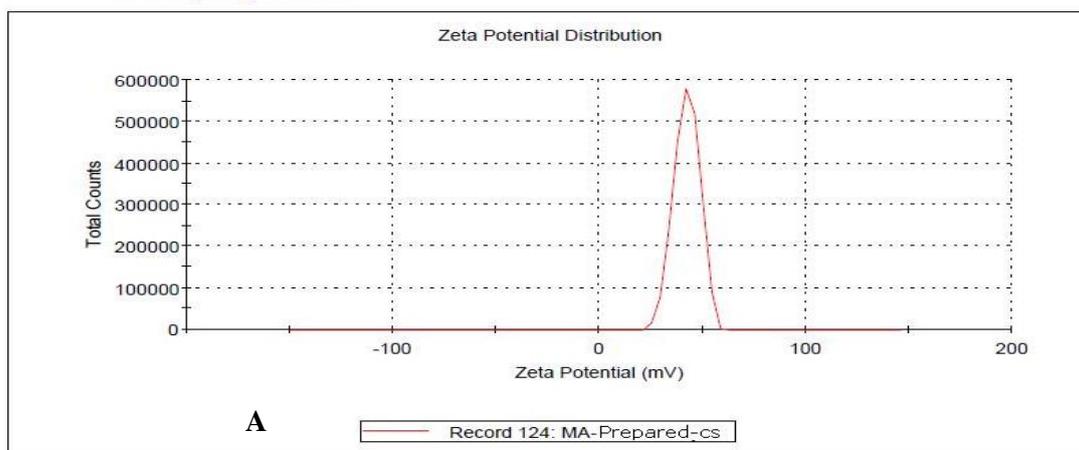
Table 1: Physicochemical properties of prepared chitosan samples

S. No.	Chitosan sample	Moisture %	% Yeild	pH	% Solubility	% DD	Crystallinity	Viscosity (cPs)
1	Sample 1 (30%NaOH)	12.49	37	8.0	55	65	1.07	82
2	Sample 2 (40%NaOH)	10.17	45	6.8	88	78	1.0	112
3	Sample 3 (50%NaOH)	9.82	50	7.2	90	80	0.95	123
4	Sample 4 (60%NaOH)	9.69	42	6.7	70	75	1.03	98
5	Sample 5 (70%NaOH)	5.98	47	6.2	70	72	1.05	86

DLS Analysis- When chitin was deacetylated with 50% NaOH, it results into chitosan with DD 80% and solubility 90%. The zeta potential and size of prepared

chitosan also matches with the commercial chitosan (Fig. 2 A to Fig.2 D).

	Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): 42.2	Peak 1: 42.2	100.0	6.16
Zeta Deviation (mV): 6.16	Peak 2: 0.00	0.0	0.00
Conductivity (mS/cm): 1.66	Peak 3: 0.00	0.0	0.00
Result quality: Good			



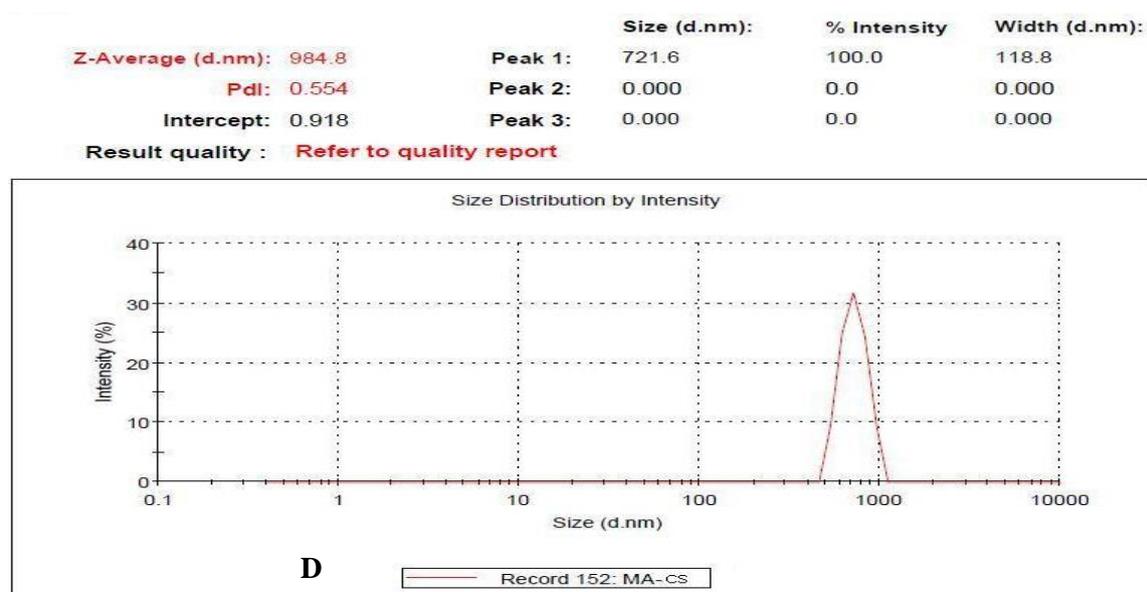
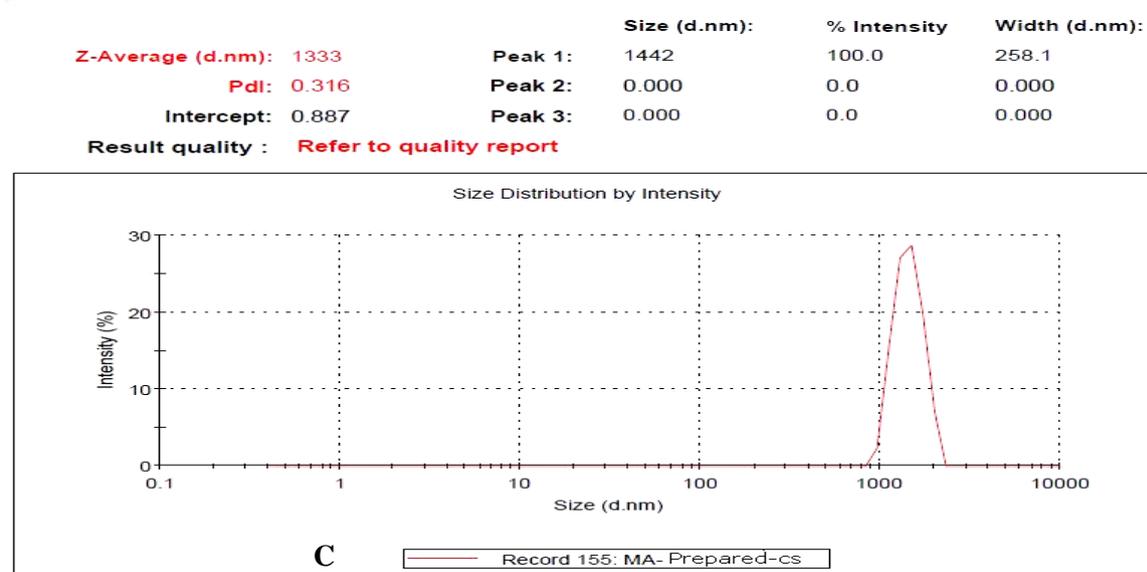
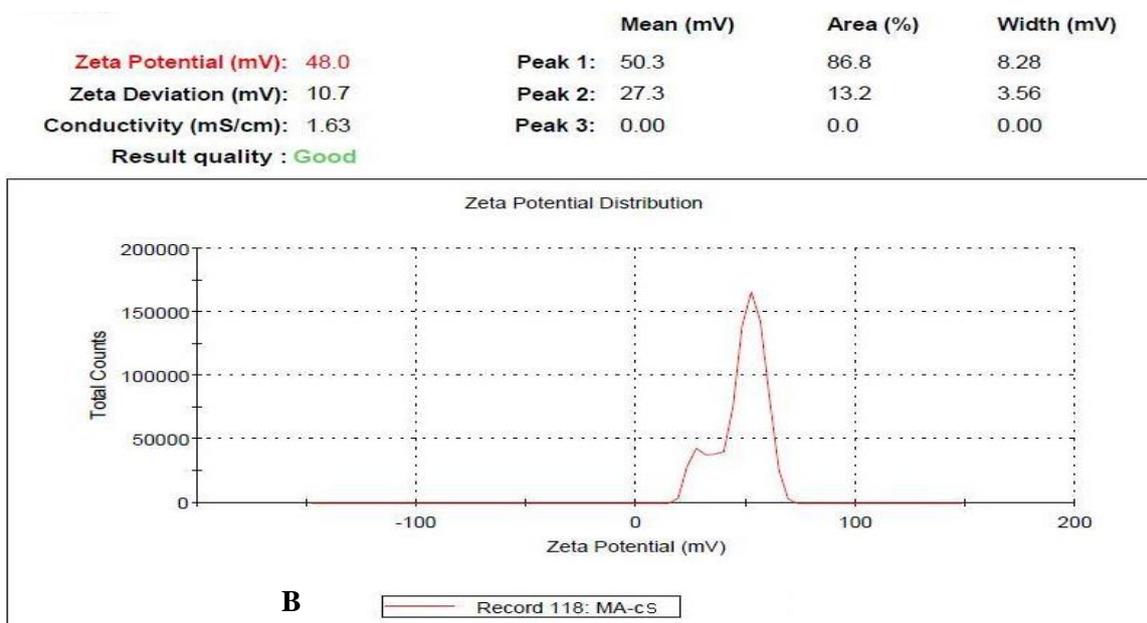


Fig. 2 (A-D): Comparison of zeta potential and size of prepared and standard chitosan; **A:** Zeta potential of prepared CS; **B:** Zeta potential of CS standard; **C:** Size prepared CS; **D:** Size prepared CS standard

SEM Analysis- The morphology was studied by SEM and it showed that chitosan had a long thin crystal structure on a smooth surface (Fig. 3).

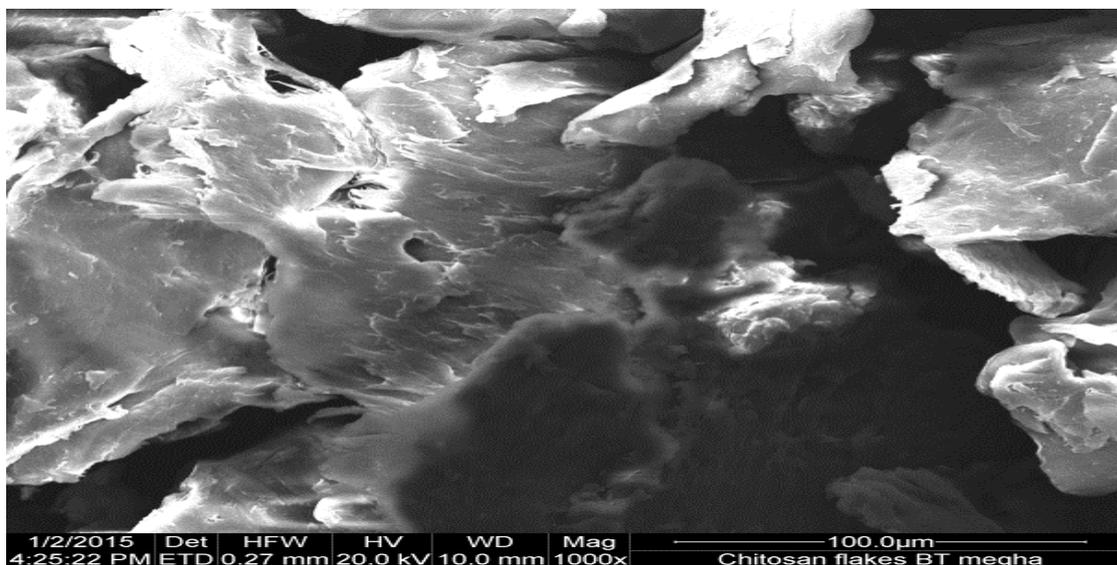


Fig. 3: SEM analysis of prepared chitosan

FTIR Analysis- The FTIR spectra of chitin at different percentage of deacetylation NaOH were analyzed. The FTIR profile of chitin extracted from crustacean shells revealed the main absorption peak in the range at 1651.63 cm^{-1} to 1622.04 cm^{-1} could be attributed to C=O stretching of amide bonds (amide-I). An absorption peak at 1552.5 cm^{-1} was assigned for the NH bending (amide-II) and the band of OH group due to O-H

stretching was observed at 3432.99 cm^{-1} . The analysis of FTIR spectra revealed that during N-deacetylation of chitin, the absorption peak at 1658.68 cm^{-1} to 1647.31 cm^{-1} of C=O (amide-I) gradually decreased while the peak at 1585.85 cm^{-1} to 1563.29 cm^{-1} increased after N-deacetylation at 121°C . The FTIR analysis showed that when chitin was deacetylated with 50% NaOH its spectrum mimic the spectrum of commercial CS (Fig. 4).

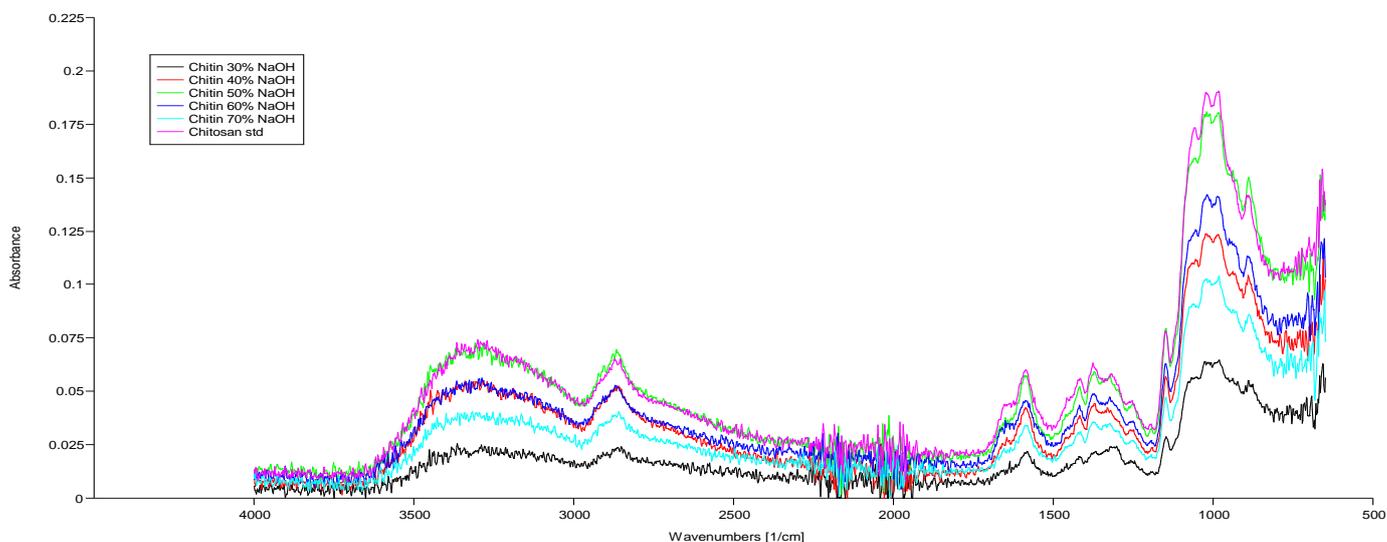


Fig. 4: FTIR spectrum of chitin at different % of NaOH

DISCUSSION

The chitosan was successfully prepared from deacetylation of crustacean chitin. The quality and physicochemical properties of prepared chitosan vary widely with the quality of crustacean chitin and methods of preparation. The % yield of chitosan obtained was in a range of 37-50%. This was due to the removal of an acetyl group from chitin during the treatment with concentrated alkali (NaOH) solution. In 2014, Paul *et al.* [7] obtained a chitosan yield 57% from chitin isolated from sea prawn [7] and Divya *et al.* [8] obtained a yield of 46% from shrimp shell waste [8]. In 2010, Nessa *et al.* [9] reported the chitosan yield 16.4-19.6%. The difference in

% yield was seen due to difference in the preparation method of chitosan as well as of chitin.

The chitosan samples had a moisture content ranging from 5.983% to 12.48%. The moisture content was the difference between the weight of the fresh and dry samples. According to Paul *et al.* [7], moisture content was 4% while Divya *et al.* [8] reported 5% moisture content. In 2010, Nessa and colleges [9] reported the moisture content in the range of 0.3–0.4%. According to Li and his group commercial chitosan product contained less than 5% moisture [10]. Chitosan is hygroscopic in nature hence it is very possible that the samples were affected by moisture absorption during storage [11]. Also,

the moisture content depends on the season, relative humidity and intensity of light ^[12].

The Degree of deacetylation (DD) was an important parameter as it affects solubility, chemical reactivity, and biodegradability. DD was calculated by using FTIR as it was fast method and does not require dissolution of the chitosan sample in an aqueous solvent ^[13]. The DD percentage was found to be in between range of 65–80%. The DD also depends on the method of preparation as well as intrinsic properties of CS. According to No and Meyers ^[14], DD ranges from 56% to 99% with an average of 80%. According to and his coworkers DD ranges from 30% to 95% ^[15].

Crystallinity ranges from 0.95 to 1.07. The Crystallinity of both chitin and chitosan was generated from hydrogen bond between corresponding hydroxyl and N-acetyl groups ^[16]. Each crystalline peak characterizes crystallographic structure, which was generated from parallel and antiparallel alignments of polymeric chains or sheets. Semicrystalline chitin and chitosan have amorphous and crystalline regions ^[17]. The crystallinity of chitosan and DDA exhibit an inverse relationship. This was due to the fact that crystallinity of chitosan decreases with an increase in deacetylation sample.

The solubility of prepared chitosan samples was varied from 55% to 90%. It was also observed that chitosan samples with higher DDA (75%) show constant rate of solubility of 90%. Chitosan showed lower solubility due to their lower deacetylation values. In 1981, Brine Austin noted that lower solubility values suggested incomplete removal of acetyl groups ^[18]. Since the solubility of chitosan depends on the removal of acetyl groups from chitin. Therefore, lower DDA value of sample could adversely affect the solubility of chitosan.

The viscosity of chitosan sample ranges from 82 to 123 cP. Viscosity increases with increase in solubility. The viscosity of chitosan solutions were reported in the literature generally ranges from 60 to 780 cP ^[19]. These ranges of viscosity were also observed by Cho *et al.* ^[20] with five commercially available chitosan.

The FTIR, SEM and DLS studies confirm the production of chitosan. The FTIR profile of chitosan samples indicated that the absorption peak in the range of 1658.68 cm^{-1} to 1647.31 cm^{-1} could be attributed to the amide I and the peak in the range at 1563.29 cm^{-1} to 1585 cm^{-1} was assigned to the amide II, and the stretched band at 3250 cm^{-1} to 3400 cm^{-1} were due to OH stretching. In the profile of FTIR appearance of specific absorption peak at 898 cm^{-1} was due to the glycosidic linkage between glucosamine and N-acetyl glucosamine, served as characteristic marker for the confirmation of polysaccharide.

SEM analysis showed that chitosan had a long thin crystal structure on a smooth surface. This was in accordance with previous data of Hwang ^[21]. Non-homogenous and non-smooth surface structure of chitosan was also seen by Muhammed *et al.* ^[22]. According to the DLS analysis size and zeta potential of prepared chitosan matches with the commercial CS. The prepared chitosan with DD 80% and solubility 90% can

be further used in pharmaceutical industries as an antimicrobial agent.

CONCLUSIONS

Chitosan was prepared from deacetylation of crustacean chitin. The prepared CS samples were characterized by various methods. The prepared CS yield was found to be in range of 37–50% and moisture content was found to be in range of 5–12%. The percent solubility of prepared chitosan samples was varied from 55% to 90%. The degree of deacetylation ranges from 65–80%. The morphology studied by SEM showed that chitosan had a long thin crystal structure on a smooth surface. The results also showed that when chitin was deacetylated with 50% NaOH its size and zeta potential matches with the standard chitosan. The characteristic of prepared chitosan was in accordance with the commercial standard. The present observations indicated that the prepared chitosan was soluble in 1% acetic acid solution. The obtained chitosan had low viscosity, high DD, high solubility and a denser crystalline structure. Chitosan with the following properties has several commercial applications and greater scope of industrial applications. It can be used in food packaging, pharmaceutical industry, drug delivery as well as water treatment.

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