

Effect of Degree of Deacetylation and Molecular Weight of Chitosan Extracted From Various Marine Sources on Its Applications

Devikrishna S¹, Remya R²

PG Student, Department of Biotechnology and biochemical Engineering, Sree Buddha College of Engineering and
Technology, Pattoor, Alappuzha, Kerala, India.¹

Assistant Professor, Department of Biotechnology and biochemical Engineering, Sree Buddha College of Engineering
and Technology, Pattoor, Alappuzha, Kerala, India.²

ABSTRACT: The biopolysaccharide is characterized as either chitin or chitosan according to the degree of deacetylation (DD) which is determined by the proportion of D-glucosamine and N-acetyl-D-glucosamine. Structurally chitosan is straight chain copolymer composed of D-glucosamine and N-acetyl-D-glucosamine which is being obtained by the partial deacetylation of chitin. Chitosan solubility, biodegradability, reactivity, and adsorption of many substrates depend on the amount of protonated amino groups in the polymeric chain, therefore on the proportion of acetylated and non-acetylated D-glucosamine units. DD and distribution of acetyl groups along the polymer chain play crucial role in chitosans molecular weight characteristics and activity. DD is determined by conductometric titration methods and molecular weight determined by Ubbelohde capillary viscometry. Both are found to be effective.

KEYWORDS: Chitosan , Conductometric titration, Degree of deacetylation, Ubbelohde capillary viscometry

I. INTRODUCTION

Among the novel families of biological macromolecules, whose relevance is becoming increasingly evident, is chitin and its main derivative chitosan. Potential and usual applications of chitin, chitosan and their derivatives are estimated to be more than 200. The wide range of applications include biomedicine, food, biotechnology, agriculture and cosmetics. The fine structure of chitosan is defined by the overall or bulk content of D-hexosamine residues as well as their distribution along the polymeric chain. Chitosan is usually isolated from exoskeletons of crustacean, molluscs, insects and certain fungi. Chitosan, a cationic polysaccharide exhibits a wide variety of biological activities, including anti tumor activities, immunostimulating activities, cholesterol-lowering effects, antimicrobial effects, wound healing effects, antifungal activities and free radical scavenging activities. Chitosan possess several unique properties such as non toxicity, biocompatibility and biodegradability, which make it an attractive candidate for pharmaceutical and biomedical applications. In this work, applications considered are, antibacterial, antifungal and heavy metal ion removal from waste water. The value of DD and molecular weight of different samples are estimated by conductometric titration method and viscometric method respectively. Both methods are found to be effective.

II. MATERIALS AND METHODS

1. RAW MATERIAL PREPARATION

The different local resources used to extract chitin are prawn shell and mollusk shells. The shells of these species were scraped free of loose tissue, washed well and dried under sunlight. Then these are crushed and grounded well to pass through a 0.315-0.5mm sieve. After this these are subjected to demineralization and deproteinization.

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 7, July 2015

a. DEMINERALISATION

Demineralisation was carried out in dilute HCl solution. All species were treated with 7% HCl solution at ambient temperature and mixed well. The treatments with HCl (7%) and their durations 24-72hr depends on the nature of species after treatment the resulting solid fraction was washed and dried.

b. DEPROTEINIZATION

Deproteinization of chitin was carried out using 10% NaOH at 60°C. The treatment was repeated several times. The absence of proteins was indicated by the absence of colour. Then the solution was washed with water to neutrality and dried.

c. PREPARATION OF CHITOSAN

Dry chitin was heated with 50% NaOH solution in the ratio of 1:15 at 90-95°C. After heating about 2hr its solubility is tested with 1% acetic acid. When the substance was completely soluble in acetic acid heating was stopped. The resulting fraction was washed and dried. The chitosan prepared from different sources were estimated by spectrophotometric methods at 410 nm. Chitosan solution was prepared from three different samples and one standard was prepared from chitosan purchased. 2g of each samples were dissolved in 100 ml of 1% acetic acid(1N) and 2ml of 1NHCl. Absorbance was checked at 410 nm in a spectrophotometer. A graph plotted between the absorbance value and source of chitosan.

2. DETERMINATION OF DEGREE OF DEACETYLATION

0.2 g chitosan sample was dissolved in 40ml HCl and titrated with 0.5 ml of NaOH. The values of conductance with the corresponding titrant volumes were plotted. Two stoichiometric points corresponds to the volume of base required.

$$\%DD = \frac{[\text{base}] (V_2 - V_1) 161}{m}$$

Where, [base] is concentration of NaOH solution in mol/l, V_2 & V_1 is volume of NaoH used in titration, 161 is molar mass of monomer and m is mass of chitosan.

3. DETERMINATION OF MOLECULAR WEIGHT

Molecular weight was determined by viscometric method. Chitosan samples of different concentrations (0.001,0.002,0.003,0.004 g/ml) were prepared in 0.1 molar acetic acid. Relative viscosity of chitosan samples were measured using Ubbelohde capillary viscometer.

$$\eta_{sp} = \frac{(\eta_{\text{solution}} - \eta_{\text{solvent}})}{(\eta_{\text{solvent}})}$$

Intrinsic viscosity, $[\eta]$ defined as reduced viscosity, obtained by extrapolating chitosan concentration zero. Molecular weight obtained from the following equation by substituting the value of intrinsic viscosity.

$$[\eta] = K (M_v)^a$$

Where, $K = 1.64 (10)^{-30} (DD)^{14}$ and $a = -1.02 (10)^{-2} (DD) + 1.82$, DD is expressed as percentage.

4. DETOXIFICATION METHOD USED FOR THE REMOVAL OF METAL IONS

10ml of waste water sample were taken in 15 conical flasks. Varying concentration of solutions say 1%, 2%, 3%, 4%, 5% were prepared in acetic acid from each chitosan sample and added to the waste water samples. This was kept undisturbed overnight and OD was measured at 510nm.

The % removal of ions was obtained by,

$$\% \text{ Removal of ions} = \frac{\text{Initial OD} - \text{Final OD}}{\text{Initial OD}}$$

5. ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY

Oxford agar cup method and disc diffusion method are used to confirm these two activities of chitosan. Bacterial culture used was E-coli and fungus was yeast. In disc diffusion, three paper discs dipped in 3 different chitosan solution (2%), were placed on to the medium in which the culture was spreaded. After incubation, zone of inhibition was noted. In oxford agar cup method, wells are bored on the medium and the chitosan solutions were added.

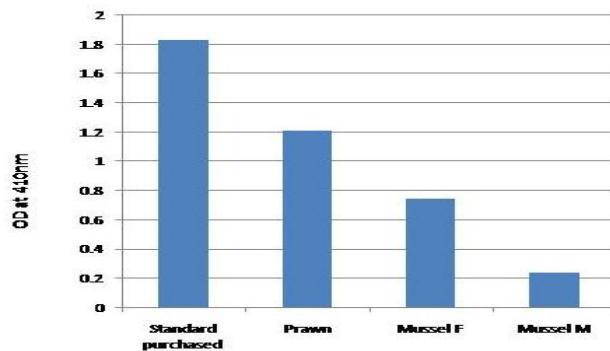
International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 7, July 2015

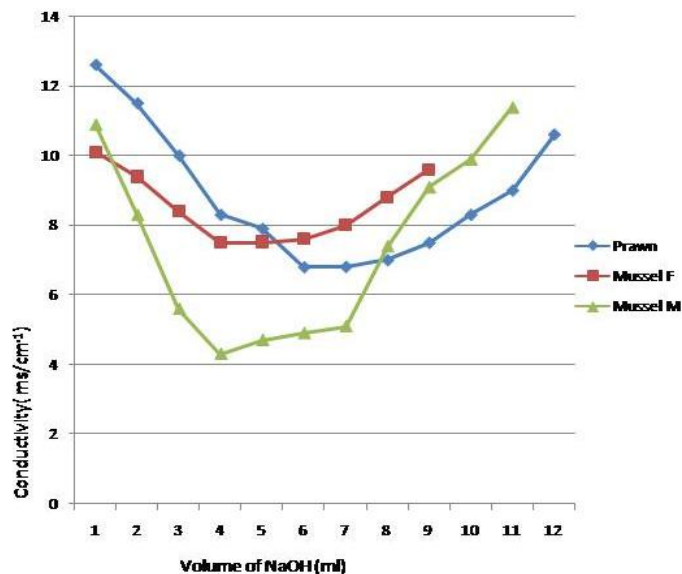
III. RESULTS AND DISCUSSIONS

1. Chitosan was obtained from three different sources like prawns, fresh water mussels and marine mussels and estimated. Graph 1 shows absorbance at 410 nm and prepared chitosan sources. Estimation of chitosan is confirmed from the absorbance noted.



Graph 1:

2. Determination of DD: The two stoichiometric points obtained are 2 and 3.6 for prawn shells and %DD=79.695. For fresh water mussels V_1 & V_2 are 2 and 2.35 and %DD = 46.48. For marine mussels V_1 and V_2 are 4 & 7, %DD=39.8. Graph 2 shows conductivity Vs Volume of NaOH. Volume from given graph is used to find the % DD values



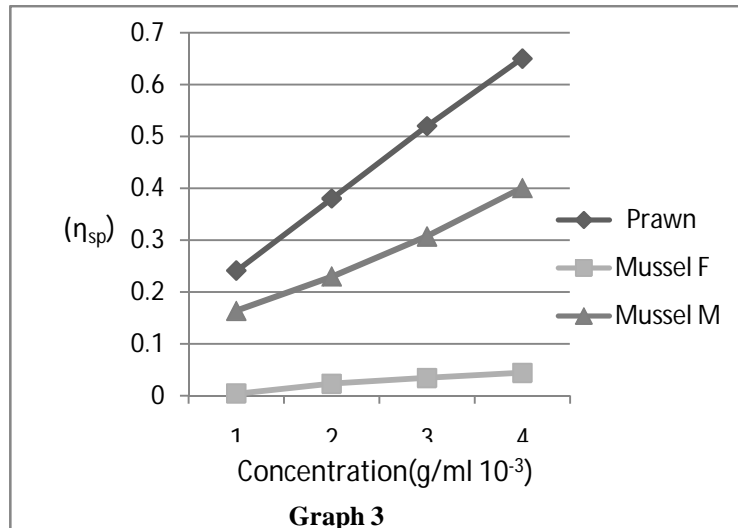
Graph 2

3. Determination of molecular weight: For prawn sample $[\eta]$ obtained was 0.17, and $M_v=239.141$. For fresh water mussel $[\eta]$ was 0.0015 and $M_v=488.197$. For marine mussels $[\eta]$ was 0.08, and $M_v=646.875$. Graph 3 shows specific viscosity Vs concentration.

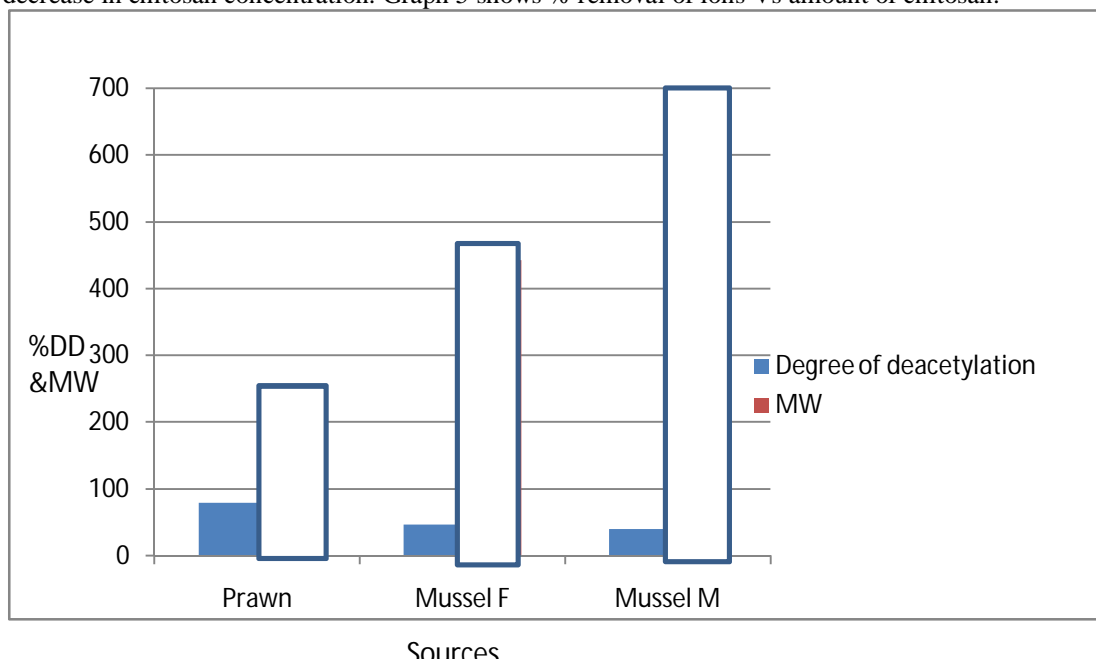
International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 7, July 2015



4. Detoxification method for removal of metal ions: The absorbance of waste water samples which were treated with varying concentrations of chitosan with different DD and molecular weight was noted by spectrophotometric method. It was observed that the maximum percentage of ion removal was obtained when chitosan with higher DD is employed. That is, when chitosan with lower molecular weight is used. Also, it was observed that the ion removal activity decreases with decrease in chitosan concentration. Graph 5 shows % removal of ions Vs amount of chitosan.



Graph 4

Graph 4 depicts variation in %DD and molecular weight with sources of chitosan and they are inversely related. Chitosan from prawns have higher %DD and low molecular weight.

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

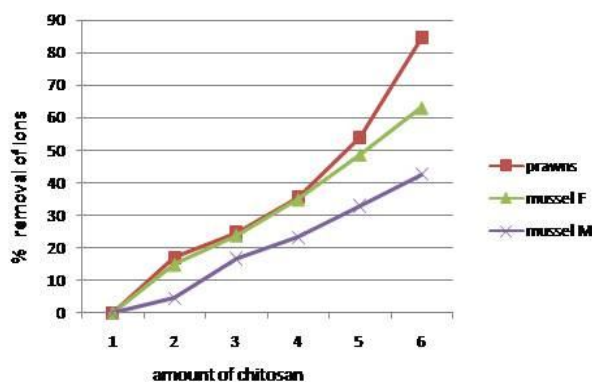
Vol. 4, Issue 7, July 2015

5. Chitosan with higher DD showed higher antibacterial activity than chitosan with low DD and high molecular weight. Chitosan with higher DD and low molecular weight showed higher antifungal activity than chitosan with lower DD and higher molecular weight. Zone of inhibition for both bacteria and fungus were found to be higher for higher DD value & for lower molecular weight. Fig 1 and 2(sample 1-Prawns, sample 2-Mussel F and sample 3-Mussel M) shows antibacterial and antifungal activity respectively of chitosan.



Fig 1

Fig 2



Graph 5

IV. CONCLUSIONS

The study showed abundant low cost biopolymer, chitosan and its ability to remove hard metal ions from the waste water. The biopolymer chitosan was successfully extracted from marine and fresh water shells. The antibacterial and antifungal activity was found to increase in chitosan solution concentration. Degree of deacetylation and molecular weight was successfully determined. Molecular weight and degree of deacetylation both are having important role in determining activity of chitosan. High molecular weight decreases the activity. Chitosan with higher DD is more active. Applications of chitosan is highly influenced by DD and molecular weight.

REFERENCES

- [1] Dos Santos Z. M, Caroni A. L P F, Pereira M R, Da Saliva D R and Fonseca J L C. "Determination of degree of deacetylation". Carbohydrate Research, Vol .344,pp.2591-2595,2002.
- [2] Duarte M L, Ferreira M C, Marvao M R and Rocha J. " An optimized method to determine the DD of Chitin and Chitosan, Vol 31, pp.1-8,2002.
- [3] Dutta P K, Ravi Kumar M N V and Dutta J. "Chitin and Chitosan for versatile applications". J M S polym rev, C42,2002.

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 7, July 2015

- [4] Kim S S, Yao K D and Zhao F Li F. "Chitin and Chitosan: Functional biopolymers from marine crustaceans". Marine biotechnology, Vol 8, pp 203-226, 2002.
- [5] Kubota N, Tastumoto N, Sano T and Toya K " Carbohydrate Research". Vol 324, pp. 268-274, 2000.
- [6] Li Q Dunn, E T Grandmaison E W and Goosen M F A. "Applications and properties of Chitosan, Applications of chitin and chitosan". 1997.
- [7] Manni L, Ghorbel-Bellaaj O, Jellouli K, Younes I and Nasri M. "Extraction and characterization of chitin, chitosan". Appl Biochem Biotechnol, Vol 162, pp. 345-357, 2010.
- [8] Rinaudo M., "Chitin and Chitosan: properties and applications" Progress in polymer science, Vol 31, pp. 603-632.
- [9] Tracey M V " Chitinase in some basidiomycetes" Biochem J, Vol 61, pp 579-586, 1955.
- [10] Zouhour Limam, Salah Selmi, Saloua Sadok and Amor El Abed. "Extraction and characterization of chitin and chitosan from crustacean by-products: Biological and physicochemical properties". African Journal of Biotechnology Vol. 10 (4), pp. 640-647, 2011.