Extraction of Chitosan and Preparation of Hydrogel and its Application in Agriculture.

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Abstract : Chitin is most important natural polysaccharides found in shells of crab, prawns and other crustaceans after cellulose. However, it is not widely utilized for industrial application till now as it is insoluble in many solvents, relatively difficult to isolate from natural sources in pristine form and to prepare in a reproducible way under good economic condition. By treating these chitin shells of shrimp and other crustaceans, chitosan is prepared. Present study was undertaken to extract chitin from waste fish scales and prepare chitosan by the sequence of chemical processes involving demineralization, deproteinization and deacetylation. Analysis and characterization of chitosan was done by FTIR and SEM. Further chitosan gel was obtained from this prepared chitosan. The successful formation of gel was confirmed through various characterization techniques like FTIR, SEM, Thermal Analysis and Textural Analysis. Chitosan gel improved the water-holding properties of soils hence making efficient use of water in agriculture. It was found that the gel improves soil permeability, reduce the need for irrigation and improve plant growth.

Index Terms **– Chitin, Chitosan gel, Agriculture, Deacetylation, Demineralization.**

I. INTRODUCTION

Many biopolymers are available now-a-days but they are not that easily accessible to the consumers because of their insufficient availability or sometimes because of their cost. While the chitosan being a second most abundant biopolymer in the nature after cellulose, it can be used. The chitosan being the biopolymer is a β (1-4) linked polysaccharide of glucosamine which can be extracted from exoskeleton crustaceans by chemical processes. With the point of view to expand its application field its study in agricultural use is going on. It is directly used in enhancing seed germination.

In recent decade a new class of polymer have been acknowledge by the scientists that are exclusively unique in their properties, performance and application. They are termed as smart polymer or stimuli polymer. Usually slight change in the environment are sufficient to induce large changes in their properties. These smart polymer includes another fascinating type of polymer with ability to retain sufficient amount of water. They are called hydrogels. Hydrogel are three dimensional hydrophilic polymer network capable of swelling in water or biological fluids, and retaining a large amount of fluids in the swollen state. Many polymer products including hydrogels are available these days but is not necessary that their natural aspects are considered. Due to the environmental awareness a new trend towards ecofriendly products is going on. Hydrogels have gained much attention in biomedical fields as carriers for drugs, protein, cells, and others because of their good biocompatibility, solute permeability and tunable release characteristics. The retaining ability of a large amount of water within their structures which results in high water content and soft-surface properties. Due to water retaining ability it can be used in agriculture for water supply to crops in controlled way.

II. EXPERIMENTAL

2.1 Raw Materials

Fish scale of Labeo Rohita (Rohu) procured from local fish market, chitosan, hydrochloric acid, sodium hydrochloride, methanol, acetone, acetic acid, deionized water and sodium dodecyl sulphate. All chemicals used were of lab grade.

2.2 Extraction of Chitosan

Preparation of chitosan include three basic step demineralization, deprotonization and deacetylation. Wash the scales and dry them in the sun for almost three days then store them in an air tight container. Deacetylaion is done to remove minerals from fish scales like calcium compounds. For this soak the dried scales in 1.0M hydrochloric acid until scales become squashy. Strain the excess acid and rinse the squashy scales with water, wash the demineralized scales with methanol and acetone and dry them at about 600C for 5 hours.

Take the dried scales add to 0.5 M NaOH solution and maintain the solution temperature at 400^0C with constant stirring for about 1.5 to 2 hours. Thus the residue so obtained is washed with water to till the pH becomes neutral. This yields the product called chitin. This basic treatment is given to scale to remove protein as their presence deviate further characterization results. The obtained chitin is then again kept in 0.25 M NaOH solution at temperature of about 500^0C for about 4 to 5 hours this yields crude chitosan. This is further purified by adding acetic acid to it and centrifuging it followed by drop wise addition of 1M NaOH solution. Collect and wash the precipitated product with distilled water to get pure chitosan.

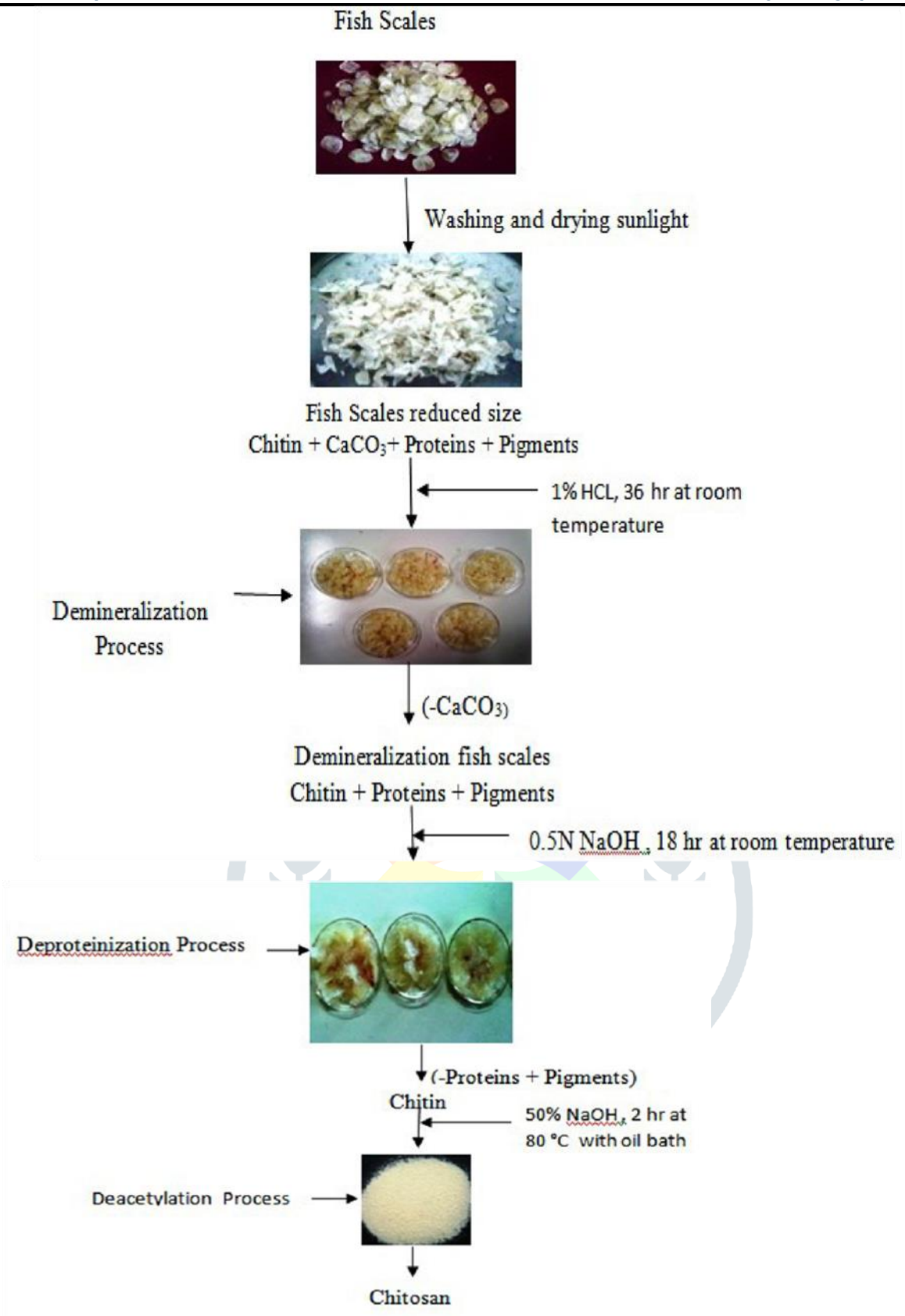

Figure 1. Flowchart for extraction of chitosan.

2.3 Preparation of Hydrogel

Weigh 0.4 and 0.6 gram of chitosan add it to 50ml of water then add acetic acid to the solution till the pH of the solution become 4. Now inject this solution to 1M NaOH (gelling solutions) to get the gel in the form of sphere. Repeat the same procedure and inject the solution to 50mM sodium dodecyl sulphate (gelling solution). Let the solution be as it is for next 6 hours thus obtaining hydrogel. Immerse the obtained hydrogel in ethanol/water solution and again let it rest for 5 to 6 hour this gives us product called alcogel. Then dry it using evaporative drying to get xerogel.

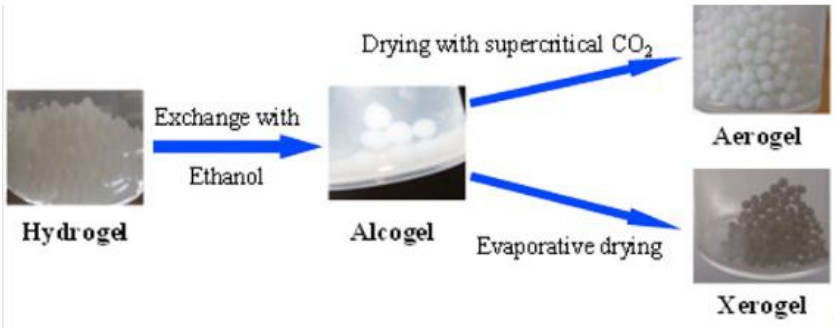

Figure 2. Synthesis of Chitosan Gel (Aerogel and Xerogel)

III. OBSERVATION

3.1 Visual Observation

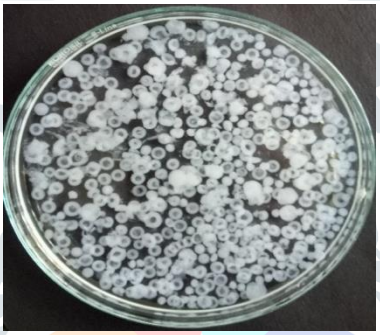

Figure 3. 0.6 NaOH Hydrogel.

While injecting the chitosan solution in NaOH and SLS we used 2 concentrations viz. 0.6% and 0.4%. We observed that while preparing chitosan solution specifically pH 4 has to be maintained to dissolve chitosan in water, with slight variation in pH, solubility changes.

The solution of 0.6 concentration, the viscosity of solution is more as compared to 0.4 concentration.

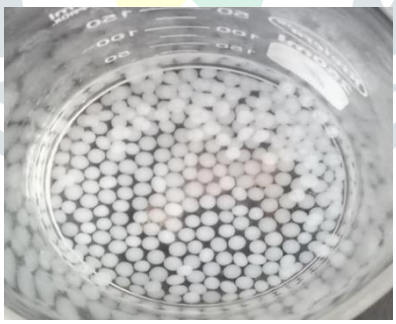

Figure 4. 0.4 NaOH Hydrogel.

The balls of 0.6 concentration formed when solution is injected in it have a spherical shape but are hazy in border and transparent in center. This can be due to viscosity difference. (Can be observed in figures).

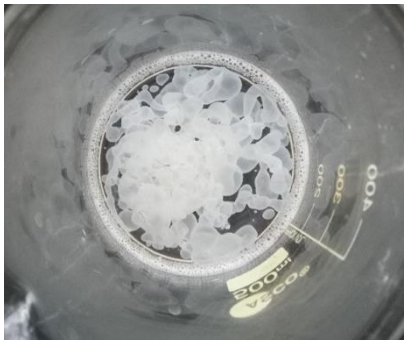

Figure 5. 0.6 SLS Hydrogel.

The balls formed are perfectly spherical and have same opaqueness allover. The drying time required for this is around 4 hours. While pouring the chitosan solutions in SLS it immidiately solidify which gave continuous flexible pipe like structure to the gel.

Figure 6. 0.4 SLS Hydrogel.

The gel so formed have a transparency in the start for few minutes and then it gets its opaqueness. This product have a little rough surface and after drying the xerogel obtained have toughness more than NaOH solution gel.

3.2 Weight Observation

Table 1. Weight Observations

Hydrogel injected in sodium hydroxide solution	
Concentration of chitosan	Weight of hydrogel obtained
0.4 % by wt.	28.76 grams
0.6% by wt.	22.5 grams
Hydrogel injected in sodium lauryl sulphate solution	
Concentration of chitosan	Weight of hydrogel obtained
0.4 % by wt.	29.67 grams
0.6 % by wt.	23.44 grams

IV. RESULTS AND DISCUSSION

4.1 FTIR of Chitosan

Fourier-transform infrared spectroscopy (FTIR) is a technique used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas. An FTIR spectrometer simultaneously collects high-spectral-resolution data over a wide spectral range. FTIR Spectroscopy, is an analytical technique used to identify organic, polymeric, and, in some cases, inorganic materials. The FTIR analysis method uses infrared light to scan test samples and observe chemical properties. The product obtained after the experimentation was analysed by FTIR in which the presence of functional groups of chitosan have been ascertained such as =C-H, C-O, C=C and –OH. The peak of 3000-5000 shows the stretch of C-H, O-H, -NH. The C=O stretch is observed to be strong at 1300. The peak value of 2250 shows the functional group –C=C- stretch.

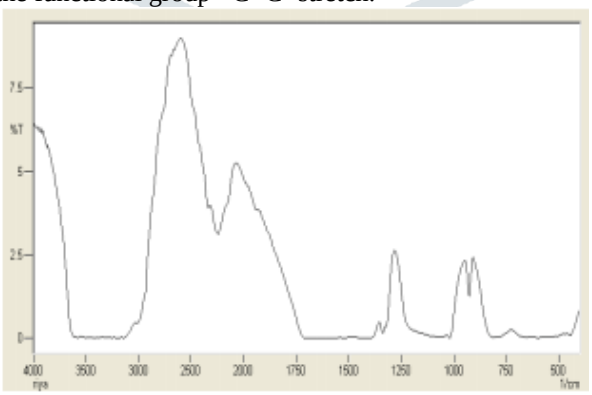

Figure 7. FTIR Spectogram of Chitosan.

4.2 SEM of Chitosan

For chitosan, SEM analysis showed that chitosan had a smooth and homogenous surface. The SEM analysis showed a three-dimensional morphology.

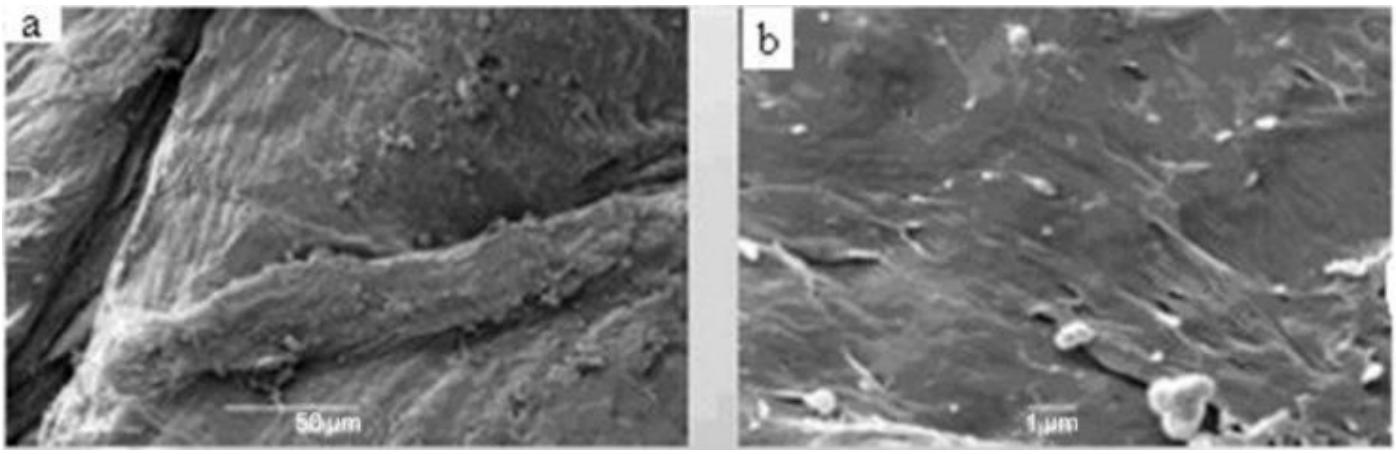

Figure 8. SEM of Chitosan.

4.3 FTIR of Hydrogel

Following figure shows the IR spectra of chitosan hydrogel (concentration 0.4). The peaks 1772,1669, 1690 cm-1 showed The carbonyl stretching absorption is one of the strongest IR absorptions, and is very useful in structure determination as one can determine both the number of carbonyl groups (assuming peaks do not overlap) but also an estimation of types such as for above values is Aldehyde C=O Stretch and Ketone C=O Stretch. The other characteristic peaks such as 997 and 809 cm-1 value shows the C-H methoxy group shifted to lower wavelength with increasing C-H content, meanwhile the characteristic absorption peaks of COOH Groups (1772 cm-1) became broader obviously and a new absorption peak appeared at 1677 cm-1.

Figure 9. FTIR of Hydrogel.

4.4 Thermal Analysis of Hydrogel

The DTA data showed for the gels obtained from chitosan gelled by sodium hydroxide a weight losses in the temperature range 100-200°C (figure 9), Thus, these gels are stable in this temperature range. For the xerogel resulting from the chitosan one notices the absence of the weight loss in the temperature range 100-200°C as shown for the chitosan. This difference is due to the degree of acetylation. The xerogel gelled by SDS (figure 10), takes a form of TGA similar to the xerogel gelled by sodium hydroxide. The only difference is at the DTA spectra where the xerogel is completely degraded towards 500°C.

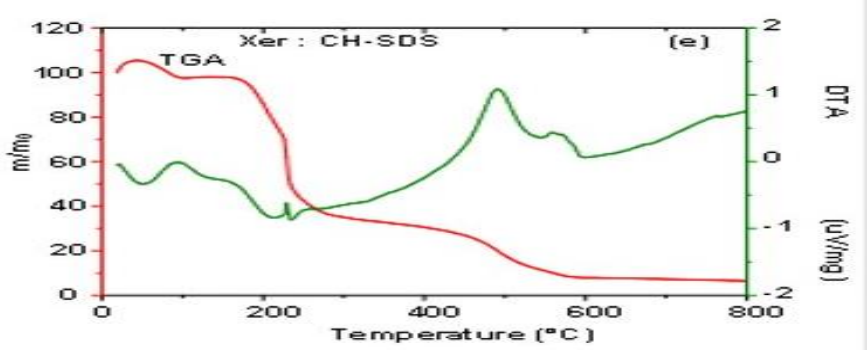

Figure 10. Xerogel: Chitosan gelled by SLS 0.4%

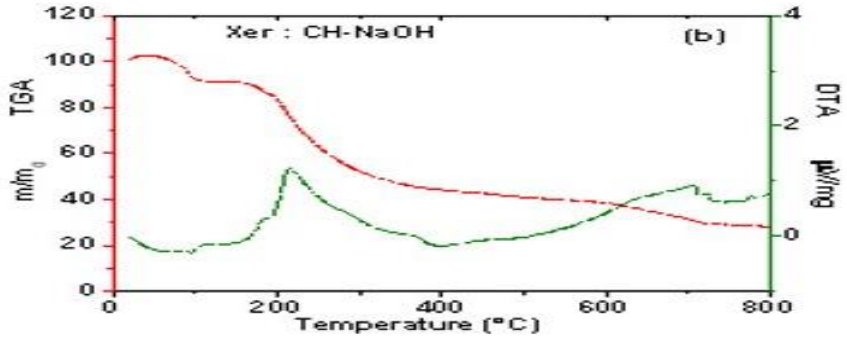

Figure 11. Xerogel: chitosan gelled by NaOH, 0.4%

V. CONCLUSION

A biodegradable polymer called chitosan is obtained by deacetylation of chitin which was extracted from fish scales by further two processes viz. demineralization and deproteinization. This polymer has application in various fields. The gelation of this polymer is quite easy and enhances its number of properties such as solubility, biocompatibility, as well as its application too. The increase in the chitosan concentration or the degree of acetylation decreases the specific surface of the chitosan gel. The chitosan concentration, the degree of acetylation (DA) of the chitosan and the drying conditions of the alcogels have an influence on the final properties of the xerogel. Chitosan gel improved the water-holding properties of soils hence making efficient use of water in agriculture. It was found that the gel improves soil permeability, reduce the need for irrigation and improve plant growth.

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