

A Novel Method for Preparation of Carboxymethyl Chitosan by Microwave Irradiation

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A novel method for the synthesis of carboxymethyl chitosan (CMC) has been adopted in the present investigation by the use of microwave irradiation. Carboxymethylation of chitosan, a renewable natural resource, has been carried out in an alkaline medium by using monochloroacetic acid. The effect of various parameters such as amount of chitosan, monochloroacetic acid, microwave power and temperature has been studied. Microwave irradiation increases the reaction speed and also results in high yield. Prepared carboxymethyl chitosan is highly soluble in water whereas chitosan is insoluble in neutral water. This water solubility property enhances the biological application of carboxymethyl chitosan. The obtained carboxymethyl chitosan is characterized by FTIR, NMR and TG analysis. Due to its wide range of solubility, carboxymethyl chitosan is extensively used in drug delivery systems.

Keywords: Carboxymethyl chitosan, Chitosan, NMR, FTIR, Microwave.

INTRODUCTION

Chitin is a naturally occurring polysaccharide which is the second most naturally occurring polysaccharide, after cellulose. Chitosan (CS) is the most important derivative of chitin. The polysaccharides chitin and chitosan are usually seen in the outer coverings/shells in many aquatic organisms, terrestrial organisms and some microorganisms. Bio wastes obtained from aquatic organisms are used for the manufacturing of chitin and chitosan. It is found that about 70 % of marine captures fisheries are used for processing and the remaining part goes waste [1]. Chitosan is commercially obtained by the deacetylation of chitin produced from aquatic animals like crabs. Normal concentrated alkali solutions at high temperatures results in chitosan. Chitosan can be prepared either by chemical method by deacetylation of chitin or by enzymatic deacetylation of chitin using acetyl xylan esterase [2,3].

Chitosan is an amino polysaccharide. Chitosan and its derivatives are highly non-toxic which increases its importance as biological active molecules. Chitosan is soluble in aqueous acidic solutions, but cannot easily dissolve in water. It is found that chitosan is soluble in aqueous acidic solution below pH 6.5. The solubility of chitosan in organic solvents is also found to be very poor and it is completely insoluble in aqueous

solution having pH beyond 7 as chitosan shows crystalline behaviour. Due to its poor solubility in water, chitosan has less application in biomedical field. Thus, modification of chitosan has been adopted by various researchers to increase the water solubility of chitosan [4].

Among various modifications, carboxymethylation of chitosan is one of the best ways of modifying the chitosan which can improve its solubility. Carboxymethylation can be done by two different ways like normal conventional method and also through microwave irradiation [5,6]. Chitosan has both amino group and hydroxyl group and carboxymethylation usually takes place at the amino and hydroxyl groups. By providing correct reaction conditions as well as proper reagents we can prepare four different substituted carboxymethyl chitosan like N-, O-, N, O- or N,N-carboxymethyl chitosan [6]. N- and N, O-substituted carboxymethyl chitosan is usually prepared at higher temperatures. N-carboxymethyl chitosan can be prepared by the reaction of chitosan with glyoxalic acid and then reducing it with sodium cyano borohydride. Thus, in the present study, an attempt has been made to prepare carboxymethyl chitosan by using microwave irradiation as this is faster in comparison to chemical modification method. In the current investigation, alkaline chitosan was added to a suspension of isopropanol/water followed by monochloroacetic acid.

EXPERIMENTAL

Biochemical reagent grade chitosan (deacetylation degree > 75 %) was purchased from Hi Media Laboratories Pvt. Ltd., Mumbai, India. Monochloroacetic acid was purchased from S.D. Fine Chem. Ltd., Mumbai, India. Sodium hydroxide, isopropyl alcohol, ethyl alcohol obtained from Sigma-Aldrich or Merck Chem. Co. and are used as received. Measurement of pH of solutions were carried out by using pH meter (PHS-25).

Procedure for preparation of carboxymethyl chitosan:

Carboxymethyl chitosan is prepared by adopting following procedure. The experiment was performed by varying the parameters like microwave power (W), temperature ($^{\circ}\text{C}$), time (min), amount of chitosan (g) and monochloroacetic acid (mL) in a microwave reactor. A specific example has been produced here for reference. 1 g of chitosan is added to a mixture of 10 M aqueous NaOH and 15 mL 2-propanol, stirred for 40 min at 40°C . 1 g of monochloro acetic acid is mixed in 12 mL of 2-propanol and the entire solution is subjected to microwave reactor. After requisite time period, the obtained product is cooled, washed with ethanol and filtered. Finally white carboxymethyl chitosan powder was obtained after drying.

Characterization: Fourier transform infrared (FTIR) spectra have been recorded using a Bruker 1600 FTIR Spectrophotometer. First the samples are finely ground and mixed with potassium bromide, KBr. The wave number range of 4000–400 cm^{-1} was used for FTIR spectral analysis. The ^1H NMR spectra of chitosan and carboxymethyl chitosan were acquired at 80°C using a Bruker AVANCE III 400, 11.75 Tesla, spectrometer operating at 400 MHz. Chitosan and CMCs were dissolved in $\text{CH}_3\text{COOH}/\text{D}_2\text{O}$ 1 % (v/v) and D_2O 1 % (v/v) respectively to get the ^1H NMR spectra. To get the chitosan and carboxymethyl chitosan spectra, a composite pulse presaturation (CPS) sequence for water signal suppression was used. The interval between pulses was 3 s, 32 scans were accumulated and the relaxation time was 7 s. The thermal stability of the samples was determined by using a Shimadzu TGA 50. Approximately 8 mg of sample was used in each measurement. All measurements were carried out under a nitrogen atmosphere with a gas flow of 50 mL min^{-1} by heating the material from room temperature to 700°C at a heating rate of $10^{\circ}\text{C min}^{-1}$.

RESULTS AND DISCUSSION

Basically, there are two steps which explain the preparation of carboxymethyl chitosan. In the first step, alkaline chitosan is treated with isopropanol. This helped as a swelling agent and as a diluents resulting in the entry of NaOH into the chitosan structure. In the second step alkaline chitosan is made to react with monochloroacetic acid [2–4]. The maximum yield obtained for CMC is 94.7 % at 100 W microwave power for period of 3 min and 50°C . The carboxymethyl group of monochloro acetic acid is substituted at the hydroxyl-oxygen and amine-nitrogen. And thus, N, O- substituted CMC is obtained [7]. The structure of chitosan and carboxymethyl chitosan were confirmed by infrared spectroscopy and NMR technique. The entire reaction is facilitated under microwave irradiation which is found to be very fast and high yielding.

The solubility of CMC has successfully checked with water [8–10]. On comparing with normal conventional methods which reported a maximum yield of 86 %, irradiation using microwave results in tremendous increase in yield *i.e.*, 94.7 % [11–15].

Fourier transform infrared spectroscopy: FTIR spectra of chitosan Fig. 1(a) shows basic characteristic absorption bands at 3421 cm^{-1} (O-H and N-H stretching), 2924 cm^{-1} (primary NH_2 at C_2) 1595 cm^{-1} (NH bending at primary amine); 1415 cm^{-1} (C-N stretching, amide III) and 1073 cm^{-1} (skeletal vibration of the C-O stretch). The carboxymethyl substituted chitosan Fig. 1(b) shows the occurrence of a strong band at 1663 cm^{-1} and a moderate band at 1587 cm^{-1} , 1386 cm^{-1} which corresponds to carboxymethyl substitution at the C_2 and carboxyl group overlaps with N-H bend, respectively, which reveals the substitution of the carboxymethyl groups in chitosan backbone [7]. These bands were attributed to symmetric and asymmetric deformation of COO^- respectively.

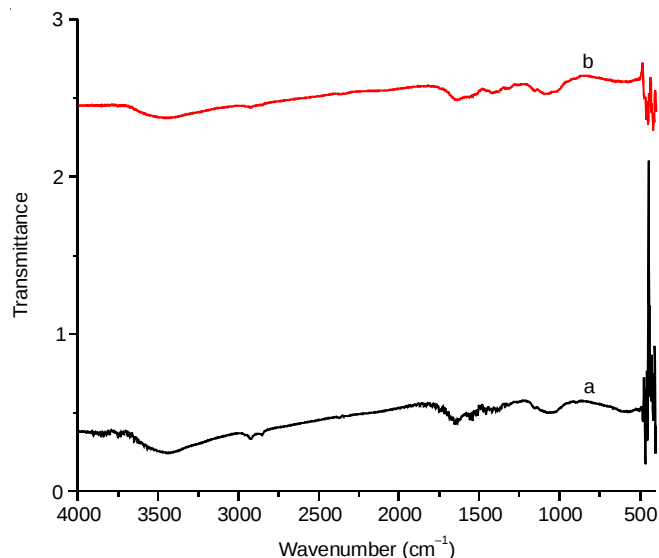


Fig. 1. FTIR spectrum of raw chitosan (a) and carboxymethyl chitosan (b)

^1H NMR analysis: The proton NMR spectrum of carboxymethyl chitosan in D_2O is shown in Fig. 2. As the CMC is substituted polysaccharide, a complex NMR spectrum is obtained for CMC. The resonance 3-acetyl-protons (2 ppm), H3–6 protons (3.6–3.9 ppm), H–2D proton (3.3 ppm) are found to be the basic peaks of chitosan resonance. This can be seen in the ^1H NMR spectrum of chitosan described previous studies. The protons resonances of substituted carboxymethyl group (O- CH_2 -COOD) of carboxymethyl chitosan can be observed between 4.46 and 4.49 ppm *i.e.* the resonance of protons of 3- and 6-carboxymethyl of carboxymethyl chitosan. The peak at 4.7 ppm is due to the solvent D_2O . The resonance signal of the proton from N- CH_2 -COOH can be seen at 3.645 ppm [3].

The experimental result proved that the amino groups and hydroxyl groups were partially substituted during the synthesis of carboxymethylated chitosan. Further, the structure of CMC was confirmed from FTIR and NMR studies

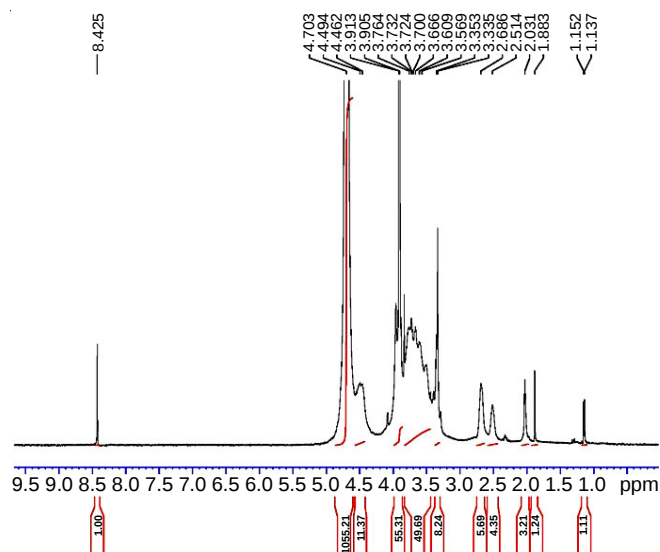


Fig. 2. ^1H NMR of carboxymethyl chitosan in D_2O

Thermogravimetric analysis: Thermogravimetric analysis (TGA) of chitosan and carboxymethyl chitosan was presented in Fig. 3. The thermal event starts at about 220°C and extends to 420°C for chitosan. TG curve of chitosan Fig. 3(a) displays three step process. In the first step, there was loss of absorbed water. The second stage mass loss up to $235\text{--}410^\circ\text{C}$ was due to the decomposition and depolymerization of acetylated and deacetylated chitosan unit. The third stage mass loss at $510\text{--}750^\circ\text{C}$ was due to the decomposition of the remaining glucosamine [13,14,16]. The TGA curve of N,O-carboxymethyl chitosan has shown two stage mass losses. In first stage the mass loss between $20\text{--}200^\circ\text{C}$ for N,O-carboxymethyl chitosan was assigned to the loss of water because polysaccharide has a strong affinity for water and thus easily hydrated. In second stage the mass loss between $200\text{--}500^\circ\text{C}$ for N,O-carboxymethyl chitosan is due to depolymerization and decomposition of the polymeric structure [16]. It has been observed that the decomposition temperature of chitosan is higher than N,O-carboxymethyl chitosan which is attributed to the reduction of amino group. Thus, it can be concluded that the thermal stability of carboxymethylated chitosan was lower than that of chitosan.

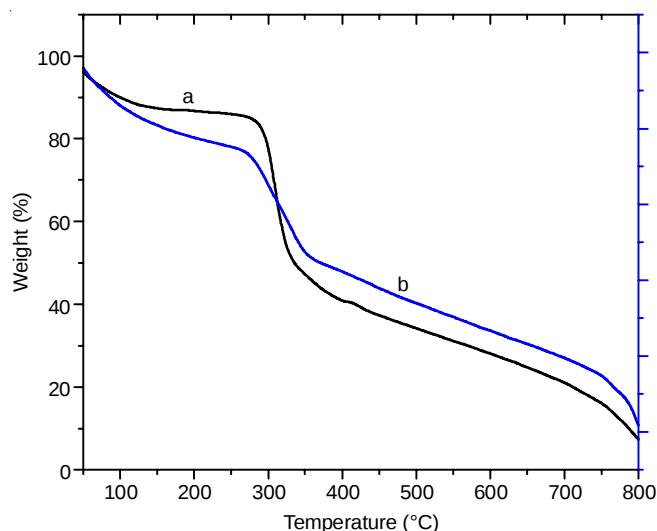


Fig. 3. TG graph of chitosan (a) and carboxymethyl chitosan (b)

Conclusion

Microwave irradiation is a more powerful tool for the conversion of chitosan to carboxymethyl chitosan as it is requiring less time period. It has been observed that different parameters are directly affecting the yield (%) of CMC. The maximum yield (wt. %) 94.7 % is obtained at 100 W microwave power at a temperature of 50°C and for 3 min working conditions. So as per our method yield is far better as well as time consumption is too less where as in conventional method many hours stirring is required. FTIR has proved that chitosan has been successfully modified and the substitution of carboxymethyl group is occurring at amine N- and hydroxyl O- of the chitosan monomeric units. Thus, the CMC synthesized in the present investigation is N, O- substituted chitosan. Further, NMR has also confirmed the structure of N, O- substituted chitosan. Thermal analysis of CMC has shown two stage degradation owing to the removal of water molecules and degradation of CMC to small molecules. The thermal stability of chitosan is more in comparison to CMC and thus, it can be used well for biomedical applications. Thus, it is concluded that the water soluble carboxymethyl chitosan is prepared which is having various biomedical applications.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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