Chapter 2

Chitosan

2.1 Introduction

Since the principal material used as flocculant in this study is chitosan, an overview of the physical and chemical nature of chitosan, its availability and suitability for use in water treatment is necessary.

Chitin is a biopolymer widely distributed in nature. In abundance, it is second only to cellulose [Muzzarelli, 1977; Shahidi et. al., 1999]. It was first described by Braconnot in 1811, who obtained it from fungi and called it ‘fungine’ Chitin is widely distributed as a major component in the tough exoskeleton of arthropods (insects like beetles, millipedes, bees, cockroaches and spiders), crustaceans (aquatic organisms such as crabs, lobsters and shrimps) and in the cell walls of fungi, yeast, and bacteria.

It is the structural material in insects, providing rigidity similar to what cellulose does in plants. It was Odier who coined the word ‘chitin’ (Gk, meaning ‘envelope’) in 1823. The chemical structure of chitin as a polymer of N-acetyl glucosamine was conclusively proved only by 1950 [Muzzarelli, 1977]. Chitosan is obtained by deacetylation of chitin using alkali, and is therefore poly(β-D-glucosamine). It is structurally very similar to cellulose, which is poly(β-D-glucose), as shown in Figure 2.1.
Figure 2.1
Structural relationship between chitin, chitosan and cellulose
2.2 Manufacture of chitosan

Chitosan is available as a commercial byproduct in those parts of the world where fishing and seafood processing are major industries. It is manufactured from the waste shells of crabs, lobsters and prawns. The composition of these waste shells is as follows [Muzzarelli, 1977]:

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
</tr>
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<tbody>
<tr>
<td>Water</td>
<td>60 to 70%</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>50% of dry weight</td>
</tr>
<tr>
<td>Proteins</td>
<td>35% of dry weight</td>
</tr>
<tr>
<td>Chitin</td>
<td>15% of dry weight</td>
</tr>
</tbody>
</table>

Extraction of chitin from the shells consist of the following steps:

1. Demineralisation by treating with dilute hydrochloric acid.
2. Removal of proteins by prolonged treatment with caustic soda.
3. Washing and drying of the residual material.
4. Grinding to the required particle size.

Chitin is then deacetylated by autoclaving with 40% caustic soda. The product chitosan is washed free of alkali, dried, powdered and sieved. It is to be noted that the raw material is a throwaway industrial waste, the processing involves only simple steps and no expensive chemicals are involved. Therefore the market price of chitosan will be dictated by demand, the price coming down with increasing demand.
2.3 Chemical properties of chitosan:

The extent of deacetylation to produce free amino groups can vary much depending on the strength of the caustic soda used and with the time and temperature of treatment. Commercial chitosan is therefore described as a partially deacetylated chitin, with the extent of deacetylation varying between 60 to 80% [Muzzarelli, 1977]. A brief outline of the chemical nature of chitosan is described below:

1. Appearance: brownish-yellow flakes.
2. Average molecular mass of the order of $10^5$.
3. Polymer structure similar to that of cellulose.
4. $\beta$-D-glucosamine monomer units. Prolonged boiling with mineral acid gives D-glucosamine.
5. Degree of polymerisation (dp): 600 to 800 monomer units.
6. Decomposes on heating above 150°C (423 K).
7. Insoluble in water and all common organic solvents.
8. Soluble in very dilute acids (0.1 M HCl or 1% acetic acid).
10. Very high metal-binding ability, especially towards Cu$^{2+}$, Pb$^{2+}$ and Hg$^{2+}$.
11. Has proven antibacterial activity.

Chitosan is presently being used in the manufacture of cosmetics such as hair sprays, nail polishes, moisturising creams and sunscreen lotions. It is used in
medicine as wound dressings, self-absorbing surgical sutures, slimming diets, artificial ligaments, diluent and binder in tablets etc. Chitosan also finds application as viscosity builder in foods and beverages, as animal and fish feeds, in the manufacture of adhesives etc. High affinity for metal ions makes it a good medium for the separation of metal ions in analytical chemistry.

2.4 Chitosan as a cationic polyelectrolyte:

Polymers containing ionisable groups on the monomer units are called polyelectrolytes. Since a basic primary amino group is present on the second carbon of each glucosamine unit in chitosan, it dissolves in dilute acids as a salt. If the polymer chain is represented by ‘R’, the change may be depicted as follows:

\[ R-NH_2\text{(s)} + HCl\text{(aq)} \rightarrow R-NH_3^+ Cl^-\text{(aq)} \]

Since the cation \(NH_3^+\) is fixed to the polymer chain, chitosan is classified as a cationic polyelectrolyte, and should be capable of binding to the negatively charged surfaces of suspended particles in water and bring about flocculation by the bridging mechanism. Thus, chitosan is expected to act as a cationic flocculant in dilute or neutral medium. In basic medium, chitosan will be insoluble.

2.5 Chitosan as a polymeric chelating ligand

The amino groups present in chitosan also make it a good chelating ligand capable of strongly binding to a variety of metal cations. The lone pairs of electrons on the nitrogen atoms and oxygen atoms are donated to the metal ion to form
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Since several amino groups and hydroxyl groups are present on the long polymeric chain, the chain can wrap around the metal ion and adopt configurations such that several amino groups are bonded to the metal atom at the same time. This type of chelation leads to the formation of very stable metal complexes. This property makes them useful for concentration of trace metals, removal of radioactive and other harmful heavy metal contaminants, and in chromatographic separation of mixtures of metal ions [Bassi et al. (2000)].

2.6 Source of chitosan for the present study

Chitosan used in the studies described in this thesis was obtained from the Central Institute of Fisheries Technology (CIFT), Kochi, and from National Sea Foods, Kochi. The samples were manufactured from waste prawn shells. The material was in the form of a pale brown powder, insoluble in water. It dissolved in 0.1 molar HCl or 1% aqueous acetic acid. A 1% solution of chitosan (w/v) was thick, syrupy and almost colourless.

2.7 Characterisation of chitosan used in the present study

The chitosan sample obtained was characterised by (1) estimating the ash content, (2) by comparing the IR spectrum of the chitosan sample with that reported
in the literature, (3) by estimating the degree of polymerisation by viscosity measurements and (4) determining the degree of deacetylation, which is a measure of the number of available free amino groups which are the binding sites, by conductometric titration, as described in section 2.7.1.

2.7.1 Materials and Methods

Experimental details of methods used for characterisation of chitosan are given below:

2.7.1.1 Estimation of ash content in chitosan:

The chitosan used in our experiments was a commercial product, the characteristics of which can vary depending on the source (shells of prawns, crab etc.) and manufacturing process conditions [Muzzarelli (1977)]. The quality of the product used was therefore beyond our control, and the material was used as received. However, since only one sample from a single production batch was procured and used throughout the work, all experiments were done using material of the same quality. Since it is customary to determine the ash content for products of natural origin, the ash content of a chitosan sample obtained from CIFT was determined gravimetrically by way of providing a characterisation. Since only one sample was used throughout the work, the ash content was determined only once (in duplicate).
About 1 g of the chitosan sample was accurately weighed into a tared silica crucible and heated cautiously using an open Bunsen flame till charring was complete. The residue was then heated strongly for about an hour on an electric Bunsen at red heat. The crucible was then cooled to ambient in a desiccator and weighed. The percentage of ash obtained was calculated based on the chitosan sample taken. Another aliquot of the same chitosan sample was soaked in an ammoniacal solution of ethylenediamine tetraacetic acid disodium salt (EDTA) for 24 hours, filtered, washed with distilled water and dried. The ash content was then determined again as before.

Since the ashes usually consist of inorganic material such as metal oxides and carbonates, a sample of the ash was subjected to inorganic qualitative analysis as given in Vogel [Svehla G., (1979)] to determine the metals present. The results are given in section 2.7.2.1 under results and discussions. No attempts were made for a quantitative estimation of these metals.

2.7.1.2 Infrared spectrum of chitosan:
A very dilute (0.1% w/v) solution of chitosan was prepared in 1% aqueous acetic acid. 10 mL of this solution was spread evenly on a horizontal glass plate so as to form a square of side 10 cm. It was allowed to dry for two days under ambient conditions. The plate was then soaked in a dilute NaOH solution for a few hours to
Figure 2.3

Infrared spectrum of chitosan (10 μm film, %T)
remove the excess acid and precipitate the chitosan. The plate was then washed repeatedly with distilled water till the washings were neutral. A very thin, perfectly transparent film of chitosan could be easily lifted off the glass plate. It was stretched on a wire mesh and allowed to dry. IR spectral scan of the film was obtained using a Shimadzu model 8101 FT-IR spectrophotometer. This is reproduced in Figure 2.3. Spectrum was also obtained using chitosan powder in the form of a KBr pellet.

2.7.1.3 Determination of molecular mass and degree of polymerisation

The bridging mechanism for flocculation described in the previous chapter depends on the chain length of the polymer; longer chains are expected to give better results. Therefore knowledge of the degree of polymerisation of the material being tested for flocculating power is of concern.

For polymeric substances, the number of monomer units present in any individual chain or strand (known as degree of polymerisation, dp) may vary to a large extent from strand to strand. Therefore, only an average estimate of the molecular mass can be given.

The number - average molecular mass \( \overline{M_n} \)

The weight - average molecular mass \( \overline{M_w} \) and

The viscosity - average molecular mass \( \overline{M_v} \)

are some values usually determined [Rabek, 1980]. It is usually observed that
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\[ \bar{M}_n < \bar{M}_v < \bar{M}_w \]

Since such polymeric substances are non-volatile and often difficult to dissolve, methods used to obtain molecular masses of small molecules are not applicable. Because of the various conformational changes possible for the chain, and due to the complicated interactions among these chains and of chains with the solvent, even colligative methods such as determination of osmotic pressure or constitutive methods like viscosity measurements can be applied only with some reservation.

In the present case, the viscosity-average molecular mass \( \bar{M}_v \) was determined for the chitosan sample used and its degree of polymerisation calculated.

Table 2.1. Important terms used in connection with viscosity measurements.

<table>
<thead>
<tr>
<th>Official names</th>
<th>Common names</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viscosity coefficient</td>
<td>Viscosity</td>
<td>( \eta )</td>
</tr>
<tr>
<td>Viscosity ratio</td>
<td>Relative viscosity</td>
<td>( \eta_{rel} = \frac{\eta}{\eta_0} )</td>
</tr>
<tr>
<td></td>
<td>Specific viscosity</td>
<td>( \eta_{sp} = \eta_{rel} - 1 )</td>
</tr>
<tr>
<td>Viscosity number</td>
<td>Reduced viscosity</td>
<td>( \eta_{red} = \frac{\eta_{sp}}{c} )</td>
</tr>
<tr>
<td>Limiting viscosity number</td>
<td>Intrinsic viscosity</td>
<td>( [\eta] = \lim_{c \to 0} (\eta_{red}) )</td>
</tr>
</tbody>
</table>
Viscosity is a measure of the resistance to laminar flow exhibited by a liquid or solution, and represented by the Greek letter \( \eta \). It is defined as the shear stress per unit velocity gradient. If \( \eta \) is independent of velocity gradient, the liquid is called a 'Newtonian liquid', and if \( \eta \) varies with velocity gradient, it is called 'non-Newtonian'. Chitosan solutions in acid behave as non-Newtonian liquids. Some common terms used in connection with the measurement of viscosity of polymer solutions are explained in Table 2.1.

Relative viscosity is the ratio of the viscosity \( \eta \) of a solution having concentration \( 'c' \), to that of the solvent or medium, \( '\eta_0' \). It is therefore a dimensionless quantity. This is easily determined with the help of an Ostwald viscometer. It makes use of the capillary flow method in which the same volume of solution and solvent are allowed to flow the same distance through the same capillary tube. Under these conditions, if both measurements are done at the same temperature, the times of flow depend only on the densities of the solutions and their viscosities. Then we have the relationship:

\[
\frac{\eta}{\eta_0} = \frac{\rho t}{\rho_0 t_0}
\]

where \( \rho \) and \( \rho_0 \) represent the densities of the solution and solvent respectively and \( t \) and \( t_0 \) their times of flow. The limiting viscosity number [\( \eta \)] is a hypothetical quantity (see Table 2.1) which will be the viscosity of the solution if it had a concentration 'zero'. It can be obtained by measuring the viscosity number for solutions of different concentrations in the same solvent, plotting the values
against concentration on the x-axis and extrapolating to meet the y-axis (zero c). The y-intercept gives the limiting viscosity number $[\eta]$. The sample concentrations should not be too large because additional effects may arise from intermolecular forces and entanglement of polymer chains.

The viscosity-average molecular mass $\overline{M}$ and the limiting viscosity number $[\eta]$ are related by the Mark-Houwink-Sakurada equation [Rabek, 1980]:

$$[\eta] = K \overline{M}^{\alpha}$$

where ‘$K$’ and ‘$\alpha$’ are constants for a given polymer at a given temperature in a given solvent. ‘$\alpha$’ depends on the thermodynamic interactions between polymer segments and the solvent molecules and is related to the solvent power and expansion factor. The value of ‘$\alpha$’ is unity for a long molecule kinked in random fashion and approaches zero for a chain coiled into a ball [Lee, 1974].

The Mark-Houwink-Sakurada equation may also be written in the form:

$$\log [\eta] = \log K + \alpha \log M$$

which is also referred to as the Staudinger equation [Muzzarelli, 1977]. Therefore a plot of $\log [\eta]$ against $\log M$ will give a straight line, the slope of which gives ‘$\alpha$’ and the y-intercept is $\log K$. This allows one to determine the values of ‘$K$’ and ‘$\alpha$’ for a particular polymer by measuring the limiting viscosity numbers of various samples of it having known uniform molecular mass $M$ (monodisperse samples).
This also requires determination of the molecular mass by some other method such as light scattering or equilibrium sedimentation. Monodisperse polymer samples are seldom available and so carefully selected fractions of the polymer are normally used. Thus Wang et. al. reported the values of ‘K’ and ‘α’ for samples of chitosan with various degrees of deacetylation in aqueous solutions of 0.2 M acetic acid and 0.1 M sodium acetate at 30°C [Wang et. al., 1991]. The values determined by them for a 69% deacetylated sample of chitosan were as follows:

\[ K = 0.104 \times 10^{-3} \quad \text{and} \quad \alpha = 1.12 \]

Chitosan in acid solutions exhibits the polyelectrolyte effect. There is an abnormal increase in the viscosity of the more dilute solutions because of an enlarged effective volume due to charge repulsion and stretching out of the molecules. When sufficient salt is added to neutralise this charge effect, the viscosity behaviour becomes normal [Muzzarelli, 1977]. For this reason, aqueous medium containing 0.2 M acetic acid + 0.1 M sodium acetate is preferred for viscosity measurements.

**Experimental:**

An Ostwald viscometer was cleaned thoroughly by passing chromic acid through it followed by rinsing several times with distilled water. It was then clamped vertically such that all of the capillary and the bulbs dipped in a water bath maintained at 30 ± 1°C.

Twelve millilitres of glacial acetic acid and 13.6 g sodium acetate trihydrate were dissolved in distilled water and made up to 1 L to get the 0.2 M acetic acid +
0.1 M sodium acetate solvent system. The bottom bulb of the viscometer was filled with this solution. The liquid was then carefully drawn by suction into the upper bulb and into the stem above the upper mark. It was then allowed to flow down freely. The time required for the meniscus to pass from the upper mark to the lower mark was measured. The measurement was replicated and the average time was taken as $t_0$ in calculations. The solution was then taken in a 10 mL density bottle and weighed accurately to determine its density $\rho_0$.

A 0.2% solution of chitosan was prepared by dissolving 400 mg of it in 200 mL of the above solvent system. The mixture was kept for some time to dissolve completely. Solutions having concentrations of 0.15, 0.1, 0.075, 0.05, 0.025, 0.0125 and 0.00625% were prepared from this by serial dilution using the solvent system. The time of flow $'t'$ and the density $'\rho'$ were determined for each of these solutions in the same manner as described above for the solvent system. All measurements were done within 2 hours of preparing the stock solution. Another set of similar readings were obtained after keeping the solution for about 18 hours.

Since the densities of the solutions and that of the solvent system were found to be the same within the number of significant figures considered for measurement, $\rho/\rho_0$ was unity for all the solutions. Therefore the relative viscosities $\eta_{rel}$ for each solution was calculated using the formula

$$\frac{\eta}{\eta_0} = \frac{\rho t}{\rho_0 t_0} = \frac{t}{t_0}$$
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Figure 2.4.

Plot of reduced viscosities of chitosan solutions against their concentrations
The following values were also calculated for each solution:

\[ \eta_{sp} = \eta_{rel} - 1 \quad \text{and} \quad \eta_{\text{red}} = \frac{\eta_{sp}}{c} \]

The values of reduced specific viscosity \( \eta_{\text{red}} \) were then plotted against the concentration \( c \). A similar plot was made using values obtained after 18 hours also. Extrapolating the curves to meet the y-axis, the limiting viscosity number \([\eta]\) for the 2-hour sample and for the 18-hour sample were read as 6.2 and 5.4 dL g\(^{-1}\) respectively (see Figure 2.4).

The molar mass \( M \) was then calculated by substituting the values of \( K \) and \( \alpha \) determined by Wang et al. and the value of \([\eta]\) determined as above in the Staudinger equation. The degree of polymerisation was obtained by dividing the molar mass of chitosan by 161, which is the molar mass of one glucosamine unit.

### 2.7.1.4 Determination of degree of deacetylation

Two different methods involving conductometric titration were used to determine degree of deacetylation, namely direct acidimetric titration and back titration.

All reagents were prepared from analytical grade chemicals. Finely powdered chitosan was used in the experiment. A digital conductometer, Century model CC 601-P with calibrated platinum sheet electrodes was used. Conductometric titration was carried out under constant speed slow stirring.

In the direct titration method, about 0.25 g of accurately weighed chitosan was added to 25 mL of distilled water in a 100 mL beaker. The suspension was
stirred using a magnetic stirrer and slowly titrated using 0.1 M HCl. To get a clearly defined titration curve, the titrant was added in 1 mL increments and conductivity measured after each addition.

In the back-titration method, about 0.25 g of accurately weighed chitosan was added to 25 mL of 0.1 M HCl in a 100 mL beaker and magnetically stirred till dissolved. The contents of the beaker were titrated using standard 0.1 M NaOH solution. Conductivity was measured after stirring for a minute after the addition of each 1 mL increment.

In both cases the measured conductivity was corrected for dilution using the equation:

\[ C_{corr} = \frac{C_{obs} (V + \Delta V)}{V} \]

where \( C_{corr} \) is the corrected conductivity corresponding to the addition of titrant, \( C_{obs} \) is the observed conductivity value, \( V \) is the initial volume of titrand and \( \Delta V \) is the cumulative volume of titrant added at the point of measurement.

In each case, the corrected conductivity values were plotted against the volume of titrant added to determine the end points of the titrations. The plot obtained for direct-titration method is given in Figure 2.5 and that for back-titration method is given in Figure 2.6. Degree of deacetylation was then calculated in each case using the formula:

\[ \text{Degree of deacetylation (\%) } = \frac{V_{t} N_{t}}{V_{in}} \times \frac{M_{G}}{m_{C}} \times 100 \]
Figure 2.5.

Titration curve for the direct method.

Volume of HCl taken up by chitosan = 12 mL
Figure 2.6.

Curve for the back-titration method.

Volume of NaOH equivalent to acid taken up by chitosan = 10.5 mL
where $V_t$ is the volume of titrant equivalent to HCl absorbed by chitosan, $N_t$ its normality, $V_m$ is the initial volume of chitosan solution, $M_G$ is the molar mass of glucosamine monomer unit and $m_C$ is the mass of chitosan per litre of the solution.

2.7.2 Results and discussion

2.7.2.1 Ash content

The original sample of chitosan was found to contain 1.2% ash consisting of magnesium, calcium, aluminium and nickel. Extraction with ammoniacal EDTA reduced the ash content to 0.27%, and the ash consisted of aluminium and nickel only. Thus calcium and magnesium were the major metal ions present, and these were preferentially removed by washing with EDTA solution. However, these results are of significance only when the chitosan is to be used as a complexing agent for metal ions or when it is ingested. Although the presence of traces of these metal ions in chitosan may block a few binding sites in connection with its flocculation properties, complete removal of these metal ions from the commercial product will be a very costly affair; application of such highly purified material will be uneconomic for its routine use as a flocculant in treatment plants. The metals present and their quantities may also vary depending on the source and manufacturing process of the chitosan. A sample analysis of the trace metals usually present, using emission spectrographic and neutron activation methods is provided by Muzzarelli (1977).
2.7.2.2 Infrared spectrum

The IR spectrum of chitosan taken as a thin film gave more defined absorption bands than that in the form of KBr pellet (Figure 2.3). The pattern and positions of the absorption bands in the fingerprint region were in good agreement with the one reported for chitosan by Muzzarelli [(Muzzarelli, 1977)], but the spectrum obtained here is better resolved and with sharper bands. There is a report that new sharp bands appear with increasing degree of deacetylation [Mima et. al., 1983].

2.7.2.3 Molar mass and degree of polymerisation

The molar mass calculations of the chitosan sample gave a value of $1.8 \times 10^4$ daltons for the 2-hour sample and $1.6 \times 10^4$ daltons for the 18-hour sample respectively. This translates into a degree of polymerisation of about 114 glucosamine units for the sample kept for 2 h after dissolution and 100 units for the one kept for 18 h. The degree of polymerization of the chitosan sample will depend very much on the concentration of the acid used and time of treatment during the manufacturing process since the polymeric chain can be randomly ruptured by acid treatment [Rege and Block (1999)]. There also appears to be great variation in the solution properties of chitosan and the Mark–Houwink constants reported by different workers [Knaul et. al. (1998), Tsaih and Chen (1999), Jiang and Han (1999), Rinaudo et. al. (1999), Sashiwa and Shigemasa (1999) Signini and Filho]
Molar masses of up to $10^6$ daltons have been reported in literature for chitosan by workers who have isolated the material carefully using mild conditions to avoid chain degradation [Muzzarelli, 1977]. Compared to these values, it appears that the commercial sample obtained by us has undergone considerable chain degradation during the manufacturing process.

However, the results also indicate that degradation of chitosan takes place even when stored as an acid solution and the degree of polymerisation decreases slowly with time of storage. Similar observations on the degradation of acid solutions of chitosan have also been reported by others (Chen et al., 1997; Vincendon, 1997). As the degree of polymerisation increases, dissolution of chitosan in the acid is slower, thus preventing immediate and reliable measurement of viscosity.

As far as application of chitosan as a flocculant in water treatment is concerned, it is desirable to have as high a value for the degree of polymerisation, considering the bridging mechanism. But it is also essential that it be possible for the chitosan to be made into a solution that can be efficiently and rapidly mixed with the bulk of the water to be treated. Since good flocculation characteristics were observed even with low molar mass samples used in this study, the performance is expected to be even better if higher molar mass samples are used. But there is also a study which reports that chitosan chains of smaller molar mass
are more extended than chains of larger molar mass [Tsaih and Chen, 1999]. Other morphological factors may also play a role (see section 2.7.2.5).

It was also noted that there was considerable variation in the degree of polymerisation among various samples of chitosan procured. One sample obtained from CIFT would dissolve in dilute acid only very slowly to give a thin gel which would not flow in the Ostwald viscometer. This sample obviously had a much higher degree of polymerisation than that for which the above calculations were made. This sample also showed a better flocculation efficiency in experiments described in the coming chapters.

2.7.2.4 Degree of deacetylation

Calculation based on the direct titration method gave a degree of deacetylation of 70.6% for the sample examined and that using the back-titration method gave a value of 69.6%. The results obtained using the two methods are in good agreement within limits of experimental error. Thus about 70% of the amino groups in the sample of chitosan are free.

2.7.2.5 Microscopic examination of precipitated chitosan gels

Microscopic examination of chitosan precipitated from a 0.1 M hydrochloric acid solution immediately and after storage for about 3 months also indicated some change in its characteristics. These are shown in Plates 1 and 2 respectively.
Plate 2.1. Chitosan precipitated from fresh solution.
(seen under the microscope with phase contrast at $\times 400$ magnification)

Plate 2.2. Chitosan precipitated from an aged solution.
(seen under the microscope with phase contrast at $\times 400$ magnification)
A Nikon Optiphot AFX-IIA light microscope at a magnification of $\times 400$ was used in this study.

In Plate 2.1, the strands of chitosan appear to be very long and closely packed in the form of a continuous mat extending in all directions beyond the field of view. But in Plate 2.2, they appear to be fragmented and aggregated into smaller clusters or loose strands.