NaAlg-PVA-g-AAm/ZnO nanocomposite hydrogel as material of wound dressing: synthesis and characterization

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Abstract. In this study, sodium alginate and polyvinyl alcohol grafted acrylamide with modified ZnO nanoparticles to nanocomposite hydrogel NaAlg-PVA-g-AAm/ ZnO have been successfully synthesized. The characterizations of hydrogels were supported by Fourier Transform Infrared (FTIR), X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM) and Tunneling Electron Microscopy and Energy Dispersive X-Ray (TEM-EDX). The image of TEM obtained shows that ZnO nanoparticle are distributed evenly with about 70 nm diameter in hydrogel nanocomposite. The results for NaAlg-PVA-g-AAm nanocomposite hydrogel modified ZnO nanoparticles was obtained the best nanocomposite at 32.21 ppm Zn-concentration. It has Zn- ion maximum loading capacity at 80 %, maximum release capacity of 25 % measured by AAS, and its water maximum swelling capacity is 230.20 (g/g). While the maximum swelling capacity for NaAlg-PVA-g-AAm nanocomposite hydrogel is 106.30 (g/g). From in-vitro antibacterial activity test, S. aureus (gram positive bacteria) has more resistance than P. Aeruginosa (gram negative bacteria) in which S. aureus inhibition percentage is 60.22 % and P. Aeruginosa is 40.10 % with the minimum inhibitory concentration of 31.25 ppm.

Keywords: acrylamide, hydrogel, nanocomposite, polyvinyl alcohol, sodium alginate

1. Introduction

Hydrogel is a network of hydrophilic polymers that can absorb substance in large amount [1]. One of the biopolymers is sodium alginate (NaAlg) isolated from brown algae with concentration about 40 % of its dry weight [2]. This biopolymer has attracted wide attention because its abundant in nature makes this biopolymer renewable and biodegradable linear polysaccharide and also non-toxic. Other unique natural properties of sodium alginate are its hydrophilicity, biocompatibility and low cost, make it suitable for many biomedical applications [3–5].

Polyvinyl alcohol (PVA) has been widely used to be mixed with alginate because it has good mechanical properties and chemical stability, low cost, non-carcinogenic, and biodegradable. PVA was composited with sodium alginate to form a nanocomposite hydrogel using grafting method with hydrophilic vinyl monomer such as acrylamide (AAm) which can increase its swelling capacity and crosslinking with N, N'-methylene-bis-acrylamide (MBA) to form a hydrophilic polymer network, insoluble in water [6].

In some previous studies, it has been known that ZnO has antibacterial properties that can inhibit gram-negative or gram-positive bacteria. Antibacterial properties are important for wound dressing materials, considering fluid wound is a medium easily overgrown with bacteria. Using a hydrogel matrix as a nanoreactor allows the release of ZnO nanoparticles and Zn ions to be more controlled [7–9]. Zinc oxide is element which effective against microorganisms such as Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Aspergillus niger. Mechanism of action is with penetrating the bacterial cell wall via diffusion leading to cell death [10].

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In this study, we have blended sodium alginate used as the back bone polymer with polyvinyl alcohol loaded with ZnO nanoparticle to form polymeric nanocomposites, based on zinc oxide, an element which effective against microorganisms as has been described previously [10]. The synthesized of hydrogel nanocomposite by free radicals graft polymerization of acrylamide (AAm) using methylenebisacrylamide (MBA) as crosslinking agent and potassium persulfate (KPS) as an initiator. The grafting of acrylamide onto sodium alginate and PVA has been reported previously [3,6]. The effects of zinc nitrate concentration on capacity of Zn\(^{2+}\) loading, the water swelling and the release Zn\(^{2+}\) the superabsorbent hydrogel were investigated. The formation of silver nanoparticles on nanocomposite was confirmed by Fourier Transform Infrared (FTIR), X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM) and Tunneling Electron Microscopy-Energy Dispersive X-Ray (TEM-EDX). The best nanocomposite hydrogels tested to antibacterial activity was done using two types of cultural bacteria, there are P. aeruginosa negative bacteria and S. aureus positive bacteria.

2. Experimental

2.1. Material
Sodium alginate (Sigma Aldrich) as backbone, polyvinyl alcohol (PT. Brataco Chemical) as an additive polymer, acrylamide (Sigma Aldrich) as a monomer, potassium persulfate (Merck co), N, N'-methylene-bis-acrylamide (MercK Co), Zn(NO\(_3\))\(_2\) (Sigma Aldrich), NaOH (Merck co), nutrient agar for microbiology (Merck Co), peptone ISO 6579 (Merck Co) and beef extract Lab-Lemco Powder (Oxoid Ltd.) for bacterial growth medium, chloramphenicol (Bernaform Pharmaceutical) as a positive control on antibacterial tests, culture of Staphylococcus aureus bacteria and culture of Pseudomonas aeruginosa bacteria.

2.2. Synthesis of NaAlg-PVA-g-AAm/ZnO hydrogel
The first step was the NaAlg-PVA-g-AAm hydrogel synthesis, by referring to the reported method [6] with modification. The second step was synthesis of NaAlg-PVA-g-AAm modified with ZnO nanoparticles using the Zn (NO\(_3\))\(_2\) precursor referred to the reported method [7] with modification. The NaAlg-PVA-g-AAm was put in the zinc nitrate precursor solution for 24 hours. Hydrogels that had been loaded with Zn\(^{2+}\) ions were washed with distilled water to remove the zinc ions attached on the surface of the hydrogel. Furthermore, hydrogel was put in 0.2M NaOH for 24 hours, then washed with distilled water, and dried in an oven at 50 °C.

In this work, the effect of the Zn(NO\(_3\))\(_2\) concentration of precursor have been studied at 0, 6.25, 12.5, 31.25 and 62.5 ppm by code H0, H1, H2, H3 and H4, respectively. The nanocomposite hydrogels that were successfully synthesized were characterized by FTIR, XRD, SEM and TEM-EDX.

2.3. Capacity of loading-swelling-release
The swelling capacity of water was determined using the gravimetric method, based on our previous study [11]. The swelling capacity is calculated using equation (1):

\[
\text{Swelling capacity (g/g)} = \frac{m_f - m_o}{m_o}
\]

where m<sub>f</sub> is the initial mass of dry hydrogel and m<sub>i</sub> is the mass of hydrogel after swelling at a given time.

The loading capacity of Zn\(^{2+}\) ionic was determined using atomic absorption spectroscopy (AAS) instrument. The loading capacity is calculated using equation (2):

\[
\text{Loading capacity (%)} = \frac{C_f - C_0}{C_0} \times 100
\]

where C<sub>i</sub> is the initial concentration of Zn(NO\(_3\))\(_2\), and C<sub>f</sub> is the concentration of Zn (NO\(_3\))\(_2\) precursor after loading at a given time.

The release capacity of Zn\(^{2+}\) ionic was determined using atomic absorption spectroscopy (AAS). The release capacity is calculated using equation (3):

\[
\text{Release capacity (%)} = \frac{C_f - C_0}{C_0} \times 100
\]

where C<sub>i</sub> is the loaded concentration of Zn(NO\(_3\))\(_2\) in hydrogel and C<sub>f</sub> is the released concentration of Zn (NO\(_3\))\(_2\) at a given time.

2.4. Antibacterial activity
An antibacterial activity test was performed on a modified ZnO nanocomposite hydrogel with a
Figure 1. The scheme of formation ZnO nanoparticle in hydrogel

variation of Zn(NO$_3$)$_2$ concentrations. The antibacterial activity in-vitro test was carried out by dilution method referred to previous study [12]. Percentage of inhibition is the percentage of bacterial cells that can be inhibited the growth in 24 hours compared to positive and negative controls. The positive controls are bacteria culture with hydrogel of chloramphenicol antibiotic loaded; meanwhile negative controls are bacteria culture without hydrogel. The measurements of turbidity are performed using optical density (OD) at a maximum wavelength 625 nm. The bacteria used were two types, Staphylococcus aureus positive bacteria and Pseudomonas aeruginosa negative bacteria. Percentage of inhibition is calculated using the equation (4):

$$\text{Percentage of Inhibition (\%)} = \left( \frac{\text{OD negative control} - \text{OD sample}}{\text{OD negative control}} \right) \times 100$$

3. Results and discussion

3.1. Hydrogel of NaAlg-PVA-g-AAm/ZnO
In this study, NaAlg-PVA-g-AAm hydrogel was utilized as nanoreactor to form ZnO nanoparticle where the product was known as hydrogel nanocomposite. Polymerization process for hydrogel synthesis was free-radical polymerization by ex-situ method. Alginate macroradical formation was initiated using potassium persulfate. Afterwards, the active site of polymeric chain would initiate acrylamide so that it causes the grafting reaction. The addition of N, N'-methylene-bis-acrylamide as crosslinking agent would create three-dimensional network called hydrogel [6,11]. The method of ex-situ was used to obtain evenly distributed ZnO nanoparticles within the hydrogel matrix. ZnO nanoparticles formation within the hydrogel matrix is explained in figure 1.

Figure 1 shows formation of ZnO nanoparticle in hydrogel. When hydrogel was swollen by Zn(NO$_3$)$_2$ aqueous solution, Zn$^{2+}$ ions entered the hydrogel matrix and filled in the carboxylate and hydroxyl groups along the alginate, polyvinyl alcohol and acrylamide chain. Then, Zn$^{2+}$ ion-loaded hydrogel was added into 0.2 M NaOH aqueous solution, OH$^-$ ions entered the hydrogel matrix and reacted with Zn$^{2+}$ ions to form zinc hydroxide. Furthermore, it was heated at 50 °C for a few hours to obtain ZnO nanoparticles; the ZnO nanoparticles were formed along with the loss of water [7].

3.1.1. Capacity of Zn$^-$ loading. The amount of Zn$^-$ ions loaded into the NaAlg-PVA-g-AAm hydrogel was calculated by measuring the difference in concentration of the remaining Zn(NO$_3$)$_2$, with the initial solution Zn(NO$_3$)$_2$ concentration. The effect of Zn(NO$_3$)$_2$ concentration on the loading capacity of Zn$^-$ ions absorbed into the hydrogel is shown in figure 2.
Figure 2. Effect of Zn(NO$_3$)$_2$ concentration on the loading of Zn$^{2+}$

Figure 3. Swelling capacity of nanocomposite hydrogel

Figure 2 shows that the greater the concentration of Zn(NO$_3$)$_2$, used as a precursor, the Zn$^{2+}$ ion loaded in matrix hydrogel will be increased. It caused the rise concentration of a solution, the greater the collision between particles that occurs so that Zn$^{2+}$ ions the more diffuse into the hydrogel matrix. It shows that at the 31.25 ppm concentration of Zn(NO$_3$)$_2$, with H3 code obtained the maximum loading capacity 80%.

3.1.2. Swelling capacity. Water swelling capacity of nanocomposite NaAlg-PVA-g-AAm hydrogel modified ZnO nanoparticles with various concentrations of Zn(NO$_3$)$_2$, is shown in figure 3. Swelling capacity is measured against time, until a balance time about 300 minutes. It can be seen that the hydrogel without ZnO (H0) has the smallest swelling capacity; this is due to the absence of ZnO nanoparticles so that the hydrogel matrix pore is not expanded. Figure 3 shows that the higher the precursor concentration of zinc nitrate, the greater the swelling capacity of NaAlg-PVA-g-AAm nanocomposite hydrogels modified ZnO nanoparticles. This is due to the more ZnO nanoparticle formed in the hydrogel matrix so that the pore will expand as explained in figure 1, so that its ability increases to absorb water [13,14]. The effect of the Zn(NO$_3$)$_2$ concentration to maximum swelling capacity at 300 minutes are 0 (H0), 6.25 (H1), 12.5 (H2), 31.25 (H3) and 62.5 ppm (H4) obtained 106.30, 146.30, 186.20, 230.20, 226.00 g/g, respectively. It shows that swelling maximum obtained on hydrogel at 31.25 ppm concentration is 230.20 g/g.
3.1.3. Release capacity. Nanocomposite hydrogel swollen at the maximum swelling capacity tested against the release capacity test of Zn$^{2+}$ ions was determined by the atomic absorption spectroscopy (AAS) as shown in figure 4. It shows that the higher the precursor concentration of zinc nitrate, the greater the release capacity of Zn$^{2+}$ ions. Release of Zn$^{2+}$ ions from the hydrogel matrix was related to its maximum swelling capacity. If maximum swelling capacity is higher, it will cause more Zn$^{2+}$ ions to be released from the hydrogel matrix. It shows that maximum release obtained at 31.25 ppm Zn(NO$_3$)$_2$ concentration is 25% at 300 minutes.

3.2. Characterization
The result shows that loading capacity of Zn$^{2+}$, water swelling and the Zn$^{2+}$ release of NaAlg-PVA-g-AAA modified with ZnO nanoparticles obtained the best hydrogel nanocomposite at 32.21 ppm Zn$^{2+}$ concentration is 80%, 230 g/g and 25%, respectively. The best nanocomposite hydrogel was characterized by FTIR, XRD, SEM and TEM-EDX.

3.2.1. FTIR analysis. Figure 5 shows FTIR spectra, in figure 5a, it shows the absorption spectra of the nano ZnO at 475 cm$^{-1}$ peak, that is the identical absorption peak of the Zn-O bond, similar to our
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previous study [5]. Figure 5b shows the spectra of the sodium alginate, the main peak at 752-855 cm\(^{-1}\) is mannuronic acid and 990 cm\(^{-1}\) and 1100 cm\(^{-1}\) are the C-O peaks of uronic acid, both of these peaks are identical band of sodium alginate. The peaks at 1480 and 1700 cm\(^{-1}\) which indicated the asymmetric and symmetric stretching of carboxylate O-C-O [2] and it can be observed in the group of C=O at 1650 cm\(^{-1}\) and C-O at 1300 cm\(^{-1}\) and O-H peak at 3000-3500 cm\(^{-1}\). Figure 5c shows the spectra of the NaAlg-PAVA-AAm hydrogel, there is a characteristic peak of alginate, PVA grafted with AAm identifies the C=O carboxylic group shifts to 1700 cm\(^{-1}\) and C-O 1350 cm\(^{-1}\), this is due to the interaction between the groups and the OH peak becomes wide and sharp at 2900-3500 cm\(^{-1}\); this is caused by a combination of OH groups from alginate, PVA, and overlap with acrylamide amine (-NH\(_2\)) monomers. Figure 5d shows the spectra of hydrogel nanocomposite modified by ZnO nanoparticles. Loading of Zn\(^{2+}\) into the NaAlg-PVA-g-AAm hydrogel network caused electrostatic interaction occur between Zn\(^{2+}\) ions and hydrophilic groups in the hydrogel pores and network. The interaction of hydrophilic groups such as O-H, N-H and carboxylate (C=O and C-O) intensity decrease [6,15]. As for O-H bond peak, its broaden and sharp band, it due to combination O-H of alginate, PVA, overlap amine (-NH\(_2\)) acrylamide monomer and zinc hydroxide remain. It can also be observed that the Zn-O peak shifts to 500 nm, this supports ZnO nanoparticles formed on the hydrogel and indicates the hydrogel has been successfully modified by ZnO.

3.2.2. XRD analysis. To ensure that ZnO nanoparticles were formed within the hydrogel matrix through ex-situ method, the sample was analyzed with X-Ray diffraction. XRD patterns hydrogels can be seen in figure 6. Figure 6a shows diffraction pattern of sodium alginate with \(2\theta =13.30^\circ\) and 21.85\(^\circ\) peaks [2]. Figure 6b shows diffraction pattern of NaAlg-PVA-g-AAm hydrogel can be seen 20 peaks: 19.20\(^\circ\) and 11.84\(^\circ\), it indicated that the peaks derived from PVA and overlap with sodium alginate peaks. Figure 6c shows diffraction pattern of ZnO nanoparticle has sharp peaks, there are 32.30\(^\circ\), 34.52\(^\circ\), 36.40\(^\circ\), 47.30\(^\circ\), 57.10\(^\circ\), 63.40\(^\circ\) and 68.35\(^\circ\), which are the characteristic of ZnO nanoparticle. Figure 6d shows diffraction pattern of NaAlg-PVA-g-AAm/ZnO, it can be observed that all peaks derived from sodium alginate, PVA and ZnO nanoparticle are appeared. However, there is shift of the peaks due to interaction between groups, this supports the results of previous characterizations.

3.2.3. Characterization by SEM. The morphology of surface by SEM is shown in Figure 7. Figure 7b shows the surface morphology of NaAlg-PVA-g-AAm hydrogel has a smooth surface and smaller pores. It shows that graft polymerization reaction had occurred among sodium alginate, PVA and acrylamide, so it caused the pores on hydrogel. Figure 7c shows the surface morphology of NaAlg-PVA-g-AAm modified hydrogel have larger pores. It is due to ZnO nanoparticle have entered into hydrogel so that the pores will be bigger, it can be seen the ZnO nanoparticle have rod-shaped. This supports the swelling capacity to rise after the hydrogel is modified with ZnO nanoparticle.

Figure 6. XRD pattern of NaAlg (a), NaAlg-PVA-g-AAm (b), ZnO (c), andNaAlg-PVA-g-AAm/ZnO (d)
3.2.4. Characterization by TEM. Figure 8 shows TEM image of NaAlg-PVA-g-AAm/ZnO hydrogel nanocomposite. Figure 8a shows that the ZnO nanoparticle are evenly distributed with about 70 nm diameter in hydrogel nanocomposite. Figure 8b shows EDX spectrum NaAlg-PVA-g-AAm/ZnO hydrogel have C, O, Na and Zn elements are 26.56, 22.51, 27.10, and 23.83 %.wt, respectively.

From the characterization of FTIR, XRD, SEM and TEM-EDX can support the formation of ZnO nanoparticles in the hydrogel matrix.

3.3. Antibacterial activity
Antibacterial activity test was done using two types of cultural bacteria, which were Staphylococcus aureus positive bacteria and Pseudomonas aeruginosa negative bacteria. Figure 9 shows the results of antibacterial activity test of NaAlg-PVA-g-AAm/ZnO hydrogel. Antibacterial activity caused by Zn ions and ZnO nanoparticles were released from the hydrogel. In figure 9, it can be seen that positive gram S. aureus bacteria was more resistant compared to negative gram P. aeruginosa bacteria. Maximum percent inhibition for S. aureus and P. aeruginosa was seen at concentration of Zn(NO₃)₂ of 31.25 ppm with inhibition percentage of 60.22 % and 40.10 %, respectively. NaAlg-PVA-g-AAm hydrogel nanocomposite modified with ZnO nanoparticles that have been successfully synthesized is more effective to S. aureus positive gram compare to P. aeruginosa gram negative bacteria.
4. Conclusion
Nanocomposite of NaAlg-PVA-g-AAm hydrogel modified with ZnO nanoparticles has been successfully synthesized. This study has obtained the best hydrogel nanocomposite with loading capacity of Zn$^{2+}$ is 80%, water swelling capacity is 230.20 g/g and the Zn$^{2+}$ release is 25% at 32.21 ppm Zn-concentration. The results were supported by FTIR, XRD, SEM and TEM-EDX characterization. Maximum percent inhibition for S. aureus and P. aeruginosa with 32.21 ppm Zn$^{2+}$ concentration are 60.22% and 40.10%, respectively. Hydrogel nanocomposite modified with ZnO nanoparticles that have been successfully synthesized is more effective for S. aureus positive gram compared to P. aeruginosa negative gram bacteria.

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