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Antimicrobial Activity of Carboxymethyl Cellulose Based Nanogels

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ABSTRACT

Carboxymethyl cellulose based hydrogel nanoparticles are synthesized by graft copolymerization of various ratios of acrylic acid and acrylonitrile onto carboxymethyl cellulose (CMC). The structures were confirmed and characterized by Fourier transform infrared spectroscopy, X-ray diffraction, and Dynamic light scattering. The swelling efficiency of the designed nanogels in various solvents is studied. Antimicrobial activities of the biocides nanogels against two pathogenic fungi and three Gram positive bacterial are examined. The prepared hydrogels showed much higher antimicrobial activities than that of CMC. The antimicrobial efficiency of tested nanogels is increased by increasing acid moiety and by increasing the degree of cross-linking in the hydrogels.

Keywords: Nanogels; Antimicrobial activity, Swelling behaviors, Graft copolymerization, Carboxymethyl cellulose.

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INRODUCTION

Graft copolymerization is used to the modify surfaces of polymers [1, 2], and it is a vital tool to change the physical or chemical properties of polymers. Grafting of vinyl monomers onto cellulose is an important tool for the modification of cellulose. The applications of cellulose graft copolymers change with the structure of polymer. Depending on the chemical structure of the monomer grafted onto cellulose, graft copolymers gain new properties such as water absorption [3], improved elasticity, hydrophilic or hydrophobic character, ion-exchange [4-10] and dye adsorption capabilities [6, 11], heat resistance [12-15], thermosensitivity [16,17], pH sensitivity [18], antibacterial effect, resistance to microbiological attack, etc. [20,21]. In order to obtain a cellulose graft copolymer with high water or moisture absorbency, hydrophilic monomers such as acrylic acid (AA), acrylamide (AAm), 2-acrylamidomethylpropane sulfonic acid (AASO3H)), N-vinyl-2-pyrrolidone or polyacrylamide grafts which are hydrophilic in nature, have high water absorption capacity [22,23].

Biomedical applications of nanomaterials have become one of the most significant trends in the nanotechnology area. The nanometer size of polymeric nanoparticles, which is much smaller than that of blood cells, allows them to readily move in biological environments. The advantage of nanogels is based mainly on their small particle size which provides large surface area with good surface properties. The small size of nanogels helps in increasing drug/protein stability and confers useful controlled release properties. Nanoparticles could cross the biological barriers to reach the target sites inside the cells due to their small size, thus achieving an improved effect as reported in the literature [24, 25]. In our previous works Farag and Mohamed [26] designed Antimicrobial Nanogels based on carboxymethyl chitosan (CMCh) and poly-(vinyl alcohol) PVA using free surfactant method. Moreover Labena et al [27] synthesized dendritic hyperbranched PAMAM and evaluated as a broad spectrum antimicrobial agent and anti-biofilm.

In the present study antimicrobial nanogels are synthesized by graft modification of carboxymethyl cellulose surfaces using various ratios of acrylic acid and acrylonitrile comonomers. Their properties are characterized via various techniques. The swelling ability at different solvent is measured. The efficiency of these nanogels to inhibit the growth of two crop-threatening pathogenic fungi and three types of Gram positive bacteria are studied.

MATERIAL AND METHOD

Materials

Acrylic acid (AAc), acrylonitrile (AN), methylene bi sacrylamide (MBA), azobis iso butyro nitril (AIBN), and aerosol (AOT) were used as pure compounds. Carboxymethyl cellulose (CMC) of commercial grade was supplied by El Nasr Pharmaceutical Chemicals Co., Egypt. Analytical grade toluene, ethanol, acetone, DEMSO, DMF, NMP and diethyl acetate solvents were used. All the chemicals were supplied from the Fluka chemical company. Two crop-threatening pathogenic fungi namely: Aspergillus fumigatus (A. fumigatus, RCMBA 06002), and Aspergillus niger (A. niger, RCMBA 06106), and three bacterial species namely: Bacillis subtilis (B. subtilis, RCMBA 6005), Staphylococcus aureus (S. aureus, RCMBA 2004), Streptococcus pneumonia (S. pneumonia, RCMB 000101) as Gram positive bacteria were provided by the Reginol center for Mycology and Biotechnology Culture Collection.

Synthesis of CMC-g-poly(AN-co-AAC) nanohydrogels

In a 50 ml three neck flask purged with nitrogen for 20 min (to remove oxygen before the polymerization), 0.5 g of (AOT) was added to 5 ml of hexane (oil phase). The solution was stirred at 300 rpm and purged with nitrogen for 10 min. A solution of CMC (1%) was prepared in a separate flask by slow addition of 10 g of CMC into 1,000 ml distilled water with continuous stirring to avoid flocculation. From this solution, a specific amount (namely 10, 30, 50, 70, 90 and 100 wt/wt of the monomer, respectively) was withdrawn and added to the reaction mixture. The solution was heated to 70°C with continuous stirring. Then the desired amount of initiator (AIBN) was added and kept at 70°C for 10 min to initiate radical formation. After cooling the reaction mixture to 40°C, the equivalent weight ratios of AN and AA (as described in Table 1) were added drop wise during 1 h. Various amounts of MBA (as mentioned in Table 1) were added to the solution. The temperature rose to 70°C and maintained for 7 h to complete the reaction [28-30]. Different grafted nanohydrogel samples were obtained with varying the CMC contents and crosslinking agent concentrations as

shown in Table 1. The grafted nanohydrogels were purified using selective precipitation with excess acetone and diethyl ether to disperse the micelles. After the purification process, the nanohydrogels were dispersed in ethyl acetate within 2 h with continuous stirring [28].

Infrared Spectroscopy

FTIR spectra were recorded in KBr discs on a Shimadzu FTIR model 8000 Testcan IR-spectrometer under dry air at room temperature within the wave number range of 4,000–500 cm−1.

X-ray Diffraction

X-ray Diffraction was done using a Brüker D8 Advance instrument, at 40 KV, 40 mA using target Cu Kα with secondary monochromator (Karlsruhe, Germany).

Dynamic Light Scatering

The particle size of the synthesized nanogels was measured using **Dynamic Light Scatering** (DLS**)** instrument (Zeta Sizer Nano, ZS-Malvern Company, UK at 25 ˚C).

Swelling Behavior of Nanogels

The equilibrium swelling was performed to characterize the swelling behavior of prepared nanogels. To determine the equilibrium swelling behavior, freeze-dried gel sample (100 mg) was dispersed into 10 mL of each solvent of distilled water, DMSO, NMP and DMF respectively. The degree of swelling was defined as the volume ratio of CMCh/PVA after and before swelling. The measurements were made in triplicates using an analytical balance, and the error % was estimated to be 10%. The equilibrium degree of swelling of the gel was calculated as:

$$\text{Swelling} = We/Wd$$

where We is the weight of gel at the equilibrium and Wd is the weight of initial dry gel were carried out till 120 min, and then all pieces were dried and re weighed again.

Antimicrobial Activity of the Prepared Nanogels

A Zone of Inhibition Test, also called a Kirby-Bauer Test, is a qualitative method used clinically to measure antibiotic resistance and industrially to test the ability of materials inhibits microbial growth. Antimicrobial activity of the nanogel samples was determined using a modified Kibry-Baur disc diffusion method [31, 32]. Briefly, the test bacteria/fungi (100 µL) were grown in fresh media (10 mL) until they reached a count of approximately 108 cells/mL for bacteria or 105 cells/mL for fungi [33].

Microbial suspension (100 µL) was spread onto agar plates corresponding to the broth in which they were maintained. Isolated colonies of each organism that might be playing a pathogenic role should be selected from primary agar plates and tested for susceptibility by disc diffusion method [34]. Of the many media available, NCCLS recommends Mueller-Hinton agar due to its good batch-to-batch reproducibility results. Disc diffusion method for filamentous fungi was tested by using standard method (M38-A) developed by researchers [35] for evaluating the susceptibilities of filamentous fungi to antifungal agents. Disc diffusion method for yeasts was developed by using approved standard method (M44-P) by NCCLS [36].

The activity of tested samples was studied against the B. subtilis (RCMBA 6005), S. aureus (RCMBA 2004), and S. pneumoniae (RCMB 000101) as Gram positive bacteria. Centrifuged pellets of bacteria from a 24 h old culture containing approximately 104–106 CFU (colony forming unit) per milliliter were spread on the surface of nutrient agar (typetone 1%, yeast extract 0.5%, NaCl 0.5%, agar 1%, 1000 mL of distilled water, pH 7.0) which was autoclaved under 121 °C for at least 20 min. Wells were created in medium with the help of sterile metallic bores and then cooled down to 45 °C. The activity was determined by measuring the diameter of the inhibition zone (in mm). One hundred microliters of the tested samples (10 mg/mL) were loaded into the wells of the plates. All compounds were prepared in DMSO, and DMSO was loaded as control. The plates

were kept for incubation at 37 °C for 24 h and then the plates were examined for the formation of zone of inhibition. Each inhibition zone was measured three times by caliper to get an average value. The test was performed three times for each bacterium culture: penicillin and streptomycin were used as antibacterial standard drugs [37].

Antifungal activities were investigated by screening the tested samples separately in vitro against A. fumigatus (RCMBA 06002), and A. niger (RCMBA 06106) fungi on sabourad dextrose agar plates. The culture of fungi was purified by the single spore isolation technique. The antifungal activity was by agar well diffusion method [38] as follows: Sabourad dextrose agar plates: A homogeneous mixture of glucose-pepton-agar (40:10:15) was sterilized by autoclaving at 121 °C for 20 min. The sterilized solution (25 mL) was poured into each sterilized Petri dish in laminar flow and left for 20 min to form the solidified sabourad dextrose agar plate. These plates were inverted and kept at 30 °C in an incubator to remove the moisture and to check for any contamination. Antifungal assay: A fungal strain was grown in 5 mL sabourad dextrose broth (glucose:peptone; 40:10) for 3–4 days to achieve 105 CFU/mL cells. The fungal culture (0.1 mL) was spread out uniformly on the sabourad dextrose agar plates by sterilized triangular folded glass rod. Plates were left for 5–10 min so that the culture was properly adsorbed on the surface of sabourad dextrose agar plates.

Small wells (4 mm × 2 mm) were cut into the plates with the help of a well cutter and the bottom of the wells was sealed with 0.8% soft agar to prevent the flow of test sample at the bottom of the well. One hundred microliters of the tested samples (10 mg/mL) were loaded into the wells of the plates. All compounds were prepared in DMSO, and DMSO was loaded as control. The plates were examined for the formation of zone of inhibition. Each inhibition zone was performed three times for each fungus.

To determine the minimum inhibition concentration (MIC) of tested samples, the agar plate method was used; two-fold serial dilutions of each sample were added to nutrient broth for bacteria (beef extract 5 g, peptone 10 g added to 1000 mL distilled water, pH 7.0) and to sabourad dextrose broth for fungi, DMSO was used as the control. Then they were heated in an autoclave at 121 °C for 25 min. The culture of each organism was diluted by sterile distilled water to 105–106 CFU/mL, a loop of each suspension was inoculation, and the plates were incubated at 37 °C for 24 h for bacteria, and at 30 °C for 3–4 days for fungi. The colonies were counted and the MIC values were obtained. The MIC was considered to be the lowest concentration that completely inhibits against inoculums compared with the control, disregarding a single colony or a faint haze caused by the inoculums [39].

RESULTS AND DISCUSSIONS

In this study, inverse microemulsion polymerization was used to synthesize CMC-g-poly(NIPA-co-AAC) hydrogel nano particles with various CMC and crosslinker concentrations as seen in Table 1. Characterization was done on the prepared nanogels via various analysis tools.

FTIR Characterization of the Hydrogels

The FTIR spectrum of CMC, poly (AN), poly (AAC) and Carboxymethyl cellulose-g-poly((AN-co-AAC) is illustrated in **Figure (1)**. In the FTIR spectrum (see Fig. 1b) of CMC a strong peak at 3443.8cm^{-1} is observed due to OH stretching vibration. The band in the range of 1000–1166cm^{-1} is assigned to the ether bonds. The absorption peak at 1635cm^{-1} is related to the carboxylate. The peak at 2926.8cm^{-1} is due to methylene.

The FTIR spectrum of p(AAc) shows the main characteristic peaks at 3350 cm^{-1} that are due to the vibration of the carboxylic –OH group of acrylic. In addition, we band at 1650 cm^{-1} for C=O of AAc.

P(AN) characteristic functional groups of nitrile (–CN) stretching band appears at 2,240 cm^{-1}. Also a sharp band appears at 1,091 cm-1characteristic for C-N stretching vibration.

FTIR spectra of the CMC-g-p (NIPA-co-AAC) superabsorbent nanogels, it has been observed that the spectrum of CMC-g-p (AN-co-AAC) shows variations in intensity and shifting of peak from 3455.9 to 3437.8cm^{-1} appear due to OH stretching vibration of CMC. The characteristic absorption bands of CMC at 1000–1166.6cm−1 are obviously weakened after reaction. These indicate the participation of hydroxyl of CMC in chemical reaction. The appearance of a sharp intensity peak at 2,246 cm-1 characterizing C:N groups

This indicates that the C:N groups are free, which means that the PAN doesn't contribute into the crosslinking process of CMC by using Agar. Also a sharp band appears at 1,091 cm-1 characteristic for C - N stretching vibration [40, 41. The spectrum of CMC-g-(NIPA-co-AAC) confirms the presence of the most important functional groups of the monomers and CMC in the polymer structure. Similar spectra were obtained for other samples of synthesized nanohydrogels.

Table (1): Symbol, compositions and particle sizes of the prepared nanogels

Sample	CMC (wt%)	AN (wt%)	AAc (wt%)	(MBA) (wt%)	AIBN (wt%)	Size (nm)
P(AN)	0	100	0	1	1	300
P(AAc)	0	0	100	1	1	300
CMC	100	0	0	1	1	400
CMC1g(AN-CO-AAc)	90	5	5	1	1	70
CMC2g(AN-CO-AAc)	70	15	15	1	1	60
CMC3g(AN-CO-AAc)	30	35	35	1	1	40
CMC4g(AN-CO-AAc)0.5MBA	10	45	45	0.5	1	20
CMC4g(AN-CO-AAc)1MBA	10	45	45	1	1	30
CMC4g(AN-CO-AAc)2MBA	10	45	45	2	1	50
CMC4g(AN-CO-AAc)3MBA	10	45	45	3	1	80

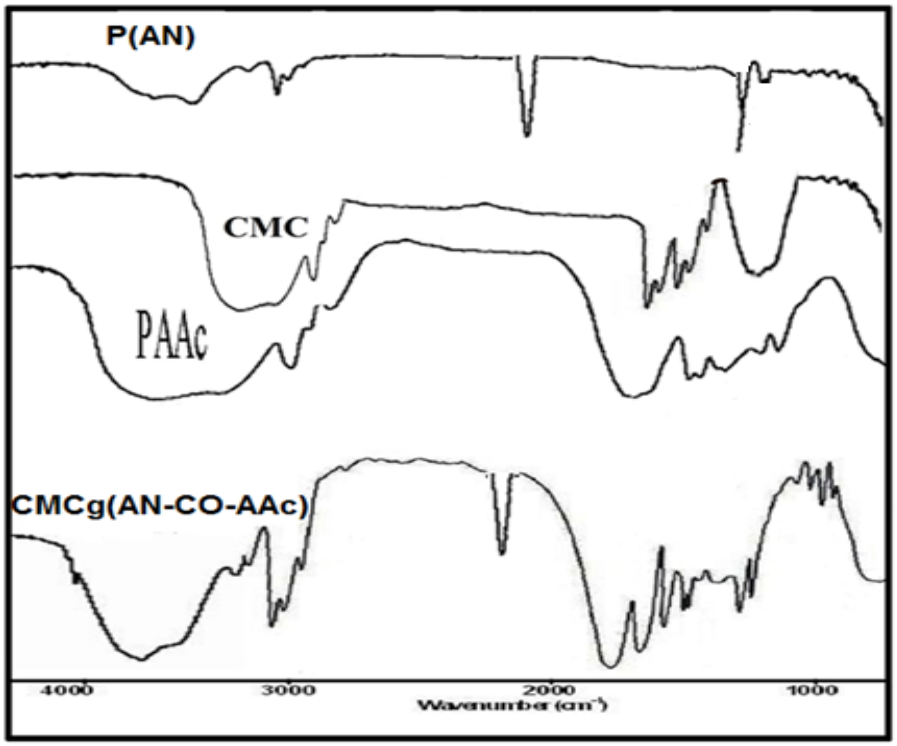

Figure 1: FTIR Spectra of P(AN), CMC, PAAc and CMC-g(AN-CO-AAc)

X-ray Diffraction

X-ray diffraction was used to study the effect of cross-linking content on the nanogels morphology. X-ray diffractograms of carboxymethyl cellulose (CMC) and its grafted nanogels namely, CMC4g(AN-CO-AAc)0.5MBA, CMC4g(AN-CO-AAc)1MBA CMC4g(AN-CO-AAc)2MBA and CMC4g(AN-CO-AAc)3MBA are existed

in Figure 2a–e. It can be seen from Figure 2a that one peak with maximum intensity at 2θ = 20°, this is indicates that CMC is highly crystalline in nature. The incorporation of the cross-linker into carboxymethyl cellulose decreases the intensity of its peak till almost no peaks are obtained with using 3wt% of MBA crosslinker as seen in Figure 3b-e. The cross-linked polymers showed an almost amorphous structure since the functional groups of CMC show significant change after cross-linking. This suggested that a large number of hydrogen bonds in the CMC powder were destroyed after crosslinking with MBA crosslinker, which destroyed the regularity of the packing of the original chains and resulted in the formation of amorphous nanogels.

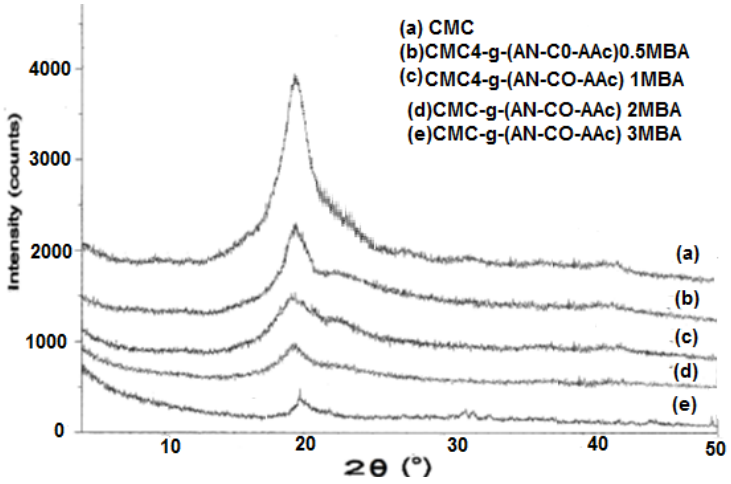

Figure 2: XRD for, CMC and CMC4g (AN-CO-AAc) nanogels crosslinked by different contents of MBA crosslinker

Dynamic Light Scattering

The particle size of the CMCg- p(AN-co-AAC) and the homo-polymers is given in Table 1. It is obvious that the particle size of the grafted polymer is much smaller than the particle size of the homo-polymers. This may be explained by that the cross-linking reduces the particle size. As the particle size depends on the degree of crosslinking, it was found that the increase in weight percent of MBA from 0.5 to 3% increases the particle size from 20 to 80 nm. This results may be explained by the fact that the more crosslinking concentration the stiffer the crosslinking network is and the smaller the cavities produced this enhance the collapse of polymer chains and made it stable in nanosize. Furthermore, the decrease in weight percent of the CMC which represents the main backbone of the polymer chain from 90% (CMC1g(AN-CO-AAc)) to10% (CMC4g(AN-CO-AAc)) decreases the particle size of the prepared nanogels from 70 to 30 nm as seen in Figure 3 which represents the DLS image of . CMC4g(AN-CO-AAc) nanogel crosslinked by 1wt% of MBA crosslinker.

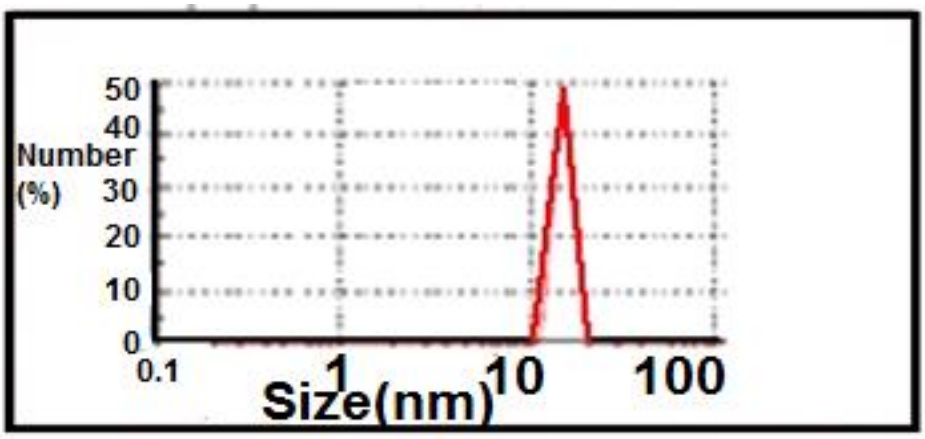

Figure 3: DLS Image of CMC4g(AN-CO-AAc) nanogels crosslinked by 1wt%MBA

Swelling behavior of the prepared nanogels

The swelling degrees of the prepared nanogels in various solvents are summarized in Table 2. The data showed that all the hydrogels are greatly swelled in all the investigated solvents, as they are much more hydrophilic in nature due their constituent which can form hydrogen bonds with the investigated solvents. The data also reveals that the swelling increase by increasing the contents of acrylic acid & acrylonitrile and by decreasing crosslinker weight percent. This indicates successful formation of cross-linked networks in these hydrogels. The highest swelling degree is observed in DMSO due to its higher polarity. Furthermore, the increased swelling capacity of P(AN) hydrogel than the swelling of P(AAc) and CMC may be attributed to the presence of pendent chains or dangling chains in the polymeric network of P(AN) [34, 41]. On increasing the cross-linking density, the swell ability decreases this may be attributed to the more crosslinker contents makes the particles themselves very tightly and hence decrease the swelling. This is well illustrated by the data reported for the swell ability in the case highly crosslinked gel as compared with that of the other hydrogels irrespective of the nature of the investigated solvents.

Table 2: Swelling degree of the parents & prepared nanogels at pH =7

Sample	Swelling Degree			
	DMF	NMP	Distilled H_2O	DMSO
P(AN)	6	8	10	15
P(AAc)	3	4	5	10
CMC	2	2	4	8
CMC1g(AN-CO-AAc)	50	55	57	60
CMC2g(AN-CO-AAc)	55	60	64	68
CMC3g(AN-CO-AAc)	60	65	70	78
CMC4g(AN-CO-AAc)0.5MBA	70	75	80	88
CMC4g(AN-CO-AAc)1MBA	65	70	75	80
CMC4g(AN-CO-AAc)2MBA	53	60	62	70
CMC4g(AN-CO-AAc)3MBA	45	50	55	62

Antimicrobial Activity of the designed nanogels

The increasing number of antibiotic-resistant bacterial strains has developed into amajor health problem. In particular, biofilms are the main reason for hospital-acquired infections and diseases. Once formed, biofilms are difficult to remove as they have specific defense mechanisms against antimicrobial agents. Antimicrobial surfaces must therefore kill or repel bacteria before they can settle to form a biofilm. In the present study, graft copolymerization of carboxymrthyl cellulose with various acrylic acid and acrylonitrile were prepared containing can kill bacteria and prevent from biofilm formation. Tables 3, 4 show the antibacterial activity of the synthesized nanogels using the inhibition zone method. Compared with carboxymethyl cellulose (CMC), all the nanogels have a higher antibacterial activity. Antimicrobial measurements were done as the average of three times on synthesized samples. P(AN) and P(AAc) have no antibacterial activity, but their graft copolymerization with carboxymethyl cellulose (CMC) and when their contents increases in the nanogels chains, it gives higher inhibition than CMC itself till it reaches the maximum value at CMC4g(AN-CO-AAc) a similar behavior was reported in literature with PAN [41]. If the designed nanogels are used in disinfection treatment of water, there will be a great variety of advantages, such as strong antibacterial activity, without residues, without disinfection by-products and avoiding recontamination of water. Several mechanisms elucidating the antimicrobial activity of CMC have been suggested. Carboxymethyl cellulose (CMC) or cellulose gum is a cellulose derivative with carboxymethyl groups (-CH_2-COOH) bound to some of the hydroxyl groups of the glucopyranose monomers that make up the cellulose backbone. The most acceptable mechanism is the interaction between CMC molecules and microbial cell membranes surfaces. This electrostatic interaction results in a twofold interference: (i) by promoting changes in the properties of membrane wall permeability, thus provoking internal osmotic imbalances and consequently inhibiting the growth of microorganisms; and (ii) by the hydrolysis of the peptidoglycans in the

microorganism wall, leading to the leakage of intracellular electrolytes such as potassium ions, and other low molecular weight proteinaceous constituents (e.g., protein, nucleic acid, glucose, and lactate dehydrgenase) [42].

Another proposed mechanism is the binding of chitosan with microbial DNA, which leads to the inhibition of the mRNA and protein synthesis via penetration of chitosan into the nuclei of the microorganisms [43]. Moreover, it can be concluded that not only the synthesized nanogels have a repulsive effect as proposed in the literature [44, 45], but also do PAAc contents with about 45 wt% have a high antimicrobial effect. This may be due to the presence of acrylic acid moiety reduced pH-value in the drop after time. On the one hand, it is known that low pH-values cause stress to the cell by disrupting cytoplasmic pH homeostasis besides impeding enzymeand transport system functions [46-48]. Prolonged exposure to acids results in membrane damage, de naturation of proteins, and de purination of DNA [49, 50]. On the other hand, small leaching acids such as fatty acids are expected to attack the cell membrane [51-54]. Finally, it is known that leaching acids can kill bacteria depending on the acid concentration and the pH-value [55]. Some acids are known also to be antiviral [56] and antifungal [57], which show the high potential of acidic materials. The activity of ferulic acid copolymers against Aspergillus niger [58] is an example of an acidic material without leaching acids. The presented poly acrylic acid moiety in the CMC chains of this study support the observation that immobilized acids is very active as anti-microbial activity depending on the acrylic acid content. This explains the observed higher antibacterial activity of prepared nanogels contains higher value of AAc and AN moiety of CMC4-g-(AN-CO-AAc) contents relative to the parent CMC. Moreover, both the CMC and its nanogels were active against the three tested bacteria as seen in Table 3. The strongest CMC4-g-(AN-CO-AAc) caused an inhibition zone diameter for B. subtilis, S. aureus, and S. pneumonia of 32.2, 30.1, and 27.8 mm, respectively. This may be attributed to their different cell walls. The cell wall of Gram-positive bacteria is fully composed of peptide polyglycogen. The peptidoglycan layer is composed of networks with plenty of pores, which allow foreign molecules to come into the cell without difficulty and allow more rapid absorption of ions into the cell.

The effect of crosslinker contents of the tested naogels as antimicrobial polymers was determined and presented in Table 4. It is seen from Table 4 that, inhibition efficiency decreases by increasing crossinker concentrations. The cross-linker moieties incorporated onto hydrophilic CMC part the CMC chains away from each other, decrease their intermolecular hydrogen bonds, and increase their solubility; this is the reason for the ease of penetration of the hydrogels into the cells of microorganisms, thereby preventing the growth of the cell by preventing the transformation of DNA to RNA to obtain a higher antibacterial activity. The different inhibitory effect of the crosslinker may be attributed to the extent of the swell ability of the nanogels which decreased with increasing cross-linker content incorporated into the hydrogels. The swell ability seems to improve the contact surface between gel and the bacteria. Also, it seems that the lower the degree of cross-linking of the hydrogels, the higher the degree of freedom of the cross-linked CMC chains that can fulfill their antibacterial activity while still covalently being immobilized into the network.

An additional evidence for the greater activity of the hydrogels against Gram-positive bacteria comes from their minimum inhibitory concentration (MIC) values. MIC is defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. The MIC values of the prepared antibacterial hydrogel are shown in Table 5. Since the MIC values of the CMC4-g-(AN-CO-AAc) hydrogel against B. subtilis, S. aureus, and S. pneumoniae were 0.75, 1.5 and 1.8 µg/mLrespectively. It is interesting to note that the CMC4-g-(AN-CO-AAc) nanogel showed the highest antibacterial activity, relative to the other hydrogels, as seen by the highest inhibition zone diameter (Table 3). On the other hand, the CMC1-g-(AN-CO-AAc) hydrogel exhibited the lowest antibacterial activity. The activities of the other hydrogels lie in between these two cases. Thus, the inhibitory effect increased with increasing the amounts of both acrylic acid and acrylonitrile monomers. This may be also attributed to the extent of the swell ability of the hydrogels, which increased with increasing AAc &AN contents incorporated into CMC chains. The swell ability seems to improve the contact surface between the hydrogel and the bacteria. The same results were found for the antifungal activities of the prepared nanogels against A. fumigatus, and A. niger as shown in Tables 3-5. The results show that all the gel had effective activities against the tested fungi, compared with the parent CMC itself. Further, for a comparable antifungal activity, the MIC values of the prepared gels. The data reveals that antifungal activity increased by increasing the contents of acrylic acid and acrylonitrile monomers. Moreover the inhibition efficiency of the nanogels decreased by increasing the crosslinker concentrations. This is attributed to the higher swelling ability of the hydrogels which is expected to enhance the diffusion of the active ingredient inside the pathogens, which may lead to a disturbance of the enzyme activities responsible

for the growth criteria, instead of the adsorption of the insoluble compounds on the fungal hyphae surface. Pentachloronitrobenzene and chlorothalonil are usually used as fungicides; however, the chloro-groups in these fungicides constitute a big problem in the environment due to their toxicity [59, 60]. The designed nonogels might be expected to induce lower pollution to the environment.

Table 3: Inhibition indices of the synthesized nanogels against tested bacteria and fungi

Sample	Inhibition zone diameter (mm/sample)				
	Gram positive bacteria			pathogenic fungi	
	B. subtilis (RCMBA 6005)	*S. aureus (RCMBA 2004)*	*S. pneumonia (RCMB 000101)*	*A. fumigates (RCMBA) 06002*	*A. niger (RCMBA 06106)*
P(AN)	-	-	-	-	-
P(AAc)	-	-	-	-	-
CMC	15.4	13.5	12.1	11.5	14.7
CMC1g(AN-CO-AAc)	22.4	20.1	18.5	18.2	20.6
CMC2g(AN-CO-AAc)	24.4	22.8	20.7	21.5	23.8
CMC3g(AN-CO-AAc)	28.2	25.6	24.4	22.1	24.7
CMC4g(AN-CO-AAc)	32.2	30.1	27.8	25.6	29.5

Table 4: The Effect of crosslinker concentration of selected CMC4g(AN-CO-AAc) nanogel sample on Minimum inhibitory concentration (MIC) (µg/mL) of the synthesized nanogels against tested bacteria and fungi

Crosslinker wt% of CMC4g(AN-CO-AAc) nanogel	Inhibition zone diameter (mm/sample)				
	Gram positive bacteria			pathogenic fungi	
	B. subtilis (RCMBA 6005)	*S. aureus* (RCMBA 2004)	*S. pneumonia* (RCMB 000101)	*A. fumigates* (RCMBA) 06002	*A. niger* (RCMBA 06106)
0.5	36.5	33.7	30.5	27.7	32.4
1	32.2	30.1	27.8	25.6	29.5
2	26.7	24.6	20.6	18.8	21.6
3	22.3	20.8	16.7	15.6	17.7

Table 5: Minimum inhibitory concentration (MIC) (µg/mL) of the synthesized nanogels against tested bacteria and fungi

Sample	Minimum inhibitory concentration (MIC) (µg/mL)				
	Gram positive bacteria			pathogenic fungi	
	B. subtilis	*S. aureus*	*S. pneumonia*	*A. fumigates*	*A. niger*
P(AN)	-	-	-	-	-
P(AAc)	-	-	-	-	-
CMC	75	125	150	250	150
CMC1g(AN-CO-AAc)	2.1	2.7	3.1	10.5	3.9
CMC2g(AN-CO-AAc)	1.4	2.1	2.7	6.7	3.5
CMC3g(AN-CO-AAc)	0.95	1.9	2.1	5.9	3.1
CMC4g(AN-CO-AAc)	0.75	1.5	1.8	3.6	2.5

CONCLUSIONS

- Hydrogel nanoparticles based on graft copolymerization of CMC with various contents of acrylic acid (AAc) & acrylonitrile (AN) monomers crosslinked by various wt% of MBA as crosslinker are synthesized.
- The synthesized nanogels show enhanced swelling capacities in different solvents.
- The equilibrium swelling degree of the prepared nanohydrogel decreases by increasing crosslinker dosage. This makes the particles themselves very tightly.
- The results showed that with increasing AAc &AN substrate amounts, the particle size slightly decreases.
- The prepared nanogels exhibited good antibacterial activities against three types of bacteria, namely: Bacillis subtilis (B. subtilis, RCMBA 6005), Staphylococcus aureus (S. aureus, RCMBA 2004), Streptococcus pneumonia (S. pneumonia, RCMB 000101) as Gram positive bacteria.
- Also Two crop-threatening pathogenic fungi namely: Aspergillus fumigatus (A. fumigatus, RCMBA 06002), and Aspergillus niger (A. niger, RCMBA 06106), are affected by the prepared nanogels.
- The antimicrobial activity of the prepared nanogels increases with the increase of AAc & AN content.
- Finally the antimicrobial activity of the designed nanogels decreases with the increase of MBA crosslinker concentration.

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