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Synthesis and characterization of novel carboxymethylcellulose hydrogels and carboxymethylcellulolse-hydrogel-ZnO-nanocomposites

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ABSTRACT

New approach for preparation of CMC hydrogels was undertaken through reacting CMC with either malic, succinic or citric acid. Characteristics of the hydrogels, as monitored by the swelling behavior, FTIR, SEM, EDX, TEM and XRD were dependent on nature and concentration of the polycarboxylic acid, time and temperature of curing. The best practice achieved from these studies was harnessed to synthesize and characterize CMC hydrogel-ZnO-nanocomposites with additional study pertaining to the antibacterial activity of the nanocomposites. CMC hydrogel with excellent swelling behavior could be prepared by adding succinic acid (0.5%) to CMC solution then drying the obtained paste at 80 °C for 5 min followed by curing at 120 °C for 3 min. Similarly, addition of $ZnNO_3$ solution to the CMC paste results in CMC hydrogel-ZnO-nanocomposites having biocidal activity to gram +ve and gram −ve bacteria.

1. Introduction

Hydrogels are defined as hydrophilic polymers which, due to their structural network, can absorb water without being soluble under physiological conditions of temperature, pH, and ionic strength. Crosslinks can be formed by covalent, electrostatic, or hydrophobic bonds, or dipole–dipole interactions (Coronado, Pekerar, Lorenzo, & Sabino, 2011) In particular, hydrogels are a class of polymers that can absorb water or biological fluids and swell several times their dry volume. Dependence of the swelling behavior of the hydrogel on changes in the external environment was reported (Chaterji, Kwon, & Park, 2007). Also reported was that the hydrogels can afford free-space between the networks in the swollen stage that serve for nucleation and growth of nanoparticles and act as nanoreactors or nanopots (Mohan et al., 2010).

By virtue of their biocompatibility and biodegradability, cellulose and sodium carboxymethylcellulose (CMC) are often used in the biomedical field (Chang, Duan, Cai, & Zhang, 2010). Na-CMC with its many hydroxy and carboxylic groups can absorb water and moisture, so the hydrogel made of it has many excellent properties, such as high water content, good biodegradation and wide source for its low cost (Nie, Liu, Zhan, & Guo, 2004).

At low pH values, CMC may be crosslinked using a lactonization reaction of free carboxylic and hydroxyl groups (Chen et al., 2011). Dicarboxylic acids (glutamic acid and succinic acid), were used simultaneously as solubilizing and crosslinking agents in the preparation of chitosan-based hydrogel. With two carboxyl groups, glutamic and succinic acids can simultaneously act as solubilizing and crosslinking agents (Chen et al., 2011).

Zinc oxide (ZnO), an n-type semiconductor, is a very interesting multifunctional material for its promising applications (Yu, Yang, Liu, & Ma, 2009), such as semi conductor diodes, ultraviolet-protection films, catalysts, sensors, ceramics, solar energy conversion, etc. (Comini, Faglia, Sberveglieri, Pan, & Wang, 2002; Lee, Bhattacharyya, Easteal, & Metson, 2008; Yang, Chen, Zhao, & Bao, 2004). The real move toward the use of ZnO as an antimicrobial agent was in 1995; recently, more and more researchers have embarked on the fundamental studies on the antibacterial activities of ZnO. These studies were concerned with the antibacterial activity of ZnO nanoparticles against *Escherichia coli, Salmonella typhimurium, Bacillus subtilis, Staphylococcus aureus*, etc. The main conclusions of these studies are that, ZnO nanoparticles are effective for inhibiting both gram positive and gram-negative bacteria. They even have antibacterial activity against spores that are high-temperature resistant and high-pressure resistant (Sawai, Kawada, & Kanou, 1996; Zhang, Ding, Povey, & York, 2008).

Many methods have been developed for preparation of ZnO nanoparticles with different morphologies and size distributions. Among these methods are the sol–gel technique, laser heating, mechanochemical process, solvent-thermal process, hydrothermal synthesis, microwave heating synthesis, and homogeneous precipitation (Chang et al., 2008; Lupan et al., 2007; Mozaffari, Ebrahimi,

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Daneshfozon, & Amighian, 2008; Pedram, Shahram, & Ali, 2010; Zheng & An, 2008). In most of these approaches, nanoparticles tend to agglomerate, due to large specific surface area as well as high surface energy. To improve the size distribution and morphology of nanoparticles without any agglomeration during the synthesis, coating the magnetite nanoparticles with a capping agent is necessary. Thermal decomposition of zinc salt is one of the facile methods to fabricate ZnO nanoparticles, in which zinc salt is coated by appropriate coating agent followed by thermal decomposition of coated zinc salt resulting in ZnO nanoparticles (Raveendran, Fu, & Wallen, 2003). Much effort is now being focused on polysaccharides used as the protecting agents of nanoparticles (Yu et al., 2009). Stabilization of metal and metal oxide nanoparticles by CMC and its hydrogel has been reported (Hebeish, El-Rafie, Abdel-Mohdy, Abdel-Halim, & Emam, 2010; Hebeish, Hashem, Abd El-Hady, & Sharaf, 2013; Pourjavadi, Barzegar, & Mahavinia, 2006).

With the above in mind, intensive investigation of the synthesis and characterization of CMC hydrogels is carried out in current work. The synthesis of the hydrogel is based on conversion of the water soluble CMC into CMC hydrogel via introduction of crosslinks in the molecular structure of CMC using bi- and multifunctional carboxylic acids. In concomitant with are the synthesis and characterization of antibacterial products. The latter refer to CMC hydrogel composite loaded with ZnO nanoparticles.

2. Materials and methods

2.1. Materials

Carboxymethyl cellulose (CMC) having high molecular weight (Mw = 10,000 Da) was used. Malic, succinic and citric acids as well as other chemical were of laboratory grade chemicals.

2.2. Methods

2.2.1. Preparation of CMC hydrogels

Definite amount of CMC was dissolved in an aqueous solution with continuous mechanical stirring until a homogeneous viscous mixture was obtained. Then different concentrations from three different polycarboxylic acids, namely, malic, succinic and citric acids at different concentrations (0.5–1%) were added separately dropwise to CMC solution with continuous stirring. The formed paste, was transferred to Petri dish, dried in an oven at 80 °C for 5 min then cured for (2–5 min) at different temperatures (120–140 °C).

2.2.2. One step process for preparation of CMC hydrogels containing ZnO nanoparticles (in situ process)

Optimum conditions for preparation of CMC/succinic acid hydrogel (obtained from Section 2.2.1) were employed to prepare hydrogel paste. To this paste, zinc nitrate solution was added dropwise till milky white color appeared. The paste was then dried in an oven at 80 °C for 15 min, following by curing at 120 °C for 3 min.

2.3. Characterization and analysis

2.3.1. Swelling behavior

The swelling behavior of the prepared hydrogel whether containing ZnO nanoparticles or not was calculated using the ratio (Q) of the gels as per the Equation [23]

$$Q = \frac{W_e}{W_d}$$

where W_e is the weight of the swollen hydrogel and W_d is the dry weight of the pure hydrogel.

2.3.2. FTIR spectroscopy

FTIR analysis was recorded on a Perkin Elmer FTIR Spectrophotometer, using the potassium bromide disk technique, in the range of 4000–400 cm^{-1}. The disk was prepared from grinded samples (2 mg) and KBr (45 mg) using 400 kg/cm^2 pressure for 10 min.

2.3.3. Scanning electron microscopy (SEM and EDX)

Surface morphology of the prepared hydrogel was examined on a JEAOL JXA-840 scanning electron microscope (SEM). The prepared hydrogel samples were coated with a thin layer of palladium gold alloy after mounting on a double sided carbon tape. An elemental analysis of the particles was implemented by a SEM equipped with an energy dispersive X-ray spectrum (EDX), which can provide a rapid qualitative and quantitative analysis of the elemental composition

2.3.4. Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) was used to determine the size of ZnO nanoparticles inside the hydrogel. The swollen hydrogels were grounded with the help of a soft ball and the resulted hydrogel containing ZnO nanoparticles was dispersed in 1 ml of distilled water for 3 day to extract the ZnO nanoparticles.

2.3.5. X-ray diffraction (XRD)

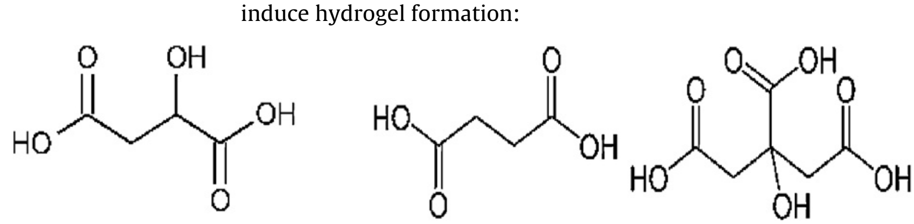
The X-ray diffraction method was used to identify ZnO nanoparticles loaded in the polymer matrix. XRD patterns recorded on a Philips PW 3050/10 model. The samples were recorded on a Philips X-Pert MMP diffractometer. The diffractometer was controlled and operated by a PC computer with the programs P Rofit and used a MoK (source with wavelength 0.70930 A°, operating with Mo-tube radiation at 50 kV and 40 mA.

2.3.6. Antibacterial activity

Antimicrobial activity of the prepared hydrogel was evaluated using agar diffusion test according to AATCC Standard Test Method 147–1988.

3. Results and discussions

The following three different carboxylic acids were used to induce hydrogel formation:



Malic acid Succinic acid Citric acid

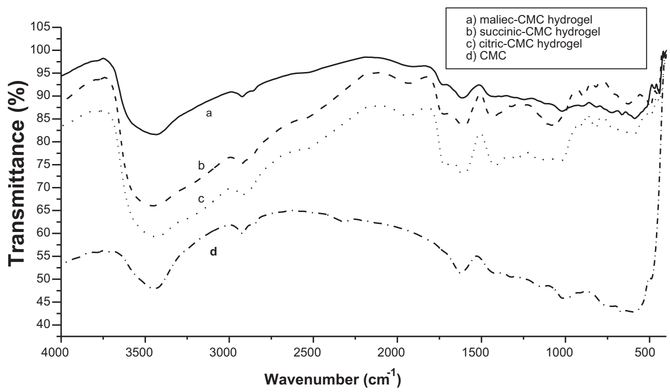


Fig. 1. Effect of concentration of polycarboxylic acid on swelling behavior of CMC hydrogel.

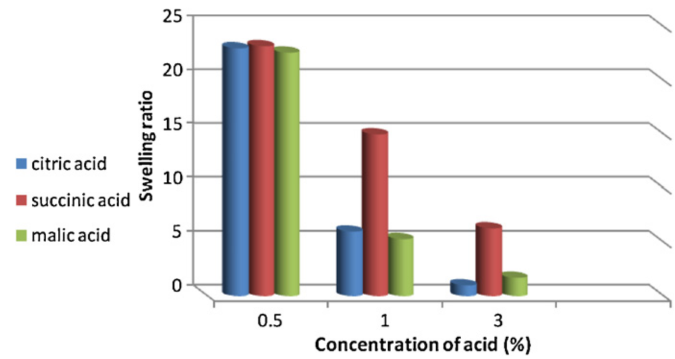
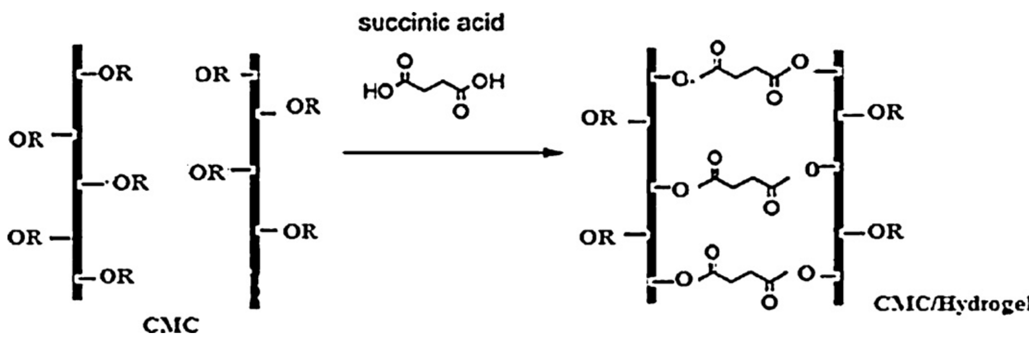


Fig. 2. Effect of curing temperature on the swelling behavior of CMC hydrogel.

3.1. Proposed mechanisms



It is assumed that crosslinking occurs via: esterification reaction between the —OH group of CMC and —COOH groups of the polycarboxylic acids and —OH groups and —COOH groups of adjacent chains of CMC, as well as dehydration between the —COOH groups and adjacent CMC molecules to give rise to strong hydrogen bonding during the thermal treatment (Fei & Gu, 2002).

3.2. Proposed mechanism of ZnO synthesis

Upon addition of zinc nitrate solution to the CMC hydrogel paste zinc nitrate reacted with water and forms zinc hydroxide (Zn $(OH)_2$), large particles may be formed via precipitation followed by a step-like aggregation process. Since polysaccharides i.e. CMC could form complexes with divalent metal ions due to their high number of coordinating functional groups (hydroxyl and glucoside groups) (Al-Hartomy & Shahl, 2011). It is likely that the majority of the zinc ions were closely associated with the CMC molecules. As the concentrations of Zn^{2+} and OH^- ions exceed the critical values, the precipitations of nuclei starts. The $Zn(OH)_2$ can be transformed into the ZnO crystals via the simple chemical reaction as suggested mentioned below (Pushpamalar, Langford, Ahmad, & Lim, 2006).

$$Zn(OH)_2 \xrightarrow{heat} ZnO + H_2O$$

Therefore nucleation and initial crystal growth of ZnO may preferentially occur on CMC. In addition, polysaccharides presented interesting dynamic supramolecular associations facilitated by inter- and intra-molecular hydrogen bonding, which could act as templates for nanoparticle growth (Pushpamalar et al., 2006).

3.3. Effect of acid concentration of polycarboxylic acid

Fig. 1 depicts the effect of polycarboxylic acid concentration on swelling ratio of the CMC hydrogel when three polycarboxylic acids, namely, citric, succinic and malic acids were independently used. It was observed that increasing the concentration of the acid as a crosslinking agent up to 0.5% enhances the swelling properties of CMC hydrogel. Crosslinker concentration higher than 0.5% decreases significantly the swelling of the hydrogel. This could be ascribed to decrease flexibility of the chains and the ability of the chains to undergo segmental motion. Diffusion of the hydrolyzing agents (water) into the polymer network also decreases thereby giving rise to more rigid structure of the polymer network

3.4. Curing temperature

The influence of curing temperature on the swelling ratio of CMC hydrogel parameters was studied by raising the reaction temperature from 120 to 140 °C. As shown in Fig. 2 elevating the thermal treatment leads to strong hydrogen bonding interactions between the COO^- groups of CMC and the hydroxyl groups of the crosslinking acid, which greatly reduce the relaxation and expansion of the molecular chains. Accordingly, the water uptakes of the hydrogels decrease by raising the temperature from 120 °C to 140 °C in case of succinic acid while increasing temperature above 130 for the other two acids has practically no effect i.e. maximum swelling ratio of the prepared hydrogel is achieved at a curing temperature of 120 °C in case of succinic acid while in case of malic and citric acid 130 °C is the most appropriate curing temperature for reaching maximum swelling ratio.

3.5. Curing time

Fig. 3 shows the dependence of the swelling ratio of the CMC/acid crosslinked hydrogel when the latter was cured for different times. Obviously, prolongation of the curing time from 1 to 3 min leads to an increase in the swelling ratio. It is also

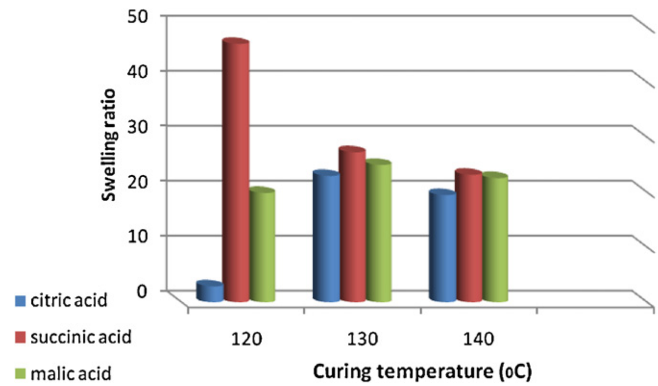


Fig. 3. Effect of cutting time on the swelling behavior of CMC hydrogel.

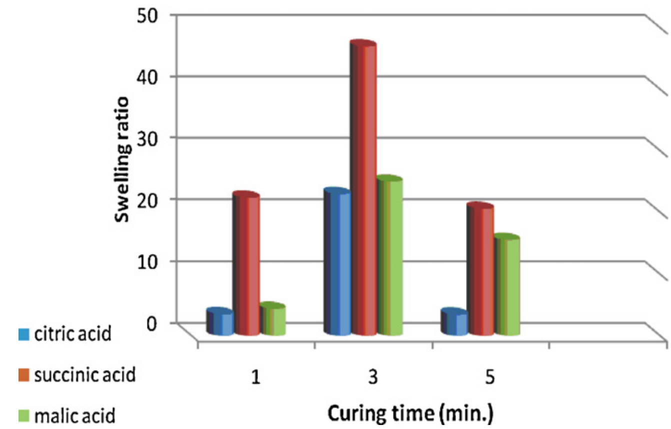


Fig. 4. IR spectrum of CMC and CMC/polycarboxylic acid hydrogel, (a) malic-CMC hydrogel, (b) succinic-CMC hydrogel, (c) citric-CMC hydrogel and (d) CMC.

obvious that, irrespective of the acid used, the swelling ratio decreases after 3 min curing time. As already stated, the crosslinking occurs via: esterification reaction between the —OH and —COOH groups of the CMC polymeric adjacent chains, dehydration between the —COOH groups forming strong hydrogen bonding during the thermal treatment (Taubert & Wegner, 2002) and between the —OH of the polycarboxylic acid and the —COOH groups of CMC. The longer the curing time the stronger will be the crosslinking.

3.6. FTIR

The IR spectrum of the pure CMC, Fig. 4d, shows a broad absorption band at 3432 cm^{-1}, due to the stretching frequency of the –OH group and a band at 2920 cm^{-1} attributable to C—H stretching vibration. The presence of strong absorption band at 1605 cm^{-1} confirms the presence of COO^- group. The bands around 1420 and 1320 cm^{-1} are assigned to —CH_2 scissoring and —OH bending

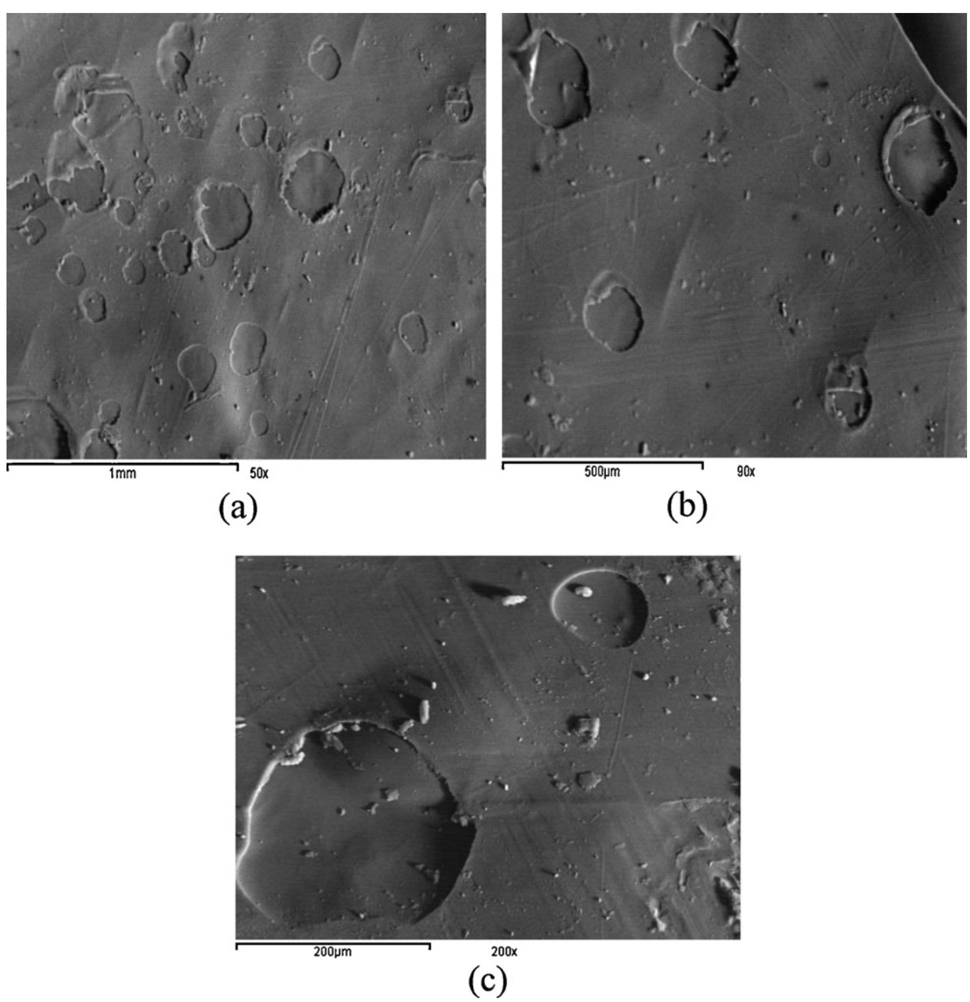
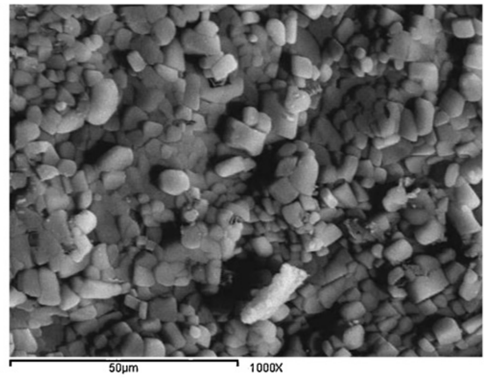


Fig. 5. SEM of CMC/polycarboxylic acid hydrogel. (a) CMC/succinic acid hydrogel, (b) CMC/malic acid hydrogel and (c) CMC/citric acid hydrogel.

Element	Weight%	Atomic %
C K	22.99	45.57
O K	23.48	34.94
Zn K	53.52	19.49
Totals	100.00	



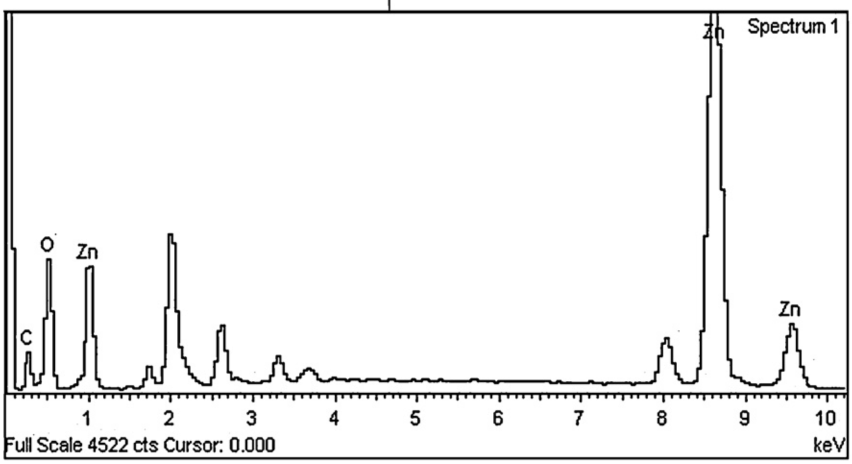


Fig. 6. SEM & EDX analysis of CMC/succincic acid hydrogel loaded ZnO nanoparticles.

vibration, respectively. The band at 1030 cm^{-1} is due to carboxymethyl ether group > CH—O—CH_2 stretching (Pushpamalar et al., 2006). The IR spectrum of CMC/polycarboxylic hydrogel (Fig. 1a–c) clearly shows the presence of the characteristic bands of its parent components. The characteristic carboxymethyl ether stretching band > CH—O—CH_2 for CMC appears at 1030 cm^{-1}. Moreover, the peak appeared at 1709 cm^{-1} and 1612 cm^{-1} were assigned to the carboxyl group vibrations.

3.7. SEM and EDX analysis

Scanning electron microscopy is the most widely employed technique used for investigation of the shape, size, morphology, and porosity of the hydrogel matrices. Fig. 5a–c shows SEM microphotographs of the dried CMC/polycarboxylic acid hydrogel. The connectivity of the pores played a crucial role in fast swelling of the hydrogels. The interconnected pore structure facilitates the diffusion of the water molecules in and out through it. Such porosity was increased in case of using succsinc acid in the prepared hydrogel. The order of porous structure follows the order: succsinic acid > malic acid > citric acid.

In other words, the porous structure is directly related to the structure of polycarboxylic acid i.e. the presence of more ester bonds in the prepared hydrogels.

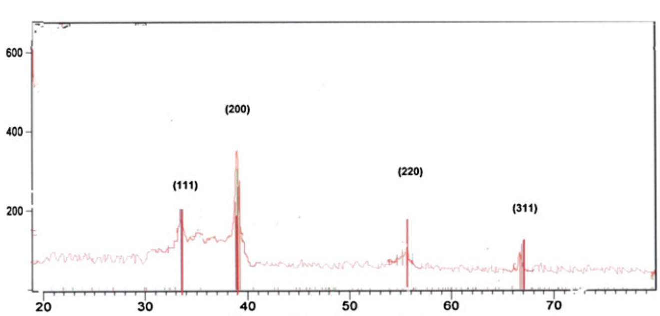


Fig. 7. X-ray diffraction patterns of CMC/succinic acid/nano ZnO hydrogel.

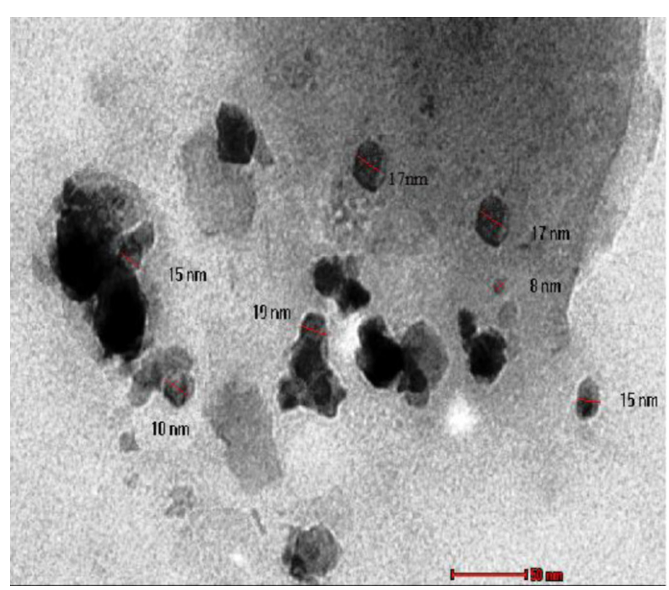
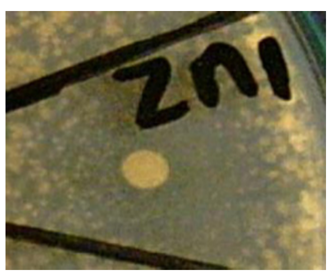
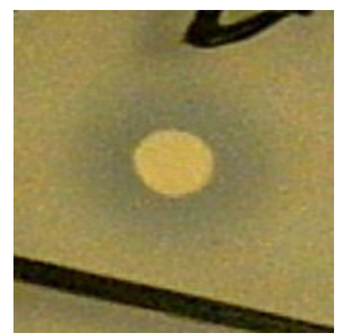


Fig. 8. TEM image of ZnO nano particles included in CMC hydrogel.

Table 1
Antibacterial effect of CMC/nano ZnO hydrogel.

Sample	Inhibition zone (mm)/1 cm sample			
	(G+ve)		(G−ve)	
	St. aureus	*B. subtilis*	*P. aeruginosa*	*E. Coli*
Dryed hydrogel loaded nano ZnO	13	16	20	16
				
Nano ZnO released form swollen hydrogel	13	14	18	14
				

The surface morphology SEM and EDX analysis of CMC/succincic acid hydrogel loaded nano ZnO shown in Fig. 6 From the prepared ZnO it is visibly judged that the particle is in nanostructure, The nanorods are hexagonal in shape as also proved by TEM and it has on track to produce but still maintained at a regular shape of hexagonal structure. The ZnO nanoparticles appeared in higher content in the prepared hydrogel matrix as seen in elemental analysis

3.8. X-ray powder diffraction patterns

The XRD pattern with Cu Ka irradiation in Fig. 7 shows that the CMC/succinic acid/nano ZnO hydrogel exhibit some broad reflections corresponding (1 1 1), (2 0 0), (2 2 0), and (3 1 1). All diffraction peaks in Fig. 7 are sharper and stronger at 33.07°, 39.54°, 55.59° and 67.01° were assigned to the (1 1 1), (2 0 0), (2 2 0), and (3 1 1) planes of cubic zinc oxide (see card No. 65-2880, JCPDS-ICDD, June 2002). The broad reflections mean that, the ZnO has nano structured size which can match with TEM images.

3.9. Transition electron microscope (TEM) images

Fig. 8 shows the TEM images of ZnO nanoparticles. One can find that the morphology of ZnO crystallites is rather hexagonal morphology, narrow distribution of sizes with a 10–20 nm diameter.

3.10. Antibacterial activity

Table 1 clearly shows the antibacterial activity of hydrogel nanocomposites in its dry form and solution form. The bioassay was carried out using two gram −ve bacteria (*E. coli* and *P. aeruginosa*) and against two gram +ve bacteria (*S. aureus* and *B. subtilis*) as per the disc plate technique. It is seen that, the prepared hydrogel containing ZnO nanoparticles has high antibacterial properties as evidenced by higher inhibition zone. This holds true regardless of the kind of bacterial used. This could be associated to the photocatalysis mechanism of nano ZnO.

Photocatalysis mechanism was as follows: when ZnO nanoparticles (E_g = 3.37 eV) were under light irradiation, the electron transition from the valence band to the conduction band resulted in the electron–hole pair in which electron (e^-) was reductive and hole (h^+) was oxidative. The hole (h+) reacted with OH^- on the surface of ZnO nanoparticles, generating hydroxyl radicals (OH^-), superoxide anion ($O_2{}^-$), and perhydroxyl radicals ($HO_2{}^-$). These highly active free radicals and actual lethal agent hydrogen peroxide (H_2O_2), produced from OH^- and $O_2{}^-$, could damage the cells of bacteria, leading to decomposition, complete destruction of its internal structure, eventually, to achieve germicidal and antibacterial effects (Abd El-Hady, 2012).

4. Conclusion

Best practice for production of green and novel CMC hydrogels with high swellability was established through a thorough investigation of crosslinking of CMC with polycarboxylic acids including malic, citric and succinic acids. Of the latter, succinic acid constitutes the most proper croolinking agent. Other characteristics of the hydrogels were determined using the most up-to date world facilities. In situ formation of ZnO nanoparticles and their inclusion in the CMC hydrogels were concomitantly prepared. The CMC-ZnO-nanocomposites so obtained displayed excellent antibacterial activity to gram +ve and gram −ve bacteria. It is envisioned that the green nature and purity of current hydrogels with or without ZnO nanoparticles advocate them for medical application.

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