Characterization and Evaluation of Antibacterial Properties of Polyacrylamide Based Hydrogel Containing Magnesium Oxide Nanoparticles

I.I. Muhamad,S. A.Asgharzadehahmadi, D. N. A. Zaidel and E. Supriyanto

***Abstract*—P**olyacrylamide based hydrogel was synthesized using sodium carboxymethylcellulose (NaCMC). N,N'-Methylenebisacrylamide (MBA) as cross-linker, ammonium persulfate (APS) and N,N,N',N'-tetra-methylethylenediamine (TEMED) as initiators. Magnesium oxide (MgO) nanoparticles were added to the hydrogel network to investigate the antibacterial activity of synthesized polymer. Hydrogels were characterized using Fourier Transform Infrared Spectroscopy (FTIR), and Field Emission Scanning Electron Microscope (FESEM). The physical and chemical characterizations of the prepared hydrogels give valuable information on the morphological structure of polymer, swelling behavior, bonding formation of gels and physical properties. The incorporation of NaCMC enhanced the hydrogel properties physically and chemically in the aspects of swelling capacity, strength and flexibility. This study also investigated the antibacterial activities of prepared hydrogels against Escherichia coli which is a Gram negative food pathogenic bacteria. For this purpose, agar diffusion test or agar plate test was carried out and inhibition area for each hydrogel was determined. Polyacrylamide hydrogel with NaCMCshowed a lowinhibition zone towards E. coli. However, interestingly, the addition of 0.03 gram of MgO nanoparticles into the hydrogel network resulted in about triple inhibitionstrength relatively.

***Keywords*—**nanoparticles, hydrogel, antibacterial activity, MgOnanocomposite.

I. INTRODUCTION

HYDROGELnanocomposites are cross-linked hydrophilic polymerscontaining nanoparticles having capacity to absorb, swell and retain large amount of water in their crosslinked networks.In particular, the hydrogel nanocomposites in which the hydrogel matrix is combined with inorganic nanoparticles (NPs) have gained much attention during the past few years. Nanostructured materials and nanoparticles are considered as significant types of materials which have obtained lots of interests in medical, catalysis, electronics, and optical applications [1]–[3].Previous findings also show that applying nanotechnology will provide preparing nano vectors for both drug delivery and gene therapy [4]. These become another promising implication to develop chemically derivatized drugs or drug delivery vectors able to target defined cells by means of specific recognition mechanisms and also able to overcome biological barriers [5].

In polyacrylamide based hydrogels, lots of applications have been found such as metal extraction and wastewater treatment. Cross-linked polymers which can imbibe large amount of water can be used in broad various fields such as biotechnology, biomedical engineering, food industry, water treatment and separation process. Due to specific properties like considerable amount of swelling in water, biocompatibility, absorbing water easily or hydrophilicity, and non-toxicity, hydrogels can be used in various fields of biologic, medical, pharmaceutics and environment. In recent years, in order to remove dyes from aqueous solutions, cross-linked polymers which have functional groups like carboxylic acid, amine and hydroxyl were applied as removing agents [5].

Bacteria are single-celled microorganisms which can exist either as independent organisms or as parasites. One of the different types of bacteria is *Escherichia coli* or *E. coli* which normally resides in the human colon. Most strains of *E. coli* are completely harmless, but some strains of it can cause diseases which sometimes lead to catastrophic results.In recent years, using metal nanosystems have been an interesting issue due to their applications in antibacterial activities [5]. The nanosize metals can be more effective in comparison with bulk metal; however, the most controversial issue is how to use these nanoparticles. In the last decades, some studies have demonstrated that the macroscopic gels can be a suitable template for in situ synthesis of nanoparticles.Among metal nanoparticles which have been used as antibacterial agents, MgO has some advantages compare to other metals. Recently, the antibacterial characteristics of MgO nanoparticles have attracted many attentions. MgO is believed to be highly inhibitive properties against bacteria [5]–[8].

Moreover, it is predicted that magnesia or its mixtures with various inorganic materials will improve properties for different applications such as antibacterial activities [7], [8]. In addition, adding sodium carboxymethylcellulose to polyacrylamide will enhance abilities of hydrogel and antibacterial actions will be increased by this new hydrogel

The objective of this work is to characterize the MgOnanocomposite hydrogel and evaluate the effect of synthesized hydrogel towards the inhibition of microbial

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activity.

II. PROBLEM FORMULATION

A. *Materials*

Acrylamide (Sigma–Aldrich), sodium carboxymethylcellulose (NaCMC) (average molecular weight of 250,000, Acros Organic), magnesium oxide nanopowder (MgO) (<50 nm (BET), Sigma–Aldrich), N,N^'methylenebisacrylamide (MBA), Ammonium persulfate (APS), N, N,N^',N^'-tetramethylethylenediamine (TEMED) Sigma–Aldrich), HCl (Qrec Grad AR), NaOH (Qrec Grad AR). Distilled water is used in hydrogel synthesis and all chemicals are used as received with no additional purification.

B. *Preparation of acrylamide hydrogel*

A typical hydrogels synthesis is as follows: 0.4 gram of acrylamide was dissolved in 2 ml of distillated water at 80 ºC and then 0.04 gram N,N'-methylenebisacrylamide (MBA) as crosslinker was added to the solution. The hot mixture was under gentle stirring for approximately 15 minutes to formulate a clear, viscous and homogenous solution with no bubbles. Finally, 0.01 gram N, N,N',N'-tetramethylethylenediamine (TEMED) and 0.01 gram ammonium per sulfate (APS) as initiator successively were added to the solution at the same temperature and the stirrer was bring out immediately, because the solution will change to gel after a few seconds[9].

C. *Preparation of acrylamide/NaCMC hydrogel*

In order to increase the swelling ability of acrylamide, it was blended with Sodium carboxymethylecellulose (NaCMC). To synthesis acrylamide/NaCMC hydrogel at first a solution of acrylamide and N,N'-methylenebisacrylamide (MBA) was prepared as it discussed in previous section. Then 0.05 gram NaCMC was poured into the solution under gentle stirring at 80 ºC. The system was put under reflux to guarantee the water content of the system. After half an hour, 0.01 gram N,N,N',N'-tetramethylethylenediamine (TEMED) and 0.01 gram ammonium per sulfate (APS) as initiator successively were added to the solution.

D. *Preparation of MgO/acrylamide/NaCMCnanocomposite*

MgO/AAm/NaCMCnanocomposites were prepared by blending the MgO Nanoparticles (<50nm) with the polymer matrix. In order to entrap MgO nanoparticles within the network, MgO nanoparticles need to be dispersed in distillated water. First, 0.01 gram of MgO was poured into 2 ml distillated water at 80 °C under rigorous stirring. After almost 10 minutes, acrylamide, N,N'-methylenebisacrylamide (MBA), Sodium carboxymethylecellulose (NaCMC), N,N,N',N'-tetramethylethylenediamine (TEMED) and ammonium per sulfate (APS) were added to the solution respectively, according to the previous section. The whole duration of synthesis takes about 40 minutes and the final disc was approximately 1.5 cm in diameter and a thickness of 40 mm.

E. *Measuring the swelling ratio of hydrogels*

Three different types of hydrogels including acrylamide, acrylamide/sodium carboxymethyl cellulose hydrogels (AAm/NaCMC) and MgO loaded hydrogel were immersed in distilled water at room temperature (25 ºC) to investigate their swelling ability. Samples were placed in a petri dish filled with 50ml of the distilled water separately. Filter paper was used to remove the surface water from swollen hydrogels prior to weighting. Then, swelling ratio (%) was determined using Equation 1.

$$Swelling\ ratio(\%) = \left[\!\left[\frac{w_t - w_0}{w_0}\right]\!\right] x100 \qquad (1)$$

wherew_t is the weight of swollen gels at predetermined time t, and W0 is the initial weight of samples. Samples were immersed in fresh distilled water after weighing. Also, the test was carried out in triplicate and the calculated swelling ratios were reported in the mean values to maximize the accuracy.

F. *Characterization of hydrogels*

The developed hydrogels were characterized for physical and chemical properties and the effect of metal nanoparticles was evaluated.For Fourier Transform Infrared Spectroscopy analysis, 3 mg of the dried samples (at 37 ºC) of blank and loaded hydrogels were ground and then mixed with 10 times as much KBr powder. Hydraulic press was used to form the sample pellets under a pressure of 500 kg/cm2. Prepared samples were analyzed using a Fourier Transform Infrared Spectroscope (FTIR) (Nicolet 670 FTIR, USA) with a 16 scan per sample in the region of 370–4000 cm-1 with 1.0 cm-1 interval and resolution of 4.

Field Emission Scanning Electron Microscopy (FESEM) analysis was done using the previous method [10] with minor modification. FESEM (Zeiss Supra 35 VP, Germany) was used to evaluate the surface characteristics of hydrogels.

G. *Antibacterial studies*

Antibacterial studies were carried out to investigate the effectiveness of MgO loaded hydrogel on gram-negative bacteria *E. coli*. To do this, *E. coli* microorganism was cultivated, then the effect of metal nanoparticles was evaluated.At first 13 grams of nutrient broth were suspended in 1000 ml deionized water. Heating was applied for fully dissolution of the medium. Autoclaving was carried out to dispense and desire the nutrient broth at 15 lbs pressure and 121°C for 15 minutes. After incubation at 36°C for 20-40 hours, the culture characteristics were observed [11]. In order to study the effect of MgO nanoparticle-loaded hydrogels on inhibition of bacteria growth, agar diffusion approach was applied. To do so, *E. coli* was cultured on nutrient broth and the assay medium was maintained at temperature of 40^{0}C for 18 hours. Then the medium was poured to a Petri dish and solidification was carried out. After that the nanoparticle-loaded hydrogel was soaked in deionized water and then it was put on microorganism surface. After one hour the Petri dish was incubated at temperature of 30^{0}C for one day and finally the diameter of gained inhibition zone was measured

[12].

III. RESULTS AND DISCUSSION

A. *Swelling Properties*

The swelling capacity of three different hydrogels in distilled water is shown in Fig. 1. Clearly, by adding NaCMC the amount of swelling was increased considerably. NaCMC has abundant carboxylic acid groups in its structure. It is a polyelectrolyte of a smart cellulose derivative that has shown pH-sensitivity, ionic-strength variations and very good swelling characteristics, as in [13] and [14]. The presence of more hydrophilic chains or hydration of functional groups on the polymeric chains (–OH) and (–COOCH3Na) can cause increasing the hydrogel swelling [15].

On the other hand, MgO nanoparticles cause a negative effect on swelling capacity. These nanoparticles can effect on structure of hydrogel network by reducing the porosity of gel. So the presence of MgO nanoparticles in the network results in a harder gel structure that hinder the swelling of hydrogel.

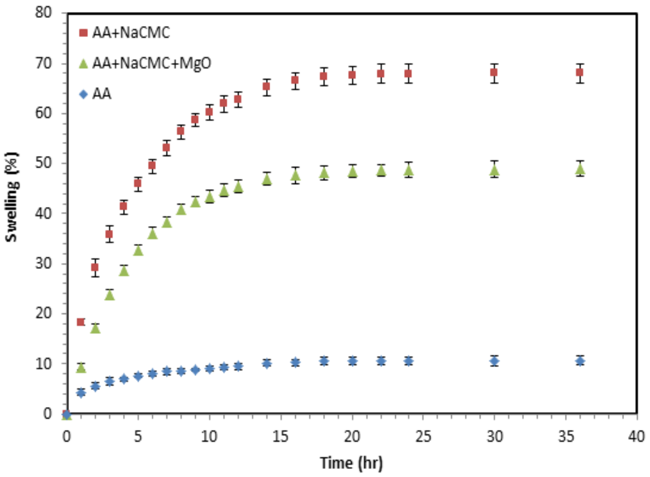


Fig.1 Swelling behavior of AAm, AAm/NaCMC and AAm/NaCMC/MgO hydrogels*versus* time.

The swelling kinetics of synthesized polymers was carried out in order to clarify the controlling mechanism of the swelling process. The data may be well fitted with a Voigt-based equation (Eq. 2)[16]:

$$S_t = S_e\left(1 - e^{(-t/\tau)}\right) \quad (2)$$

where St (g/g) is the swelling at time t, Se is equilibrium swelling (power parameter, g/g), t is time (hr) for swelling, and τ (hr) stands for the rate parameter. For calculating the rate parameter, by using the above formula (after a little modification), one can plot Ln(1-St/Se) versus time (t).The slope of the fitted straight line (slope = -1/τ) gives the rate parameter. The rate parameter for swelling of the three hydrogels is showed in table 1.

Table 1 Swelling rates of acrylamide, acrylamide/NaCMC and acrylamide/NaCMC/MgO hydrogel

Hydrogels	**τ (hr)**	**R^2**
AAm	3.8332	0.9615
AAm/NaCMC	4.6764	0.9953
AAm/NaCMC/MgO	4.1561	0.9983

The τ value is a measure of swelling rate and according to Equation 2, low amounts of τ value demonstrates higher rate of swelling. It means that polyacrylamide hydrogel has the highest rate in comparison with others, as shown in table 2. The second hydrogel (AAm/NaCMC) although has the highest amount of swelling capacity, but represent the lowest amount of swelling rate among these three polymers. Less amount of swelling rate although does not show any important effect on this work but sometimes could be considered as a negative parameter in different applications.

B. *Characterization of Hydrogels*

FTIR analysis was carried out to study the chemical bondings formed in the hydrogel nanocomposite and also to determine the effect of incorporation of substance on the structural changes of polymers [17]. It was noted in reference [18] that the usefulness of this vibrational spectroscopy technique is mainly attributed to its speed and economy against other analytical techniques. Moreover, it offers the possibility to perform on-line analysis of analytical targets and allows the use of deriving information from complex samples because of the development of chemometrics. But, not being a technique that allows separation of the analytes, its application in analysis of related substances is limited. The infra-red spectra ofblank, NaCMC loaded and MgO hydrogel nanocomposite are presented in figure 2.

For MgOnanocomposite hydrogel the broad band formed in the 3600-3800 cm^{-1} range is due to -OH symmetric stretching vibrations of the polyacrylamide/NaCMC hydrogel. This peak in both loaded and unloaded nanocomposites appears at 3733 cm^{-1} which indicates the interaction between the MgO nanoparticles and the network [19].A peak at 1114.25 cm^{-1} and another at 1414.21 cm^{-1} appeared that indicates the interaction of MgO nanoparticles in the network. In addition, appeared peak at 2917 cm^{-1} is intensified when MgO nanoparticles are loaded.

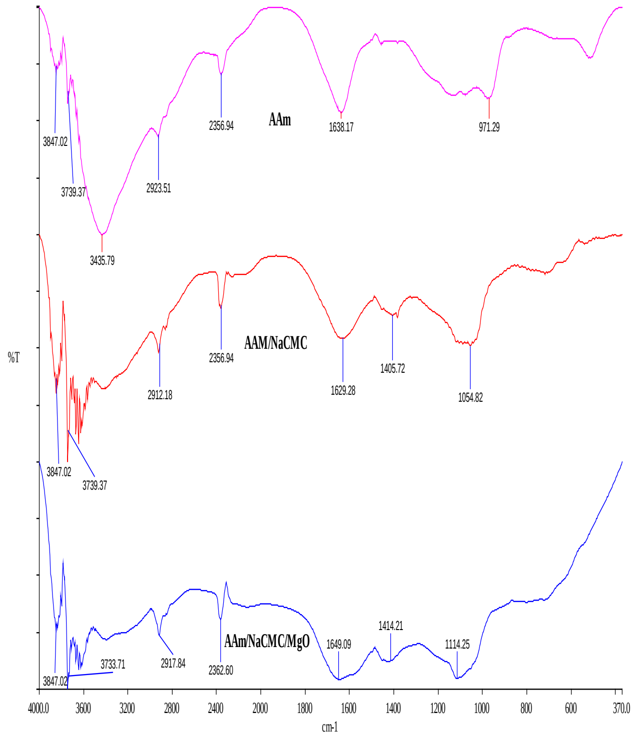
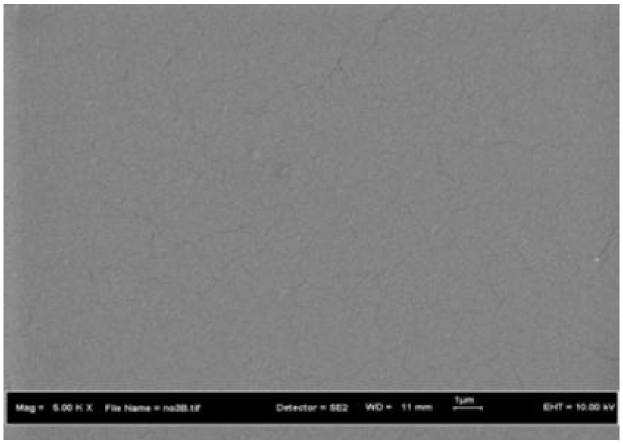
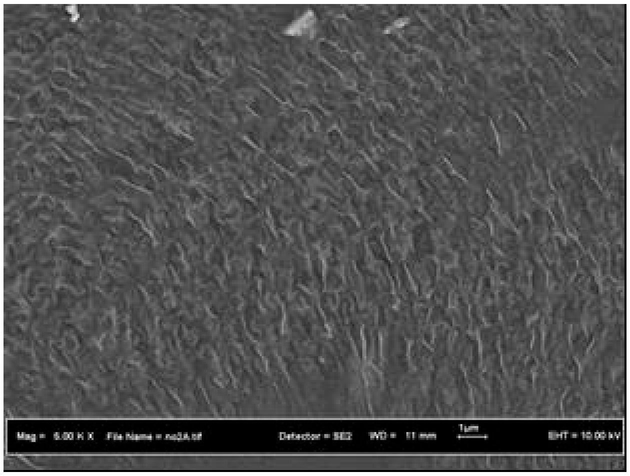


Fig.2.FTIR spectra of AAm, AAm/NaCMC and AAm/NaCMC/MgO hydrogels

Figure 3(a) shows the surface micrograph of polyacrylamide network with NaCMC whereas figure 3(b) demonstrates that of the same polymer with MgO nanoparticles. The micrograph of the first hydrogel shows a somewhat compact matrix with some whitish granule on the surface. This reveals characteristic pattern that correspond to the uniform distribution acrylamide throughout the polymer network. However, as can be seen from Figure 3(b), the addition of MgO nanoparticles has changed the surface morphology of polymer and it is expected the porosity of nanocomposite is also increased.



(a)

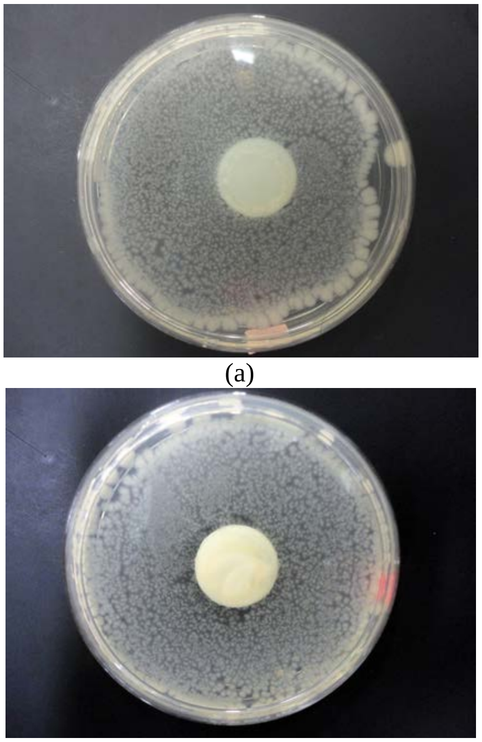


(b)

Fig. 3 The surface micrograph obtained by FESEM for (a) AAm/NaCMC (b) AAm/NaCMC/MgO hydrogels

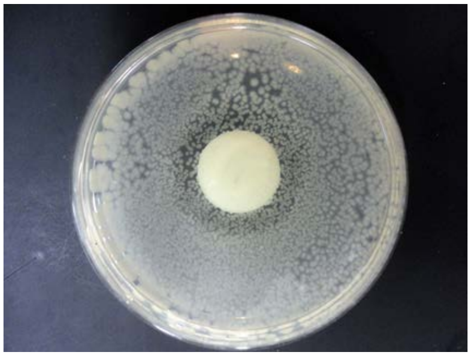
C. *Antibacterial Studies*

Agar diffusion test or agar plate test also known as zone inhibition essay was performed as a preliminary step to screen the antibacterial activity of all hydrogels, in an effort to select a hydrogel with high bactericidal activity against test bacteria [20]. Figure 4 shows the observed inhibitory effect from all the hydrogels towards *E. coli*.which indicated by clear zones surrounding the hydrogel films.

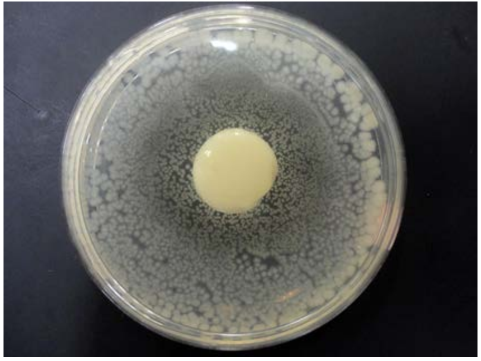


(a)

(b)



(c)



(d)

Fig. 4 Zones of inhibition towards E. coli by(a) AAm; (b) AAm/NaCMC; (c) AAm/NaCMC/MgO$_{0.01}$ and (d) AAm/NaCMC/MgO$_{0.03}$ hydrogels

Antibacterial activity of several hydrogels against Gram negative pathogenic bacteria, *Escherichia coli*, was expressed in term of zone inhibition. The agar diffusion test simulates wrapping of foods, and therefore can be used to estimate how much the antimicrobial agent migrates from the film to the food when the film contacts contaminated surfaces [21].

Table 2 lists calculated inhibition area for each plate tests. The blank polyacrylamide hydrogel showed no inhibition area and colonies were formed all over the plate. Those other hydrogels only could kill the bacteria in its contact surface with the cells.

Table 2 Inhibition of *Escherichia coli* on agar plates expressed as an area (cm^2) of inhibition zone

Hydrogel	***Escherichia coli* inhibition area (cm^2)**
AAm	-
AAm/NaCMC	4.52
AAm/NaCMC/MgO(0.01)	7.06
AAm/NaCMC/MgO(0.03)	15.19

The antibacterial activity of polyacrylamide/ NaCMC hydrogel showed a little inhibitory growth against bacteria, which is because of increase in the amount of swelling and as a result, more contact surface of hydrogel with bacteria. Several hydrogels with different concentrations of MgO were tested against *E.coli* bacteria, however only two of them were considered in this study, because little changes in concentrations usually do not show a considerable effect on inhibition zone. The polyacrylamide hydrogel containing 0.01 gram hydrogel in its instruction successfully inhibited higher growth of bacteria as showed in table 2. However, the highest inhibition growth corresponds to the hydrogel contained 0.03 gram of MgO nanoparticles in its network. Hence increasing the amount of MgO nanoparticles will increase the antibacterial activity of hydrogel, but synthesizing higher amount of MgO in the network was not possible.It can be concluded that the incorporation of MgO nanoparticles into hydrogel network enhance the antibacterial activity towards selective gram positive bacteria.

Amoxicillin is presently the most commonly used semi synthetic antibiotic. It is an analog of ampicillin, with a broad spectrum of bactericidal activity against many gram-positive and gram-negative microorganisms. The presence of a benzyl ring in the side chain extends the antibacterial activity to gram-negative bacteria [22]. The action mechanism of these antibiotics has not been unequivocally established, but it is thought they may interfere with peptidoglycan bacterial cell wall synthesis in the effected organisms. The application of a validated method in analyzing samples from a bioequivalence study involving this MgO-incorporated polyacrylamide based hydrogel is also necessary.

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