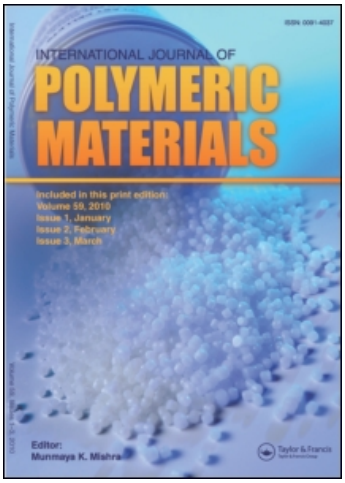

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Grafting Study and Antifungal Activity of a Carboxymethyl Cellulose Derivative

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Grafting Study and Antifungal Activity of a Carboxymethyl Cellulose Derivative

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Graft copolymerization of appropriate monomers onto cellulose and its derivatives can enhance their characteristics and consequently expand their potential applications. Carboxymethyl cellulose (CMC) was prepared and characterized by FTIR spectroscopy and XRD. Graft copolymerization of acrylic acid sodium salt (AAs) onto CMC using ammonium persulfate (APS) as a free radical initiator was carried out under nitrogen atmosphere in aqueous solution. Occurrence of grafting was confirmed by comparison of FTIR spectra of CMC and the graft copolymers as well as the XRD patterns and thermal analysis. The effects of concentration of AA, temperature, concentration of APS and reaction time on the grafting yield were investigated by determining the grafting percentage and grafting efficiency. With other conditions kept constant, the obtained optimum grafting conditions were: CMC = 0.2 g, [AAs] = 2 mM, [APS] = 7.5 mM, temperature = 70°C and reaction time = 2 h. A preliminary study was then carried out to evaluate the antifungal activity of the prepared graft copolymer. This preliminary investigation of the prepared graft copolymers showed that they may be tailored and exploited to expand the utilization of these systems in medical applications.

Keywords: antifungal, carboxymethyl cellulose, grafting

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INTRODUCTION

The last few decades have witnessed a significant body of research on the structure–function relationship of polysaccharides [1,2]. The research has shown that polysaccharides play a significant role in the process of many diseases. In addition, research in polysaccharides has attracted increasing attention because of their low toxicity, biocompatibility, biodegradability, renewability and their prospective pharmaceutical and biomedical applications.

Cellulose is one of the most abundant biopolymers in nature and estimated to be at levels approaching 10^{11} tons annually [3]. Chemical modification of cellulose is an important route for the production of multifunctional materials. Considerable interest has been focused on chemical modification by graft copolymerization of vinyl monomers onto cellulose [4–6]. This graft copolymerization onto cellulose can impart desired properties or enhance the existing properties, and consequently expand the area of its potential applications via the selection of the appropriate types of side chains. Recently, a wide range of initiator systems has been employed to initiate the graft copolymerization. These initiators include free-radical initiators such as ceric ammonium nitrate (CAN), potassium persulfate (KPS) and ammonium persulfate (APS) which produce free-radical sites on the polymer backbone [7–10].

The solubility characteristics of water-soluble polysaccharides depend on the presence of certain functional groups (such as OH, COOH, and NH_2). Sodium carboxymethyl cellulose (CMC), also known as cellulose gum, is a water-soluble anionic cellulose ether and it is available in a wide range of substitution [11]. The CMC structure is based on the β-(1–4)-D-glucopyranose polymer of cellulose. Different methods of preparation of CMC may lead to different degrees of substitution, but it is commonly in the range 0.6–0.95 carboxymethyl groups/monomer unit [12].

In addition to the various superior characteristics of CMC, including its low cost, its chemical modifications can offer additional desired properties, enhance its overall properties and increase its potential applications in many fields. Recently, some trials have been reported in the literature for grafting some monomers onto CMC using variable initiation systems and for different applications [13–19].

In this study, CMC has been prepared by etherification of cellulose using monochloroacetic acid in alkaline medium followed by the graft copolymerization of acrylic acid sodium salt (AAs) onto its backbone in the presence of ammonium persulfate (APS) as a free-radical initiator. The effects of reaction conditions such as reaction temperature,

reaction time, concentration of monomer (AAs) and concentration of initiator (APS) on the yield of graft copolymerization were investigated. Then, the antifungal activities of the prepared CMC-g-AAs copolymer at different graft levels (G%) and concentrations were investigated **in vitro** against the *Aspergillus flavus* strain.

EXPERIMENTAL

Materials

Pure cellulose powder was supplied by Whatman W&R Balston Ltd., England. The monochloroacetic acid 99% was obtained from Cambrian Chemicals and used without further treatment. Ammonium persulphate (APS) was purchased from Sigma (USA) and acrylic acid (AA) (99%) was supplied by Aldrich. All other reagents were of analytical grade and used without further purification.

METHODS

Preparation of CMC

Water-soluble CMC was prepared by a modified method described by Pushpamalar et al. [20]. In a typical procedure, 2 g of cellulose were put in 500 ml round-bottomed flask and suspended in 60 ml isopropyl alcohol at room temperature for 3 h. To the swollen cellulose suspension, 75 ml aqueous NaOH solution (60% w/v) were added and then the whole mixture was refluxed at 100°C for 3 h. Then 100 ml of monochloroacetic acid solution (60% w/v) was added to the reaction mixture over a period of 10 min. The mixture was heated for 2 h at 60°C with stirring. Then the resulting CMC was precipitated in methanol. The product was filtered, washed several times with a mixture of CH_3OH/H_2O (1:1) and dried.

Preparation of CMC-g-AAs

The grafting reactions were carried out in a 500 ml two-necked flask using 0.2 g CMC. Before the addition of the predetermined volume of AA monomer, the monomer was neutralized using NaOH (2 M) and then made up to the volume of 20 ml with distilled water. The components were mixed and stirred for 20 min with bubbling of a slow stream of nitrogen gas. The flask was then placed in a thermostated bath at the desired temperature (50–90°C). Finally, the precalculated concentration of the initiator, APS (2.5–12 mM based on the total

volume of reaction mixture) dissolved in 10 ml of distilled water was added dropwise with stirring. The used monomer concentrations based on the total volume of reaction mixture, 30 ml, were in the range of (0.3–2.9 mM). After the copolymerization was carried out for the predetermined period (30–180 min), the reaction was stopped by letting air into the flask and rapidly cooling down the reactor. The products were precipitated by pouring the reaction mixture into acetone. The precipitate was filtered off, washed with acetone and the crude product was dried and weighed. The formed homopolymer (PAAs) was extensively extracted in a Soxhlet apparatus with methanol for 6 h. The residual graft copolymer obtained was washed with methanol, dried and weighed. The percent grafting (G%) and the grafting efficiency (GE%) of the copolymers were calculated as follows [21]:

$$G\% = [(W_g - W_0)/W_0] \times 100$$

$$GE\% = [W_g/(W_g + W_h)] \times 100$$

where W_g, W_h, and W_0 are the weights of graft copolymer, homopolymer and carboxymethyl cellulose, respectively.

Characterization

Both the CMC and CMC-g-AAs were characterized by FTIR (Mattson 5000 FTIR spectrometer) using KBr discs in the range of 600–4000 cm^{-1}. The differential scanning calorimetry (DSC) of CMC and CMC-g-AAs copolymers of different grafting yields were performed in a duPont model 2000 DSC analyzer. The samples (3–4 mg) were weighed into aluminum sample pans and sealed. An empty aluminum pan of approximately equal weight was used as a reference. The applied heating rate was 10°C/min up to a maximum temperature of 400°C under nitrogen atmosphere. All peaks were determined and the areas were converted into enthalpy values. The X-ray diffraction (XRD) patterns of cellulose, CMC and the prepared copolymers were determined using a Philips apparatus PW 105 diffractometer using Ni-filtered Cu K_α radiation ($\lambda = 1.540$Å) at operating voltage of 40 KV.

Antifungal Evaluation

Five samples of CMC-g-AAs copolymer of different concentrations and different G% were investigated **in vitro** for their antifungal activities against *Aspergillus flavus* strain using the agar diffusion technique [22]. The fungi were maintained on dry potato dextrose agar plates.

Then, after 48 h of incubation at 28°C, the diameter of the inhibition zone (Iz, mm) was measured.

RESULTS AND DISCUSSION

Characterization of CMC and CMC-g-AAs Copolymer

The CMC was prepared by a method adapted from Pushpamalar et al. [20]. The structural changes of cellulose, CMC and CMC-g-AAs were confirmed by FTIR (Figure 1). In the FTIR spectrum of cellulose (Figure 1a), the strong peak at 3439 cm^{-1} is due to the O-H stretching vibration and the intermolecular H-bonds of the polysaccharide moieties. The FTIR spectrum of CMC (Figure 1b) shows a strong new peak at 1728 cm^{-1} representing the carboxylate C = O asymmetric stretching. The signal at 1392 cm^{-1} could be assigned to the symmetric

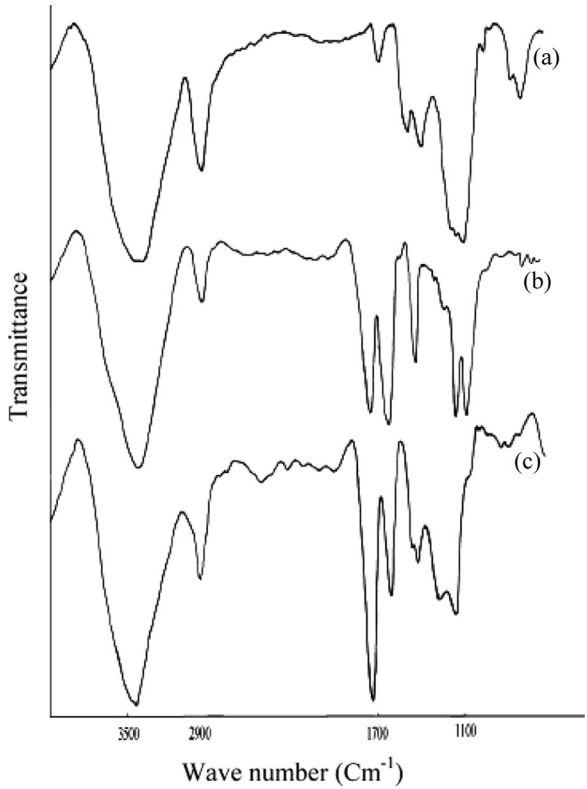
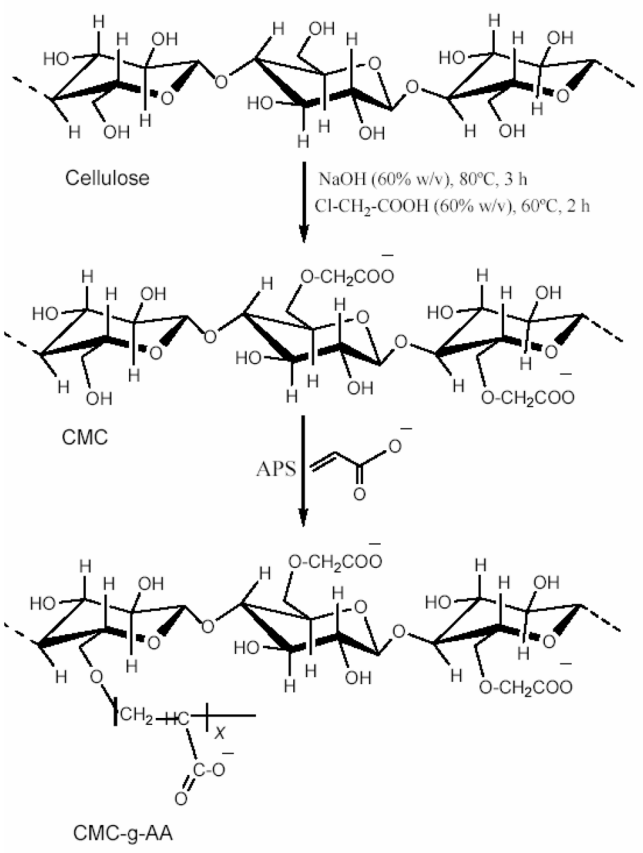


FIGURE 1 FTIR spectra of (a) cellulose, (b) CMC, (c) CMC-g-AAs (G%: 625%).

stretching vibration of carboxylate C = O. In case of IR spectrum of CMC-g-AAs (Figure 1c), there was a new absorption peak at 1264 cm^{-1}. No clear absorption due to vinyl unsaturation was observed at about 1640 cm^{-1}, and also no clear bands appeared in the range of 1400–1420 cm^{-1}, representing vinylic double bond in conjunction with carbonyl group. This tends to indicate the disappearance of the vinylic double bond of AAs due to grafting. The preparation of CMC and CMC-g-AAs copolymer are shown in Scheme 1.

Proof of Grafting

The higher weight of the prepared graft copolymer than that of the starting CMC after the extensive removal of the homopolymer can



SCHEME 1 Preparation of CMC and CMC-g-AAs.

be taken as evidence of grafting. Besides, the FTIR spectra of CMC-g-AAs, as discussed in the above section, had both characteristic peaks of CMC and AAs. Also, as shown in Figure 2, the intensity of C = O absorption at 1728 cm^{-1} was increased with increasing the G%, which can be considered additional experimental evidence of grafting.

Occurrence of the graft copolymerization was also confirmed by thermal analysis. The DSC of cellulose, CMC and CMC-g-AAs copolymer are shown in Figures 3a and 3b, respectively. Cellulose shows a strong exothermic transition at 330°C (Figure 3a–i) while CMC shows

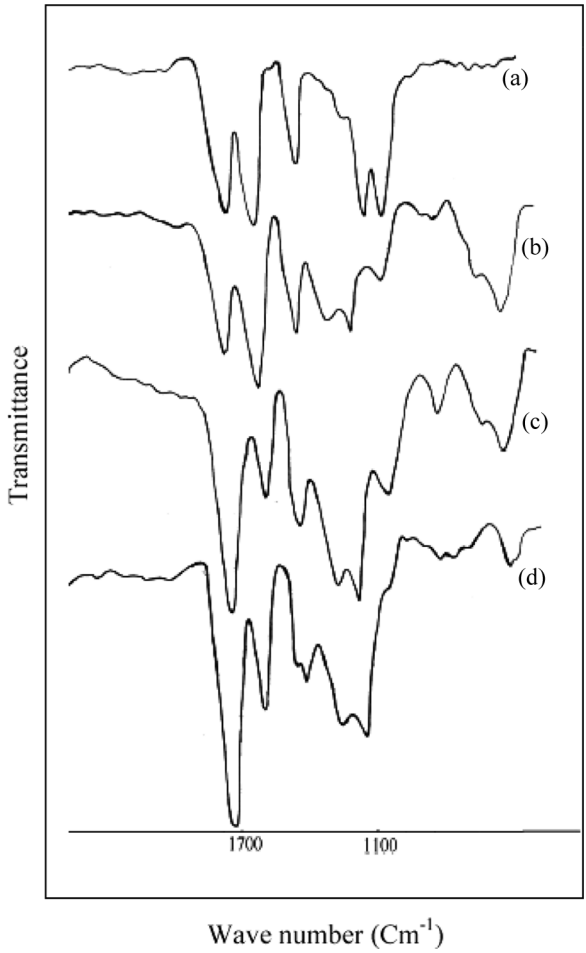
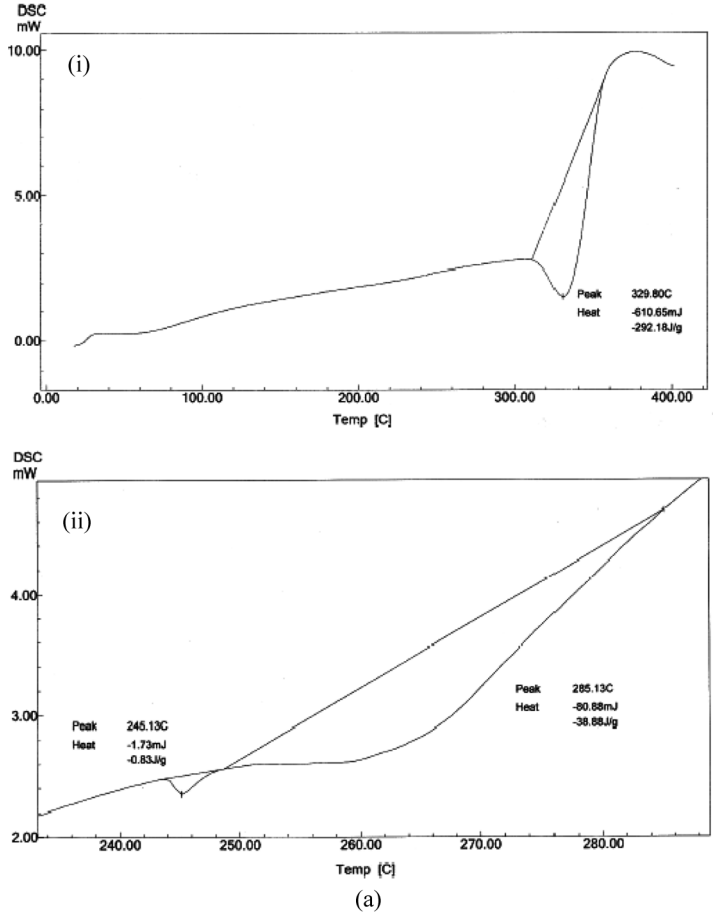


FIGURE 2 FTIR spectra of CMC (a) and CMC-g-AAs of different G%, (b) 100%, (c) 365%, (d) 625%.

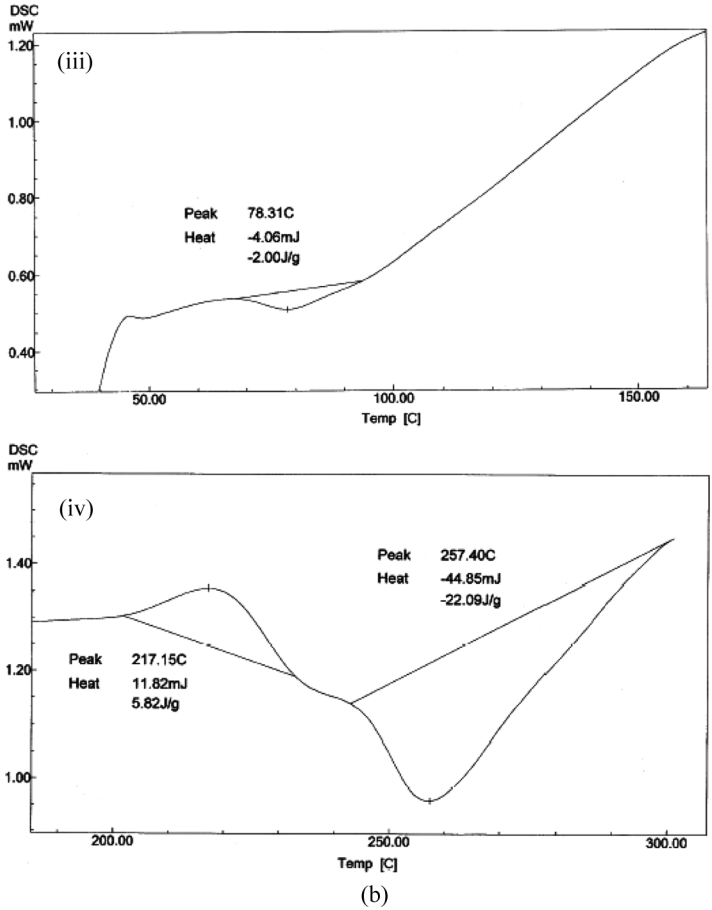


(a)

FIGURE 3 (a) DSC of (i) cellulose and (ii) CMC. (b) DSC of CMC-g-AAs (G%: 625%).

a sharp band at 245°C and a broad exotherm at 285°C (Figure 3a–ii). In case of CMC-g-AAs copolymer (Figure 3b), a new broad exothermic transition appeared at 78°C, which may be due to the loss of bound water. In addition, an endothermic peak appeared at 217°C and a strong exotherm was found at 257°C. These changes in the pattern of thermal transitions in the CMC-g-AAs copolymer, when compared to cellulose and CMC, confirm the grafting process.

Figure 4 shows the XRD patterns of CMC and CMC-g-AAs copolymer. In both Figures 4a and 4b, the halos centered around 2θ value of 12–13° represent the patterns of the amorphous CMC. In



(b)

Figure 3. Continued.

Figure 4b, the new halo at 2θ value of 20–22° can be attributed to the AAs side chains (inter-sodium distances) and its intensity reflects the level of grafting. The sharp reflections appearing at much higher values of 2θ in both diffractograms may be due to either the sample holder or some inorganic salt residues. The existence of all these sharp and intense reflections should be disregarded since they are representative of neither the CMC nor the CMC-g-AAs.

Effect of Initiator Concentration

The effect of the concentration of initiator (APS) on the grafting extent is shown in Figure 5. With other reaction conditions kept

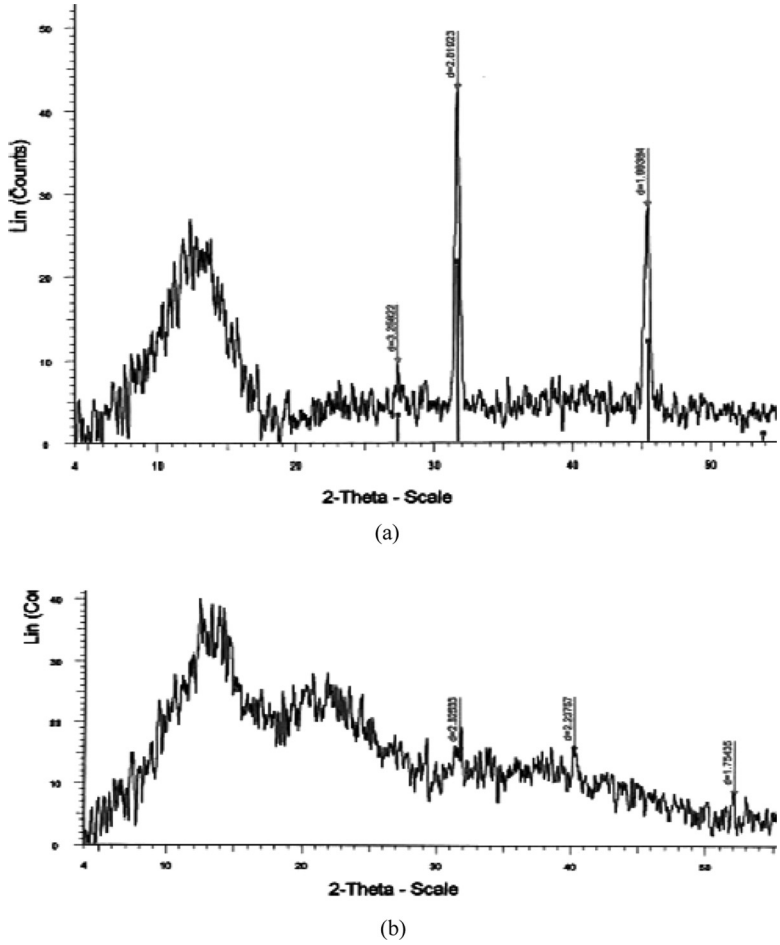


FIGURE 4 The XRD patterns of (a) CMC and (b) CMC-g-AAs (G%: 625%).

constant, both G% and GE% increased with increasing concentration of APS, in the range from 2.5 to 12 mM, reaching a maximum value at 7.5 mM, then decreased again. This behavior may be attributed to the generation of more CMC macroradicals with increasing the concentration of APS. Thus, more active sites of CMC could react with the monomer AAs, leading to increasing G% and GE%. However, the excessive APS may engender plenty of radicals, which could terminate the propagation of graft copolymerization. This resulted in the decrease of G% and GE%.

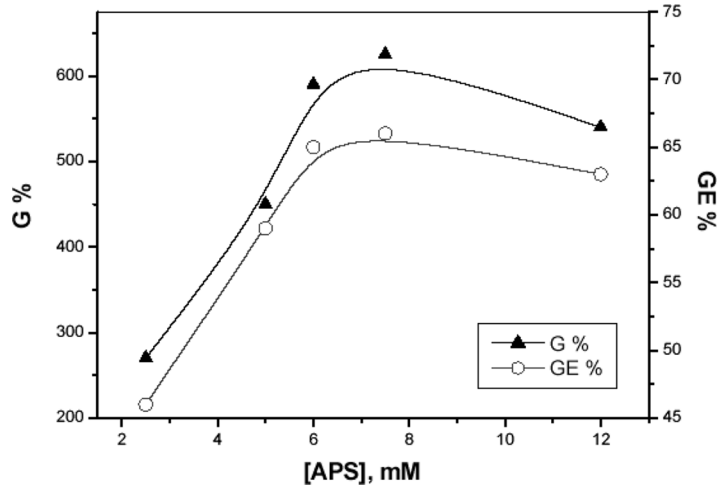


FIGURE 5 Effect of initiator concentration (mM) on grafting parameters; CMC: 0.2 g; [AAs]: 1.2 mM; Temp.: 70°C; Time: 120 min.

Effect of Monomer Concentration

Figure 6 shows the effect of monomer concentration on the yield of graft copolymerization. It is apparent that there is an increase in both G% and GE% upon increasing the monomer concentration up to a certain value (2 mM of AAs under the experimental conditions) followed by a gradual decrease with the monomer further increasing. This behavior may be due to the limited number of active centers available for grafting on the polymer backbone than upon increasing the monomer concentration. More competition occurs between the monomer units for the same sites, leading to increasing the grafting extent until saturation of the backbone. At higher concentrations of monomer, however, the excess of monomer units could initiate much more chain transfer and termination reactions, leading to more homopolymerization instead of grafting.

Effect of Reaction Time

Figure 7 illustrates the effect of reaction time on the grafting parameters. The effect of time was investigated through checking the values of G% and GE% at different times from 30 to 180 min. Both G% and GE% increased with increasing reaction time and reached a saturation value of grafting at the optimum time of about 120 min. As the reaction time increases, both free radicals and the amount of monomer in the reaction are reduced, leading to levelling off the grafting parameters.

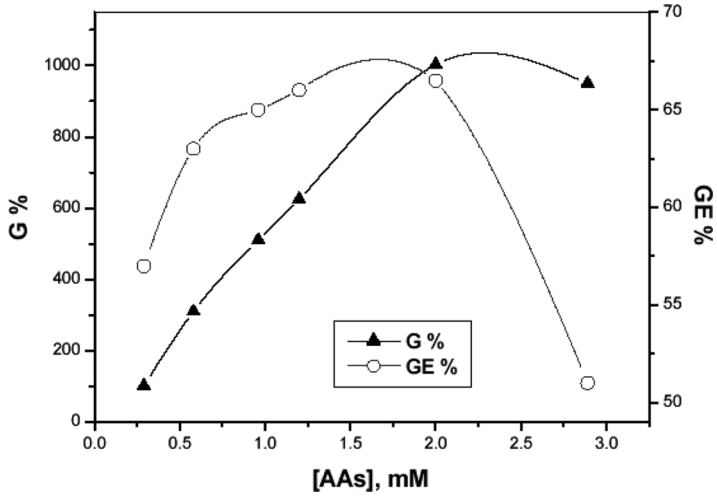


FIGURE 6 Effect of monomer concentration (mM) on grafting parameters; CMC: 0.2 g; [APS]: 7.5 mM; Temp.: 70°C; Time: 120 min.

Effect of Temperature

The effect of temperature on the grafting yield is demonstrated in Figure 8. As appeared from this figure, as the temperature

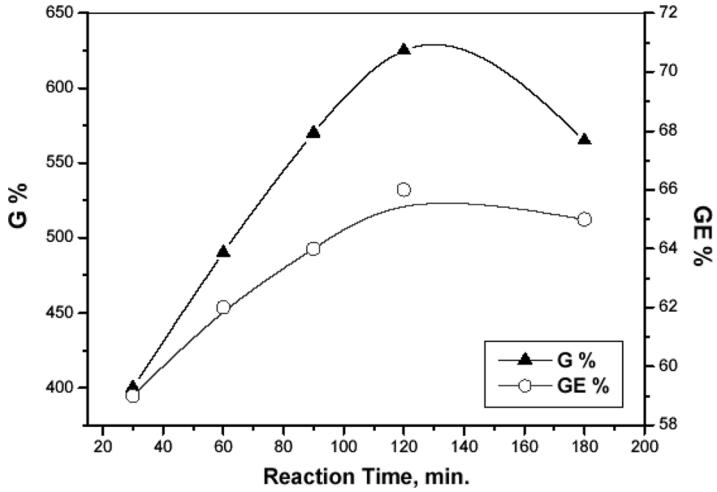


FIGURE 7 Effect of reaction time on grafting parameters; CMC: 0.2 g; [AAs]: 1.2 mM; [APS]: 7.5 mM; Temp.: 70°C.

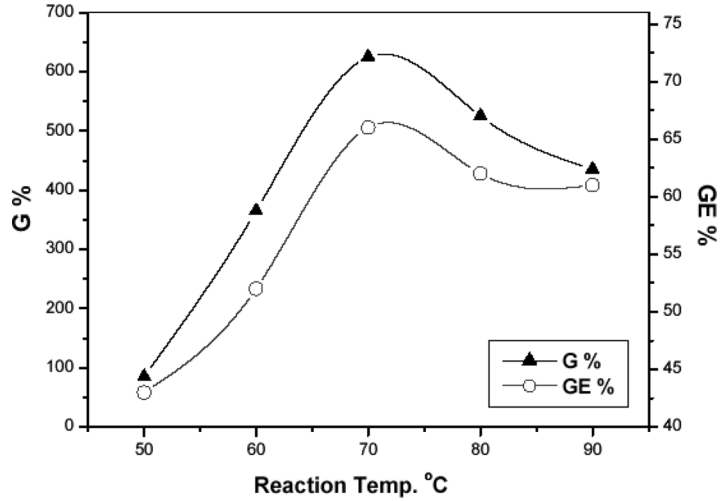


FIGURE 8 Effect of reaction temperature on grafting parameters; CMC: 0.2 g; [AAs]: 1.2 mM; [APS]: 7.5 mM; Time: 120 min.

increases, both G% and GE% increase as a result of increasing the decomposition rate of initiator until the reach maximum values at 70°C. With further increase in the temperature beyond 70°C, more chain transfer and chain termination reactions may occur, leading to a decrease in the grafting yield. Also, with increasing reaction temperature, an oxidative degradation of CMC chains caused by sulfate radical anions originating from the initiator may occur.

Antifungal Activity of the CMC-g-AA Copolymer

In this study *Aspergillus flavus* was used as the test fungi to examine in vitro the antifungal activity of the prepared CMC-g-AAs copolymer in comparison to CMC. As shown in Table 1, CMC itself (S_0) showed a kind of antifungal activity (inhibition zone diameter, Iz 14 mm). In the case of CMC-g-AAs, increasing the G% from 101% (S_1) to 625% (S_2) has led to a significant increase in the inhibition zone. Further increase in the G% up to 1003% (S_3) slightly increased the zone of inhibition (Iz 24 mm). These results may reflect a role played by the AAs in increasing the antifungal activity of the copolymer. Also, from Table 1, increasing the concentration of the copolymer solution was accompanied by an increase in the zone of fungal inhibition. For instance, increasing the concentration from 0.10 mg/ml (S_3) to 0.15 mg/ml (S_4) has increased the diameter of inhibition zone (Iz) from

TABLE 1 The Antifungal Activity of the Prepared CMC-g-AAs as a Function of Concentration and G%

Sample Code	G%	Concentrations (mg/ml)	Iz* (mm)
S_0	–	0.10	14
S_1	101	0.10	15
S_2	652	0.10	23
S_3	1003	0.10	24
S_4	1003	0.15	26
S_5	1003	0.20	28

*Iz: Mean diameter of inhibition zone (mm).

24 mm to 26 mm. With further increase in the concentration up to 0.20 mg/ml (S_5), the inhibition diameter reached 28 mm. Within the range of the samples studied (S_0–S_5), the CMC-g-AAs copolymer with G% equal 1003% and concentration of 0.20 mg/ml (S_5) has showed the most effective antifungal ability. Figure 9 illustrates the fungal inhibition zones produced from the samples S_0–S_5. This preliminary investigation showed that the prepared CMC-g-AAs copolymers may be tailored to expand the utilization of these systems in medical applications.

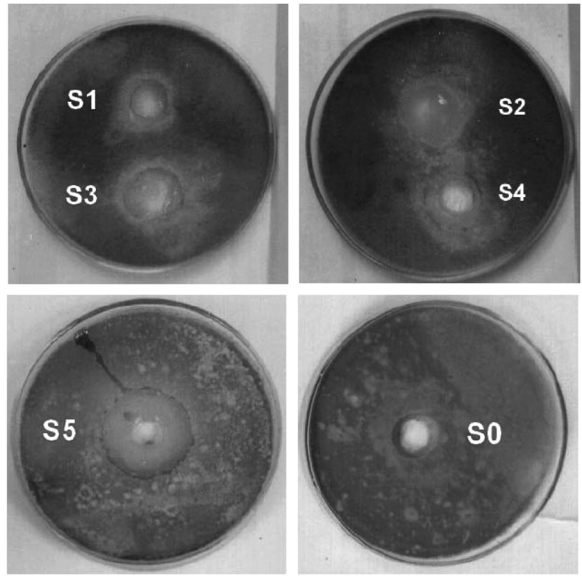


FIGURE 9 The fungal inhibition zones (mm) produced from the CMC-g-AAs samples (S_0–S_5).

CONCLUSION

In this study, CMC was prepared [20] and characterized. Then, graft copolymerization of acrylic acid sodium salt onto its backbone was carried out under nitrogen atmosphere in aqueous solution using a free-radical initiator. Grafting was confirmed by various tools and the effects of monomer concentration, temperature, initiator concentration and reaction time on the grafting yield were investigated. Then, a preliminary investigation was carried out to evaluate the antifungal activity of the prepared graft copolymers. This preliminary investigation showed that these copolymers may be tailored to work as good candidates in medical applications.

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