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# Synthesis and Characterisation Of Carboxy Methyl Chitosan

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## ABSTRACT

*Chitosan is a naturally occurring polysaccharide of marine origin consists of a linear (1-4)) linked 2-amino-2-deoxy-D glucan. It can be prepared from naturally occurring chitin by treatment with alkali at elevated temperature. Chitosan is a nontoxic, biodegradable and biocompatible polymer. The main objective of the present research is development and characterizationofa novel carboxymethyl chitosan. Prepared carboxymethyl chitosan is soluble in water which enhanced its properties like exhibiting enhanced aqueous solubility, excellent biocompatibility, controllable biodegradability, osteogenesis ability and numerous other outstanding physicochemical and biological properties. The obtained carboxymethyl chitosan is characterized by Scanning Electron Microscopy, XRD, FTIR and NMR Spectroscopy. It can load hydrophobic drugs and displays strong bioactivity which shows its suitability and extensive usage for preparing different drug delivery and tissue engineering formulations respectively.*

***Key words: Carboxymethyl chitosan, Scanning Electron microscopy, XRD, NMR spectroscopy***

## Introduction

Chitin and Chitosan are naturally occurring biopolymers. They are non-toxic, biodegradable and biocompatible in nature. The advantages of these biomaterials are such that, they can be easily converted into different forms such as membranes, sponges, gels, scaffolds, micro particles, nanoparticles and Nano fibers for a variety of biomedical applications such as drug delivery. Both Chitin and Chitosan are abundantly present in nature. Chitosan is a biodegradable polysaccharide composed of primarily d-glucosamine repeating units also it has many good bio properties as well as some particular physiochemical characteristics. Therefore has been widely used in many biomedical applications, especially in the field of drug delivery system. But its poor solubility in aqueous solutions restricts its applications. Recently, there have been many attempts to make chitosan soluble and widen its application. Carboxy methylation of chitosan using monochloroacetic acid is one of the modifications that has been extensively used to overcome this problem. Carboxymethyl chitosan is used various fields pharmaceutical, drug delivery, textile industry, etc. due to its low toxicity and biocompatibility.

The carboxymethyl derivative of chitosan, carboxymethyl chitosan, has a wide range of applications such as in food and nutrition, material science, biotechnology, pharmaceuticals, agriculture and environmental protection. The net cationicity and the presence of multiple functionalities in the molecule make CS a sought after biomolecule. The latter offers scope for manipulation for preparing a broad spectrum of derivatives for specific end use applications in diversified areas. The biomedical and therapeutic uses of both Chitin and Chitosan derivatives has a great significance all over the world. The abundant hydroxyl groups (one primary hydroxyl at C-6 and one secondary hydroxyl at C-3), amino groups (at C-2) or its N-acetyl counterpart with a tendency for intra and intermolecular hydrogen bonds result in the formation of linear aggregates with extensive crystallinity.

Chitosan is only soluble in some dilute acid solutions due to its inter- and intra-molecular hydrogen bonds network. This insolubility nature greatly limits its applications. Thus, chemical modification of chitosan is done in order to improve its solubility and also to increase its applications. Among the various modified products, Carboxymethyl chitosan is a water -soluble derivative of chitosan with several improved biological

properties, such as antimicrobial, antioxidant and inhibitory activities. Carboxymethyl chitosan is not only soluble in water, but has unique chemical, physical and biological properties such as high viscosity, large hydrodynamic volume, low toxicity, biocompatibility and film, gel-forming capabilities, all of which make it an attractive option in connection with its use in food products and cosmetics. And it has been considered as a novel biopolymer for different biomedical applications, including wound healing, tissue engineering, drug delivery, gene therapy and bio imaging.

## 2. Experimental section

### 2.1. Materials and equipments.

Biochemical reagent grade chitosan (deacetylation degree>75%) Hi Media Laboratories Pvt. Ltd., Mumbai, India was used. All other chemicals were of reagent grade and were used without purification. A Samsung CE74JD model microwave oven was used in these studies. Measurement of solution pH was done on a pH meter (PHS-25).IR spectra were recorded on a Bruker FT-IR spectrometer, SEM analysis was done by Zeiss ,EVO 18 SEM and the pattern of X-ray diffraction of the samples was obtained by using an X-Ray diffractometer (General Instrument Co. Ltd., Beijing XD-3) at 40 kV with the scan range from 5 to 70◦ and a scan rate of 2 ◦/min. Nuclear magnetic resonance (NMR) analyses were carried out using an AC-400 Bruker spectrometer.

### 2.2 Preparation of Carboxymethyl Chitosan

Carboxymethyl Chitosan is prepared by the following procedure. 1g of Chitosan is added to a mixture of 10M aqueous NaOH and 15ml 2-propanol, stirred for 40min at $50^0$C.10g of Monochloro acetic acid is mixed in 12ml of 2-propanol, added drop wise to the above mixture and subjected to heating by using a hotplate. The obtained product is cooled, washed with ethanol and filtered. Finally white Carboxymethyl chitosan was obtained after drying.

### 2.3 Characterization

#### 2.3.1. Fourier Transform infrared (FTIR) Spectroscopy

Fourier transform infrared (FT-IR) spectra were recorded using a Perkin-Elmer 1600 FTIR Spectrophotometer. Samples were finely ground and mixed with potassium bromide, KBr. The mixture was then compressed into pellet form. FTIR spectral analysis was carried out within the wave number range of 400–4000 $cm^{-1}$

#### 2.3.2. X-ray diffraction (XRD)

The pattern of X-ray diffraction of the samples was obtained by using an X-Ray

Diffractometer (General Instrument Co. Ltd., Beijing XD-3) at 40 kV with the scan range from (2θ) of $5^0$ to $90^0$ and a scan rate of 2 ◦/min. The XRD method is widely used to examine the crystallographic nature of a material.

#### 2.3.3. Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) was performed with a Cold Field Emission S-4800 Scanning Electron Microscope (Hitachi, Tokyo, Japan) operated under an acceleration voltage of 5 kV. The samples were mounted on a double sided carbon tape and then coated with a thin layer of platinum.

#### 2.3.4.$^1$H NMR Spectroscopy

The $^1$H NMR spectra of chitosan and carboxymethyl chitosan were acquired at 80 ◦C using a Bruker AVANCE III 500, 11.75 Tesla, spectrometer operating at 500.13 MHz for 1H. CS and CMCs were dissolved in $CH_3COOH$/D2O 1% (v/v) and $D_2O$ 1% (v/v) respectively to get the $^1$H NMR spectra. To get the chitosan and carboxymethyl chitosan spectra, a composite pulse presaturation (CPPR) sequence for water signal suppression was used. The interval between pulses was 3 s, 32 scans were accumulated and the relaxation time was 7 seconds. The spectral window used was 10.0 ppm and the FID size was 32 K.

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## 3. Result and Discussion

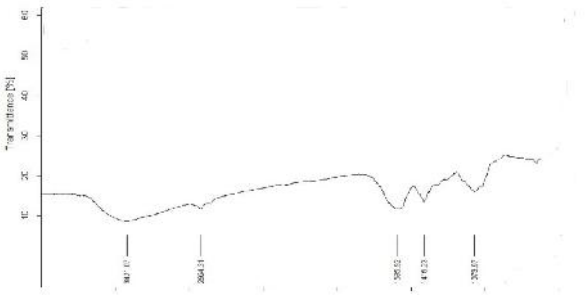
The synthesis of Carboxymethyl chitosan involved two reaction steps. In the first step, chitosan was treated with NaOH, in the presence of isopropanol, which acted both as a swelling agent and as a dilutant facilitating the penetration of NaOH into the chitosan structure. The second step consisted of the irradiation reaction of alkaline chitosan and monochloroacetic acid using a microwave. The occurrence of carboxymethylation and the presence of characteristic functional groups of chitosan and Carboxymethyl chitosan were confirmed by Infrared Spectroscopy and NMR.

### 3.1. Fourier Transform infrared (FTIR) Spectroscopy.

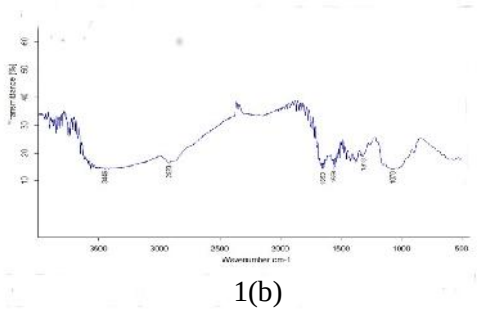
FTIR spectra of Chitosan Fig1(a) shows basic characteristic absorption bands at 3421 $cm^{-1}$ (O-H and N-H stretching),2924 $cm^{-1}$ (primary $NH_2$ at $C_2$) 1595$cm^1$ (NH bending at primary amine);1415 $cm^{-1}$ (C- N stretching, amide III) and 1073 $cm^{-1}$ (skeletal vibration involving the C-O stretch). The carboxymethylation substituted structural changes which were clearly identified by comparing the infrared

Spectra of chitosan and Carboxymethylchitosan. The FTIR spectrum of Carboxymethyl chitosan Fig 1(b) shows the occurrence of an intense band at 1663 $cm^{-1}$ and a moderate band at 1588 $cm^{-1}$, 1388 $cm^{-1}$ is attributed to carboxymethyl substitution at the $C_2$ and carboxyl group overlaps with N-H bend, respectively, which confirms the introduction of the Carboxymethyl groups in Chitosan. These bands were attributed to symmetric and asymmetric deformation of COO−, respectively.

Figure:1 shows the FTIR spectrum of raw Chitosan (a) and of Carboxymethyl Chitosan (b)



1(a)

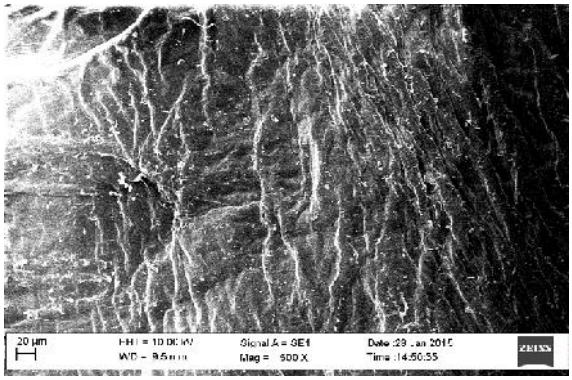


1(b)

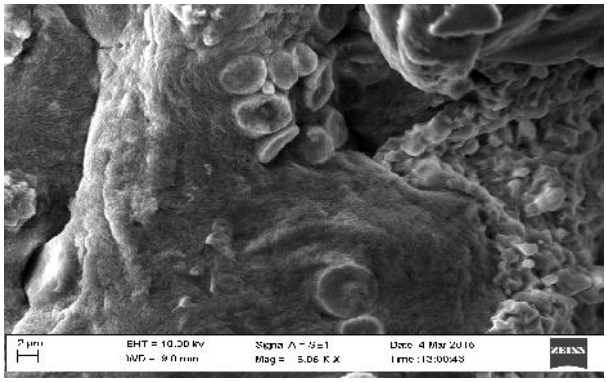
### 3.2. SEM Analysis

Scanning electron microscopy is used for inspecting topographies of both chitosan and Carboxy methyl chitosan. Fig 2(a) shows the surface appearance of raw chitosan and Fig2 (b) that of Carboxy methyl chitosan. SEM analysis clearly explains the porous structure of Carboxy methyl chitosan.

Figure 2: SEM images of raw Chitosan (a) and Carboxy methyl chitosan (b)



**2(a)**



**2(b)**

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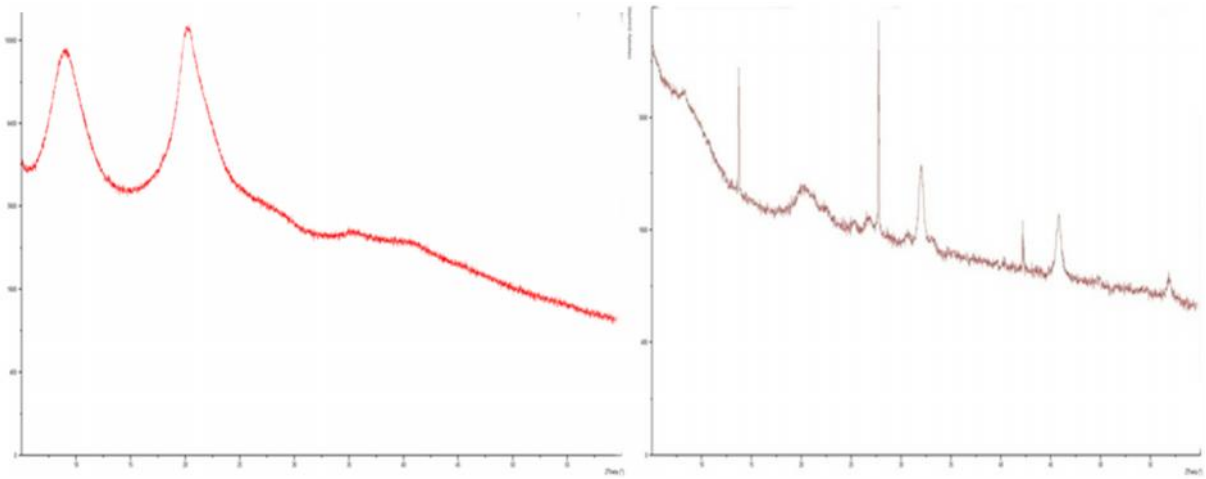
### 3.3. XRD Analysis

According to the literature, Carboxymethyl chitosan had a less ordered arrangement compared to Chitosan. Therefore Carboxy methyl chitosan bears more amorphous nature .Thus XRD analysis was conducted to compare the crystanillinity of chitosan and Carboxymethylated derivative as presented in Fig 3(a) is unmodified chitosan has a main peak at 2θ = 20.0° related to the reflection of (200) plane and other at 2θ =10.4° corresponding to the (020) plane. For the chitosan derivatives Fig 3(b), the peak at 10.4° and the peak at 20.0° significantly decreased. These results suggest that the arrangement of the polymer chains in the solid state has changed upon the derivatization of chitosan and that its degree of order was significantly decreased as a consequence of the introduction of a large number of bulky and charged substituent which disrupt hydrogen bonding and impart an important steric hindrance.The reason was due to the less number of hydrogen bond formation between Carboxymethyl chitosan molecules than chitosan. Presence of carboxymethyl groups which substitute the hydrogen atoms of the hydroxyl and amino groups of chitosan decrease the formation of hydrogen bonds.These observations confirmed that chitosan had a more ordered arrangement than Carboxy methyl chitosan products. Also chitosan had a higher degree of order than that of Carboxy methyl chitosan. Therefore, the crystal structure (d spacing) of Carboxy methyl chitosan was different from chitosan.

Figure3: XRD images for raw Chitosan 3(a) and Carboxy methyl chitosan 3(b)

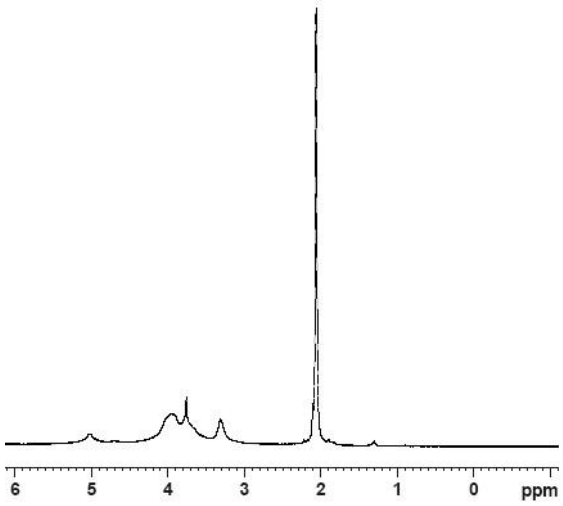


**3(a)** **3(b)**

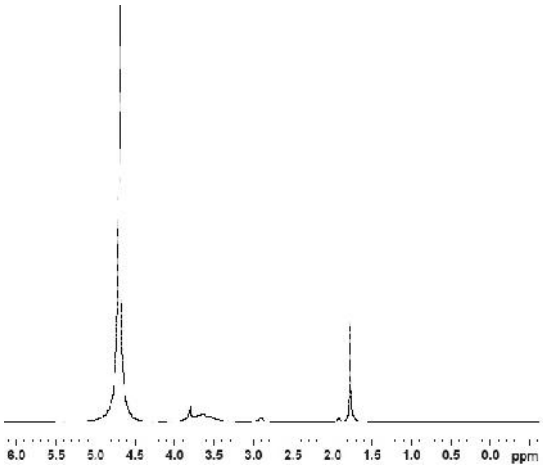
### 3.4. $^{1}$H NMR Analysis

The proton NMR spectrum of raw Chitosan and Carboxy methyl chitosan in $D_2O$ shown in Fig. 4(a) and 4(b). The resonance 3acetyl-protons (1.77 ppm), H3–6 protons (3.6–3.9 ppm), H–2D proton (3.305 ppm) are the basic assignments of chitosan resonance which can be found in the 1H NMR spectrum of chitosan described previously. The protons resonances of substituted carboxymethyl group of Carboxy methyl chitosan can be observed between 4.69 ppm. The result proved that the amino groups were partly carboxymethylated along with the hydroxyl groups. This indicates that our product complies with the previously reported parameters of Carboxymethyl chitosan.

Figure4: $^{1}$H NMR of Chitosan (a) in $CD_3COOD/D_2O$ and $^{1}$H NMR of Carboxymethyl Chitosan in $D_2O$.




4(a)




4(b)

## CONCLUSION

In conclusion, the carboxymethylation was successfully prepared from Chitosan.Different analyses were made to confirm the structures of the prepared derivatives like; SEM, FTIR and XRD. The occurrence of *N*-carboxymethylation was proved by FTIR and $^{1}$H NMR spectroscopy which revealed that *N*, *O*Carboxymethylchitosan was produced. The prepared carboxymethyl chitosan is found to be soluble in water which increased its application.

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