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Full Paper

THE EFFECT OF ZnO NANOPARTICLES ON THE PHYSICAL, MECHANICAL, AND ANTIBACTERIAL PROPERTIES OF CHITOSAN/GELATIN HYDROGEL FILMS

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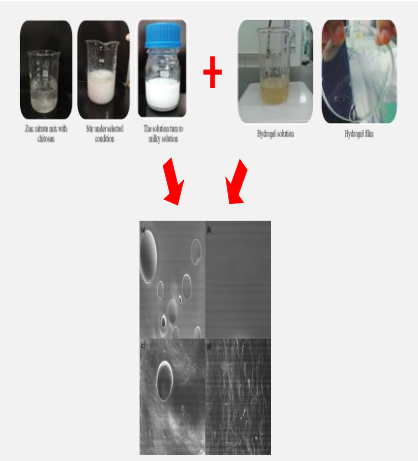
Graphical abstract



Abstract

Apart from biocompatibility, hydrogel films with good physical, morphology, swelling, and antibacterial properties are required for biomedical applications. In this study, the effects of nanofiller (i.e., zinc oxide (ZnO) nanoparticles) on physical, morphology, swelling, and antibacterial properties of hydrogel film were investigated. The new chitosan/gelatin/ZnO hydrogel films were synthesized using solution casting method by blending the chitosan and gelatin solutions with ZnO nanoparticles. The use of glycerol (plasticizing agent) could enhance the durability and flexibility of the hydrogel film. From the FTIR results, it was found that chemical reactions of gelatin occurred at between 1500 cm-1 to 800 cm-1. On the other hand, the SEM results showed that the pore size distribution within the hydrogel film varied from 20µm to 700µm. The antibacterial properties ZnO hydrogel film against bacteria such as Staphylococcus aureus (S. aureus) and Escherichia coli (E. coli) were evaluated using the zone of inhibition method. The antibacterial activity (against the S. aureus bacteria) of the new ZnO hydrogel film was more promising than that of the nanoparticle-free hydrogel film. The current findings showed that the new chitosan/gelatin/ZnO hydrogel film could be used in biomedical application.

Keywords: Chitosan, gelatin, hydrogel film, ZnO nanoparticles, antibacterial

Abstrak

Filem hidrogel dengan ciri-ciri yang bagus dan bioserasi yang sangat baik adalah penting untuk aplikasi bioperubatan. Dalam kajian ini, kesan nanopartikel zink oksida (ZnO) sebagai pengisi nano pada sifat-sifat fizikal, morfologi, bengkak dan antibakteria telah dikaji. Filem hidrogel kitosan/gelatin telah disediakan melalui kaedah acuan larutan dengan menggabungkan larutan kitosan dan gelatin dengan nanopartikel ZnO. Penambahan gliserol sebagai agen pemplastik meningkatkan ketahanan dan kelenturan filem hidrogel sementara kalium klorida (KCl) digunakan sebagai agen penghubung untuk memperkuat filem hidrogel. Hasil FTIR menunjukkan bahawa tindak balas kimia berlaku antara -NH_2 dari kitosan dan –COOH dari gelatin di antara 1500 cm^{-1} hingga 800 cm^{-1}. Manakala analisis SEM menunjukkan saiz liang filem hidrogel adalah dari 20µm hingga 700µm. Filem hidrogel ZnO diuji ke atas *Staphylococcus aureus* (*S. aureus*) dan *Escherichia coli* (*E. coli*) untuk mengkaji aktiviti antibakteria menggunakan kaedah zon larangan. Filem hidrogel ZnO menunjukkan aktiviti antibakteria yang besar terhadap bakteria *S. aureus* berbanding filem hidrogel tanpa nanopartikel. Dari penelitian ini menggambarkan bahawa filem hidrogel kitosan/gelatin/ZnO boleh digunakan untuk aplikasi bioperubatan.

Kata kunci: Kitosan, gelatin, filem hidrogel, nanopartikel ZnO, antibakteria

1.0 INTRODUCTION

Hydrogels are gaining popularity due to their excellent response to changes of parameters such as temperature modulation, ionic strength, pH, and solvent exchange that can lead to the in-situ formation of hydrogel [1]. Hydrogels are a soft and wet material that can be used in different fields of pharmaceutical and biomedical engineering, drug delivery devices, artificial skin, and wound dressing production [2].

Chitosan and gelatin are primary components in hydrogel prepared for wound-dressing film applications [3]. Chitosan is widely used in wound-dressing due to its promising hemostatic properties. It is biodegradable, biocompatible, non-antigenic, and non-toxic. In addition, it has a regenerative effect on connective tissue, by promoting tissue granulation and accelerating wound healing via attracting inflammatory cells (e.g., polymorphonuclear leukocytes and macrophages) to the wound site [4, 5]. Chitosan is a positively charged bio-adhesive that is suitable to form gel. On the other hand, gelatin is a proteinaceous hydrocolloid biopolymer that is biodegradable, biocompatible, swellable (high water absorbance), and can easily undergo chemical modification. Chitosan/gelatin composite is now popular. Both exhibit healing properties with chitosan exhibiting antimicrobial and gelatin exhibiting cell adhesion properties [5]. It also exhibits promising cell adhesion, migration, differentiation, and proliferation properties which are important for wound healing and tissue engineering [6–8]. Previous studies have reported that the addition of silver nanoparticles into the chitosan/gelatin composite have improved mechanical and antibacterial activities [9, 10].

However, chitosan alone with gelatin is difficult to apply into hydrogel because the film will be rigid and needs the plasticization. Glycerol is frequently used in the production of polymer blends. Glycerol could improve the flexibility and ductility of polymer by blending with the rigid polymer. It has many attractive properties, such as a wide range of molecular weights, excellent solubility in an aqueous medium, low toxicity and biocompatibility [11]. Several cations such as K^+ (KCl), Na^+ (NaCl), and Ca^{2+} (CaCl2) have been used as a gelation promoter in previous studies among them potassium chloride (KCl) showed the highest improvement in mechanical and chemical stability of gel [12, 13].

ZnO nanoparticle is an excellent antibacterial agent [14]. In fact, the antibacterial properties of biopolymers could be improved by adding a small amounts of ZnO nanoparticles [15, 16]. Thus, ZnO nanoparticles have been used in the biomedical and cosmetics industries (e.g., wound dressing and toothpaste [17, 18]. In this study, we introduced a green method to synthesize chitosan/gelatin hydrogel film filled with ZnO nanoparticles. The physical, mechanical, thermal, and antibacterial properties of the new hydrogel film were investigated. The Gram-positive and Gram-negative bacteria used were Staphylococcus aureus (S. aureus), and Escherichia coli (E. coli), respectively.

2.0 METHODOLOGY

2.1 Material

In this experiment, all chemicals used were of analytical grade (used without further purification). The zinc cation and the hydroxide anion precursor were zinc nitrate hexahydrate ($Zn(NO_3)_2$. $6H_2O$, 98%, Aldrich) and sodium hydroxide (NaOH, 98.9%, Bendosen), respectively [19]. Acetic acid was used to dilute chitosan which was used as a stabilizing agent, and distilled water was used to prepare the solution.

2.2 Preparation of ZnO Nanoparticles

Chitosan solution of 1.0% v/v was prepared by dissolving chitosan in acetic acid solution of 1.0% v/v. After the chitosan was fully dissolved, 5mL of chitosan solution was mixed with 50mL of $Zn(NO_3)_2$. $6H_2O$ and 50mL of NaOH. A milky solution was then obtained upon the reduction process of zinc ions. The mixture was subjected to microwave radiation (600W) for 6 minutes.

2.3 Formulation of Chitosan/Gelatin Hydrogel

The chitosan/gelatin hydrogel solution was prepared by employing the solution casting method. Here, 20ml of 2% v/v of chitosan was stirred using a magnetic stirrer with 1ml of glycerol (plasticizer). The gelatin solution was prepared by dissolving 10g of gelatin powder in 50ml of distilled water. The mixture was then subjected to stirring for 30 minutes at 40°C. Next, 1ml of glycerol was added into the solution. Both chitosan and gelatin solutions were mixed and stirred for 12 hours at 40°C. Next, cross-linking agent, i.e. 1M of KCl (3ml) was added into the mixture of chitosan and gelatin. Then, ZnO nanoparticles (6.4 ml) were added, and the mixture was stirred for 1 hour at room temperature. The mixture was then poured on a petri dish, and cooled at 4°C to form a film.

2.4 Testing and Characterization

2.4.1 FTIR of Hydrogel Film

The FTIR absorption spectra of wave number ranging from 4000 cm^{-1} to 400 cm^{-1} were scanned using the FTIR model: iD7 ATR Nicolet iS5 Spectrometer (Thermo Fisher, USA).

2.4.2 Morphology of Hydrogel Film

The morphologies of ZnO nanoparticles were observed using SEM (JSM-7800F, JEOL, USA) at high magnification (150 and 500).

2.4.3 Mechanical Testing

Tensile strength was measured using the Instron universal testing machine (cross-speed of 10 mm/ min). All films were cut into 10.0 mm x 30.0 mm x 1.0 mm size. The Young modulus was calculated from the slope of the linear portion of the stress-strain curve. For each sample, the test was repeated thrice.

2.4.4 Swelling Ratio Testing

In order to calculate the film swelling ratio (SR), the pre-weighed film samples (2.5 cm × 5 cm) were immersed in distilled water. SR can be calculated in the following way:

$$SR(\%) = \frac{W_2 - W_1}{W_1} \times 100$$

where W1 and W2 are the weights of the film before, and after swelling, respectively.

2.4.5 Antibacterial Activity by Zone of Inhibition Method

The antibacterial activities of the ZnO nanoparticles were tested against Gram-positive (S. aureus), and Gram-negative (E. coli) bacteria. Firstly, a total of 50µL of 108 CFU/ml bacteria suspension was spread on the tryptone soya agar (TSA) plate. Next, the freshly prepared ZnO nanoparticles (30 µL) were deposited on a sterile disc which was then placed on the surface of the agar and incubated overnight at 37°C. Finally, the diameter of the zone of inhibition was measured [20].

3.0 RESULTS AND DISCUSSION

3.1 FTIR Analysis

The chemical interaction between chitosan/gelatin, and ZnO nanoparticles was studied using FTIR spectroscopy. The results are shown in Figure 1. Some characteristic peaks were apparent in the range of 4000cm^{-1} to 400 cm^{-1}. Chitosan (denoted by(a)) was represented by two typical peaks, i.e. 3277 cm^{-1} and 1636cm^{-1} attributed to OH and NH_2, respectively. For chitosan/gelatin mixed with ZnO nanoparticles, the broadening of peak was found to be within the range (3277cm^{-1} to 3284cm^{-1}), and was attributed to the intermolecular hydrogen bonding between ZnO and chitosan [21, 22]. By comparing chitosan/gelatin hydrogel (b) and chitosan/gelatin/ZnO hydrogel (c), the formation of new peaks in the range of 1500cm^{-1} to 800cm^{-1} was due to the interaction between the carboxylic acid groups in gelatin and the amine groups in chitosan. Our results exhibited the same patterns as those reported by Chang and Xiao [23] and Ahmad *et al*. [9]. Both amino and carbonyl bands were shifted in the spectra of hydrogel, indicating the formation of complex hydrogen bonding between chitosan and gelatin [24]. The formation of band within 1034cm^{-1} and 1048 cm^{-1} was due to the interaction between OH group of glycerol and gelatin[25, 26]. The emergence of ZnO nanoparticles at 522cm^{-1} was also detected [21].

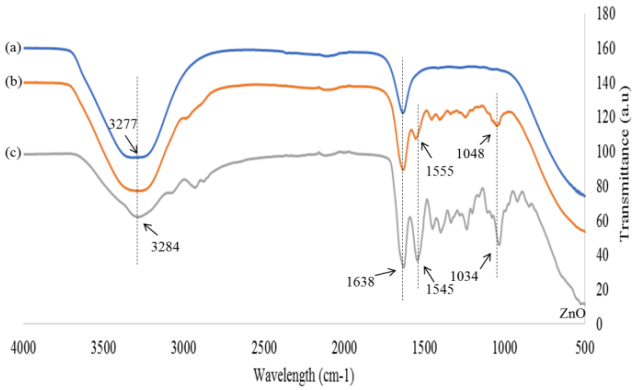


Figure 1 FTIR spectra of chitosan and chitosan/gelatin hydrogel films

3.2 Morphology and Structural Properties of Hydrogel Films

The surface morphology of hydrogel film was examined using SEM (see Figure 2). Water molecules could diffuse easily within the inter-connected pore structure of hydrogel films as shown in Figure 2(a) and Figure 2(c). For chitosan/gelatin hydrogel, the pore size ranged from 40µm to 700 µm. On the other hand, the pore size of chitosan/gelatin/ZnO hydrogel ranged from 20µm to 700µm as shown in Figure 2(b). The addition of ZnO nanoparticles also can act as a nanofiller, which can enhance the density of hydrogel film, and leads to the porosity of hydrogel film being reduced [27]. The presence of pores in the hydrogel film could allow good oxygen, nutrient permeabilities and excellent retention of tissue fluids, which could offer tremendous benefits in biomedical application [28]. As shown in Figure 2(d), the surface of the chitosan/gelatin hydrogel was smoother than that of the chitosan/gelatin hydrogel with ZnO nanoparticles. The presence of ZnO nanoparticles would roughen the surface. No agglomeration of ZnO nanoparticles was found. The surface morphology was comparable to those where nanoparticles were added into polymer matrices such as gelatin, agar, and carrageenan [29–31].

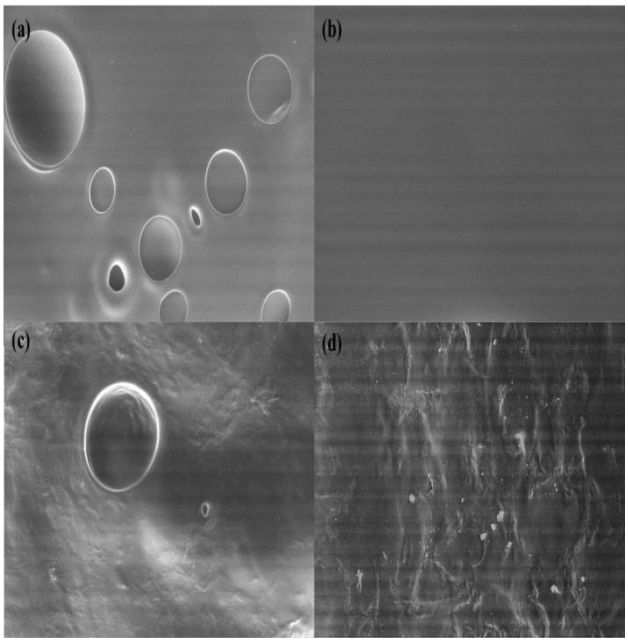


Figure 2 SEM images of the surface (left side) and cross-section (right side) of chitosan/gelatin and chitosan/gelatin/ZnO hydrogel film

3.3 Mechanical Properties of Hydrogel Films

The tensile properties of the hydrogel films such as tensile strength (TS) and elongation at break (E) are presented in Table 1. The TS of chitosan/gelatin hydrogel film was recorded at 26.44 kPa. Upon adding the ZnO nanoparticles, the TS of the hydrogel film increased significantly to 80.00 kPa. As shown in the SEM results (Figure 2(C)), ZnO nanoparticles enhanced the mechanical properties of hydrogel film by blocking the pores within the hydrogel [27]. The addition of ZnO nanoparticles into hydrogel film resulted in the fast deterioration of elongation at break of the hydrogel film. A possible reason for this phenomenon is related to moisture content. Although water can play a plasticizing role in the biopolymers matrix, increasing the plasticizer content increases the flexibility of the films [32].

When ZnO nanoparticles were mixed with hydrogel film, new hydrogen bonds were formed between chitosan and gelatin, thus weakening the intermolecular hydrogen bond between chitosan and gelatin. A close comparison between the FTIR curves of the systems studied, revealed that the intensities of hydrogen characteristic bands increased. The increment was found to be more pronounced after the addition of chitosan/ZnO nanoparticles. Indeed, the relative increase in the intensity of hydrogen bond peaks could be interpreted as evidence of saturation and decrease of crosslink density [33]. Therefore, the movement of macromolecular chain was less rigid (easier to break) [28].

Table 1 Mechanical properties of hydrogel films

Hydrogel films	TS (kPa)	E (%)
Chitosan/gelatin	26.44	54.36
Chitosan/gelatin/ZnO	80.00	60.52

3.4 Swelling Ratio of Hydrogel Films

The swelling capacity is an important parameter describing the ability of hydrogel to absorb fluids in a reversible manner. In order to study the absorption behaviour, the films were immersed in water and the new weights were measured after 30 minutes. The swelling ratios (SR) of chitosan/gelatin hydrogel film and chitosan/gelatin/ZnO hydrogel film are presented in Table 2. The SR of Chitosan/gelatin hydrogel film was 21.85%. Meanwhile, the SR of chitosan/gelatin/ZnO hydrogel was very high at 232.15% due to the improved mechanical properties in flexibility, which provided sufficient mechanical strength to maintain the stability when the hydrogel absorbed the fluids [34]. The new hydrogel film could maintain its integrity during water absorption [26]. Besides that, the open pore structure (shown in SEM) was favorable for faster swelling and greater water retention [35].

Table 2 Swelling properties of hydrogel films

Hydrogel films	SR (%)
Chitosan/gelatin	21.85
Chitosan/gelatin/ZnO	232.15

3.5 Evaluation of Antibacterial Activity by Zone of Inhibition Method

The antibacterial activities of hydrogel films against Gram-positive bacterium (S. aureus), and Gram-negative bacterium (E. coli) are presented in Figure 3. The inhibition zones of Chitosan/gelatin/ZnO hydrogel films towards S. aureus and E. coli were comparable, and similar to that observed by Mohandas *et al.* [22]. Nevertheless, the inhibition zone observed on the S. aureus stain was more prominent, suggesting that the new hydrogel film (chitosan/gelatin/ZnO) was more effective against S. aureus [30].

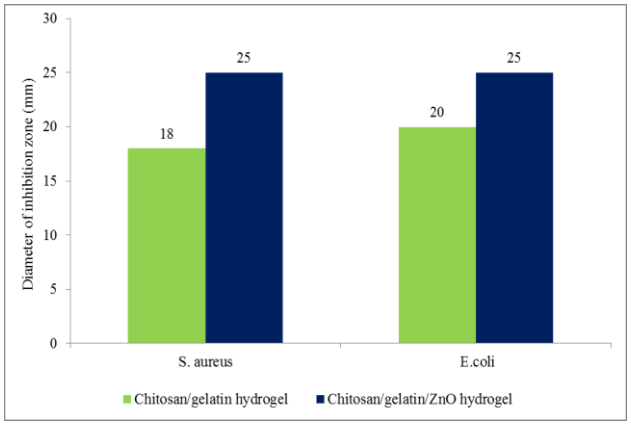


Figure 3 Inhibition zone of Gram-positive (*S.aureus*) and Gram-negative (*E. coli*) for hydrogel films of chitosan/gelatin and chitosan/gelatin/ZnO

Some previous studies also reported the same results. Anitha *et al*. (2013) reported on antibacterial properties of cellulose acetate (CA) membrane embedded with ZnO nanoparticles. Their result showed that the CA membrane exhibited stronger antibacterial activity against Gram-positive bacteria (*S. aureus*) than Gram-negative bacteria (*E. coli*). Nafchi *et al.* (2012), on the other hand, studied the antibacterial properties of sago starch films filled with nanorod-rich ZnO oxide. Their result showed that the film was more active against S. aureus than E. coli. It is believed that antibacterial activity of film incorporated with ZnO nanoaprticles mainly depends on the cell wall structure of Gram-positive and Gram-negative bacteria. Gram-positive bacteria have a thick cell wall structure with multilayers of peptidoglycan, while Gram-negative bacteria have a complex cell wall structure with thin peptidoglycan layer surrounded by an outer membrane [36, 38, 39]. The presence of hydrogel film with embedded ZnO nanoparticles directly bind with the outer cell wall of Gram-positive bacteria, which contains plenty of pores to make easy penetration of ZnO nanoparticles into the cells, thus causing leakage of intracellular contents and leads to cell death [40]. But in the case of Gram-negative bacteria, the ZnO nanoparticles initially bind with bacterial outer cell membrane which contains lipoprotein, lipopolysaccharide, and phospholipids that may reduce the attachment of ZnO nanoparticles [36]

4.0 CONCLUSION

In this study, chitosan/gelatin based hydrogel films incorporated with ZnO nanoparticles were synthesized using solution casting method. The FTIR results showed good compatibility between ZnO nanoparticles and the polymer matrix. The pore size range of the new hydrogel film is wider and no agglomeration of ZnO nanoparticles was found (homogeneous distribution). The tensile strength and percentage of elongation of the new film were higher than those of the hydrogel film without ZnO. Water holding capacity of the new hydrogel film has also improved. In addition, the new hydrogel film is more effective against S. aureus bacteria. In general, the new hydrogel film proposed in this study could be employed in biomedical applications.

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