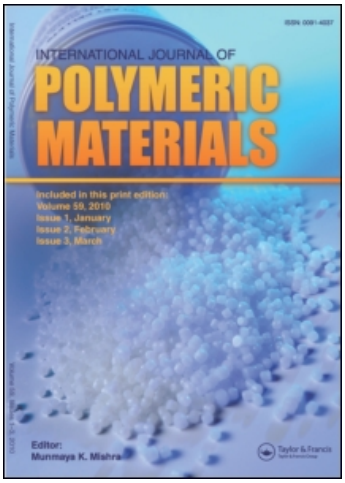

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Synthesis, Characterization and Optimization of Water-Soluble Chitosan Derivatives

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Synthesis, Characterization and Optimization of Water-Soluble Chitosan Derivatives

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Chitosan was chemically modified using monochloroacetic acid at various reaction conditions. Chemical structure was confirmed by Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and x-ray diffraction (XRD). The carboxymethyl chitosan (CM-chitosan) was prepared at different temperatures, water/isopropanol (IPA) ratios and alkali concentrations. Reaction conditions have great influence on the degree of substitution (DS) and, in turn, the solubility. The water solubility of chitosan derivatives depended upon modification conditions and degree of substitution.

Keywords: carboxymethyl chitosan, chitosan, degree of substitution, SEM, XRD

INTRODUCTION

Chitin is one of the most abundant organic materials. It is found in animals, particularly in crustacea, mollusks and insects, where it is a major constituent of the exoskeleton, and in certain fungi, where it is the principle fibril polymer in the cell wall. Chitin has a crystalline structure and it constitutes a network of organized fibers. This structure confers the rigidity and resistance to organisms that contain it. Chitin is a poly [β-(1–4)-2-acetamido-2-deoxy-D-glucopyranose]; its

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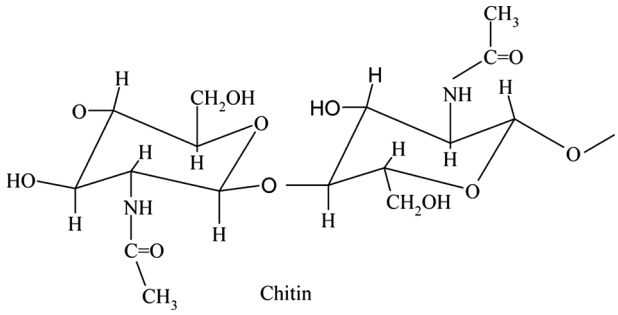
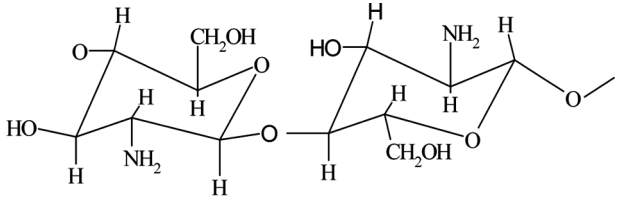
structure is shown below:



Chitin

The principal derivative of chitin is chitosan, produced by alkaline deacetylation of chitin. Chitosan also occurs in nature in certain fungi, in limited amounts. Chitosan is poly [β-(1–4)-2-amino-2-deoxy-D-glucopyranose] and its idealized structure is shown below. Approximately 85% deacetylation is obtained in commercial chitosan [1,2].



Chitosan

Owing to its good biocompatibility, biodegradability and capacities to form membrane, fiber, gel and microspheres, chitosan has found many biomedical applications [3,4]. In spite of potential applications of chitin and chitosan, it is necessary to establish efficient appropriate modifications. Poor solubility of chitosan resulted in practical difficulties, such as heterogeneity of the reaction mass and, therefore, poor extent of reaction. Recently there has been a growing interest in chemical modification of chitosan to improve its water solubility and widen its applications, in particular in drug delivery [5–13]. Chitin and chitosan have been modified via variety of chemical modifications. The references cited for the chemical modification includes acylation, hydroxyalkylation [1,14,15], nitration, phosphorylation, xanthation [16], Schiff's base formation and alkylation [14,17–19]. Roberts has explained the modification reactions in his sourcebook *Chitin Chemistry* [17].

One of the major difficulties is to dissolve chitosan in water at higher pH. The difficulty was overcome by chemical modifications, such as PEG-grafting [20], sulfonation [21], quaternarization [22], N- and O-hydroxylation [23] and carboxymethylation of chitosan (CM-chitosan) [24]. Among the water-soluble chitosan derivatives, CM-chitosan is an amphoteric ether derivative, containing –COOH groups and NH_2 groups in the molecule. The structure, antibacterial activity, toxicity and membrane properties of N- and O-carboxymethyl chitosan have been reported previously [25–28]. The water solubility of CM-chitosan is dependent on the nature of the solvent and degree of substitution.

The present work deals with the preparation of carboxymethyl chitosan at optimized condition, and its solubility study. Characterization of CM-chitosan has been carried out using FTIR, SEM and XRD.

MATERIALS AND METHODS

Materials

Chitosan (molecular weight 8.4×10^4; the degree of deacetylation 85%) was provided by Central Institute of Fisheries Technologies, India. Monochloroacetic acid was supplied by S.D. Fine Chemical, India. Sodium hydroxide was obtained from National Chemical, India. All other reagents were of analytical grade and were used after proper purification.

Preparation of Carboxymethylchitosan

Chitosan (10 g), sodium hydroxide (12.5 g) and isopropanol solvent (100 ml) were suspended in a flask to swell and alkalize at room temperature for 1 h. The temperature was maintained at 25°C in a water bath. The monochloroacetic acid (13 g) was dissolved in isopropanol, and added to the reaction mixture dropwise within 30 min and then reacted for 4.5 h at 55°C. Then the reaction was stopped by adding a few drops of acetic acid to neutralize the reaction mass and the isopropanol was decanted. Ethyl alcohol (80%) was added and the solid product was filtered and rinsed with 80% ethyl alcohol to desalt and dewater. The product was then vacuum dried at 40°C. The reaction scheme is shown in Figure 1.

The degree of substitution (DS) of CM-chitosan was determined by pH-titration [29].

Characterization

IR spectra of chitosan derivatives were recorded using a Perkin Elmer Fourier transform infrared (FTIR) spectrometer at room temperature

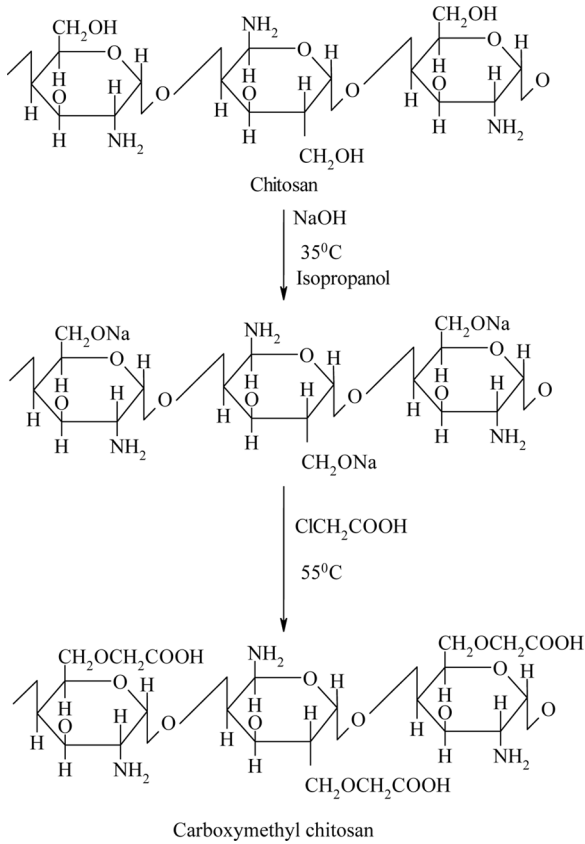


FIGURE 1 Reaction scheme of carboxymethyl chitosan.

using KBr pellets. Scanning electron microscopy (SEM) of chitosan and CM-chitosan was obtained using SEM XL-Series of Philips, (The Netherlands) at 15 kV. Powder x-ray diffraction patterns of chitosan and CM-chitosan were obtained by using Xe-filled counteract solid-state liquid nitrogen cooled detector, Xpert-Philips instrument equipped with a θ-θ goniometer under the following operation conditions: 40 kV and 35 mA with Cu Kα_1-radiation at λ 1.54056 Å. The relative intensity was recorded in the scattering range (2θ) of 0–167°.

Water Solubility

The water solubility of the CM-chitosan was evaluated in 0.1 M acetic acid buffer (pH-4.0), 0.01 M phosphate buffer saline (PBS) (pH-7.2)

and 0.2 M sodium carbonate buffer (pH-10.0). A sample was soaked in each buffer at a concentration of 2 mg/ml and solubility after 5 h was observed.

RESULTS AND DISCUSSION

The CM-chitosan was prepared at different reaction conditions. The effect of each parameter is discussed as follows.

Effect of Solvent

The CM-chitosan samples were prepared at different solvent ratios as shown in Table 1. In 100% isopropanol (CM-chitosan-2) the percentage yield was only 50%, which may be attributed to the fact that chitosan was not alkalized in the nonaqueous solvent. The maximum percentage yield was obtained when the ratio of water/isopropanol was 2:8. As the water content in the water/isopropanol ratio increases the percentage yield decreases, and at 100% water (CM-chitosan-6) the yield was 5.46%. The reason was that the previously formed CM-chitosan was easily swelled in water to form a gel. The gel formed a coat outside the chitosan particle and inhibited the reaction.

Effect of Temperature

The CM-chitosan samples were prepared at different reaction temperatures. The percentage yields (CM-chitosan 1–2) were very low at 5–15°C, but as the temperature increases above 25°C the percentage yields were much higher (CM-chitosan 2–6). The highest yield 88.9% was obtained at 55°C. Hence, higher temperature enhanced the CM-chitosan percentage yields as shown in Table 2.

TABLE 1 Effect of Solvent Ratios on Percent Yields of CM-Chitosan

Samples	Water/isopropenol (v/v)	DS	Yields (%)*
CM-chitosan-1	1/9	0.62	78.22
CM-chitosan-2	0/10	0.75	50.89
CM-chitosan-3	2/8	0.82	88.90
CM-chitosan-4	5/5	0.68	81.67
CM-chitosan-5	8/2	0.55	18.54
CM-chitosan-6	10/0	0.40	5.46

*% yield = water-soluble CM-chitosan (g)/raw product (g) × 100.

TABLE 2 Effect of Reaction Temperature on Percent Yields of CM-Chitosan

Samples	Temperature °C	DS	Yields (%)*
CM-chitosan-1	5	0.30	8.4
CM-chitosan-2	15	0.35	18.43
CM-chitosan-3	25	0.50	68.71
CM-chitosan-4	35	0.62	71.90
CM-chitosan-5	45	0.77	75.67
CM-chitosan-6	55	0.82	88.90

*% yield = water-soluble CM-chitosan (g)/raw product (g) × 100.

Effect of NaOH Concentration on Degree of Substitution (DS)

The CM-chitosan samples were prepared at different alkali concentrations as shown in Table 3. The alkali concentration can be considered to be one of the important factors to regulate carboxymethylation yield on chitosan. Takura et al. demonstrated that the DS value of CM-chitosan increased with an increase in the NaOH concentration [30]. When the concentration of NaOH increased from 20 to 50%, the DS value increased from 0.12 to 0.82. A 50% NaOH concentration seems to be the optimum alkali concentration for carboxymethylation reaction. At lower NaOH concentration, the rigid crystalline structure of chitosan was difficult to disrupt, ensuring penetration of the $ClCH_2COOH$ into the interlocking polymer chains [31]. Whereas in a high alkali concentration, i.e., 60%, it promotes a side reaction between NaOH and $ClCH_2COOH$ [32], and the $ClCH_2COOH$ concentration decreases accordingly. When the NaOH concentration was 40% or 60%, a small part of the resulting products were not soluble, but they did swell in water.

TABLE 3 Effect of Alkali Concentration on Degree of Substitution (DS)

Samples	Concentration of NaOH (%)	DS
CM-chitosan-1	20	0.12
CM-chitosan-2	30	0.31
CM-chitosan-3	40	0.63
CM-chitosan-4	50	0.82
CM-chitosan-5	60	0.64

Water Solubility

The CM-chitosan was prepared at various solvent ratios and temperatures. The solubility of CM-chitosan was examined at various pH ranges shown in Tables 4 and 5. All the CM-chitosan samples were soluble in acidic pH buffer. Furthermore, some CM-chitosan which had higher DS value was soluble at neutral and alkaline buffers. The good solubilities in PBS may provide many possibilities for the application of chitosan in the biomedical field.

Effect of Solvent Ratio and Temperature on DS

Figure 2 shows that the degree of substitution depends on the solvent ratio (water/isopropanol). In isopropanol alone, the degree of carboxymethylation was 0.62. In water-containing solvents, the degree of carboxymethylation had the highest value. In water alone the degree of carboxymethylation was at its lowest value.

Figure 3 shows that the degree of substitution depends on the reaction temperature. As the temperature increases from 15 to 55°C, the degree of carboxymethylation increased. The higher reaction temperature favored the substitution of the carboxymethyl on the –OH group.

Characterization of Chitosan Derivatives

Structural changes of chitosan and CM-chitosan were confirmed by FTIR spectroscopy. The IR spectrum of chitosan (Figure 4) shows peaks assigned to the saccharide structure at 1152, 1080.4 and

TABLE 4 Solubility of CM-Chitosan in Water of Various pH, and in Relation to the Preparation Solvents

		Solubility*		
Samples	Water/ isopropenol (v/v)	0.1 M CH_3COOH pH = 4.0	0.01 M PBS pH = 7.2	0.2 M Na_2CO_3 pH = 10.0
Chitosan	-	+++	(−)(−)	(−)(−)
CM-chitosan-1	0/10	+++	+(−)	+(−)
CM-chitosan-2	1/9	+++	+(−)	++
CM-chitosan-3	2/8	+++	+++	+++
CM-chitosan-4	5/5	+++	+++	+++
CM-chitosan-5	8/2	+++	+++	+++
CM-chitosan-6	10/0	+++	+(−)	+++

*(−)(−) insoluble, +++ soluble, ++ partially soluble and +(−) swelling.

TABLE 5 Solubility of CM-Chitosan in Water at Various pH and Preparation Temperatures

Samples	Temperature °C	Solubility* 0.1 M CH_3COOH pH = 4.0	0.01 M PBS pH = 7.2	0.2 M Na_2CO_3 pH = 10.0
CM-chitosan-1	5	+++	++	++
CM-chitosan-2	15	+++	+(−)	++
CM-chitosan-3	25	+++	+(−)	+++
CM-chitosan-4	35	+++	+++	+++
CM-chitosan-5	45	+++	+++	+++
CM-chitosan-6	55	+++	+++	+++

*(−)(−) insoluble, +++ soluble, ++ partially soluble and +(−) swelling.

897 cm^{-1}, and a strong amino characteristic peak at around 3420 cm^{-1} peaks at 1655.7 cm^{-1} and 1325.3 cm^{-1} are assigned to amide I and II bands, respectively [33]. In the IR spectrum of CM-chitosan (Figure 5), the strong peak at 1412.3 cm^{-1} could be assigned to the symmetrical stretching vibration of COO^-. The asymmetrical stretching vibration of COO^-(1900–1550 cm^{-1}) overlapped with the deforming vibration of NH_2 at 1599.3 cm^{-1} to obtain a very strong peak. C-O absorption peak of the secondary hydroxyl group became stronger and moved to 1074.1 cm^{-1}. The results indicate that the substitution occurred at C_6 position.

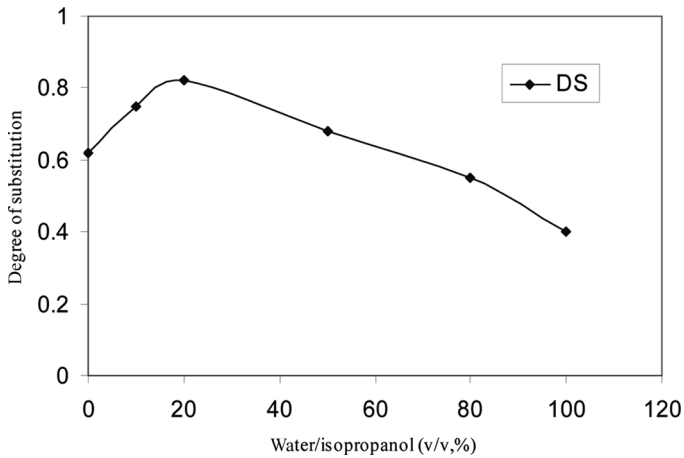


FIGURE 2 The effect of water/isopropanol ratio on the degree of substitution (DS) of CM-chitosan.

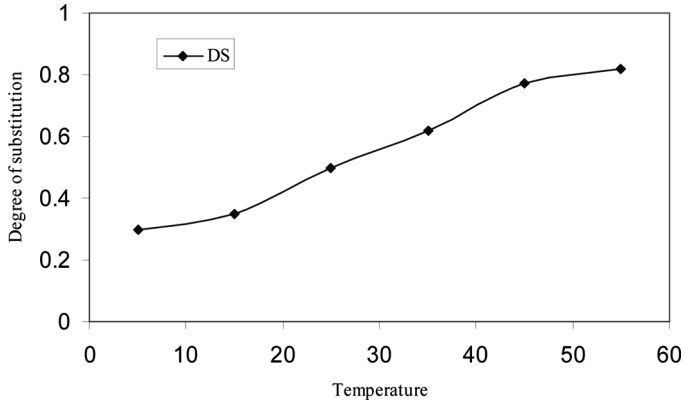


FIGURE 3 The effect of temperature on the degree of substitution (DS) of CM-chitosan.

The scanning electron micrographs (SEM) of chitosan and CMCH are shown in Figures 6 and 7, respectively. By carboxymethylation the surface morphology and also the physical and chemical characteristics of chitosan were modified. Figure 8(a, b) shows the powder x-ray diffractograms obtained for chitosan and carboxymethyl chitosan, respectively. In Figure 8a, two peaks showing the maximum intensity

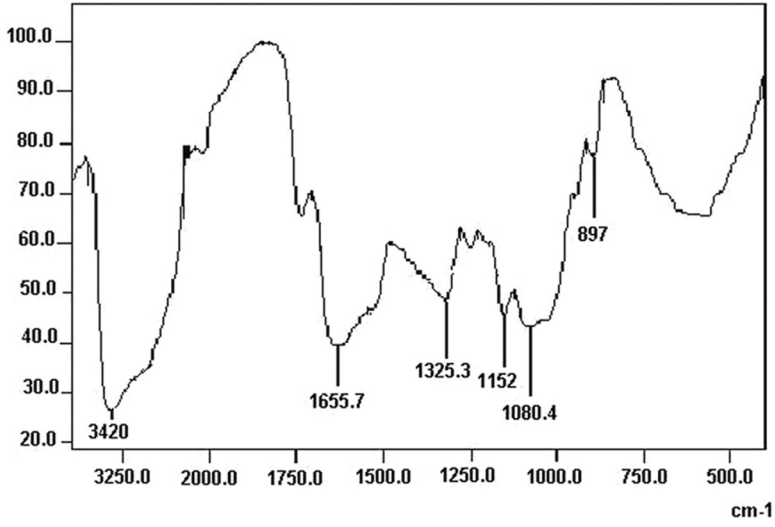


FIGURE 4 IR spectrum of chitosan.

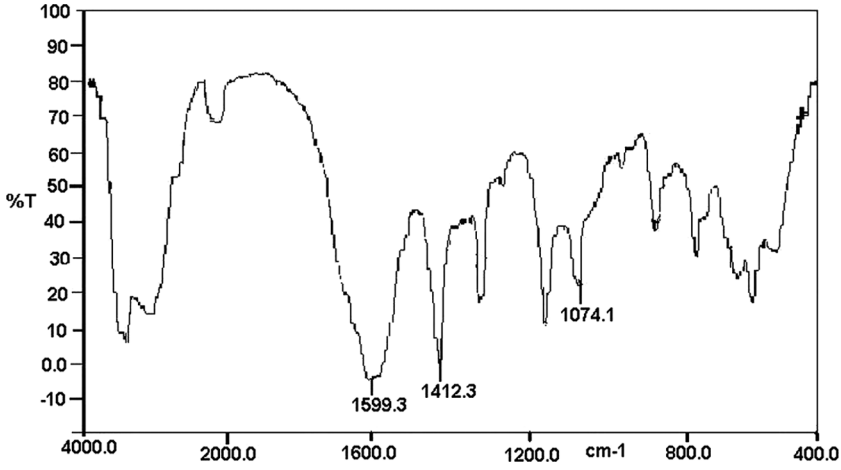


FIGURE 5 IR spectrum of CMCH.

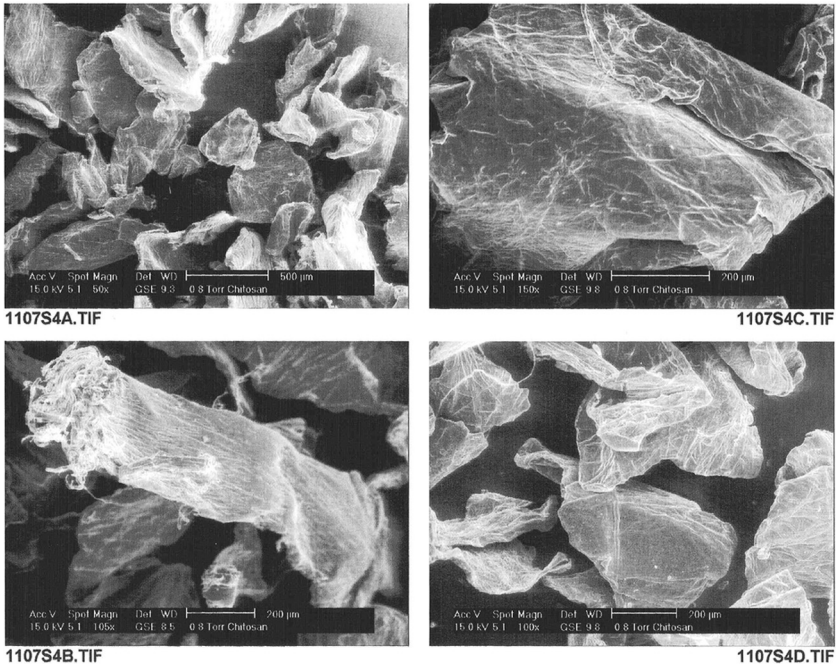


FIGURE 6 SEM of chitosan.

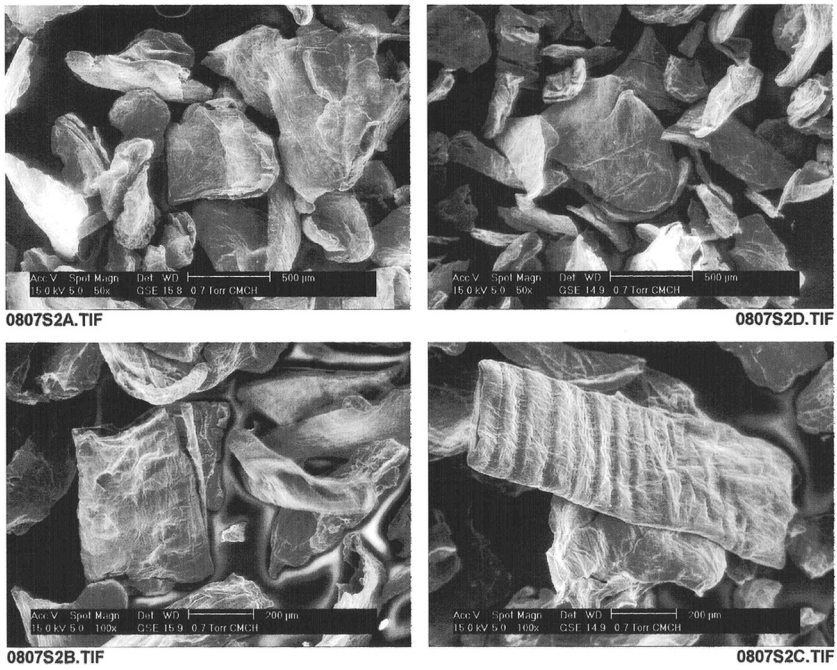


FIGURE 7 SEM of CMCH.

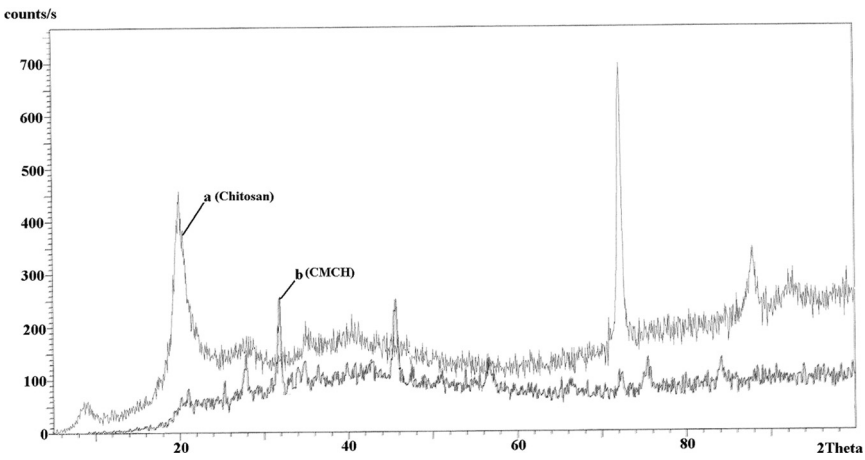


FIGURE 8 XRD of (a) chitosan and (b) CMCH.

obtained, at $2\theta = 20^\circ$ and $2\theta = 72^\circ$, which is matches with values reported in the literature [34], indicating chitosan is highly crystalline in nature. After carrying out carboxymethylation the intensity of these two peaks is decreased. From that we can conclude that after carboxymethylation chitosan loses its crystalline nature, which is clearly shown in Figure 8b.

CONCLUSIONS

The yields of CM-chitosan prepared in the mixed solvents were higher than in water alone or in isopropanol alone. The highest yields were close to 90% at water/isopropanol ratios between 1:4 and 1:1 at 55°C. The carboxymethyl groups were mostly substituted on the –OH groups, with a small amount on $–NH_2$ groups. The solubility in water depended on conditions like water/isopropanol ratio and temperature used for the modification. Carboxymethylation process improved the water solubility of chitosan, keeping the main skeleton intact.

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