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# Ceric ammonium nitrate induced grafting of polyacrylamide onto carboxymethyl chitosan

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**Abstract**

*O*-Carboxymethyl chitosan (CMCH) was prepared and characterized by FTIR spectroscopy and X-ray diffraction. Grafting of acrylamide (Am) onto CMCH using ceric ammonium nitrate (CAN) as an initiator was carried out under nitrogen atmosphere in aqueous solution. Evidence of grafting was confirmed by comparison of FTIR spectra of CMCH and the grafted copolymer as well as scanning electron micrography (SEM) and X-ray diffraction of the products. The effects of concentration of CAN, Am, reaction time and temperature on graft copolymerization were studied by determining the grafting percentage, grafting efficiency and percentage add-on. With other conditions kept constant, the optimum grafting conditions were obtained as follows: CMCH = 2 g, CAN = 0.2 M, and Am = 0.563 mol/L, reaction temperature = 40 °C, and reaction time = 4.5 h.


*Keywords:* Carboxymethyl chitosan; Acrylamide; CAN; Graft copolymerization

## 1. Introduction

Chitosan is a partially deacetylated polymer of acetyl glucoseamine obtained after alkaline deacetylation of the chitin. It displays interesting properties such as biocompatibility, biodegradability (Felt, Buri, & Gurny, 1998; Hirano, Seino, Akiyama, & Nonaka, 1989; Ravi Kumar, 2000; Ravi Kumar, Muzzarelli, Muzzarelli, Shashiwa, & Domb, 2004) and its degradation products are non-toxic, and non-immunogenic and non-carcinogenic (Bersch, Nies, & Liebrndorfer, 1995; Muzzarelli, 1997). Therefore chitosan has prospective applications in many fields such as biomedicine, waste water treatment, functional membrane, and flocculation. However chitosan is only soluble in few dilute acid solutions, which limits its wide applications. Recently there has been a growing interest in chemical modification of chitosan to improve its water solubility and widen its applications (Heras, Rodriguez, & Ramos, 2001; Sashiwa & Shigemasa, 1999; Sridhari & Dutta, 2000; Sugimoto, Morimoto, & Sashiwa, 1998; Terada et al., 1999). Among various methods, graft copolymerization is most attractive because it is useful technique for modifying the chemical and physical properties of natural polymers. Chitosan bears two types of reactive groups that can be grafted. First, the free amino group on deacetylated units and secondly, the hydroxyl groups on the $C_3$ and $C_6$ carbons on deacetylated units. Grafting of a chitosan allows the formation of functional derivatives by covalent binding of a molecule, the graft, onto chitosan backbone. Recently researchers have also shown that after primary derivatization followed by graft copolymerization; chitosan obtained much improved water solubility and bioactivities such as antibacterial antioxidant properties (Xie, Xu, Wang, & Lu, 2001, 2002a). Grafting of chitosan is a common way to improve its properties such as increasing chelating (Yang & Yuan, 2001) or complexation properties (Chen & Wang, 2001), bacteriostatic effect (Jung, Kim, Choi, Lee, & Kim, 1999), and enhancing the absorption properties (Kotze et al., 1997; Thanou, Verhoef, & Junginger, 2001). Although, grafting of chitosan modifies its properties, it is possible to maintain some interesting characteristics such

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as mucoadhesivity (Hoffman et al., 1997), biocompatibility (Ono, Saito, Yura, & Ishikawa, 2000; Tasker, Connell, Ross, & Elson, 1998), and biodegradability (Singh & Ray, 1998). Many investigations have been carried out on the graft copolymerization of chitosan in view of preparing polysaccharide-based advanced materials with unique bioactivities and thus widening their applications in biomedicine and environmental fields. The potential applications of grafted chitosan are in various fields such as controlled drug delivery, biomedical, and tissue engineering.

Graft copolymerization of vinyl monomers onto chitosan and other natural polymers can introduce desired properties and enlarge the field of potential application of them by attaching various selected types of side chains. In recent years numbers of initiator systems have been developed to initiate graft copolymerization. Redox system, such as ceric ammonium nitrate (CAN), potassium persulfate (KPS), and ammonium persulfate (APS) usually produce free radical sites on polymer. However, the properties of grafted chitosan have been improved but not so much because of its regular structure and the strong intermolecular hydrogen bonds. Recent researchers showed that grafting onto pre-modified chitosan would obtain much improved water solubility and bioactivity (Xie, Xu, Wang, & Lu, 2002b). But there are very limited reports about the graft copolymerization of pre-modified chitosan derivatives (Sun, Xu, Liu, Xue, & Xie, 2003; Xie, Xu, Wang, & Liu, 2002).

In this paper, multiple-derivatized chitosan (CMCH-g-Am) was prepared by etherification of chitosan with monochloroacetic acid followed by the graft polymerization of acrylamide, and the effects of reaction conditions on graft copolymerization were investigated. Such graft copolymers are widely used in controlled drug delivery system.

## 2. Experimental

### 2.1. Materials

Chitosan (molecular weight $8.4 \times 10^4$; the degree of deacetylation 85%) provided by Central Institute of Fisheries Technologies, India. Ceric ammonium nitrate (CAN) of analytical grade reagent was supplied from S.D. Fine Chemical, India. Acrylamide was obtained from National Chemical, India. All the other reagents are analytical grade and used without further purification.

### 2.2. Preparation of carboxymethyl chitosan

To synthesize carboxymethyl chitosan, chitosan (10 g), sodium hydroxide (13.5 g) and solvent isopropenol (100 ml) were suspended into a flask to swell and alkalize at room temperature for 1 h. The temperature was maintained in a water bath. The monochloroacetic acid (15 g) was dissolved in isopropenol, and added into the reaction mixture drop wise within 30 min and reacted for 4 h at 55 °C. Then the reaction was stopped and isopropenol was discarded. Ethyl alcohol (80%) wad added and solid product was filtered and rinsed with 80–90% ethyl alcohol to desalt and dewater, and vacuum dried at 50 °C. The degree of substitution (DS) of CMCH was determined by pH-titration (Eyler, Kludge, & Diephius, 1947) and found to be 0.38.

### 2.3. Graft copolymerization

A small amount of CMCH (2 g), a predetermined amount of acrylamide, and 120 ml double distilled water were charged into a three necked round bottom flask in a constant temperature water bath maintained at a 40 °C. Nitrogen gas was bubbled for 30 min to remove the dissolved oxygen under stirring. 0.2 M CAN dissolved in 10.0 ml of 0.3 M $HNO_3$ was slowly added to the three necked flask to initiate graft copolymerization. Reaction products were neutralized by 10% NaOH, precipitated in methanol, filtered, washed with methanol and methanol/$H_2O$ (90:10), so that all the unreacted CMCH and Cerric salt were removed and dried under vacuum at 45 °C. Homopolymers were extracted with water for 24 h, and dried at 40 °C for 24 h. The reaction scheme of the graft copolymerization is shown in Fig. 1 (Joshi & Sinha, 2006). The grafting parameters were calculated in the following manner (Fanta, 1973):

$$\text{Grafting percentage (G\%)} = [W_1/W_0] \times 100, \quad (1)$$

$$\text{Grafting efficiency (GE\%)} = [W_1/W_2] \times 100, \quad (2)$$

$$\text{Percentage add-on (\%add-on)} = [W_1 - W_0]/W_1 \times 100, \quad (3)$$

where $W_0$, $W_1$, and $W_2$ denote the weight of CMCH, weight of pure graft copolymer and weight of crude graft copolymer, respectively.

### 2.4. Characterization

IR spectra of chitosan derivatives were recorded with a Perkin Elmer Fourier-transform infrared (FTIR) spectrometer using KBr pellets. Scanning Electron Microscopy (SEM) of chitosan, CMCH and graft copolymer were obtained by using SEM XL-Series from Philips, (The Netherlands) at 15 kV. Powder X-ray diffraction patterns of chitosan and graft copolymers were obtained by using Xe filled counteract solid state liquid nitrogen cooled detector, X″ pert-Philips instrument equipped with a $\theta$–$\theta$ goniometer under the following operation conditions; 40 kV and 35 mA with Cu K$\alpha_1$-radiation at $\lambda$ 1.54056 Å. The relative intensity was recorded in the scattering range ($2\theta$) of 0–167°.

## 3. Results and discussion

### 3.1. Characterization of chitosan derivatives

Structural changes of chitosan and its derivatives were confirmed by FTIR spectroscopy (Figs. 2 and 3). The IR spectrum of chitosan shows peaks assigned to the saccharide structure at 1152, 1080, 1028, and 897 $cm^{-1}$, and a

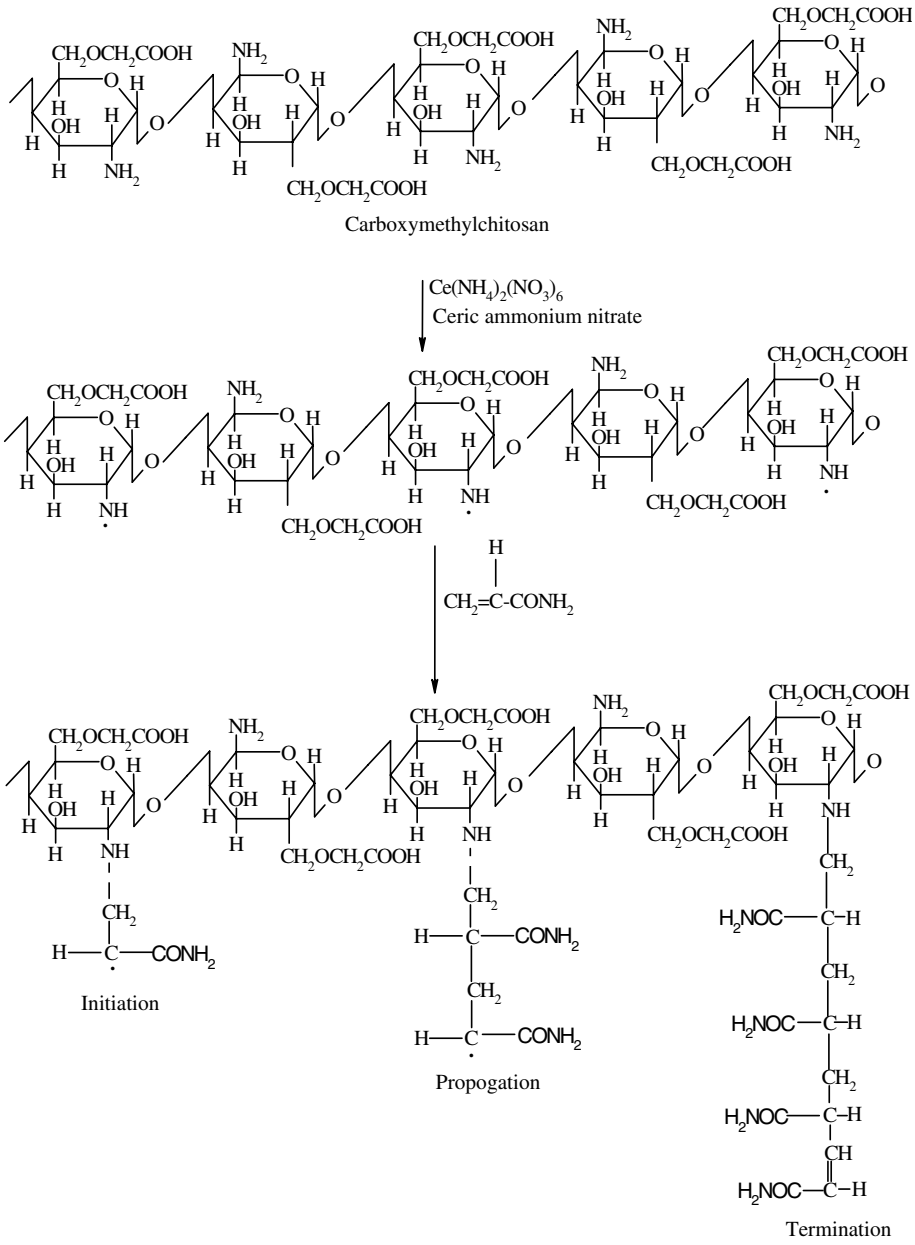


Fig. 1. Reaction scheme of CMCH-g-Am.

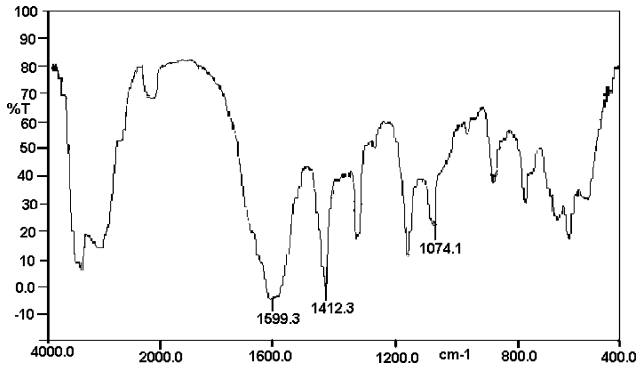


Fig. 2. FTIR of CMCH.

strong amino characteristic peak at around 3420, 1655, and 1325 $cm^{-1}$. Overlapped peaks around 1665 and 1550 $cm^{-1}$ are assigned to amide I and II bands, respectively (Brugnerotto et al., 2001). In the IR spectrum of CMCH, 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 1600 $cm^{-1}$ to obtain a very strong peak. And C—O absorption peak of secondary hydroxyl group became stronger and moved to 1074.1 $cm^{-1}$. The results indicated that the substitution occurred at $C_6$ position (Xie et al., 2002). On comparing the IR spectrum of CMCH and CMCH-g-Am following graft copolymerization additional peaks in the spectra of CMCH-g-Am have been observed. The absorption band

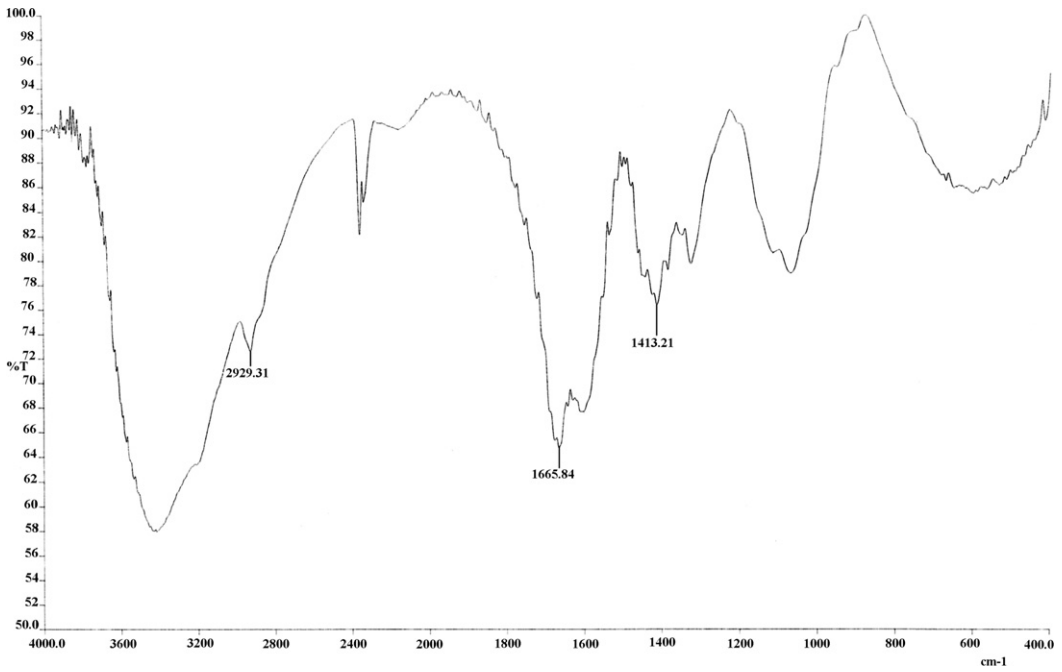


Fig. 3. FTIR of CMCH-g-Am.

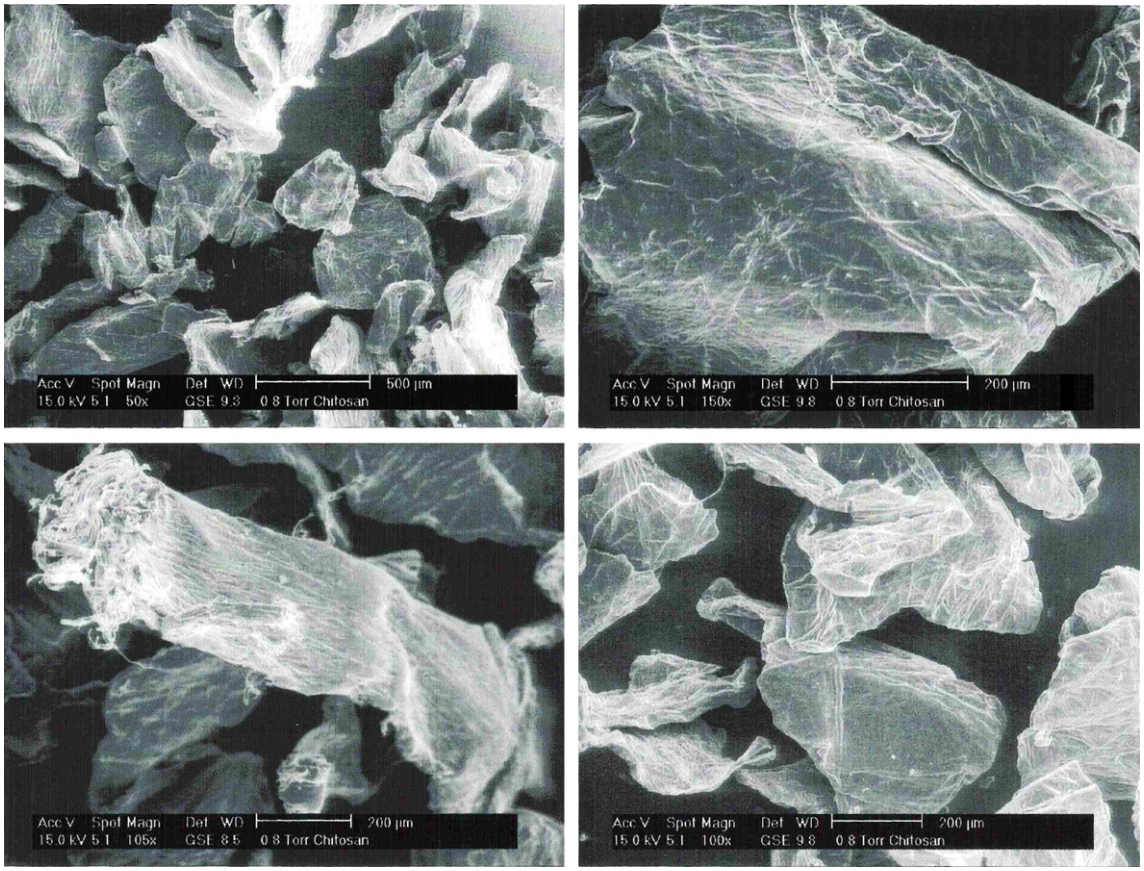


Fig. 4. SEM of chitosan.

due to C=O stretching vibrations of amide was observed at 1665.84 $cm^{-1}$, the peak at 1413.21 $cm^{-1}$ due to $COO^-$ stretching vibrations and the absorption peak at 2929.31 $cm^{-1}$is due to the C—H stretching vibrations. From the IR data it is clear that the grafted copolymer CMCH-g-Am had characteristic peaks of polyacrylamide and of chitosan and its derivative, which could be effective evidence of grafting.

The scanning electron micrograph (SEM) of chitosan, CMCH, and its graft copolymer are shown in Figs. 4, 5, and 6, respectively. Carboxymethylation and graft copolymerization modified the surface morphology and also its physical and chemical characteristics of chitosan. It is clearly seen from Figs. 4 and 5 that flaky nature of chitosan was little modified in carboxymethylation process. The fibrous nature of CMCH was totally modified in the graft copolymer, wherein distinct morphological differences were discernible in their surface topography. Fig. 6, CMCH-g-Am showed the clustered irregular structure.

Fig. 7a, b, and c shows the powder X-ray diffractograms obtained from chitosan, carboxymethyl chitosan and CMCH-g-Am, respectively. From panel a, the two peaks showing the maximum intensity obtained at $2\theta = 20°$ and $2\theta = 72°$, very well matching with values reported in literature (Yui, Imada, Okuyama, & Ogawa, 1994), which indicates chitosan is highly crystalline material. After carrying out carboxymethylation, the carboxymethyl chitosan does exhibit some crystallinity as is clearly shown in panel b. Compared to chitosan and carboxymethyl chitosan, the grafting decreases intensity of both the peaks i.e. almost no peak is observed which is clearly visible in panel c. The graft copolymerized samples become almost amorphous. The grafting of acrylamide is takes place randomly along the carboxymethyl chitosan chain, giving rise to a random copolymer. This will efficiently destroys the regularity of the packing of the original carboxymethyl chitosan chains, which results in the formation of amorphous copolymer.

### 3.2. Effect of initiator concentration

When the other reaction conditions were kept fixed, the effect of CAN concentration on the extent of graft parameters as in Fig. 8. The %G, %GE, and %add-on increase with increase in the initiator concentration and reaches at a maximum value. This is because of CAN directly attacks the characteristic group $NH_2$ of CMCH backbone producing free radicals; those initiate the graft copolymerization process. It was observed that formation of homopolymer

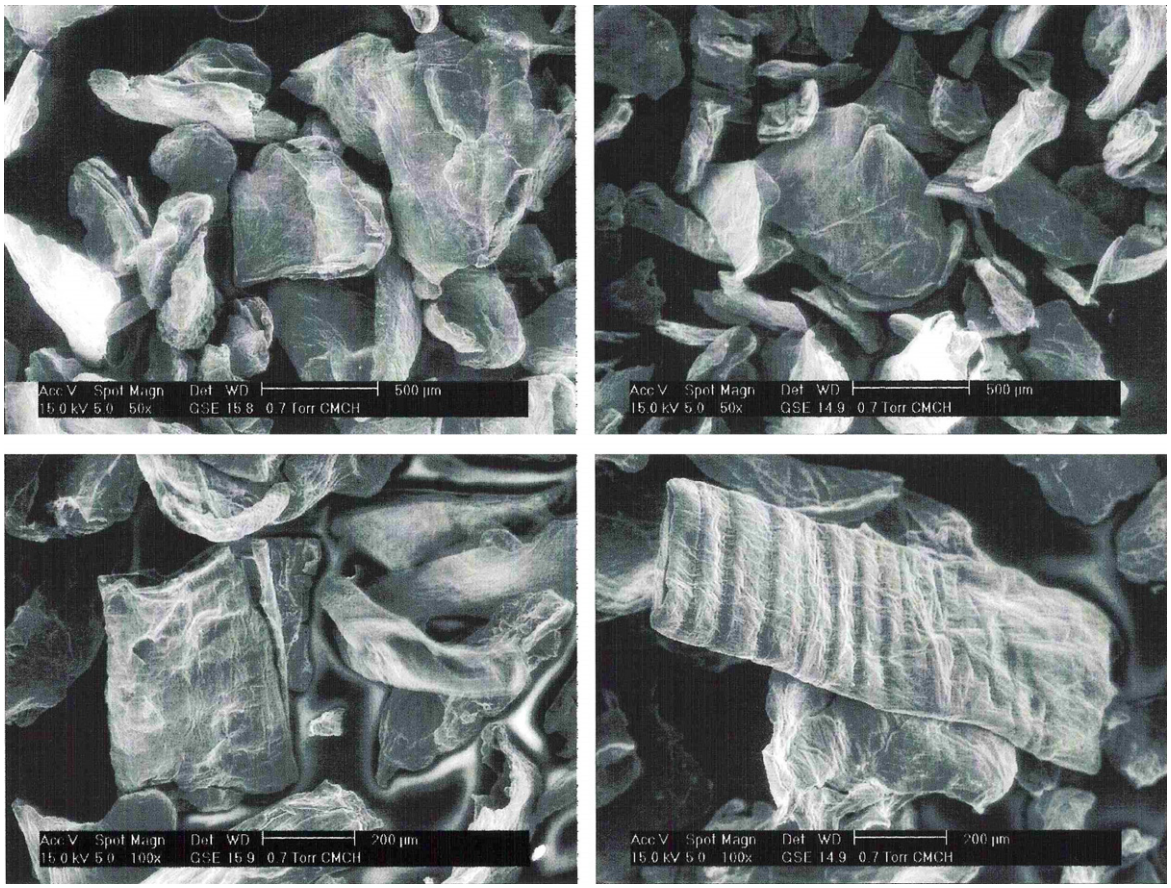


Fig. 5. SEM of CMCH.

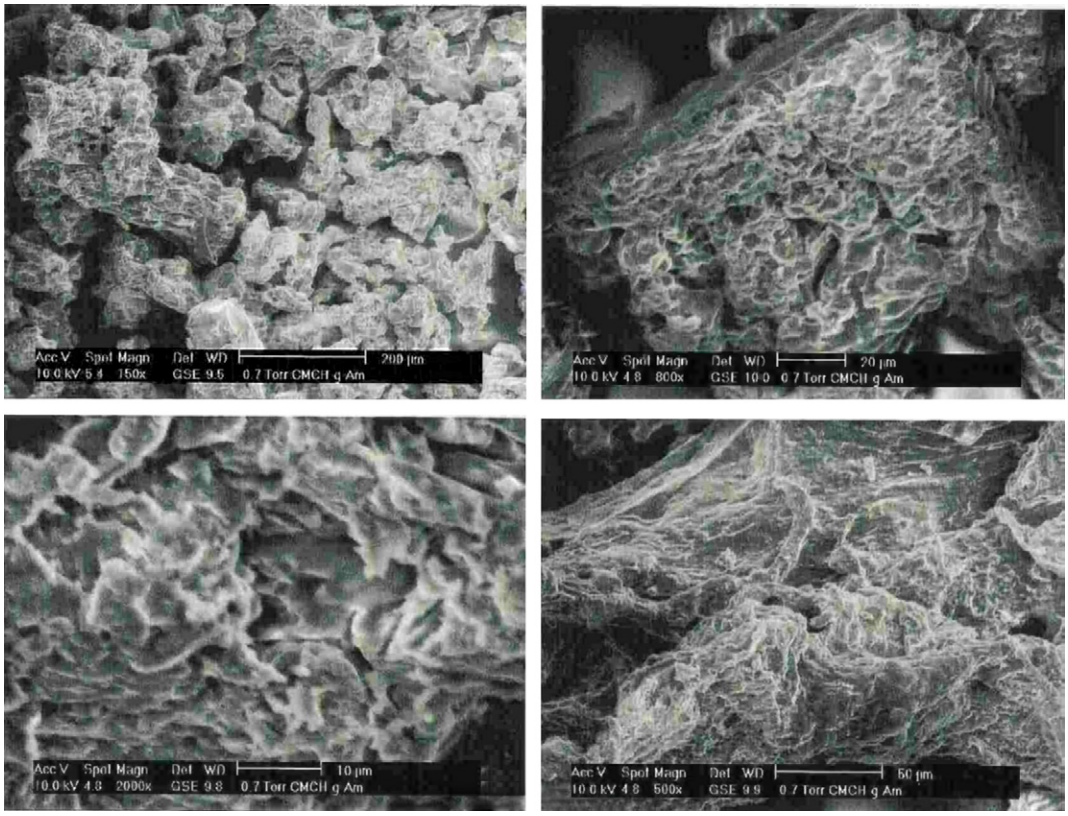


Fig. 6. SEM of CMCH-g-Am.

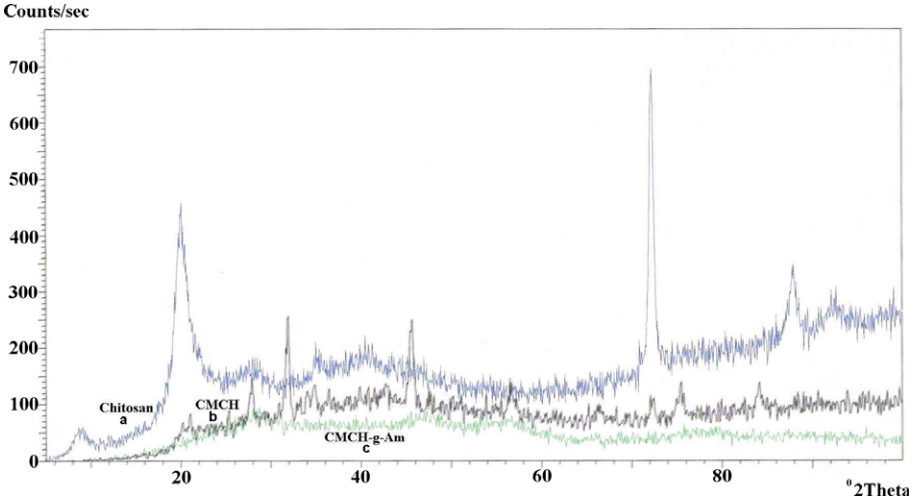


Fig. 7. XRD of CMCH-g-Am.

was considerably less at low initiator concentration while there was a significant homopolymer formation beyond a certain value. Increase the initiator concentration further resulted in a decrease of the %G, %GE, and %add-on. A relatively high concentration of the initiator may cause a reduction of %G, %GE, and %add-on due to increase in the number of CMCH free radicals terminated prior to acrylamide addition. Furthermore, the formation of homopolymers can be expected due to the non-availability of sites on CMCH on which CAN can generate radicals, and thus unutilized CAN can lead to increase in the rate of homopolymerization (Table 1) (Shukla & Sharma, 1987).

### 3.3. Effect of monomer concentration

Fig. 9 shows the effect of concentration of acrylamide (Am) on grafting parameters. With increase in concentration

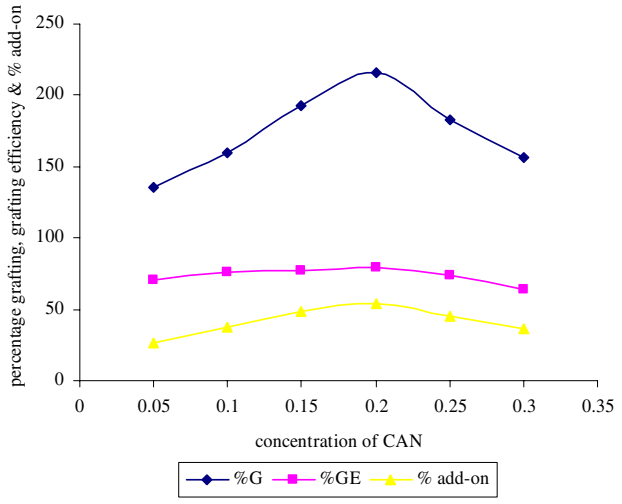


Fig. 8. Effect of initiator concentration (CAN) on percentage grafting (%G), grafting efficiency (%GE), and %add-on.

Table 1
Effect of initiator (CAN) concentration on %G, %GE, and %add-on

| Sr. Number | Mole of CAN (mol/L) | %G | %GE | %Add-on |
|---|---|---|---|---|
| 1. | 0.05 | 135.8 | 70.20 | 26.38 |
| 2. | 0.1 | 159.9 | 75.62 | 37.48 |
| 3. | 0.15 | 192.7 | 76.88 | 48.11 |
| 4. | 0.20 | 215.8 | 79.03 | 53.67 |
| 5. | 0.25 | 183.2 | 73.35 | 45.42 |
| 6. | 0.30 | 156.1 | 64.02 | 35.93 |

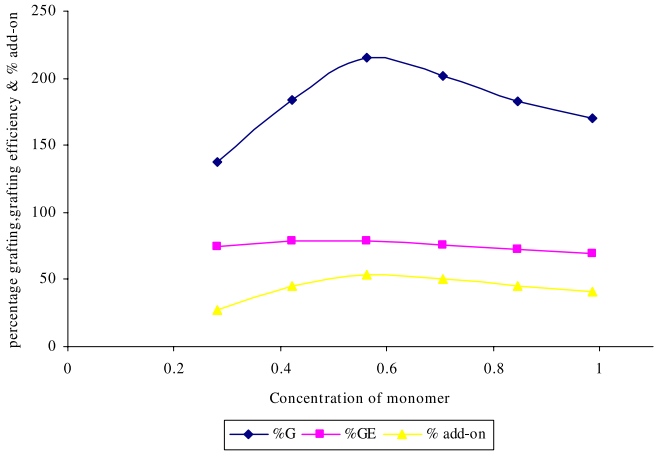


Fig. 9. Effect of monomer concentration (Am) on percentage grafting (%G), grafting efficiency (%GE), and %add-on.

of Am, %G, %GE, and %add-on increased continuously, reached the maximum value when the concentration of acrylamide was 0.563 mol/L, and then decreased. This behavior could be explained by the fact that an increase of monomer concentration leads to the accumulation of monomer molecules in close proximity to the CMCH backbone. The decrease of %G after saturation could be associated with depletion in the available acrylamide concentration as well as a reduction in the active sites on the CMCH backbone as graft copolymerization proceeds. With the higher monomer concentrations the primary radicals attack the monomer instead of reacting with the backbone polymer. It can also be noted that once the graft copolymer radical has formed, the excess monomer will shield the graft copolymer which may inhibit the rate of graft copolymerization. In addition to this, excess monomer will be available for initiator radicals to initiate the homopolymerization reaction and there by decrease in the %GE and %add-on (Table 2).

### 3.4. Effect of reaction temperature

The effect of temperature was studied by changing the reaction temperature from 20 to 70 °C and keeping the other reaction condition constant. It can be seen from Fig. 10 that %G, %GE, and %add-on increase with rise in temperature form 20 to 40 °C but decrease with further rise in temperature to 70 °C. The dependence of %G on temperature can be ascribed to the swelling of CMCH and the enhancement of the rate of diffusion of monomer. With any further increase in temperature the graft copolymerization occurs with poor selectivity, and various hydrogen abstraction and chain transfer reaction might be accelerated and thus lead to a decrease in %G as well as %add-on (Mishra & Dogra, 1980). The decrease in %GE at higher temperature may be attributed to the solubility of monomer in the aqueous phase and also to the acceleration of the termination reaction which leads to the formation of more homopolymer (Table 3).

Table 2
Effect of monomer (Am) concentration on %G, %GE, and %add-on

| Sr. Number | Moles of Am (mol/L) | %G | %GE | %Add-on |
|---|---|---|---|---|
| 1. | 0.281 | 137.2 | 74.18 | 27.11 |
| 2. | 0.422 | 183.4 | 78.51 | 45.47 |
| 3. | 0.563 | 215.8 | 79.03 | 53.67 |
| 4. | 0.704 | 201.2 | 75.68 | 50.31 |
| 5. | 0.845 | 182.4 | 72.78 | 45.17 |
| 6. | 0.985 | 170 | 69.82 | 41.17 |

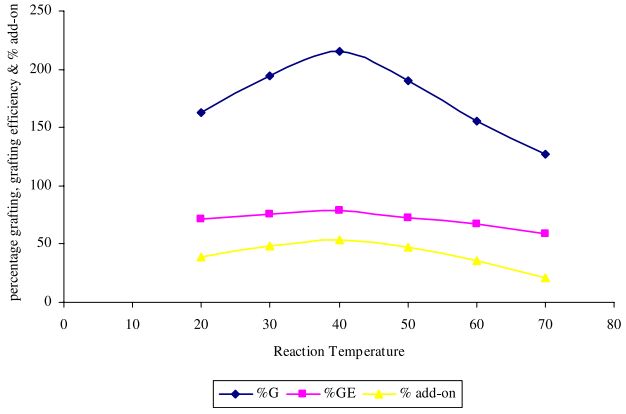


Fig. 10. Effect of reaction temperature on percentage grafting (%G), grafting efficiency (%GE), and %add-on.

Table 3
Effect of reaction temperature on %G, %GE, and %add-on

| Sr. Number | Temperature (°C) | %G | %GE | %Add-on |
|---|---|---|---|---|
| 1. | 20 | 163.3 | 71.66 | 38.76 |
| 2. | 30 | 194.4 | 75.64 | 48.55 |
| 3. | 40 | 215.8 | 79.03 | 53.67 |
| 4. | 50 | 190.6 | 72.50 | 47.54 |
| 5. | 60 | 155.9 | 66.90 | 35.87 |
| 6. | 70 | 127.35 | 58.80 | 21.47 |

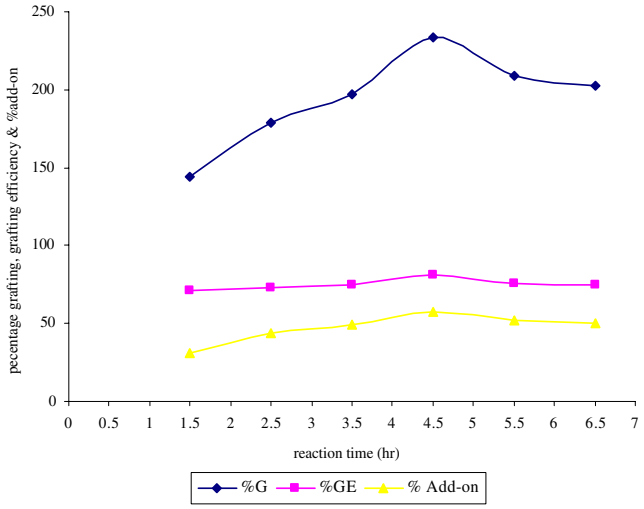


Fig. 11. Effect of reaction time on percentage grafting (%G), grafting efficiency (%GE), and %add-on.

Table 4
Effect of reaction time on %G, %GE, and %add-on

| Sr. Number | Time (h) | %G | %GE | %Add-on |
|---|---|---|---|---|
| 1. | 1.5 | 149.3 | 73.75 | 33.04 |
| 2. | 2.5 | 183.9 | 76.91 | 45.63 |
| 3. | 3.5 | 197.7 | 77.21 | 49.41 |
| 4. | 4.5 | 215.8 | 79.03 | 53.67 |
| 5. | 5.5 | 201.9 | 76.78 | 50.47 |
| 6. | 6.5 | 187.8 | 72.23 | 46.76 |

### 3.5. Effect of reaction time

Fig. 11 shows the effect of reaction time on the %G, %GE, and %add-on. It is clear from figure that as reaction time increases grafting parameters increased gradually. The decrease in the %G, %GE, and %add-on with time could be attributed to decrease in concentrations of initiator and the monomer as well as a reduction in the number of free radicals accessible for grafting as reaction proceeds. The higher value of %G can be attributed to the fact that the presence of bulky groups, such as $-CH_2COOH$ in the CMCH, may open up its structure, thereby increasing the diffusion of the initiator and monomer into CMCH (Table 4).

## 4. Conclusions

With the intention to obtain the potential substrate as a carrier for binding the potential drugs, the purpose of this work was to prepare a polymer to be used in the controlled drug delivery system. Chitosan, having better absorptivity in the animal system, after carboxymethylation has enhanced solubility into the water as well as grafting efficiency. In other words, it gives an in vitro binding system for the potential drugs.

In the present work, the feasibility of grafting acrylamide onto CMCH by CAN as the redox initiator has been demonstrated. The reaction condition such as initiator concentration, monomer concentration, reaction time, and reaction temperature had great influence on graft copolymerization. The study of FTIR spectra, SEM, and XRD provides evidence that graft copolymerization do take place. Such chitosan derivatives as this have wide use as binders in controlled drug release tablets.

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