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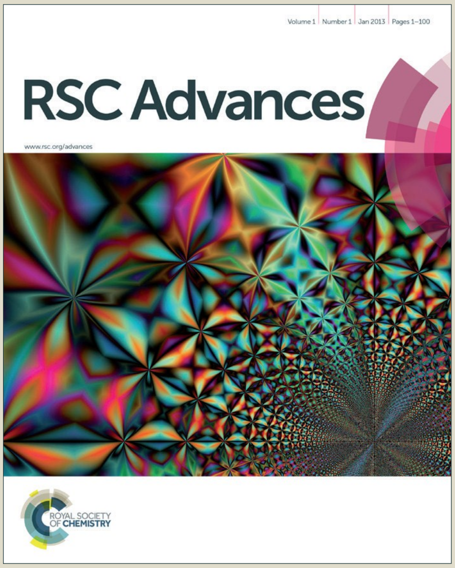

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Novel nanocomposites hydrogel for wound dressing and other medical applications

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Abstract

Graft copolymerization of diallyldimethylammonium chloride (DADMAC) vinyl monomer together with N,N'- methylene-bis-acrylamide (MBA) crosslinking agent onto water soluble carboxymethyl cellulose CMC was carried out using ammonium persulfate (APS) initiator . The copolymerization resulted in hydrogels. Characterization and properties of these hydrogels were dependent on conditions affecting the copolymerization reaction and, in turn, control the pore size and porous structure of the hydrogels. Thus increasing the monomer concentration causes outstanding enhancement in the swelling ratio of the hydrogel provided that monomer is used at a concentration of 40% or more .The opposite holds true for initiator concentration; the swelling ratio of the hydrogel decreases significantly by increasing APS concentration from 0.05 to 0.25 mole/L. With respect to MBA crosslinker, maximum swelling ratio of 30 could be achieved with hydrogel prepared using MBA at concentration of 0.1 mole/L; hydrogel prepared in presence of MBA at 0.05 mole/L exhibits zero swelling ratio while hydrogel prepared using MBA at 0.3 mole/L displays swelling ratio of 10%, maximum swelling ratio for hydrogel could achieved at pH7 .Striking decrease in the swelling ratio of hydrogel is observed within pH range 2-6 as well as at pH 8. The hydrogel could also be successfully attached to modified cotton fabric, namely partially carboxymethylated cotton (PCMC) through ionic crosslinking The in situ formation of CuO nanoparticles inside the matrix of CMC-DADMAC hydrogel nanocomposites attached to cotton fabric was also investigated .The formation of CuO nanoparticles in the hydrogels was confirmed using X-ray diffraction and scanning electron microscopy studies. Furthermore, the functional performance of the novel CuO nanocomposite hydrogel as wound dressing was tested for antibacterial activities; the nanocomposite hydrogels demonstrated excellent antibacterial effect. The work was further extended to include synthesis and

characterization of Ag/CMC-DADMAC hydrogel nanocomposites. The latter displays high antibacterial activity.

1. Introduction

Hydrogels, known also as super absorbents, are preferably synthesized by grafting vinyl monomers onto natural polysaccharides and then compounding with inorganic nano-scale metals. This approach forms the basis of the method of choice because it affords unique environmental and commercial advantages. Up to now, most dual temperature and pH-sensitive hydrogels that can swell in acidic pH surrounding and deswell in alkaline pH are necessary in certain cases, such as drug release and dye adsorption [1-4]. For example, the drug (chloramphenicol) must be released more rapidly from hydrogel in a pH 1.4 (close to the pH of the stomach) buffered solution than in a pH 7.4 (close to the pH of the intestine) [1], in which the drug release is controlled by the swelling/deswelling behavior of the hydrogel. To achieve such functions, cationic hydrogels are needed. Diallyldimethylammonium chloride (DADMAC) is a water-soluble quaternary ammonium compound that can be cyclopolymerized to its corresponding polymer and is widely used in water treatment, paper manufacturing, mining, and biology [5]. It was found in previous studies that polyDADMAC hydrogel could absorb several hundred times of water [6,7]. Furthermore, quaternary ammonium compound is antibacterial which takes advantage to serve in medical application.[8]

Among all cellulose ethers, only carboxymethyl cellulose (CMC), available as the sodium salt (NaCMC), is a polyelectrolyte, and thus a smart cellulose derivative which shows sensitivity to pH and ionic-strength variations, plus good swelling capability [7,9]

As textiles become more functional, stimuli-responsive polymers have also found their application in the creation of intelligent or smart textiles. These environmentally responsive fabrics can be tailored by chemical modification of the textile's surface using polymeric chains. Smart textiles may provide us with considerable convenience, support, and even pleasure, in our daily activities. [10]

Hydrogel-based hybrid materials incorporating an inorganic phase in the form of nanoparticles (NPs) are receiving an increasing amount of attention, thanks to the synergic properties of the hydrogels and their inorganic components [11]. Different types of inorganic nanoparticles have been incorporated to prepare hydrogel-based hybrid systems with tailored mechanical or functional properties [12–14] .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. This approach was established by Wang et al. [15] and Murali Mohan et al. [16] to obtain 3–5 nm sized gold and silver nanoparticles within the poly (N-isopropylacrylamide) (PNIPAM) based hydrogel networks. Much research and development efforts have been devoted to the production of hydrogels containing metal nano-particles which are highly suitable for biomedical applications [17]

Very recently development of different stimuli-response hydrogels were the subject of our research activities for instance , thermal responsive hydrogels based on semi interpenetrating network of poly (NIPAm) and cellulose nanowhiskers brought into focus [18]. Investigation into the synthesis and characterization of novel CMC hydrogels and CMC hydrogel-ZnO –nanocomposites were performed [19]. We have also reported on development of CMC hydrogels loaded with silver nanoparticles for medical applications [17].

Current research is undertaken with a view to develop smart textile with tunable water absorbance change with environment. Development of such a textile hydrogel is based on radical solution polymerization of DADMAC monomer on to carboxymethylcellulose (CMC) using ammonium persulfate (APS) as an initiator and N, N methylenebisacrylamide (MBA) as a crosslinker. To our knowledge this copolymer is not used before in textile application as well as a carrier for nanoparticles. Copolymerization to achieve hydrogel formation is carried out under a variety of conditions and so does the application of the hydrogels to partially carboxymethylated cotton (PCMC) fabric .State of the art facilities were used for analysis and characterization of the products obtained .Preparation of a novel wound dressing containing CuO/CMC-DADMAC hydrogel nanocomposites as well as preparation of Ag/ CMC-DADMAC nanocomposite hydrogel.

2. Experimental

2.1Materials

- Fabric: mill-scoured and bleached cotton fabric was kindly supplied by Misr Co. for Spinning and Weaving, Mehala El kubra, Egypt

- Carboxymethyl cellulose (CMC) of molecular weight (Mw = 10,000 Da) diallyldimethylammonium chloride (DADMAC) 97% (Merck chemical company, Germany), N, N‘- methylene-bis-acrylamide (MBA), 99%, ammonium persulfate, (APS) 98% and all other chemicals were of laboratory grade.

2.2Method

2.2. 1Synthesis of CMC –DADMAC copolymer hydrogel through graft polymerization

Graft copolymerization of DADMAC onto CMC was effected using APS as a free radical initiator. In a 100-mL flask, definite weight of CMC was dissolved in 10 mL of degassed distilled water. The flask was placed in a water bath at a temperature 65°C A given amount of monomer, DADMAC (20-70%), was added to the flask and the mixture was stirred for 10 min. Then the initiator solution APS (0.05-02 mole/L) and MBA (0.05-0.3mole/L)were added simultaneously to the mixture, the mixture was left for 30 minutes in a shaking water bath for the gelation to occur. After gelation was completed, the gels were cut into disks with 10 mm in diameter and 2 mm in thickness, and then immersed in an excess of deionized water for 4 days to remove the residual unreacted monomer. The swollen hydrogels were dried at room temperature for 2 days

2.2.2. Preparation of Partially carboxymethylated cotton fabric (PCMC)

The aim of textile material activation is to impart ionic character to cotton fabric the incorporation of cationic hydrogel system. Among several possibilities]. The carboxymethylation was done in order to make surface activation to cotton fabric. Cotton fabric was partially carboxymethylated by a method similar to those previously reported [20]. Accordingly, bleached cotton fabric samples were impregnated with 5M aqueous NaOH for 10 min at room temperature followed by squeezing to a wet pick up of 100%. Samples were dried at 60 °C for 5 min. Thus alkali-treated samples were steeped in aqueous solution of sodium salt of monochloroacetic acid (0.3 M), for 5 min at room temperature. These samples were then squeezed to 100% wet pick up, sealed in plastic bags and heated at 80 °C for 1 h. Samples were then washed and dried at room temperature.

2.2.3 Surface Functionalisation of cotton fabric with hydrogels

As mentioned in section 2.2,that the aim of cotton fabric functionalisation was to impart ionic character to cotton cellulose by chemical methods. Anionic cotton has been produced by reaction with monochloroacetic acid (CAA) to give partially carboxymethylated cellulose. The preparation and polymerization reaction of the hydrogel was carried out in presence of (PCMC) sample until thin film of hydrogel polymer was formed thereon by ionic crosslinking between the fabric and the formed hydrogel . At this end the hydrogel coated samples were dried at 30 ◦C,

2.2.4 Preparation of cotton fabric loaded with CuO /CMC nanocomposite hydrogels

Preparation of CMC nanocomposite hydrogels containing CuO attached to cotton fabric surface were prepared as follow Typically1 g fabric containing CMC hydrogel was immersed in copper sulphate solutions for 24 h. Cotton fabric loaded with copper ion CMC hydrogels were washed with distilled water to remove copper ions attached to the hydrogel surface. Following cleaning, the fabric containing CMC hydrogel loaded with Cu ion –CMC hydrogel was placed in 100 ml of 0.2 M NaOH solution with heating at 100 °C for 10minutes, to oxidize Cu^+ to CuO. After oxidation of the bound Cu ions the fabric will contain hydrogels loaded with CuO nanoparticles with greenish brown color. At this end, the fabric were washed with distilled water and finally dried at ambient condition

2.2.5 Preparation of silver nanoparticles within hydrogels

Another purpose for this research to prepare a novel composite hydrogel with antibacterial activity .Fifty milligrams of dry hydrogel discs were equilibrated in distilled water for 2 days and the swollen discs were transferred to a beaker containing 50 ml of $AgNO_3$ (0.01 mole/L) aqueous solution and then allowed to equilibrate for 1 day. During this equilibration stage, the silver ions were exchanged from solution to the gel network through their free-space between the cross-linked networks or anchored to the $–COO^-$, $–NH_2$, –OH groups of polymeric chains of the hydrogel.

Then, the silver salt-loaded hydrogels were wiped off using a tissue paper and transferred to a beaker containing 50 ml of cold aqueous $NaBH_4$ solution (0.1 mole/L). The beaker was left in the refrigerator (4 °C) for 2 h in order to reduce the silver ions into silver nanoparticles and the hydrogel–silver nanocomposites were separated from $NaBH_4$ solution. The silver nanoparticles produced in the hydrogels are often termed as hydrogel–silver nanocomposites. During this process, there was no change in the shape and size of hydrogels in all the samples. It is noted that all the hydrogels and hydrogels loaded with silver salt, and hydrogel–silver nanocomposites are stored in a refrigerator until their use. **[21]**

2.3 Characterization and analysis

2.3.1 Swelling behavior

The swelling behavior of the prepared hydrogel was calculated using the ratio (Q) of the gels as per the equation **[19]**

$$Q = W_e/W_d$$

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 X- Ray Diffraction (XRD)

The X-ray diffraction method was used to identify CuO and Ag 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.

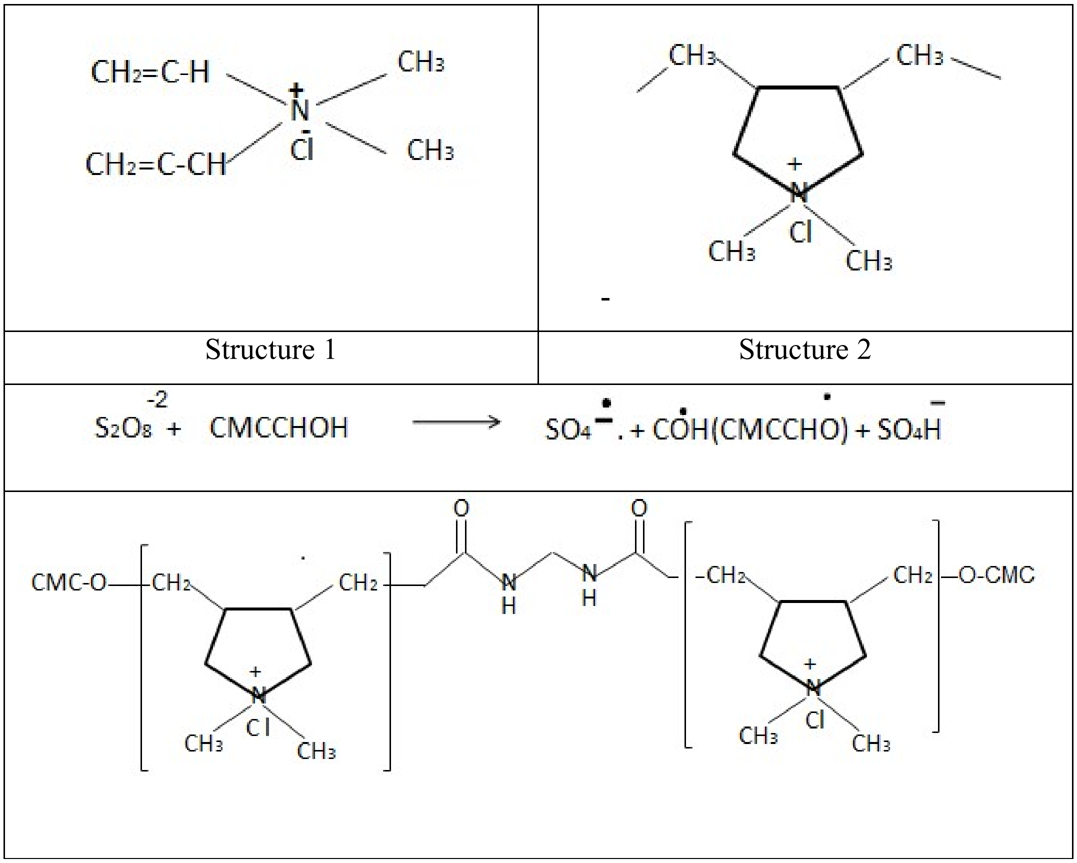
2.3.5 Antibacterial activity

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

3. Results and discussion:

3.1Mechanism of hydrogel formation

Initially, the persulfate initiator is decomposed under heating to generate sulfate anion-radicals. The radicals extract hydrogen from the hydroxyl group of the sodium carboxymethylcellulose to form alkoxy radicals on the substrate. The monomer molecules, which are in close vicinity of the reaction sites, become acceptor of carboxymethyl cellulose radicals resulting in chain initiation and thereafter themselves become free radical donor to neighboring molecules. In this way grafted chain grows (22, 23). Since a cross linking agent, i.e. MBA is present in the system, the end vinyl groups of crosslinker MBA may react synchronously with polymer chains during the chain propagation. The copolymer consists of a crosslinked structure. Keeping in mind that DADMAC takes place mostly via five member ring formations. Given below are the two structures, i.e structure 1 and structure 2, suggested for DADMAC monomer. Below also is the mechanism of CMC-poly(DADMAC) crosslinked copolymer hydrogel along with reactions involved therein



Scheme 1 represents the mechanism of CMC-poly(DADMAC) hydrogel synthesis were structure 1and 2 represent the proposed structure of DADMAC monomer

3.2.Hydrogels characterization

3.2.1 FTIR spectral analysis of hydrogels grafted to cotton

FTIR analyses of CMC and DADMAC monomer, as well as hydrogels prepared therefore are shown in figures 1,2,3. Figure 1 depicts that the characteristic band of DADMAC appear at 3413,3025,2980,1639,1479,1159,609cm^{-1} as numbered in figure 1(1,2,3,8,10,17,19). A comparison between figure 1 and those of figure 2 for CMC and figure 3 for CMC grafted with DADMAC signifies that the hydroxyl group band appears in both figures at 3427cm-1 shifts in intensity from 59 to 21.15 .This lowering in intensity suggests that breakage of hydrogen bonds takes place due to presence of lower amount of hydroxyl groups by virtue of their involvement in the graft polymerization reaction .We can also notice the presence of new band at 960cm-1 which is the characteristic band for CH_2 bonded with the quaternary ammonium group of DADMAC.

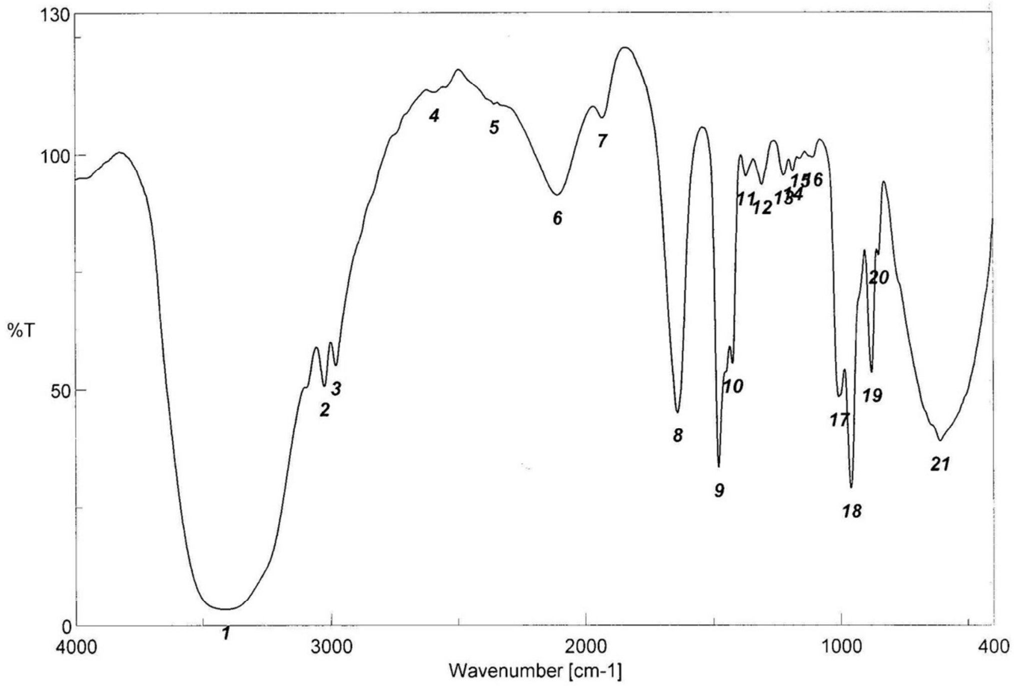


Figure 1 FTIR for Diallyldimethyl ammonium chloride monomer

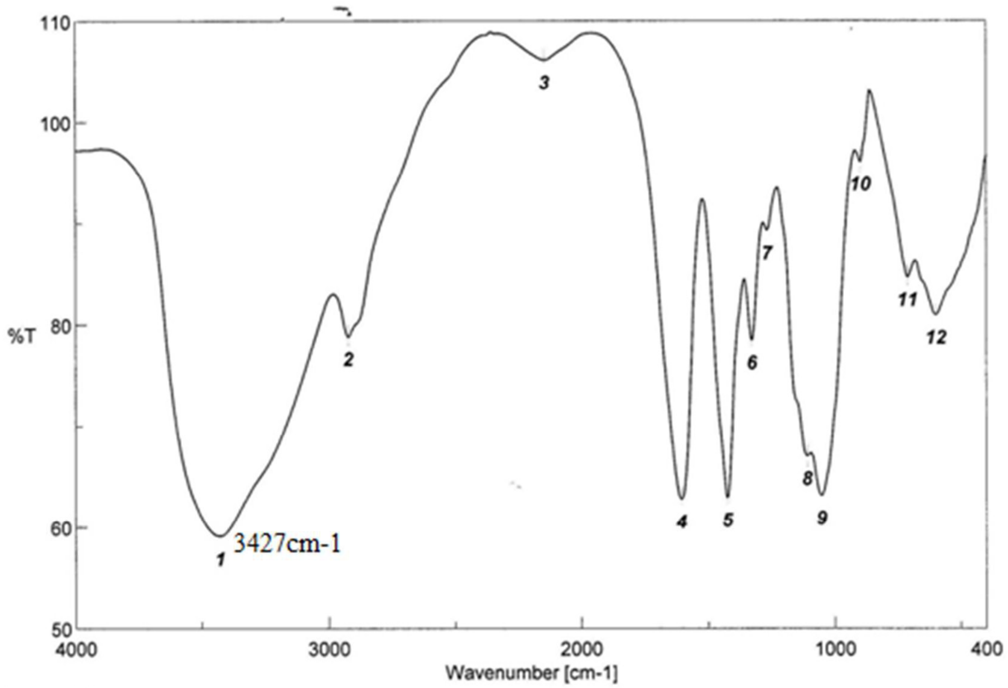


Figure 2: FTIR of carboxymethylcellulose (CMC)

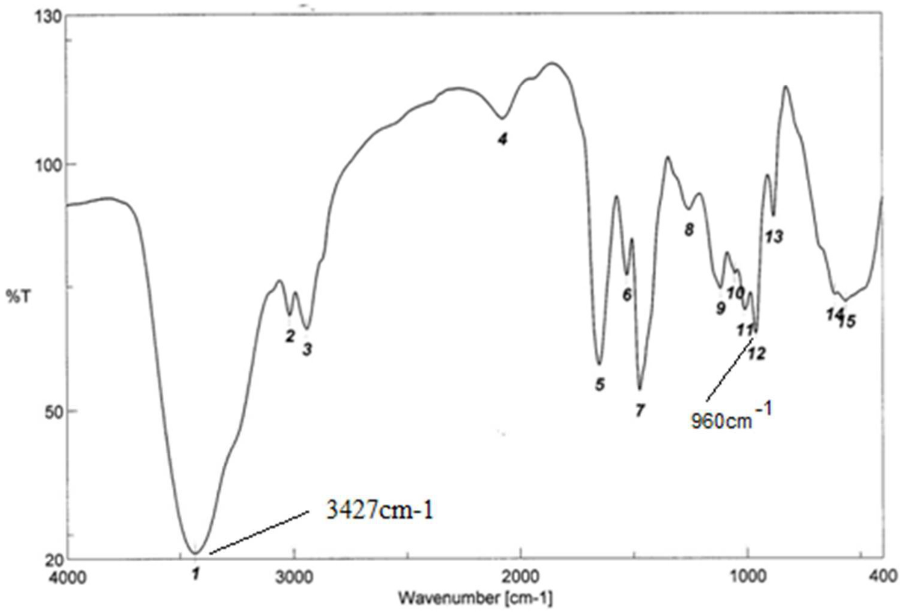


Figure 3: FTIR for CMC-DADMAC copolymer hydrogel

3.2.2 Morphology of CMC-DADMAC copolymer hydrogel using SEM

Figure 4 shows the SEM image of the surface and cross-sectional morphologies of CMC-DADMAC copolymer hydrogels. It is observed (figure 4a) that the plain hydrogel is characterized by a clear and flat surface. On the other hand, figure4b shows that the freeze dried hydrogel has porous structure; .three dimensional network structures are also formed. These pores are supposed to constitute the regions of water permeation .It is well established that porous surface is essential for the transport of the oxygen from outside to inside for example wound dressing, meanwhile a three dimensional structure is equally crucial to absorbing and keeping large amount of water in the hydrogel materials

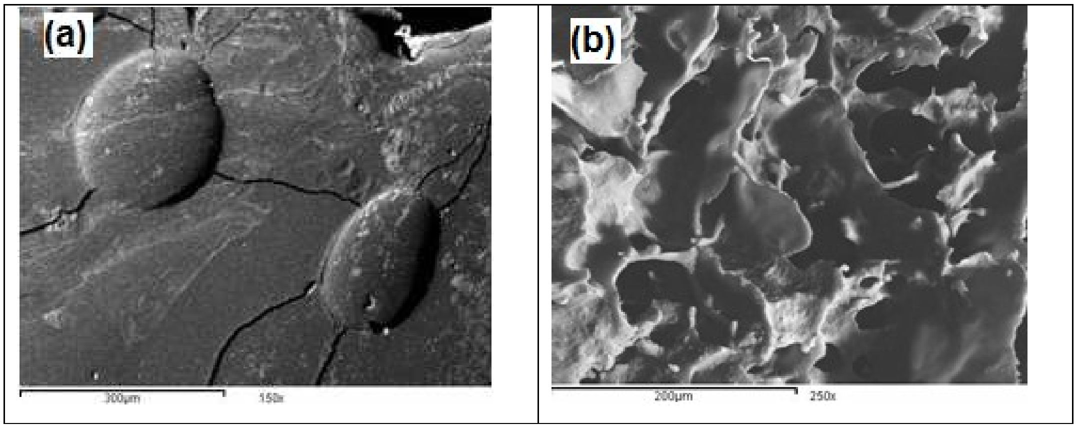


Figure 4: SEM for CMC/DADMAC hydrogels where figure4a : SEM micrographs of the CMC/DADMAC hydrogels outer surface Figure 4b represents the SEM image of freeze-dried sample of hydrogel (cross section).prepared at pH=7

3.3. Synthesis of CMC /DADMAC hydrogels: Effect of process parameters

3.3.1 Monomer concentration:

Figure 5 shows the effect of concentration of DADMAC monomer on the swelling ratio of hydrogel prepared as per the procedure previously described for copolymerization of this monomer on CMC in presence of MBA crosslinking agent, , .As is evident (figure5), the crosslinking and copolymerization were carried out in highly concentrated aqueous solutions of the monomer mixture .This is due to the interesting aspect of DADMAC polymerization as this monomer can be polymerized only in highly concentrated solutions, because of the strong Coulombic repulsion between the quaternary ammonium groups, as evident from Butler’ s pioneering work on the cyclopolymerization of DADMAC monomer [23]. Because of this reason, the terpolymerization reactions were performed at high monomer concentrations (40% w/w) at 65 °C .Particularly notable is that increasing the monomer concentration above 40% causes outstanding enhancement in the swelling ratio provided that the monomer is used at a concentration of at least 40%. This means that concentration of DADMAC monomer plays a key role in the values of swelling ratio of the hydrogel. The pore size and the porous structures of the hydrogels under investigation are a direct impact of DADMAC concentration

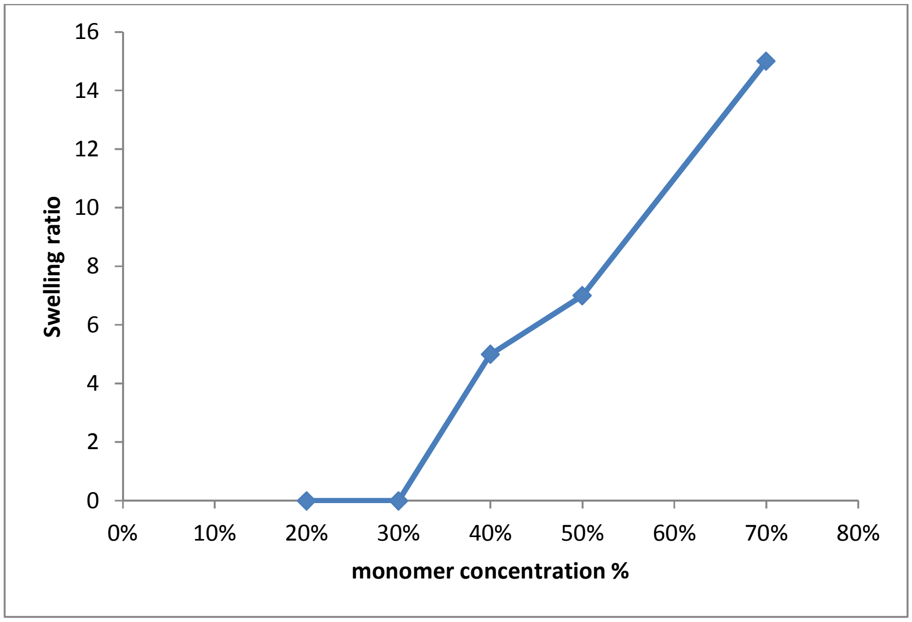


Figure 5 Effect of concentration of DADMAC monomer on the swelling ratio of CMC-poly (DADMAC) crosslinked copolymer

Reaction condition, 0.5gm CMC, 0.16mole/L MBA;0.04 mole/L APS;65°C ;2hrs

3.3.2 Initiator concentration

Figure 6 discloses the effect of APS concentration on swelling ratio of CMC-poly (DADMAC) crosslinked copolymer .Obviously, the increase in APS initiator concentration is accompanied by a decrease in swelling efficiency. This state of affairs refers to the serious impact of the initiator concentration on the morphology, porous structure in particular, of the hydrogel. As started earlier initiator above certain concentration would lead to decreased grafting due to fast termination rate, and in turn, lower molecular weight of the graft, With this in mind, it is logical in current work that the molecular weight of poly (DADMAC) branches grafted chains and sequence of these branches on the CMC backbone would rely much on the initiator (APS) concentration. Besides decreasing the molecular weight of the grafted chains, higher concentration of the initiator causes oxidation of the CMC backbone thereby leading to CMC with lower molecular weights as a result of glucosidic bond scission, meanwhile extra carboxyl and/or aldehydic groups are created by oxidation of CMC hydrogels. In short, at higher initiator concentration the grafted chains and CMC backbone undergo changes in their molecular structure during synthesis of the hydrogel brought about thereof and, these changes affect the pore size and porous structure of the hydrogel in as such that swelling ratio of the hydrogel decreases. Once this is the case, the pore size and porous structure of the hydrogels will differ accordingly. The onset of such changes in the physical and chemical structure of the hydrogel would certainly be reflected on the swelling ratio of the hydrogels in questions.

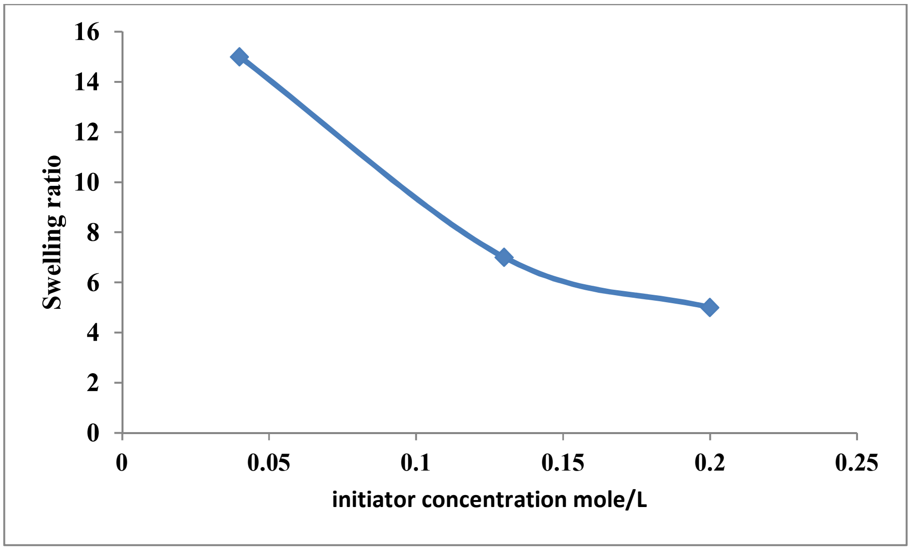


Figure 6 Effect of APS initiator concentrations on the swelling ratio of CMC-poly (DADMAC) crosslinked copolymer

Reaction condition 0.5gm CMC; 60% monomer concentration; 0.16 mole/L MBA; 65°C, 1hr

3.3.3 MBA concentration

Of the factors affecting synthesis of the hydrogel, crosslinker concentration is the most determining factor on water absorption of the hydrogel .As can be seen from

Fig. 7, the swelling ratio increases to reach maximum at 0.09 mole/l then the water absorption rapidly decreases by increasing the concentration of MBA crosslinker from 0.09 to 0.3 mol/L. This is rather in conformation with Flory's theory [24] the increase of MBA concentration results in an increase of crosslinking density which in turn, diminishes the network voids of water holding thereby decreasing the pore sizes of the hydrogels. As a consequence the swelling tendency of the hydrogel to swelling decreases

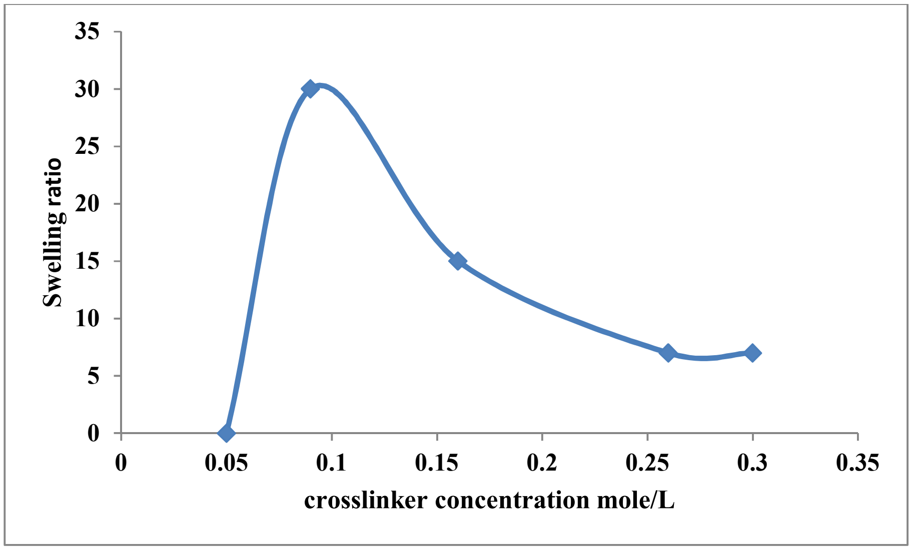


Figure 7 Effect of concentration of MBA crosslinking agent on the swelling ratio of CMC-poly (DADMAC) (copolymer) hydrogel

Reaction condition 0.5gm CMC,60% monomer concentration ,0.04 mole/L APS,65°C ,1hr

3.3.4. Effect of medium pH:

Figure 8 shows variations of the water absorption expressed as swelling ratio of CMC–DADMAC graft copolymer with different pH solutions. As is evident, no real apparent **variations** in the swelling ratio of the hydrogel by changing the pH of the swelling medium from pH 2 to pH 6. On the contrary sharp increase in swelling ratio is observed at pH7 followed by abrupt decrease at pH 8.

In neutral water as the swelling medium, CMC is a negatively charged polyelectrolyte in the swelling system, and the strong electrostatic repulsions among CMC carboxylate anions (COO^-) could result in a more expanded network of hydrogel. The latter assumes the highest swelling ratio at pH 7,a point which could be associated with increasing number of ionic groups in the hydrogels which causes an increment in their swelling capacity due to additional osmotic pressure provided by counter ions inside the gel. However, the swollen gel rapidly shrinks because of protonation of -COO$^-$ groups under acidic pH's (pH < 5), where most of the carboxylate anions are protonated. On the one hand, the hydrogen-bonding interaction among carboxylate groups is strengthened and additional physical crosslinking is generated; As a result, the network tends to shrink and consequently swelling values are decreased. The decreased absorbency at higher basic pH's (pH > 8) is related to 'screening effect' of excess cations in the swelling media

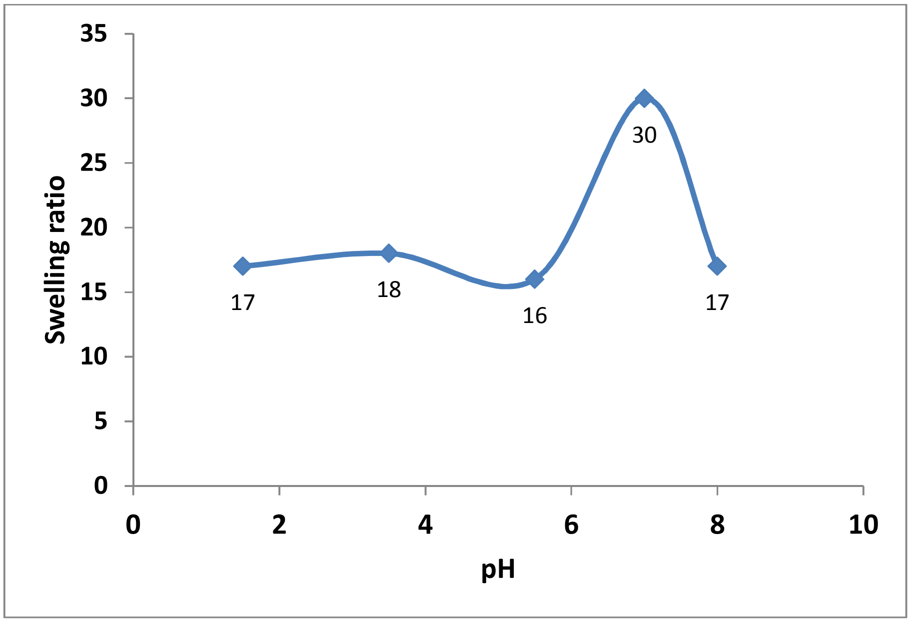
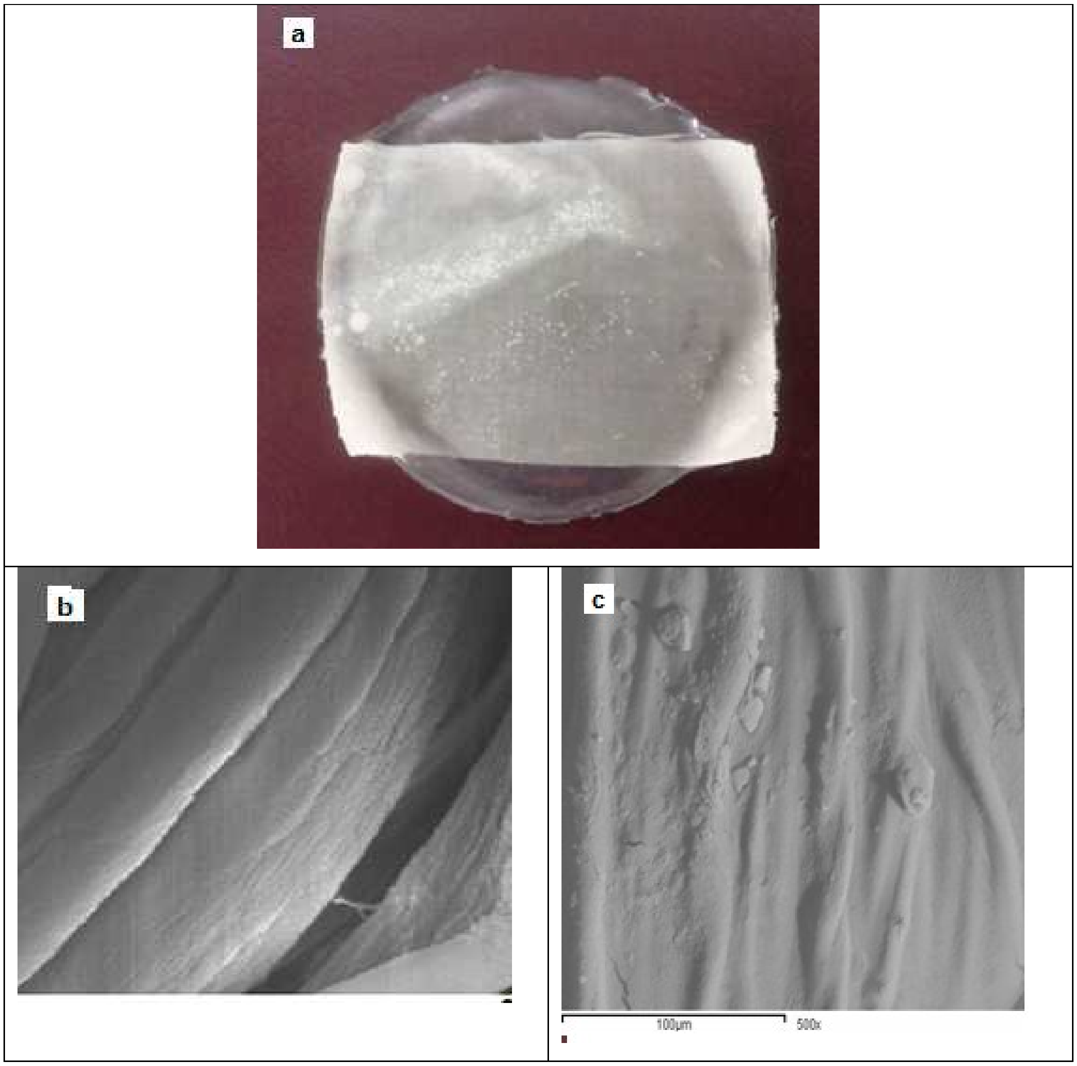
"  \

Figure 8: swelling ratio of CMC- DADMAC copolymer hydrogel versus pH of the aqueous swelling medium

3.4 Functionalization of cotton textile by CMC-DADMAC copolymer hydrogels

The main challenge of developing smart textile materials is confined to techniques for successful attachment of the hydrogel layer to the textile substrate. Recent research disclosed that hydrogel particles can be covalently bonded to cotton using appropriate crosslinking agents [25]. In current work, hydrogel was attached to the surface of partially carboxymethylated cotton (PCMC) fabric, through ionic crosslinking. Anionic (PCMC) fabric was synthesized by reacting it with monochloracetic acid in alkaline medium. This method not only keeps the elastic form of hydrogel but also confirm its attachment to cotton fabric. Presence of hydrogel on the surface of PCMC fabric is indeed visually confirmed by SEM.

The surface morphology PCMC fabric coated with the hydrogel is examined and shown in figure 9.As we can see from figure 9 a,b,c the fibers are covered with irregular fragments, rendering surface of fabrics rough and homogeneous. It means a thin hydrogel layers are coating the fabric.Figure9 illustrate the surface morphology (cross section) of fabric coated with thin layer of hydrogel.



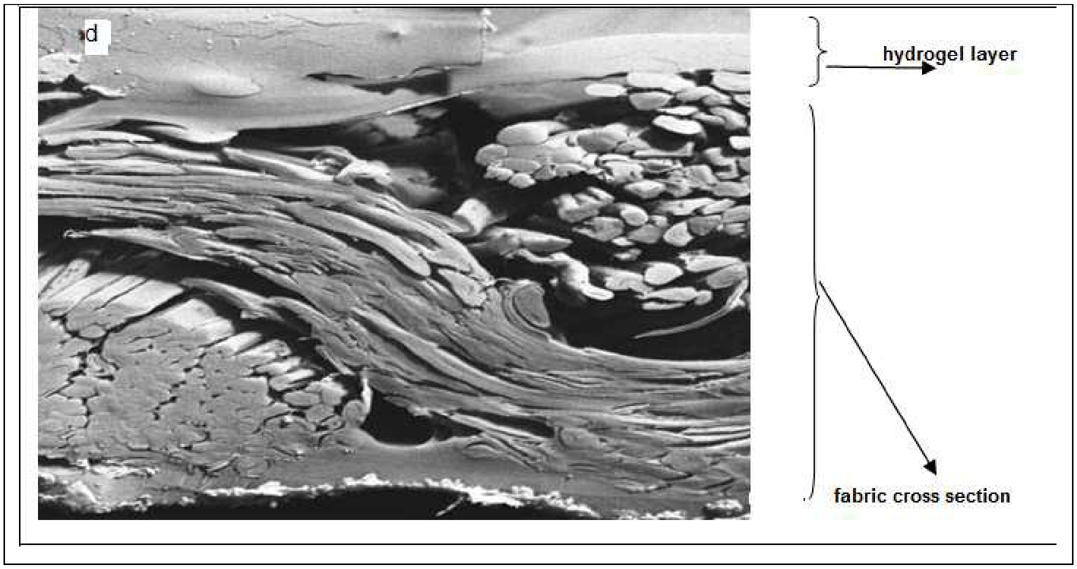


Figure 9: The surface morphology of fabric coated with thin layer of hydrogel

By studying the swelling character of cotton –hydrogel samples we have concluded that the incorporation of hydrogel into cotton fabric surface causes significant changes in its swelling behavior. Most probable the ionic crosslinking between PCMC and the cationic hydrogel decreases the affinity of the hydrogel towards water and the swelling ratio reduces to a value of 10 at PH7 .while in both acidic (pH 5) and alkaline (pH 9) environment the swelling value decrease to value of 8 .This decrease at particular pH values can be attributed to the action of the concentrated solution of caustic soda during the cotton surface functionalisation. However, the cotton coated hydrogel still acquires good swelling character.

4 Preparations of carboxymethylcellulose/CuO bio-nanocomposite hydrogels

CMC interacts with many metal cations, including Al^{3+}, Cu^{2+}, Co^{2+}, Mo^{6+}, and Zn^{2+}[26],due to the porous structure of hydrogels and the existence of carboxylate groups (–CO2−), thus the CMC hydrogels can easily bind to the Cu^{2+}cations in aqueous solutions of copper sulphate via electrostatic interactions. With the suitable basic agent such as NaOH, copper ions are oxidized to CuO nanoparticles. The reaction process can be expressed as follows

$$Cu^{2+} + 4NH_3 \cdot H_2O \rightarrow [Cu(NH_3)_4]^{2+} + 4H_2O \qquad (1)$$

$$[Cu(NH_3)_4]^{2+} + 2NaOH \rightarrow Cu(OH)_2\downarrow + 4NH_3 + 2Na^{2+} \qquad (2)$$

$$Cu(OH)_2 \rightarrow CuO + H_2O \qquad (3)$$

4.1Characterization of cotton loaded with hydrogel containing CuO nanoparticles

The main challenge here is to entrap CuO nanoparticles within the matrix of hydrogel and to provide a proof for its synthesis .To achieve the goal, samples of cotton fabric loaded with hydrogel containing CuO nanoparticles were submitted to XRD analysis, SEM examination and EDS spectral analysis. Results obtained are given under

4.1.1. X-Ray Diffraction (XRD) analysis

The XRD CuO/ CMC nanocomposite hydrogel on fabric in the 2ɵ range of 2–70◦is shown in Fig. 10. The diffractgram of CMC/CuO nanocomposite hydrogel is assigned to diffractions at 2ɵ values of about 35◦, 38◦and 49◦,53°,58°,62° which are assigned to the (110), (002) ,(112) (020),(202)and (¯311) diffractions of CuO crystals, respectively. All the peaks match well with those of monoclinic-phase CuO crystals and confirm the formation of CuO particles in the CMC hydrogel matrix

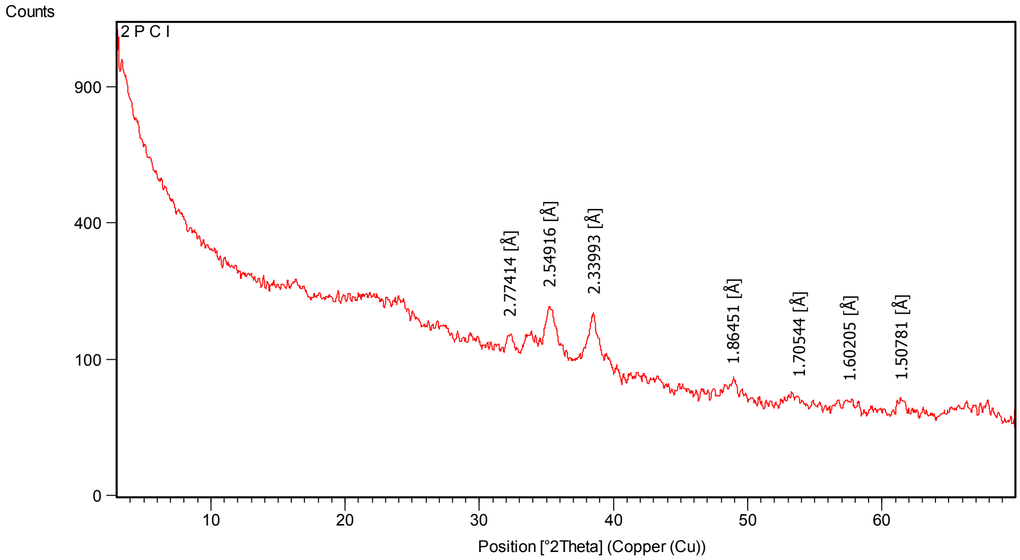
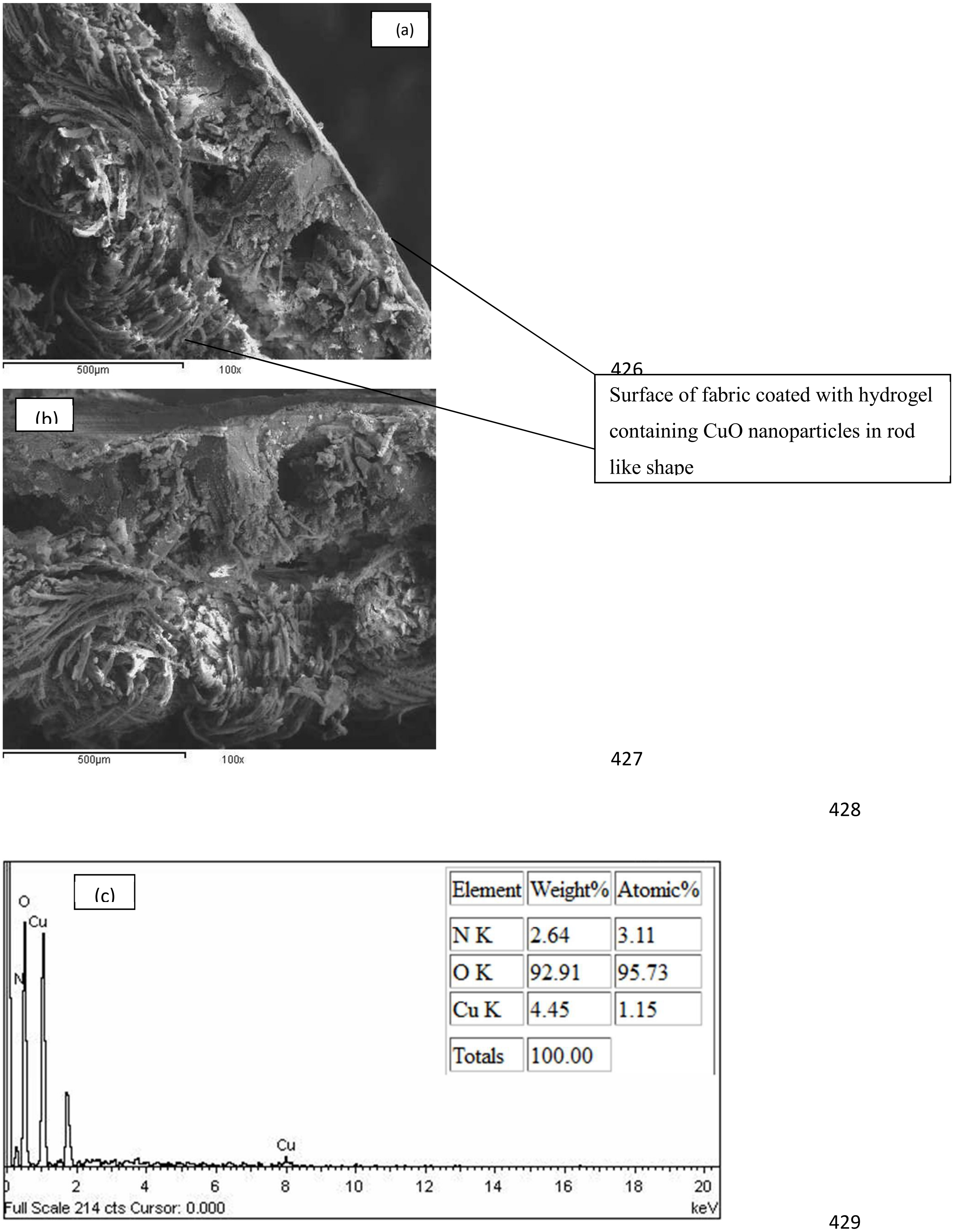


Fig. 10 **XRD patterns of CuO nanoparticles inside the matrix CMC hydrogel**

4.1.2 Scanning electron microscopy (SEM)

Figure 11 show the SEM Comparing surface morphology of the cotton coated with hydrogel layer as shown in figure 9d and that of cotton fabric coated with CMC hydrogel containing CuO nanoparticles as shown in figure11.It was observed that a large amount of needle like structure aggregate of CuO nanoparticle is trapped within the hydrogel matrix forming in homogenous layer on the surface of cotton fabric and at depth inside it



Surface of fabric coated with hydrogel containing CuO nanoparticles in rod like shape

Element	Weight%	Atomic%
N K	2.64	3.11
O K	92.91	95.73
Cu K	4.45	1.15
Totals	100.00	

Figure 11a,b Scanning electron microscope (SEM) images where 11 c EDS spectra of CuO nanoparticles

4.1.3 EDS analysis

EDS spectra of CuO nanoparticles (shown in Fig. 11c) clearly demonstrate the presence of Cu and O peaks with weight prcent 4.45 and high weight prcent of oxygen 92.9 confirm presence of CuO nanoparticles which is consistent with the XRD

5 Synthesis and characterization of silver hydrogel Nanocomposite (Ag/CMC-DADMAC)

When a fully swollen CMC-DADMAC hydrogel in the form of disk is put in the aqueous $AgNO_3$ solution, Ag^+ ions replaces the H^+ or Na^+ ions in the CMC hydrogel. Therefore, Ag^+ ions are still accessible for reduction into nano-silver by sodium borohydride solution forming silver nanoparticles within the swollen network as shown in figure 12

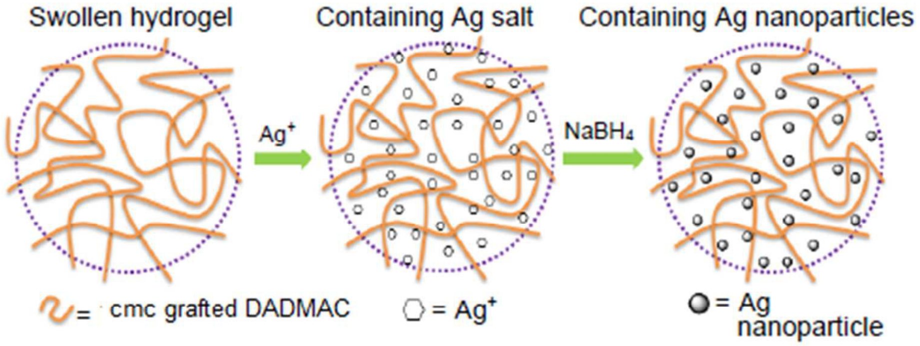
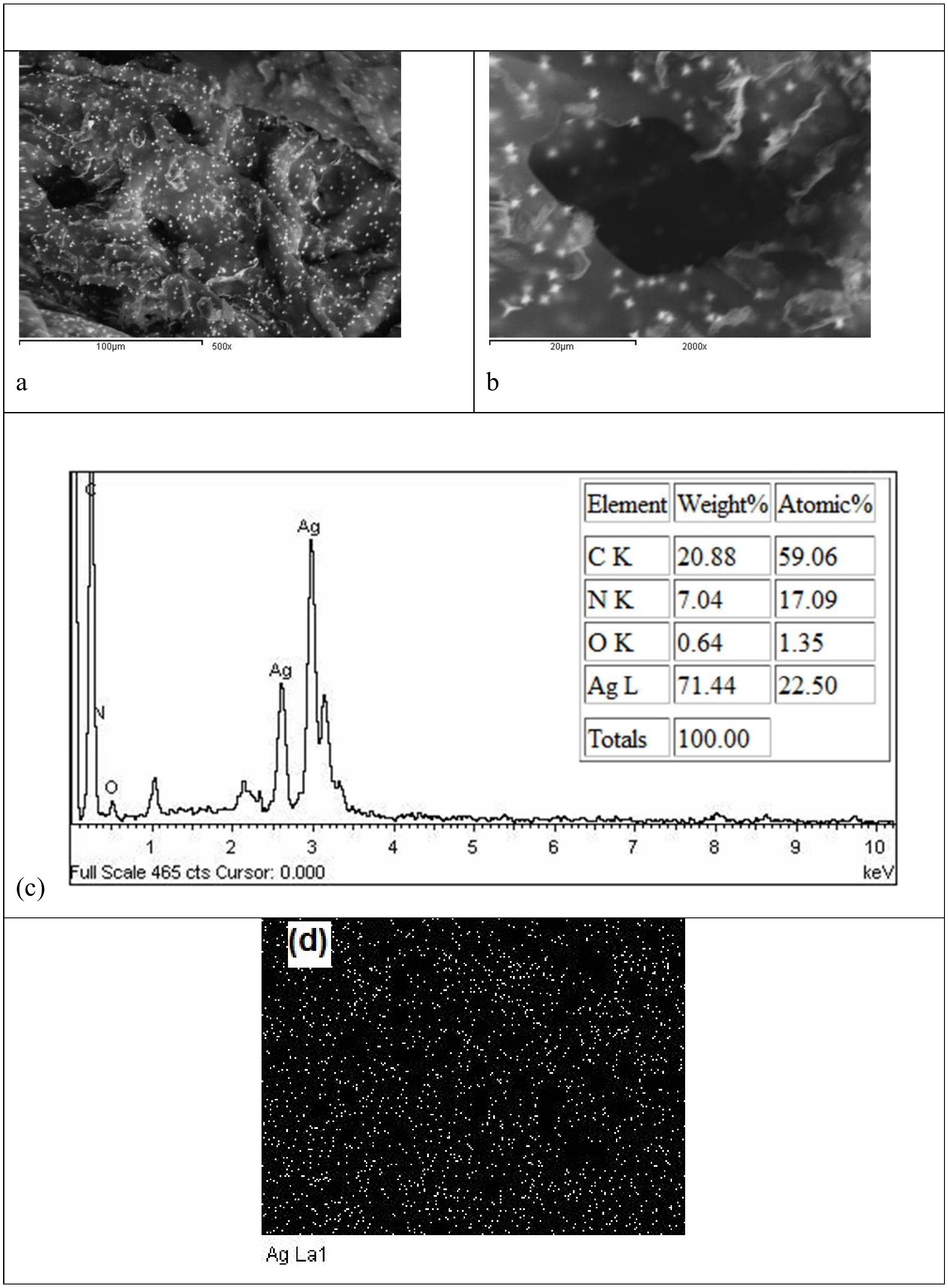


Figure 12: schematic representation of steps involved in preparation of Ag/CMC-DADMAC hydrogel nanocomposite

5.1.Characterization of Ag/CMC-DADMAC hydrogel nanocomposite

The scanning electron micrographs of Ag/CMC-DADMAC hydrogel nanocomposite are shown in figure 13. It is seen that silver nanoparticles are clearly visible not only on the surface of the Ag/CMC-DADMAC hydrogel nanocomposite (Fig. 13a,b) but also inside the network as is visible in the cross-sectional view in Fig. 13 a,b The SEM shows that no serious aggregation of the nanoparticles is formed

This can be interpreted in terms of a stable network structure in the hydrogels in addition to strong interaction between the silver particles and the copolymer hydrogel



Element	Weight%	Atomic%
C K	20.88	59.06
N K	7.04	17.09
O K	0.64	1.35
Ag L	71.44	22.50
Totals	100.00	

Figure 13 The SEM image of a freeze-dried sample of the prepared hydrogel at pH=7.0 and (a),b image of silver nanoparticles grown inside the hydrogel network,, while c the EDS spectrum of the freeze-dried sample of the prepared hydrogel containing silver nanoparticles Figure 13d:The Energy

Dispersive X-ray Spectroscopy (EDS) maps of the CMC–DADMAC hydrogel loaded with Ag nanoparticles

Figure 13 b.c supports also that the silver nanoparticles are formed throughout the network and along with the polymeric network in addition to being exist in free-spaces in the networks. This means that the hydrogel acts as reactor for silver nanoparticles that grow as bright dots distributed between the gel networks with the help of the polymeric chains .This can be seen all over where the silver nanoparticles are overlying on CMC-DADMAC copolymer chains in the hydrogel network .The Energy Dispersive X-ray Spectroscopy (EDX) of the freeze-dried sample of the prepared hydrogel containing silver nanoparticles (Figure 14 c,d) signifies that Ag nano particles are loaded inside the matrix of the hydrogel with high content71% with uniform spatial distribution of Ag nanoparticles on CMC–DADMAC hydrogel nanocomposite

5. Antibacterial activity

Antibacterial activity of CuO/CMC-DADMAC dressing, Ag/CMC-DADMAC hydrogel nanocomposite and CMC-DADMAC (control sample) were studied against Gram-positive and Gram –negative bacteria. The antibacterial activity was determined in terms of inhibition zone on agar medium. It was observed that the control sample did not display any antibacterial activity despite the presence of the quaternary ammonium groups in the copolymer hydrogel (27-29).The effectiveness of the latter as antibacterial seems to be abolished through its intimate association and interaction with CMC in the copolymer hydrogel .that is why the control sample fails to induce antibacterial to CMC-DADMAC copolymer hydrogel .On the other hand , CuO/ CMC-DADMAC nanocomposite hydrogel and Ag/CMC-DADMAC hydrogel nanocomposite can release Cupper and silver nanoparticles into the pathogenic environment (30),thereby producing highly efficient antibacterial activity as may be realized from table 1 .

Results of table 1 show decisively that both CuO/CMC-DADMAC and Ag/CMC-DADMAC hydrogel nanocomposite acquire high antibacterial activity. Inhibition zone around this silver hydrogel nanocomposite exhibit values of 19 and 20 mm/cm sample for CuO nanocomposite hydrogel dressing and 17 and 19 mm/cm sample for Ag nanocomposite hydrogels upon using *Ecoli*(G –ve) and *P.aeuognos*(G-ve)bacteria and 20 , 18 for CuO nanocomposite hydrogel dressing while 18 and 17 mm/cm sample in case of Ag nanocomposite hydrogels upon using St. *aureus*(G +ve) and *B.subtilis* (G +ve).Within this range of studies, it is logical to assume that the antibacterial activity of the materials under investigation relies, in essence, on nature of nanoparticles under investigation as well as the bacteria which ,in turn ,determine the speed and the mechanism of the release of Copper oxide or silver ions from nanoparticles of the hydrogel nanocomposite on one side and interaction of the released ions with cell wall of the bacteria. On the other side, in case of CuO nanoparticles it could be explained in term of attachment of CuO nanoparticles to the cell wall of bacteria which damages the cell wall and causing leakage of proteins and other intracellular constituents and ultimately causes cell death [30-32]. While in case of presence of Ag nanoparticles in the hydrogel matrix, it was reported that (33,34)silver nanoparticles penetrate the cell wall of Gram –ve bacteria. As a result a structural change in the cell membrane occurs. This could lead to an increase in the cell permeability which ,in turn, lead to uncontrolled transport through cytoplasm membrane and ultimately the death of the cell .Another mechanism based on free radical formation followed by free radical-induced damage of the cell membrane .It is also likely that silver ions move into the cell and ,as a result, production of reactive oxygen species takes place which can damage cell wall .It is further reported that greater tendency of silver ions to interact with thiol groups of vital enzymes as well as phosphorous containing bases(35) and with the presence of silver nanoparticles inside the cells (36), it is logical that certain damage could be realized through interactions with compounds such as DNA .This interaction may stop cell division and DNA replication and ended by death of the cell .

Table 1 Antibacterial Activity of CuO/CMC-DADMAC and Ag/CMC-DADMACThe antibacterial activity of silver nanocomposite hydrogel

All experimental test data are done in triplet, and take average value

Inhibition zone diameter (mm/cm) sample				
Sample	***E.coli*** **(G -ve)**	***P.aeuognos*** **(G -ve)**	**St. *aureus*** **(G +ve)**	***B.subtilis*** **(G +ve)**
CMC-DADMAC dressing hydrogel	Zero	Zero	Zero	Zero
CuO/CMC-DADMAC dressing hydrogel	19	20	20	18
Ag/CMC-DADMAC hydrogel	17	19	18	17

Conclusion

Hydrogels with unique properties were synthesized through copolymerization of CMC with DADMAC in presence of APS initiator and MBA crosslinker. Pore size and porous structure of thus obtained hydrogels could be controlled by making use of variables affecting the hydrogels formation. Briefly, CMC-DADMAC copolymer hydrogels having great swelling ratio could be achieved at higher DADMAC monomer concentration. The opposite holds true for either APS or MBA where the hydrogels display low swelling ratio .Particularly notable is the plot of the results of swelling ratio versus pH .Swelling ratio of the hydrogel exhibits striking decrease within a pH range 2-6 as well as at pH8 .On the contrary, the hydrogel with maximum swelling ratio could be achieved at pH 7. The hydrogels under investigation forms also the base for production of wound dressing. The hydrogel is attached to PCMC fabric via ionic crosslinking. Furthermore, antimicrobial activity of the novel hydrogel was examined on (Gram-negative) and (Gram-positive) bacteria according to agar diffusion test. The CuO/CMC-DADMAC nanocomposite hydrogels has shown higher antibacterial activity than Ag /CMC- DADMAC nanocomposite hydrogels against Gram-positive and Gram-negative bacteria. Based on these findings, the prepared nanocomposite hydrogels can be used in different medical fields i.e drug delivery, wound dressing as well as wound healing.

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