High efficiency antimicrobial cellulose-based nanocomposite hydrogels

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ABSTRACT: Carboxymethyl cellulose hydrogels were developed through crosslinking process using maleic, succinic, or citric acids. The swelling capacities of the obtained hydrogels have been controlled through variation of the crosslinking reaction conditions. Further enhancement of the swelling properties was achieved by additional blending step of the carboxymethyl cellulose with polyethylene glycol. Biofunctionalization of the hydrogels was achieved through "*in situ*" incorporation of the silver nanoparticles during the crosslinked reaction. Chemical structure verifications and morphological characterizations of the hydrogels were performed using FTIR, XRD, EDX, and SEM analyses. Finally, the antimicrobial activity of the loaded silver hydrogels against G+ve, G−ve, and yeast *Candida albicans* was demonstrated. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 42327.

INTRODUCTION

Hydrogels are hydrophilic three-dimensional networks that can be formed by chemical crosslinking or physically entangled with excellent water absorption. On the molecular level, water is either bonding with polar hydrophilic groups as bound water or filling the spaces between chains, pores, or voids as free water.[1] Rapid developing of the hydrogels has focused on their preparation, characterization, and applications.[2] They have been used in many fields such as food additives, pharmaceuticals, and biomedical applications.[3–5] The network components of the hydrogels can be manufactured from either synthetic or natural polymers that are able to absorb and maintain large quantity of water.[6,7] Natural polymers such as cellulose, chitosan, and their derivatives have received increasing interest for preparing environmentally responsive hydrogels due to their high hydrophilicity, biocompatibility, nontoxicity, and biodegradability.[8,9] Carboxymethyl cellulose (CMC) is a representative derivative of cellulose with excellent water solubility, biocompatibility, and nontoxicity.[10] The advantageous properties of CMC allow it to be used in both the biotechnological and the industrial fields due to abundant reactive carboxylic and hydroxyl groups existing on the chains of CMC.[11,12] The use of cellulose derivatives for the hydrogels' preparation has been preferred due to the facile crosslinking step through physical and chemical processes. In case of the physical crosslinked hydrogels, there is no covalent bond formation or breakage, and the networks of the gel can be formed by ionic bonds, hydrogen bonds, or associative intermolecular interaction between the polymer chains.[13] The physical

crosslinking is responsible for gelation of the hydrophobically modified cellulose (methyl cellulose (MC) and hydroxymethylpropyl cellulose through heating effect to obtain thermoreversible hydrogels.[14] The hydrophobic groups such as methyl or methylpropyl, which partially replace the hydroxyl groups of cellulose, prevent the intensive hydrogen bonds formation. So, the cellulose derivative becomes soluble resulting in physically reversible hydrogel at definite temperature. There is evidence that the gelation behavior of some hydrophobic modified cellulose derivatives can be resulted from mixed contribution of both the hydrophobic interactions of the hydrophobic groups and the intermolecular hydrogen bonds among the hydroxyl groups at C6 position.[15] Use of chemically crosslinking route is necessary to have hydrogel networks stable structure with efficient swelling properties. Some difunctional compounds (e.g., divinylsulphone (DVS)) are used to bind cellulose derivatives, for example, CMC or hydroxyethyl cellulose (HEC), through covalent bond to form three-dimensional hydrophilic networks.[16] These hydrogels display high absorption water capacity which can be enhanced by inserting macromolecule spacer chains (e.g., polyethylene glycol [PEG]) when DVS is used as crosslinker. For applications in food industry or in pharmaceutical industry, as delivery vehicles of drugs, the crosslinker included in the hydrogels must be nontoxic, so DVS must be avoided. Recently, intensive research has been carried to find new biocompatible crosslinkers for developing of cellulose-based hydrogels.[17] For example, super absorption hydrogel of CMC/HEC is obtained by using citric acid as crosslinker to overcome the toxicity and cost problems in comparison with the former

used toxic crosslinker. The proposed mechanism can explain the crosslinking role of citric acid due to formation of cyclic anhydride units by heating. The anhydride moiety of citric acid reacts with hydroxyl group in the main cellulosic chains to form ester bond. This esterification step results in emerging two carboxylic groups in citric acid molecule and then, new intramolecular anhydride moiety of citric acid is formed by heating. Another esterification reaction can take place as a result of reaction between the second anhydride moiety of citric acid with hydroxyl group through intermolecular crosslinking. On the other hand, silver nanoparticles (AgNPs) are being considered as a nontoxic environmental friendly antibacterial material, but due to their poor binding characteristic with surfaces, their utility has been restricted. Therefore, polymer-stabilized nanoparticles and nanoparticles embedded in hydrogel networks are outstanding approaches for antibacterial applications. Incorporation of AgNPs in polymeric system exhibits high biomedical applications, particularly, to control bacterial infections.[18–20] The effect of the high surface area of AgNPs resulting in an increase of silver particles (in nanosize) inside the bacterial cell makes AgNPs superior to the bulk silver particles as antibacterial agent. Also, the antibacterial effect of AgNPs (even with very low molar concentration) is more efficient than the effect of silver ions. The gel system with loaded AgNPs is a quite useful approach to prepare antimicrobial hydrogels. AgNPs can be incorporated into the gel networks by mixing of the nanoparticles during the gelation process or by adding to the swelling medium where AgNPs can be entrapped in the hydrogel matrix during absorption.

Our study focuses on the development of antimicrobial cellulose derivative (CMCNa)-based hydrogel crosslinked via nontoxic agents, such as polycarboxylic acids, as a current approach, and simultaneously functionalized with AgNPs via *in situ* and simple loading techniques during the crosslinking stage.

EXPERIMENTAL

Materials and Methods
CMC (DS 0.70–0.85) was purchased from Fluka Biochemika in the form of sodium salt. Low molecular weight PEG (600LR), maleic, succinic, and citric acids as well as other chemical were purchased from Laboratory Rasayana.

Preparation of the CMC Hydrogel
Preparation of the CMCNa-based hydrogel was carried out by using different polycarboxylic acids as crosslinkers. In a typical experiment, keeping the solution total volume constant (100 mL), 1 g of CMCNa was dissolved in distilled water. Subsequently, the acid (maleic, succinic, or citric) was then added to the CMCNa solution in concentration ranged from 0.5% to 5.0% based on the weight of CMCNa. The mixture was then heated at 80°C for 30 min under mild stirring. At the end of the reaction time, the sample was poured into a Petri dish and was placed in oven at 45°C for 3 h. After complete drying, the sample was washed by ethanol followed by ether for complete drying. The optimum acid concentration (crosslinker) was achieved through monitoring of the samples' swelling properties. Further enhancement of the swelling capacities was explored through addition of PEG to the optimum concentration mixture of CMCNa and crosslinker acids. Different

concentrations of PEG were used ranging from 5% to 20% based on CMCNa weight.

Loading of AgNPs into Hydrogel
The AgNPs were prepared *in situ* during the preparation of hydrogel by adding 0.05 mmol of $AgNO_3$ to the crosslinking reaction for 2 h, and then tri-sodium citrate solution (20 mg dissolved in 25 mL water) was added. The loaded hydrogel was allowed to dry. Reduction of Ag^+ ions to AgNPs within the preparation of hydrogel could convert the color of hydrogel into yellowish transparent matrix.[21]

Release of Silver from Hydrogels
Silver release was detected for the prepared AgNPs loaded hydrogel of different crosslinking acids: maleic, citric, and succinic. For a typical run, 0.2 g of AgNPs loaded hydrogel was immersed in 10 mL deionized water for different time intervals ranging from 1 to 24 h at ambient temperature. The immersed sample was separated from the deionized water, and certain volume of deionized water including AgNPs was drawn for analysis by atomic absorption (Agilent Technologies [200 Sevies AA]), and the measurements were the average of four times.

The mole percentage of AgNPs release was determined by calculating mmole of AgNPs released (detected from the instrument), and the AgNPs loaded in the hydrogel sample used for analysis (considering the stiometery of reduction reaction of silver cation).

$$\text{Mole percentage of AgNPs release} = \frac{\text{mmole of AgNPs release}}{\text{mmole of loaded AgNPs in test sample}} \times 100$$

Characterization and Analysis
Water Uptake Test. Predetermined dried weight hydrogel sample was transferred into 100 mL glass vial and fixed volume of distilled water was added, then the sample was left to swell at RT for 24 h. Excess of water was removed out, and the swelled sample was directly weighted. The hydrogel was characterized through evaluation of the water uptake and gel fraction (%) according to the following equations:

Water uptake = (weight of swelled sample − weight of dried sample)/weight of dried sample

Gel fraction % = (weight of dried sample after immersing in excess water for long time/initial dry weight) × 100

Initial dry weight is the dry weight of the sample consisting of the percentage of PEG in addition to the weight of CMC.

Weight of CMC = 1.5 g − (PEG g)

Infrared Spectral Analysis. Fourier transform infrared (FTIR) spectra of nonloaded and loaded AgNPs were recorded by using KBr disc technique in the range of 400–4000 cm^{-1} on Shimadzu 8400S FTIR Spectrophotometer.

X-ray Diffraction. X-ray diffractograms (XRD) of hydrogels were obtained using an X-ray diffractometer with Cu Kα radiation at 40 kV and 50 mA in a differential angle range of 5–60°2θ.

Scanning Electron Microscopy. The surface morphology of hydrogel and AgNPs containing hydrogel were analyzed by

using electron microscope FEI INSPECTS Company, Philips, Holland, environmental scanning with gold coating.

Energy Dispersive X-ray Measurement. Energy dispersive X-ray (EDX) analysis was carried out to be supporting information confirming the presence of AgNPs in the hydrogel networks.

Assay of Antibacterial Activity

The disc diffusion method was used to evaluate the antimicrobial activity of the prepared hydrogel. A volume of 0.1 mL of the tested microorganisms grown in Brian heart infusion broth (at 42°C for 24 h, 108–109 cells/mL), was incubated on Brian heart infusion media, and then spread on the entire surface of the dish using a sterile spatula. Subsequently, sterile discs were placed onto agar at certain intervals by passing gently. After the plates were incubated at 42°C for 24 h, the inhibition zones around the discs where no growth occurred, were measured in millimeters, the experiments were repeated in duplicated for all of the test strains.

## RESULTS AND DISCUSSION

Reaction Mechanism of Hydrogel Formation

Formation of anhydride moiety of citric acid (due to heating) facilitate the esterification reaction between the hydroxyl group of CMC resulting in crosslinking with liberating of carboxylic group that formed another anhydride moiety. The second anhydride moiety enhanced an esterification leading to crosslinking with hydroxyl group in another CMC chain.[17] It is not reasonable to adopt the mechanism of crosslinking via two cyclic anhydride intermediate for interpretation of the crosslinking stage in cases of maleic and succinic acids. So, we attempted to clarify how the crosslinking occurred through maleic and succinic

acids. In case of maleic acid, a cyclic anhydride can be formed through heating effect, which allowed forming half ester with hydroxyl group of CMC chain. Formation of ester moiety may activate the double bond included in maleic acid molecules by creation of electron deficiency site due to conjugation with the ester group. The carbon atom, which is electron deficient site, is attacked by the lone pairs of electron exciting on oxygen atom of hydroxyl group of CMC chain and then ether link is formed. It is difficult to accept the formation of succinic anhydride through crosslinking process because succinic acid can form anhydride near its melting point which is far from our reaction temperature. So, we can suggest two routes of crosslinking by succinic acid. One of them is direct esterification of both carboxylic groups of succinic with two hydroxyl groups at different CMC chains. The second route may take place by ionization of both carboxylic groups of succinic acid (through cation exchange of sodium on CMC chain), and then coordination bonds are formed among each of anionic carboxylate with two hydrogen atoms of hydroxyl groups on different CMC chain (Scheme 1).

Effect of Crosslinkers

The water uptake characters of the hydrogels are mainly dependent on the type and concentration of the crosslinker agent. So, the study focused on the use of three different polycarboxylic: maleic, succinic, or citric acid as crosslinker for CMCNa. The gel fraction and the water uptake of produced hydrogels were influenced by the concentration of acid used during the gelation process. As shown in Table I, increasing the crosslinker acid concentration resulted in increasing of the gel fraction until certain concentration of crosslinker acid after which the gel fraction of the prepared hydrogels was decreased.
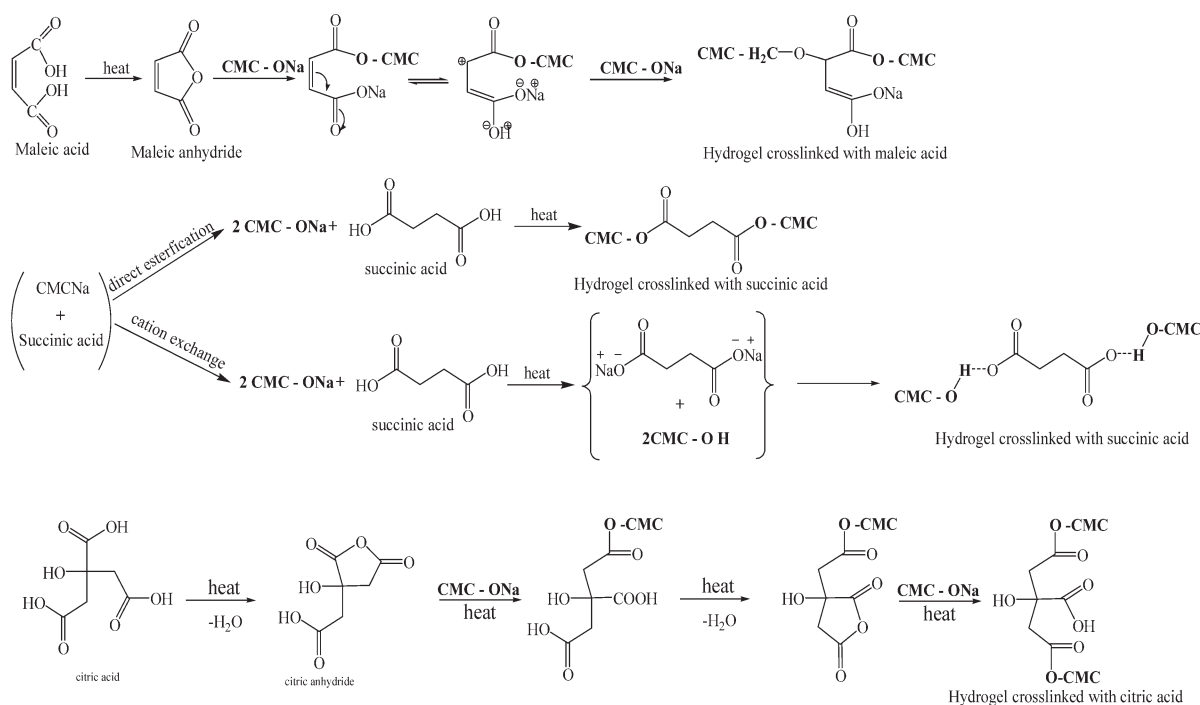
Scheme 1. Possible crosslinking reaction mechanism of different acids with CMC.

Table I. Effect of Crosslinkers on Gel Fraction and Swelling Hydrogels

Acid Conc. (%)	Gel fraction % (based on CMC weight)			Swelling ratio		
	Maleic acid	Succinic acid	Citric acid	Maleic acid	Succinic acid	Citric acid
0.5	67.0	74.0	78.5	10.15	08.27	09.96
1.0	78.5	82.5	78.5	11.50	10.49	17.00
2.0	81.5	89.0	89.5	15.60	11.41	12.50
3.0	80.5	94.0	84.0	15.00	10.20	12.00
5.0	76.5	88.5	84.0	14.79	09.78	08.27

The maxima values of gel fraction for maleic, succinic, and citric acids were 81.5%, 89%, and 89.5%, respectively. The decreasing of gel fraction values after reaching a maximum can be attributed to increasing the acidity of the reaction medium with increasing the crosslinker acid concentration, leading to partially changing the sodium salt form of CMC into acidic form which is insoluble in the reaction medium, and accordingly the chance of crosslinking points among CMC chains was lowered. This concluded as an obvious reduction of the hydrogel gel fraction. Although the gel fraction is considered an indicator of the hydrogel stability, the optimum concentration of the crosslinker was selected on the basis of water uptake capacity of the prepared hydrogel. Table I shows the water uptake of the prepared hydrogels by using maleic, succinic, and citric acids as crosslinkers. From the table, it is clear that the highest water uptake in general was achieved at low concentration of each crosslinker acid, where the highest water uptakes for maleic and succinic were 15.60 (g/g) and 11.41 (g/g) that obtained by using 2% acid concentration. On the other hand, the highest water uptake for prepared hydrogel was achieved via 1% citric acid crosslinking; 17.00 (g/g).

It was noted that the water uptake decreased after the optimum acid concentration used may be due to increasing the acidity of the medium which led to decreasing the chance for crosslinking (CMC was partially in an insoluble form) and as result that the water uptake of produced hydrogel tended to decrease. Otherwise the increasing of acidity may cause intensive crosslinking leading to increasing crosslinking density. Consequently, decreasing of the swelling ratio may be referred to the increasing of the rigidity of high dense crosslinking matrix.

Effect of PEG

Our study attempted to enhance the water uptake capacity of the prepared gel by the blending of PEG with CMCNa, and then the blended molecules were subjected to gelation reaction using the optimum concentrations of crosslinker acids (1% for citric and 2% for maleic and succinic). Table II shows the effect of various PEG concentrations used on the water uptake capacity and gel fraction of the prepared hydrogel. Generally, the gel fraction increased with increasing the amount of PEG added in cases of using maleic, succinic, or citric acid as crosslinker. Increasing the gel fraction (at optimum acidity) may be attributed to enhancing the chance for crosslinking networks formed as a result of different types of intermolecular crosslinked such as CMC–CMC, PEG–PEG, and CMC–PEG. The

highest gel fractions were 86.7%, 91.20%, and 92.22% for maleic, succinic, or citric acid respectively. On comparison, the blended hydrogel of PEG–CMCNa reaches to maxima of gel fraction values, which are higher than that for CMCNa hydrogels for the acids used. Table II shows the water uptake of the CMCNa hydrogels prepared using different amounts of PEG. For each crosslinker acid used, increasing the blended amount of PEG led to increasing of the water uptake capacity of the prepared hydrogel until certain amount of PEG after which the water uptake diminished. It was noted obviously that the optimum amount of blended PEG was 15% (based on CMCNa weight). The water uptake capacities of hydrogel crosslinked by maleic, succinic, and citric acids were 18.8%, 20.3%, and 23.77%, respectively. Beyond 15% PEG, the produced hydrogel was less able to absorb water, where the higher amount of PEG may cause more dense networks due to the crosslinking via chemical bond with the crosslinker acid (esterification) or formation of intermolecular hydrogen bonding with the main cellulosic chains.[22] PEG succeeded to improve the water uptake capacity of the unblended hydrogel.

Table II. Effect of PEG Addition During Crosslinking Reaction Using Different Crosslinker Acids

| Crosslinkers	PEG % (based on CMC)	Gel fraction % (based on total initial dry weight)	Swelling ratio
2% maleic acid	0	81.50	15.60
	5	80.30	15.80
	10	83.50	15.90
	15	85.50	18.80
	20	86.70	17.50
2% succinic acid	0	89.00	11.40
	5	88.50	12.60
	10	90.04	18.50
	15	88.80	20.30
	20	91.20	16.40
1% citric acid	0	78.00	17.00
	5	80.10	17.77
	10	87.90	18.77
	15	89.88	23.77
	20	92.22	19.10

Table III. Effect of Loading of AgNPs on Gel Fraction and Swelling of Hydrogels

Crosslinkers	Gel fraction % (based on total initial dry weight)		Swelling ratio	
	Without AgNPs	With AgNPs	Without AgNPs	With AgNPs
Maleic acid	85.50	82.80	18.80	18.00
Succinic acid	91.80	87.55	20.30	19.00
Citric acid	89.88	86.50	23.77	20.00

PEG and acid concentration are 15% and 2% (based on CMC weight), respectively.

Loading of AgNPs

The incorporation of AgNPs was *in situ* performed during the preparation of hydrogels under optimum conditions of crosslinker acid concentration and blended amount of PEG. The water uptake behavior of the prepared hydrogels with loaded AgNPs was shown in Table III. There is no big change in the gel fraction values of produced hydrogels without and with loaded AgNPs. In the absence of AgNPs, the water uptake capacities of the hydrogels loaded with AgNPs has diminished slightly as shown in Table III. This can be attributed to decrease of the available pores surface inside the gel networks which partially occupied with the AgNPs. The mechanism of crosslinking stage via polyfunctional carboxylic acid (e.g., citric acid) was illustrated in Scheme 1. In our study, we can adopt the mechanism of *in situ* incorporation of AgNPs inside the hydrogel networks. First, the silver cation is bounded physically and/or chemically with hydroxyl or carboxyl groups. A reduction process is carried out by addition of tri-sodium citrate resulting in conversion of silver cation into silver metal deposited on the CMC chains formed the hydrogel networks.[23]

$$4Ag^+ + C_6H_5O_7Na_3 + 2H_2O \rightarrow 4Ag^\cdot + C_6H_5O_7H_3$$
$$+ 3Na^+ + H^+ + O_2 \uparrow$$
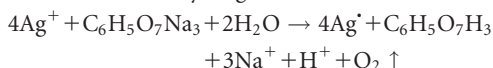
Release of Silver from Hydrogels

Freshly prepared AgNPs loaded hydrogel of different crosslinking acids: maleic, citric, and succinic samples were used for the investiga-
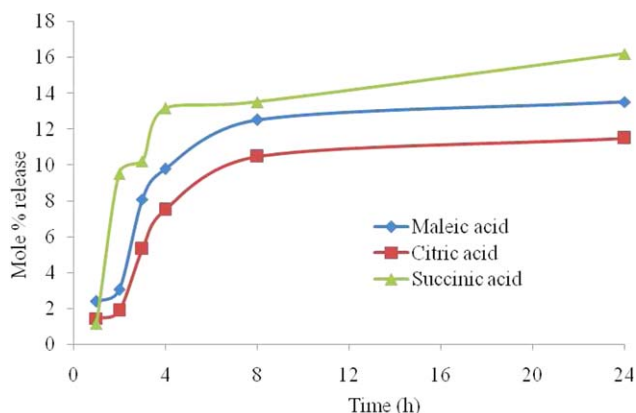

Figure 1. Silver release of hydrogels, prepared with different crosslinking acids, PEG and acid concentration are 15% and 2% (based on CMC weight), respectively. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

tion. As shown in Figure 1, in the case of succinic sample, the release of silver is rapid in the beginning and then slowly increase after 4 h.

For both maleic and citric acids as crosslinking agent, the rate of silver release passes through three phases. At early time (within 2 h) the rate of release is characterized by low rate. The time interval from 4 to 8 h is consider the bulk of silver release. For long time interval from 8 to 24 h the rate of silver release becomes very slow.

Characterization of the Prepared Hydrogel

The produced hydrogels have yellowish-white color that converts into deep brown by incorporation of AgNPs (Figure 2).

FTIR Spectroscopy

As shown in Figure 3, we can detect the characteristic absorbance for the carboxyl, methyl, and hydroxyl groups as absorbance due to stretching at 1618, 1426, and 3300 cm^{-1}, respectively. The absorbance band around 2800 cm^{-1} was attributed to C—H bond stretching. The bands around 1300 and 1400 cm^{-1} were assigned to —CH$_2$— and O—H due to bending vibrations, respectively. The FTIR spectra of CMC, crosslinked CMC blended/unblended with PEG and/or AgNPs is shown in Figure 3. It can be noted that the absorption band of CMC at 1022 cm^{-1} (stretching vibration of C—OH groups) became less intensive peak after crosslinking reaction. The absorption bands of CMC at 1115 and 1063 cm^{-1} (asymmetric vibration of C—O—C) are still present after the crosslinking reaction. The new band at 1700 cm^{-1} (asymmetric vibration of carbonyl group in —COO) is emerged in crosslinked samples. This indicates formation of carbonyl of ester, which proved the possibility of crosslinking via esterification. The absorption band of CMC at 1424 cm^{-1} (asymmetric vibration of —COO$^-$) was shifted to 1560 cm^{-1} in case of the crosslinked samples. The characteristic bands for CMC (3619 and 1630 cm^{-1} for stretching and bending vibrations of —OH group) were notably less intensive after the crosslinking reaction. Thus, the esterification (for crosslinking) was mainly carried out with the —OH group at C6 of CMC chain.[24,25]

Scanning Electron Microscopy

The scanning electron micrographs (SEM) of pure CMC, hydrogels with and without PEG, and incorporated AgNPs hydrogels were shown in Figure 4. CMC showed individual microchains and crosslinking of these microchains resulted in emerging branching networks with existing of micropores. It was obvious that the pattern of branching and size of pores of the produced networks were dependent on the crosslinker acid used. The
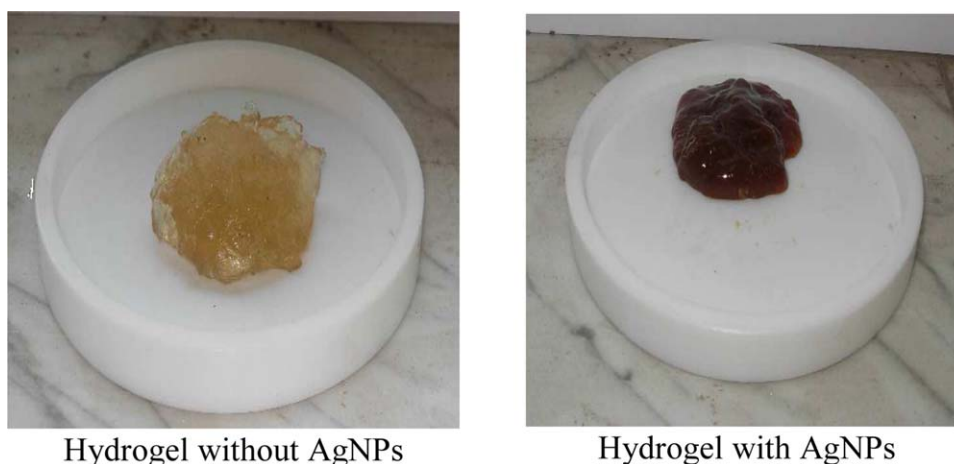
Figure 2. Photographs of hydrogel without and with AgNPs. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary. com.]

maleic acid crosslinker produced wide pores with less intense crosslinking. The crosslinking processes tended to form smaller pores with more intense networks with using succinic and citric acids. It was remarkably noted that citric acid crosslinker pro-duced more uniform pores and more homogenous branching of the produced networks. The presence of PEG blended with CMC enlarges the pore size especially in cases of succinic and citric acids. Thus, the enlarging of pores confirmed the role of PEG which may elongate the spaces between crosslinked CMC chains. The micrographs for incorporated silver hydrogels showed shrunken surface and uniform distribution of the AgNPs inside the crosslinking networks as shown in Figure 5. The incorporation of AgNPs was achieved along CMC chains throughout the hydrogel networks, especially using succinic and citric crosslinkers. As a result of the wide pores emerged with using maleic crosslinker, AgNPs dispersed through the exposed surface of pores included in the networks. According to EDX analysis, the highest load of AgNPs was obtained by using citric and succinic acids crosslinkers, whereas the lowest load of AgNPs was obtained in case of maleic acid crosslinker. One can conclude that the control of loading and the pattern of the AgNPs spreading were dependent on the architecture of the hydrogel networks.
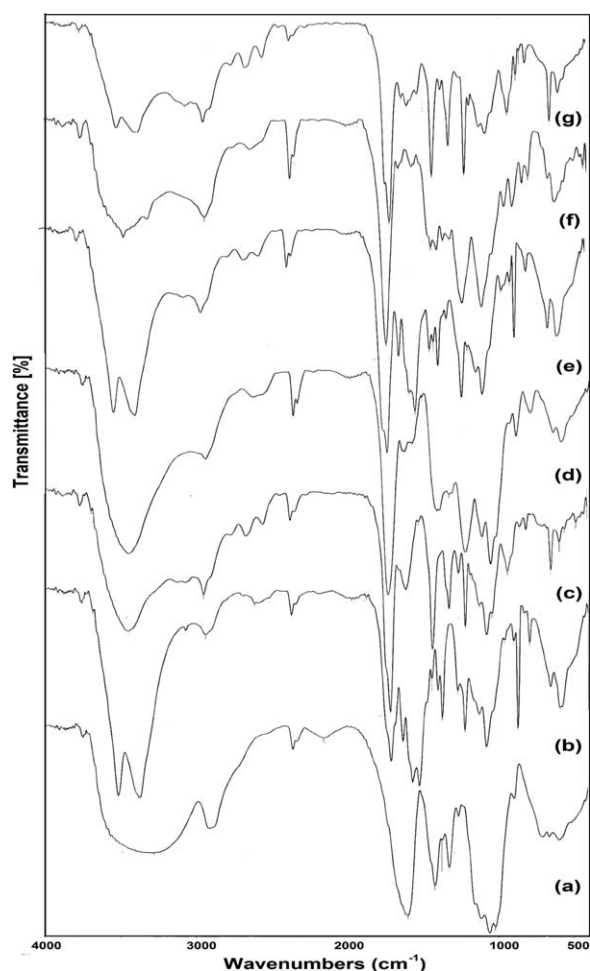
X-ray Diffraction Study

The structure of AgNPs has been investigated by XRD analysis. Typical XRD patterns of the loaded AgNPs in hydrogels using maleic, succinic, and citric acids as crosslinkers are shown in the Figure 6. Average particle size of AgNPs loaded hydrogel can be estimated by using Debye-Scherrrer formula:[26,27]

$$D = 0.9\lambda / W \cos\theta$$

where λ is the wave length of X-ray (0.1542 nm), W is the full width at half maximum, θ is the diffraction angle, and D is particle diameter (size). According to the values of W and θ which are 0.09θ (0.00157 radians) and 22.33 2θ, respectively, the average particle size of AgNPs for the three used acids cases is calculated to be around 95.5 nm.

Antimicrobial Assay

The antimicrobial effects of silver salts have been noticed since ancient times,[28] but with the advent of nanotechnology, the use of silver in nanoparticle form has opened new treatment avenues. The mechanism of the growth-inhibitory effects of AgNPs on microorganisms has not been well understood. One
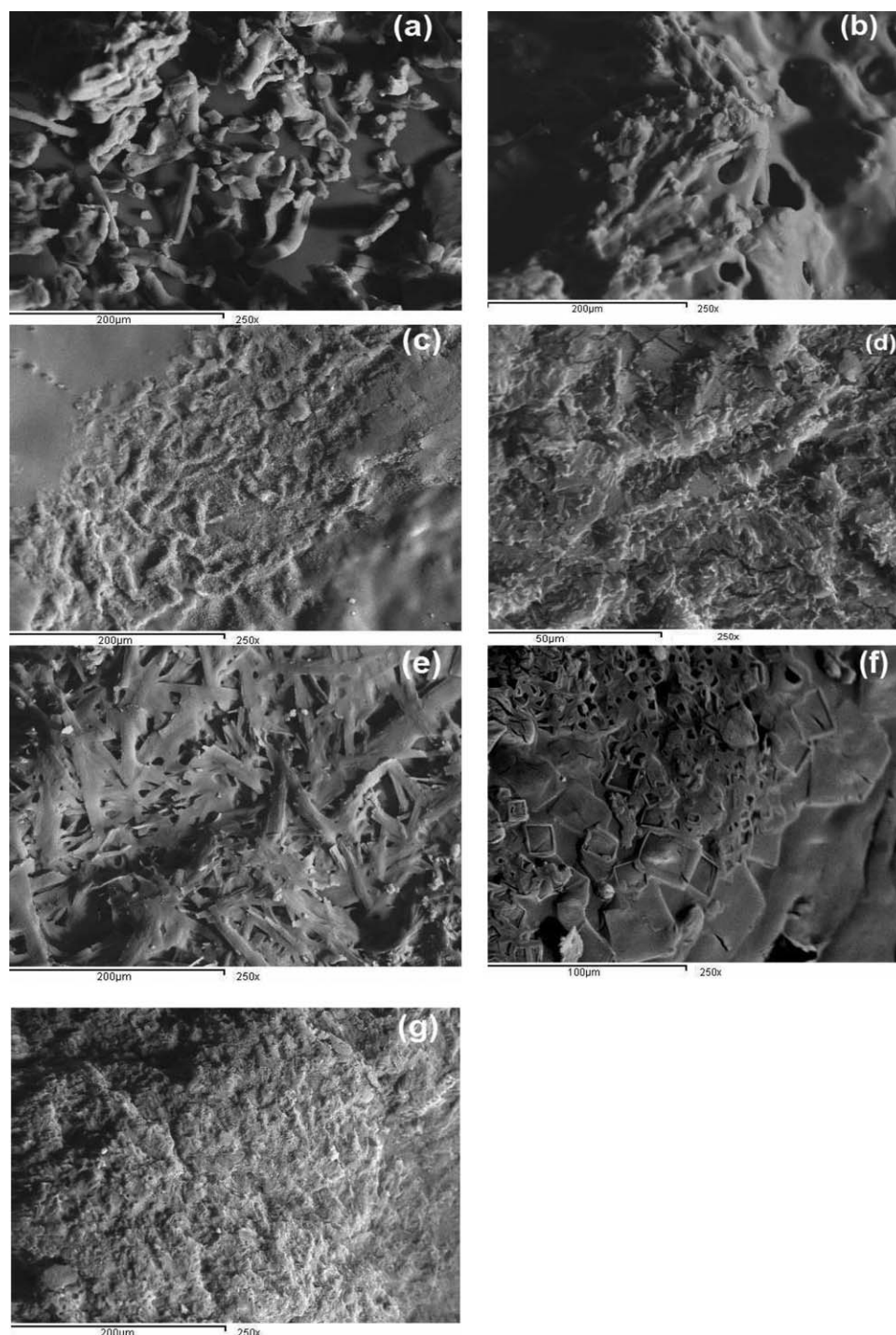

Figure 3. FTIR spectra of (a) CMC, crosslinked with (b) maleic acid, (c) succinic acid, and (d) citric acid. Blended hydrogels with 15% PEG and crosslinked with (e) malic acid, (f) succinic acid, and (g) citric acid.

Figure 4. SEM pictures of (a) CMC, hydrogels crosslinked with (b) maleic acid, (c) succinic acid, and (d) citric acid. Blended hydrogels with 15% PEG and crosslinked with (e) malic acid, (f) succinic acid, and (g) citric acid.

possibility is that the growth inhibition may be related to the formation of free radicals from the surface of Ag. Uncontrolled generation of free radicals can attack membrane lipids and then lead to a breakdown of membrane function.[29] The antibacterial activities of hydrogels loaded with AgNPs were investigated against Gram-positive *Staphylococcus aureus*, Gram-negative *Pseudomonas aeruginosa* and *Candida albicans* by agar disc diffusion method. The abilities of the hydrogels to inhibit the growth of the test bacteria are shown in Table IV and Figure 7. Zone of inhibition was measured from this microbiology assay. When the bacterial *S. aureus* was studied, the nanoparticles show a lower growth-inhibitory effect as compared with bacterial *P. aeruginosa* and *C. albicans*. These results suggest that the antimicrobial effects of AgNPs might be associated with the characteristics of certain bacterial species. By comparing the effect of hydrogels loaded with AgNPs and crosslinked with
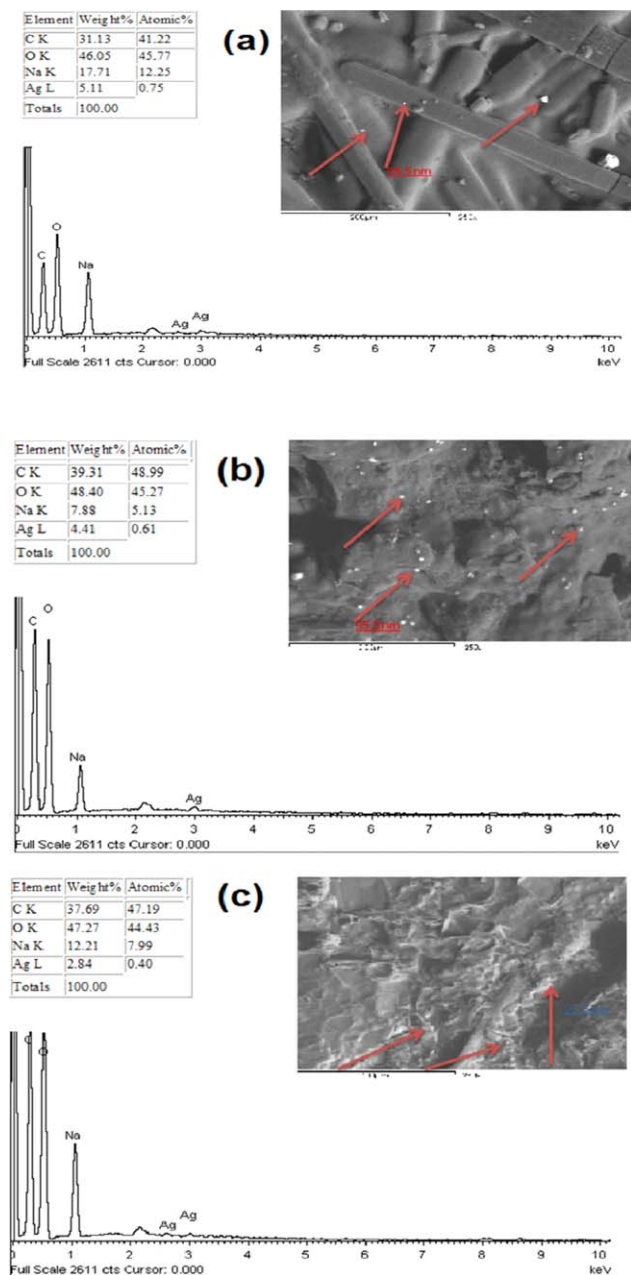
Figure 5. EDX analysis of blended hydrogels with 15% PEG loaded with AgNPs with (a) citric acid, (b) succinic acid, and (c) maleic acid as crosslinkers. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure 6. XRD of blended hydrogels with 15% PEG and loaded with AgNPs with (a) maleic acid, (b) citric acid, and (c) succinic acid as crosslinkers. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

different carboxylic acids on the same bacteria, we found that, crosslinked hydrogel with citric acid is more effective than succinic or maleic acid and this is in agreement with increasing the amount of AgNPs loaded with using citric than succinic than maleic acid (Figure 5). The composition of the prepared hydrogel manifested superior antibacterial activity than hydrogel based on CMC in previous studies.[30] The higher antimicrobial activity of the produced samples was demonstrated by high value of inhibition zone (32 mm) which exceeded the others in previous work which ranged 13–15 mm.[30]
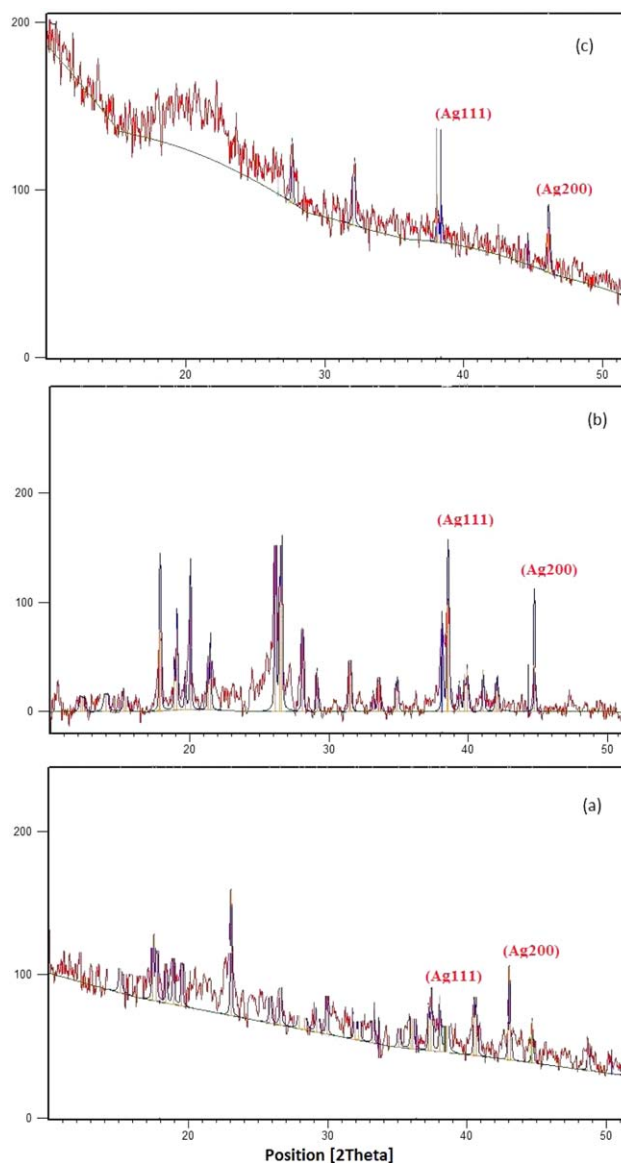
Table IV. Diameter of Zone of Inhibition of Hydrogels Loaded with AgNPs Against G+ve Bacterium *S. aureus*, G−ve Bacterium *P. aeruginosa*, and Yeast *C. albicans*

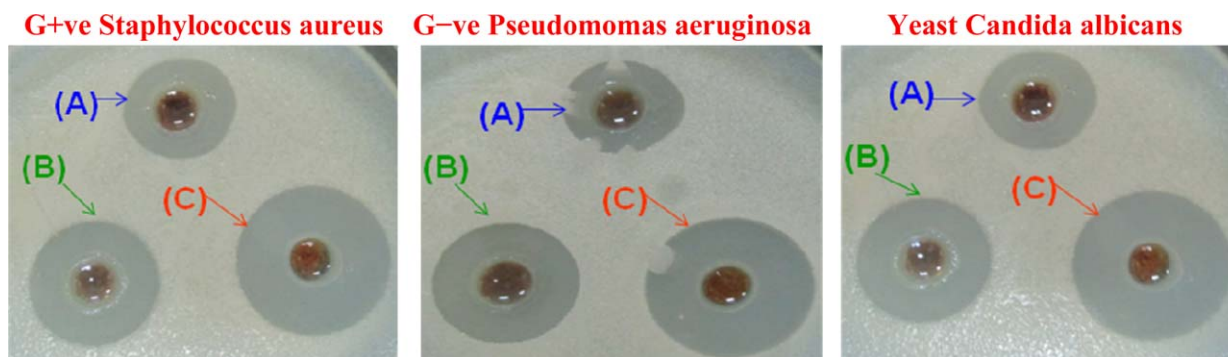| Crosslinkers	*S. aureus* (G+ve bacteria)	*P. aeruginosa* (G−ve bacteria)	*C. albicans* (yeast)
Maleic	19	20	20
Succinic	22	23	24
Citric	27	28	30

Figure 7. Antibacterial activities of hydrogels loaded with AgNPs against (A) G+ve bacterium *S. aureus*, (B) G−ve bacterium *P. aeruginosa*, and (C) yeast *C. albicans*. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

CONCLUSIONS

The study succeeded to develop cellulose-based hydrogels by using polycarboxylic acids as nontoxic crosslinkers. Crosslinking with citric acid produced hydrogels with highest water uptake capacity among other acids used as crosslinkers. The water uptake capacities of hydrogels were improved by blending CMC with PEG.

AgNPs were incorporated *in situe* during the formation of hydrogel networks produced by using maleic, succinic, and citric acids as crosslinkers.

The highest antimicrobial activity was detected for AgNPs loaded hydrogels in case of citric acid as crosslinker. It can be concluded that the control of loading and the pattern of spreading of AgNPs are dependent on the architecture of the hydrogel networks.

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