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Study on Optimization of Carboxymethylation of Chitosan Obtained from Squilla Chitin

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The physico-chemical characteristics of chitin, chitosan and their derivatives differ with crustacean species and preparation methods. The relationship between process conditions and characteristics of chitin and chitosan products are monitored to achieve uniformity and proper product quality control. Chitin, chitosan and its derivatives are mainly obtained from prawns and crab. In the present study alternative source squilla, a by-catch of Indian ocean fisheries is studied for preparation of chitin, chitosan and its water soluble derivative carboxymethyl-chitosan having unique chemical, physical and biological properties. With green chemistry approach further work was carried out to minimize the chemicals going to effluent. The carboxymethylation of chitosan was optimized by variation in solvent, mole ratio of NaOH to Chloro-acetic acid and reaction parameters like temperature and time. Carboxymethylchitosan sample prepared was checked by gel permeation chromatography.

Key Words: Chitosan, Carboxymethylchitosan, Degree of substitution, Squilla, Recycling.

INTRODUCTION

Chitin, poly-β-(1-4)-N-acetylglucosamine, is the second most abundant natural polysaccharide prepared from shells of crustacean and insects. Chitosan, poly-β-(1-4)-D-glucosamine, is readily prepared from chitin by deacetylation with alkali. Despite much research on their utilization, poor solubility in water and common organic solvents have limited their widespread applications[1]. This difficulty can be overcome by reaction of chitosan with chloroacetic acid (Fig. 1) in presence of alkali to produce a water soluble derivative known as carboxymethylchitosan.

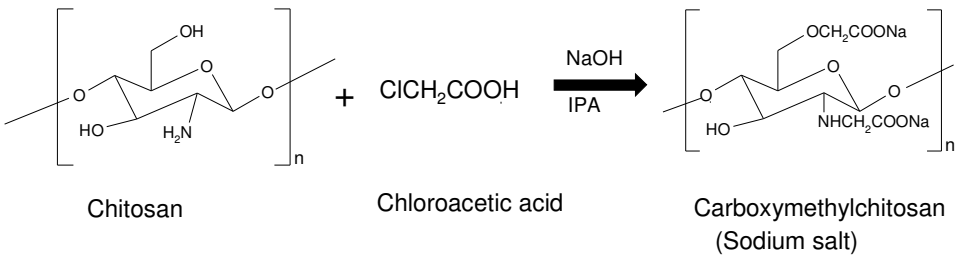

Fig. 1. Reaction Scheme for preparation of sodium carboxymethylchitosan

Carboxymethylchitosan[2,3] is a polyampholyte and has unique chemical, physical and biological properties such as high viscosity, large hydrodynamic volume, low toxicity, biocompatibility and film, gel forming capabilities which makes it useful in its use in food products and cosmetics.

The physico-chemical properties of chitin, chitosan and their derivatives differ with crustacean species and preparation methods. Thus the relationship between process conditions and characteristics of chitin and chitosan products must be monitored to achieve uniformity and proper product quality control.

Squilla (Fig. 2) is a by-catch obtained while fishing for prawns. Squilla doesn't have any food value therefore it is generally considered as a waste. In Indian context, work on conversion of this biowaste in to valuable product like carboxymethyl-chitosan acquires special significance as India is endowed with more than 7000 kms of coastline.

Fig. 2. Squilla

EXPERIMENTAL

Sun dried squilla was collected from the Dabhol port of Konkan region in Maharashtra state. Squilla was converted to chitin and chitosan in our laboratory. Chitin was prepared from the squilla by carrying out decalcification and deproteinization. Chitin was deacetylated with 50 % sodium hydroxide to obtain chitosan. The degree of deacetylation (DDA) of chitosan obtained is found to be 82 % by titration method[4]. In the similar way chitin and chitosan was prepared from prawns shells. The degree of deacetylation (DDA) of chitosan obtained is found to be 84 %. Iso-propanol and chloroacetic acid of LR grade were procured from SD Fine chemicals, Mumbai.

The degree of substitution (d.s.) is defined as average number of substituents per anhydroglucose unit. The degree of substitution is used for evaluations of the influence of reaction parameters used for carboxymethylation. Degree of substitution was checked by conductometric[5] method. Viscosity of 1 % aqueous solution of the sodium carboxymethylchitosan samples were checked by Brookfield viscometer. The sample of carboxymethylchitosan was characterized by using FT-IR Perkin-Elmer 1640 instrument. The carboxymethylchitosan is obtained as a sodium salt.

Gel permeation chromatography was done using Agilent HPLC. The quality of carboxymethylchitosan obtained from squilla was compared with that obtained from prawn's shell which is commonly used source.

Carboxymethylation procedure: Chitosan (10 g), was dispersed in 100 mL of solvent and 30 mL of 10 M NaOH solution. Reaction mass was stirred at 25-30 ºC for 1 h. Then solution of chloroacetic acid (13 g in 50 mL of solvent) was added over period of 0.5 h at 25-30 ºC. Temperature was raised to 60 ºC stirred for 8 h. Samples were drawn at 2, 4, 6 and 8 h to check the degree of substitution. Reaction mass was cooled to 25 to 30 ºC. The pH of the reaction mass is adjusted to 7-8 using glacial acetic acid. The product was filtered and washed twice with 100 mL of 70 % aqueous methanol. Then the product was washed with methanol (50 mL) and dried at 60 ºC for 4-5 h.

Preparation of carboxymethylchitosan by recycling of filtrate: Chitosan (10 g), was suspended in 175 mL of mother liquor and 3.0 g of NaOH. The reaction mass was stirred at 25-30 ºC for 1 h. Then solution of chloroacetic acid (13 g in 50 mL of isopropanol) was added over period of 0.5 h at 25-30 ºC. Temperature was raised to 60 ºC and continued stirring for 3 h. Reaction mass was cooled to 25 to 30 ºC. The pH of the reaction mass is adjusted to 7-8 using glacial acetic acid. The product was filtered and used for next recycling experiment (Fig. 3). The wet cake was washed twice with 100 mL of 70 % aqueous methanol. Then the product was washed with methanol (50 mL) and dried at 60 ºC for 4-5 h.

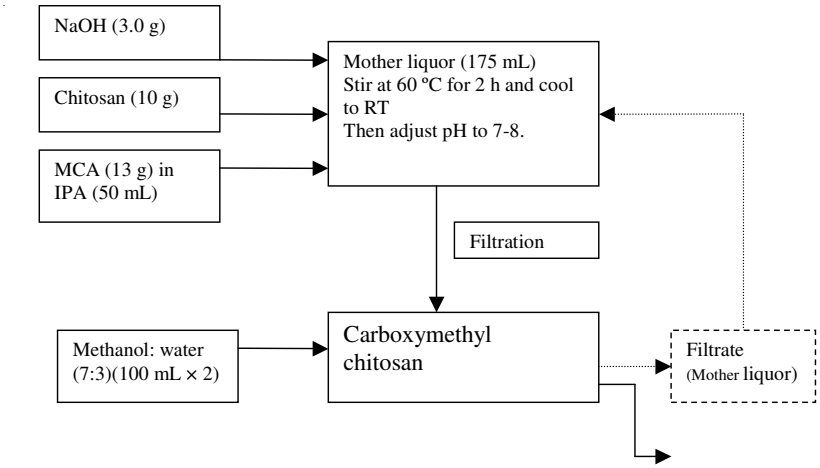

Fig. 3. Recycling of mother liquor for preparation of carboxymethyl chitosan

RESULTS AND DISCUSSION

The use of alternative source of chitosan produced from squilla chitin was studied for the carboxymethylation. As the source of biopolymer has significant impact on process conditions, quality of product obtained, the carboxymethylation parameters were studied to arrive at the optimized procedure. The parameters like type of organic

solvent, temperature, NaOH/monochloro acetate ratio were studied. The effects of reaction conditions on the carboxymethylation were studied by varying one parameter at a time while others were maintained at standard conditions.

Solvent screening studies: In literature for carboxymethylchitosan of shrimp or crab chitosan isopropanol is commonly used[6], therefore it was compared with other alcoholic solvents like methanol, ethanol. Reaction was also carried out on DMSO. The graph of degree of substitution was plotted against samples drawn at different time points for carboxymethylation in different solvents. From the graph it is evident that isopropanol is the better solvent and the degree of substitution is 0.86 whereas for products with solvent ethanol, DMSO or methanol the degree of substitution is 0.71, 0.6 and 0.17, respectively (Fig. 4).

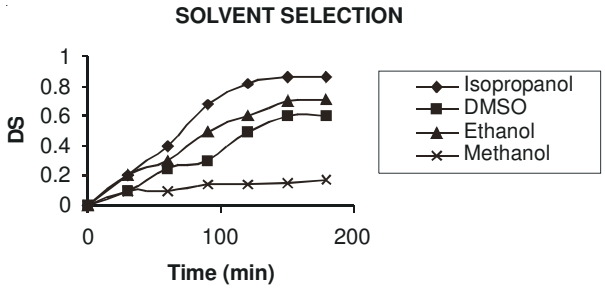

Fig. 4. Plot of degree of substitution (DS) *versus* time

Influence of temperature: Carboxymethylation was carried out at 30, 45, 60 and 90 ºC using isopropanol as solvent. The reactions were stirred at these temperatures for 3 h. Then the products obtained were checked for d.s. It is observed that at 60 ºC the d.s. value is 0.86 whereas at 30 and 45 ºC, d.s. values are 0.52 and 0.69, respectively. However the product obtained at 90 ºC is gelatinous and expected to have higher d.s. value. Based on these results, it may be concluded that the carboxymethylation at 60 ºC is the optimum temperature.

Molar ratio of chloroacetic acid and NaOH: The work by Tokura and co-workers[7] demonstrated that the degree of substitution value of carboxymethylchitosan increased with NaOH concentration from 20 to 40 %.

The NaOH concentration above 60 % promotes side reaction between NaOH and $ClCH_2COOH$ and chloroacetic acid concentration decreases gradually[8]. We have used 40 % NaOH for our experiment and varied the molar ratio of chloroacetic acid and NaOH to check its influence on degree of substitution. The effect of n_{NaOH}/n_{MCA} ratio has significant effect on the rate of reaction and final degree of substitution of carboxymethylchitosan. For proper comparison the n_{NaOH}/n_{MCA} ratios were applied (1, 2 and 3), all by varying NaOH quantity at constant sodium monochloro acetate (2.2 mole per mole of chitosan monomer) using isopropanol as solvent at 60 ºC. From the graph (Fig. 5) it can be seen that at n_{NaOH}/n_{MCA} ratio of 1 degree of substitution reaches a maximum of 0.58, whereas ratios of 2 and 3 can give

carboxymethylated products of degree of substitution 0.82 and 0.84, respectively. Therefore the ratio of 2.0 is optimum for carboxymethylation. When the reactions were carried out at ratios above 5.0 a gelatinous product was obtained.

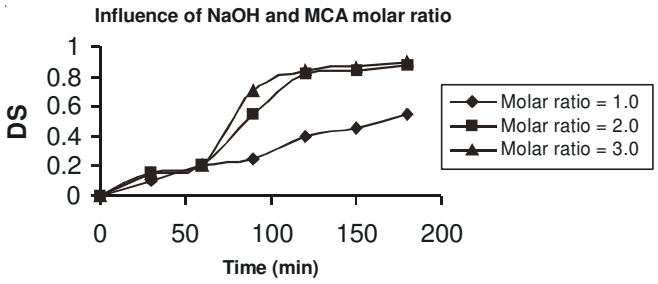

Fig. 5.

According to Okimasu[9] the activation energy of carboxymethylation is 22.4 kcal, being the same as that of decomposition of sodium monochloroacetate with sodium hydroxide; therefore it is not possible to inhibit the side reaction by controlling only the temperature of reaction. The concentration of sodium hydroxide in the reaction medium has greater effect on the velocity of the carboxymethylation than on that of the side reaction. These results may be explained when considering various reactions taking place during the carboxymethylation process.

Main reaction:

$$ROH + NaOH \rightarrow RONa + H_2O$$

$$RONa + ClCH_2COONa \rightarrow ROCH_2COONa + NaCl$$

Side reaction is the formation of sodium glycolate from sodium monochloroacetate and sodium hydroxide according to:

$$NaOH + ClCH_2COONa \rightarrow HOCH_2COONa + NaCl$$

The presence of infrared absorption frequency at 1740 cm^{-1} indicates carboxy group and 1599 and 1401 cm^{-1} indicate carboxy group (overlaps with N-H bend) and carboxymethyl group. Compared to chitosan absorption frequencies of carboxymethylchitosan at 1599 and 1324 cm^{-1} increase indicating carboxymethylation has occurred on both amino and hydroxyl group (Fig. 6).

The quality of carboxymethylchitosan prepared from squilla chitosan was compared with sample prepared from prawn's chitosan which is the commonly used source in Table-1.

TABLE-1

Carboxymethylchitosan sample	Viscosity* (cps)	Degree of substitution (DS)
NaCMC-1 (Squilla source)	96	0.81
NaCMC-2 (Squilla source)	104	0.84
NaCMC-3 (Prawn shells source)	131	0.86
NaCMC-4 (Prawn shells source)	139	0.82

*1 % chitosan solution in 1 % acetic acid aqueous solution.

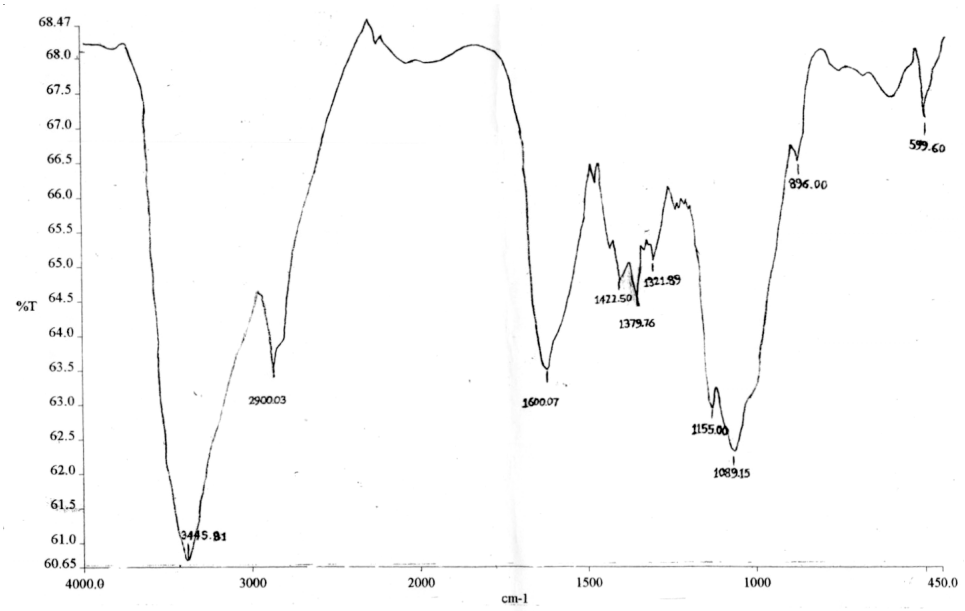

Fig. 6. IR spectra of chitosan (prepared from squilla)

Gel permeation chromatography was used to analyze carboxymethyl chitosan prepared from squilla and determine its molecular weight. The chromatogram is given in Fig. 7.

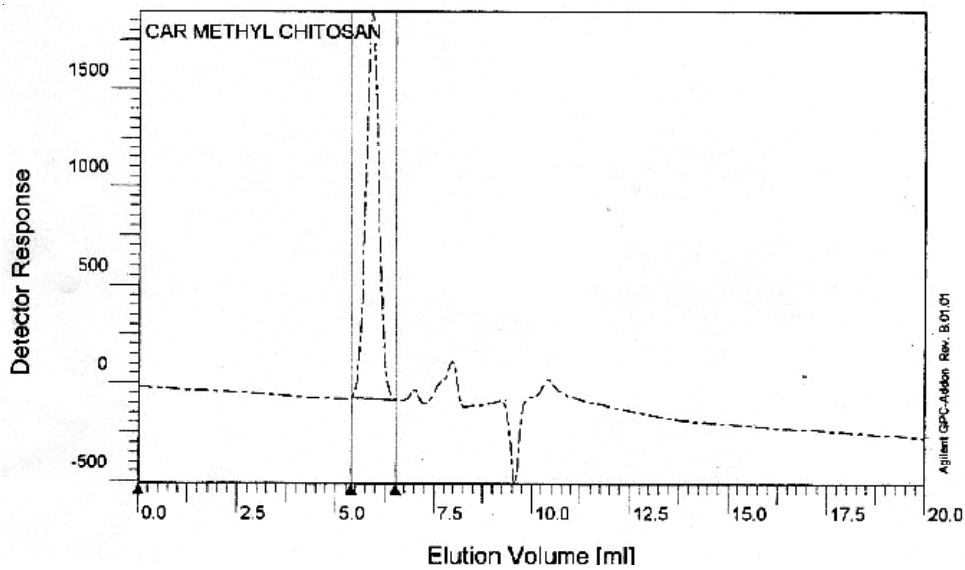

Fig. 7. GPC of carboxymethyl chitosan (prepared from squilla)

Calibration using GPC standards provided by Agilent technologies were used to calibrate GPC.

Red vial: standard molecular weight range from 615 to 1258000 (4 standards)

Blue vial: standard molecular weight range from 194 to 909500 (4 standards)

Green vial: Standard molecular weight range from 106 to 442800 (4 standards)

These standards were dissolved in water and 50 µL of each standard was injected on column PLaquagel-OHMIXED 8 µm, 300 × 7.5 mm, flow rate of 1 mL/min using RI detector and water as a mobile phase. A plot of retention time against log m was drawn within the software.

Sample preparation: Carboxymethyl chitosan sample was prepared by diluting 0.02 g of CMC with 100 mL of water, the sample was stirred for nearly 16 h to make a homogeneous solution. This sample (50 µL) was then injected under the similar conditions that used for calibration. The calibration file was down loaded for the determination of MW of carboxymethyl chitosan. The molecular weight was found to be 5×10^6 g/mol. The molecular weight[2] for carboxymethylchitosan prepared from Euphausia superba chitosan is reported to be 4.5×10^6 g/mol.

Recycling of filtrate for carboxymethylation: In order to reduce the use of isopropanol and sodium hydroxide to make cost effective process as well reduce the effluent burden, the carboxymethylchitosan obtained by three recycle experiments (Fig. 2) was checked for the degree of substitution and found to be comparable. In each recycle experiment ¼ th NaOH quantity was added to compensate the loss of NaOH in the carboxymethylation. Table-2 gives the comparison of carboxymethylated product obtained by recycling of filtrate.

TABLE-2

Recycle experiment	Viscosity* cps	Degree of substitution (DS)
NaCMC (Squilla source) R1	93	0.81
NaCMC (Squilla source) R2	85	0.75
NaCMC (Squilla source) R3	96	0.74

*1 % chitosan solution in 1 % acetic acid aqueous solution.

Conclusion

In this work we have optimized the reaction conditions for carboxymethylation of chitosan obtained from squilla. The process of optimization was carried out by applying green chemistry approach by which the chemicals going to effluent were minimized by resorting to recycling of the chemicals. The quality parameters like degree of substitution, 1 % aqueous solution and viscosity for the carboxymethylchitosan samples produced by recycling of the mother liquor (three recycles) were comparable to the product without recycling.

The optimum parameters for carboxymethylation were deduced and the degree of substitution of the product formed was comparable to carboxymethylchitosan prepared from chitosan of the prawn source. The viscosity of the 1 % aqueous solution of carboxymethylchitosan prepared from squilla chitosan is lower than

that produced from prawn shells chitosan. The studies on the recycling of filtrate for carboxymethylation with partial sodium hydroxide compensation was feasible and would bring down the consumption of sodium hydroxide and isopropanol used in the process, thus reducing the effluent burden and cost of the process.

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