Effect of glycerol on mechanical and physical properties of silver-chitosan nanocomposite films

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Abstract. The effect of using glycerol as plasticizer on mechanical and physical properties of silver-chitosan nanocomposite films have been studied. The nanocomposite films were prepared via three steps consisting of silver-chitosan colloidal nanocomposites preparation, adding of glycerol to colloids and silver-chitosan nanocomposites films formation. During the first step, silver ions were reduced by glucose and accelerated by sodium hydroxide (NaOH). Chitosan of 1 % (v/v) act as stabilizing agent. Glycerol with volume variation of 0.2, 0.4, 0.6, 0.8 and 1.0 mL was added colloidal nanocomposites of 60 mL on the second step. On the third step, colloidal nanocomposites were cast on the polypropylene plate and dried at room temperature. The as-prepared films were then neutralized by NaOH and rinsed with distilled water until the filtrate reached the pH of 7. The colloidal nanocomposites were characterized by UV-Vis spectroscopy and transmission electron microscopy (TEM). The film were characterized by X-ray diffraction (XRD) and thermogravimetric analysis (TGA). The mechanical properties, swelling capacity, water vapor permeability (WVP) of the films were also studied. The results indicated that the addition of different amounts of glycerol on colloidal nanocomposites effects on mechanical and physical properties of the resulted nanocomposite films. The elongation and tensile strength were gradually increased as the glycerol amount. Meanwhile, the swelling capacity, WVP, and crystallinity of the film also showed enhancement at increasing glycerol amount. However, the thermal stability decreased.

1. Introduction
Chitosan is available natural polymer derived by deacetylation of chitin, which is the second most abundant polysaccharide found in nature [1]. Compared to other polysaccharides, chitosan has several important advantages, including biocompatibility, biodegradability, and no toxicity[2]. Moreover, several studies have indicated that chitosan possesses antibacterial [3,4,5] and antifungal[6] activities. This resulted in widespread use of chitosan in food, pharmaceutical, and environmental industries [7].

Like the film, chitosan finds specific applications such as wound dressings and food packaging. Chitosan is insoluble in most common solvents (including water), but it can be dissolved in dilute aqueous acidic solutions [7]. It has been established that aqueous acetic acid is one of the most suitable solvents for film preparation resulting in good film properties such as tensile strength, strain-at-
break (extensibility), resistance to water and water vapor permeability [8]. Several researchers have incorporated chitosan with nanoparticles metal to form nanocomposite films to improve its antibacterial [9,10,11,12]. One of the widely studied nanoparticles is silver (Ag).

Recent work has shown that combining chitosan or modified chitosan with Ag resulted in films and hydrogels materials with enhanced antimicrobial activity, increased tensile strength but decreased water vapor permeability [9,13,14]. It was also found, that combining low molecular weight chitosan with Ag nanoparticles yielded materials with high antibacterial activity against Gram-positive bacterium of *Staphylococcus aureus* and Gram-negative bacterium of *Escherichia Coli* as well as displayed promising wound healing characteristics [5].

Unfortunately, chitosan films are rigid and need plasticizers to reduce frictional forces among polymer chains, such as hydrogen bonds or ionic forces, in order to improve mechanical properties. Several plasticizers have been used to improve the mechanical properties film, including sorbitol, PEG, and glycerol [14]. Glycerol is a polyol compound which is widely used in a diverse range of industries, for example, in food and cosmetics industries. It is added as a solvent, plasticizer, humectant, emollient, and lubricant. The addition of glycerol can improve film biopolymer film properties. The addition of glycerol reduced the tensile strength of the chitosan film [15]. The presence of glycerol resulted in less resistant, more elastic and more permeable films. It is also observed that WVTR decreased, but the elasticity improved [16].

In this study, the nanocomposite silver-chitosan films were prepared by adding glycerol as plasticizer. The effect of the presence of various amount of glycerol as plasticizer on mechanical and physical properties such as tensile strength, elongation at break, crystallinity, swelling, water vapor permeability (WVP) and thermal properties of the film is described.

2. Experimental

Chitosan with a molecular weight (MW) of 1,077,919.28 Da and degree of deacetylation (DD) of 73.44% was purchased from Biotech Surindo Cirebon Indonesia. Silver nitrate (AgNO$_3$), acetic acid (CH$_3$COOH), glucose (C$_6$H$_12$O$_6$) sodium hydroxide (NaOH) and glycerol were purchased from Merck.

A solution of chitosan (1% w/v) in acetic acid solution (1% v/v) was firstly prepared by stirring and then kept overnight at room temperature. The solution was filtered to remove any impurity before use. 1.0 mL of aqueous solution of AgNO$_3$ (0.012 g/mL) was added to 25 mL of Chitosan (1% w/v) in a flask. The solution was stirred at room temperature for 10 min, and then an aqueous solution of glucose was added so that the molar ratio of AgNO$_3$/glucose is 1:4. The solution was stirred for 10 min. After that, 2.5 mL of aqueous solution of NaOH (2M) were added. The gel was immediately formed in the solution, and then the color was turned brown. The reaction was continued for 25 min under stirring. Then, the resulted gel was dissolved in 35 mL chitosan (1% v/v) and stirred to achieve complete dissolution forming a solution of silver-chitosan colloidal nanocomposites. Glycerol with volume variation of 0.2; 0.4; 0.6; 0.8 and 1.0 mL were added to the colloidal solution and stirred for 30 min. Then, the colloidal solutions were cast onto polypropylene plates and dried at room temperature for 6 days to obtain the composite film. To get a neutral film, the films were neutralized using NaOH then rinsed with distilled water until the filtrate reached pH of 7. The films dried on glass board until dry. The films were denoted as Ag/CG1, Ag/CG2, Ag/CG3, Ag/CG4, Ag/CG5.

Localized surface plasmon resonance (LSPR) colloidal nanocomposite was determined by Shimadzu UV3150 UV-Vis spectrophotometer, operating in the absorbance mode. UV-Vis absorption spectra of the samples were recorded in the wavelength range of 300 to 600 nm. For colloidal nanocomposite, the sample was diluted 10 times before analysis. The nanocomposite film, the sample was placed in the cuvette holder.
Size and distribution of particles were characterized by Transmission electron microscopy JEM-2000EX at an accelerating voltage of 120 kV. Particle size analyses were performed using ImageJ 1.43u software.

The tensile strength (TS) and elongation at break (E) of the films were measured with Zwick Universal Testing Machine type of Z 0.5. Tensile strength values were reported as measured maximum load (N) divided by film cross-sectional area (mm$^2$) with a unit of MPa. Elongation values were obtained by recording elongation at break divided by initial length of the specimen and multiplied by 100 [17].

The X-ray diffraction method was used to identify the formation of nanoparticles in the films and their crystallinity. These measurements were carried out for dried and finely grounded samples on XRD-6000 Shimadzu. Measurement of X-ray diffraction method is done by using Cu Kα radiation source ($\lambda = 1.54060$ Å) at a voltage of 40 kV and current of 30 mA, nickel metal filter, the scanning rate of 5/min at 3-80° 2θ region.

The swelling ratio (Q) was determined by gravimetric method [18]. Dried films were swollen in (100ml) phosphate buffer (pH 7.4) solution at 25 °C. The weight of swollen films was measured at equilibrium swelling after removing the surface solution with filter paper. The swelling ratio (Q) was calculated using Equation (1):

$$Q = \frac{W_e}{W_d}$$  \hspace{1cm} (1)

where $W_e$ is the weight of the swollen film at equilibrium and $W_d$ is the dry weight of the film.

Thermal degradation processes were investigated using TGA Parkin Elmer at 25.00°C to 700.00°C with the heating rate of 10 °C/min under nitrogen atmosphere.

Water vapor permeability (WVP) was determined gravimetrically according to the standard method ASTM E 96-95 with some modifications [19]. The films were fixed on top of test cells containing a desiccant (silica gel). Test cells then were placed in a relative humidity chamber with controlled temperature and relative humidity (28 °C and 75% RH). After steady-state conditions had been reached, the weight of test cells was measured every 1 h over 8 hours. The water vapor transmission rate (WVTR) was determined using Equation (2).

$$WVTR = \frac{G}{A} \cdot \frac{t}{h} \cdot \frac{mm}{m^2}$$  \hspace{1cm} (2)

where $G$, weight change (g); $t$, time (h) and $A$, test area (m$^2$). WVP was then calculated using Equation (3).

$$WVP = \frac{WVTR \cdot d}{\Delta p} \cdot \frac{mm}{kPa \cdot m^2}$$  \hspace{1cm} (3)

where $d$, film thickness (mm) and $\Delta p$, the the partial pressure difference across the films (kPa).

3. Result and discussion
Silver-chitosan nanocomposite films were prepared by casting silver-chitosan colloidal nanocomposites at room temperature. The silver-chitosan colloidal nanocomposites have been performed at room temperature by chemical reduction using NaOH as an accelerator, glucose as the reducing and chitosan as stabilizer agent. The color of the colloidal solution was from colorless to brown indicating the formation of Ag nanoparticles.

The brown color was due to the localized surface plasmon resonance (LSPR) absorption of silver nanoparticles [20]. The surface of a metal is like a plasma, having free electrons in the conduction band and positively charged nuclei. LSPR is a collective excitation of the electrons in the conduction band near the surface of the nanoparticles [20]. The electrons are limited to specific vibrations modes by the particle’s size and shape. Metallic nanoparticles have the characteristic of optical absorption spectrums in the UV-Vis region [21].

The UV–Vis absorption spectra of silver-chitosan colloidal nanocomposite solution and film are shown in Figure 1 with the related value of $\lambda_{max}$ (nm) and absorbance. The result silver nanoparticles displayed absorption peaks, centered at 404.6 and 424.02 nm for colloidal solution and film respec-
The observed single LSPR band indicates that the silver nanoparticles are spherical in shape [20]. It is confirmed by the TEM images (Figure 2).

**Figure 1.** The UV-Vis spectra and image of silver-chitosan nanocomposite (a) colloidal solution and (b) film (Note: the colloidal nanocomposite was diluted 10 times before analysis)

**Figure 2.** (a) TEM images of silver nanoparticles and (b) the corresponding particle size distribution

Figure 1 shows UV–Vis absorption spectra of silver-chitosan nanocomposite films prepared from the colloidal nanocomposites sample. The film also showed a strong band in the visible region due to the characteristic surface plasmon resonance of silver nanoparticles. Compared to the colloidal nanocomposites, the film shows red-shifted significantly that might be caused by increase particle size of silver due to aggregation during the evaporation process.

The mechanical properties of silver-chitosan nanocomposite films were improved by adding glycerol as plasticizer. Figure 3 shows the tensile strength and elongation at break of silver-chitosan nanocomposite films at various glycerol amounts. The results show a slight increased at the initial addition of glycerol (for sample Ag/C to Ag/CG3 ) result in a slight increase in tensile strength and elongation. But, at higher plasticizer amount of plasticizer (for sample Ag/CG4 and Ag/CG5) tensile strength and elongation of films significantly increase. The increasing both tensile strength and elongation indicated that plasticizer of glycerol strengthens the film structure and integrity. Glycerol
molecule in the film may construct the crystalline formation during the process and strengthen intermolecular hydrogen bonding among chitosan molecule.

![Graph](image1)

**Figure 3.** The effect of plasticizer concentration on mechanical properties of silver-chitosan nano-composite films ((a) tensile strength and (b) elongation)

The swelling capacity of the films is one of the important criteria for maintaining a moist environment. Figure 4 shows the effect of glycerol content on nanocomposite films. It is observed that swelling capacity of the films increases increasing glycerol content in the films. This could be due to the formation of the hydrophilic structure of nanocomposite films with the addition of glycerol. Three hydroxyl groups in glycerol structure may contribute to enhanced hydrophilicity. The presence of more hydrophilic groups in the film network will assist in improving the swelling characteristic of the film [22].

However, the water vapor permeability (WVP) of the films do not significantly increase as the glycerol amount increase (Figure 5). WVP is a value for water permeation at a given temperature. The permeability of a film depends on its chemical structure and morphology, the nature of permanent and temperature of the environment [23]. The significant increase of WVP is observed for the silver-chitosan-glycerol film in comparison to the film without glycerol. This could be due to an additional hydroxyl group in polymeric chains. Hydrophilicity induces an increase in water vapor permeability.

![Graph](image2)

**Figure 4.** Swelling capacity of nanocomposite films at various plasticizer amounts

**Figure 5.** WVP of nanocomposite films at various plasticizer amounts.
The X-ray diffraction (XRD) is used to confirm the formation of silver nanoparticles. The XRD pattern of chitosan film, silver-chitosan nanocomposite film and silver-chitosan nanocomposite film plasticized by glycerol are shown in Figure 6. The amorphous structure of chitosan film is confirmed by broad peaks appeared at a diffraction angle of 9.88°, 19.86°, 20.96°, 22.06°. While, the XRD pattern of silver-chitosan nanocomposite films also exhibits a sharp peak at 38.08°, which is consistent with the (111) plane diffraction of the silver crystal [24,25]. Narrow and sharp peaks of chitosan structure are indicating improved crystallinity of the film are observed for the films using glycerol as plasticizer. This indicates that glycerol makes more good arrangement in the structure of chitosan film.

**Figure 6.** XRD pattern of chitosan film (a), silver-chitosan nanocomposite film (b), silver-chitosan-glycerol nanocomposite film (c).

Thermal degradation of the chitosan film, silver-chitosan nanocomposite film and silver-chitosan nanocomposite film plasticized by glycerol are shown in Figure 7. It can be seen that the films have similar thermal properties. Three weight losses areas are observed in the films. The initial weight loss observed at 30 – 100 °C was attributed to the water molecules. The second, weight loss at 200 – 400 °C may be related to the decomposition of amine and –CH₂OH group [26]. The third stage, at 500 – 600 °C for chitosan and silver-chitosan may be due to total degradation of the glucopyranose of chitosan. However, silver-chitosan nanocomposite film plasticized by glycerol degrades at a lower temperature of 420 – 500 °C. This indicates that the presence of glycerol in the chitosan structure causes the chitosan polymer is easier to break than that without glycerol. This is reasonable since the glycerol reduces the intermolecular hydrogen bonding of chitosan backbone, thus weakening the bond between the polymer chains.

**Figure 7.** TGA thermogram of chitosan film, silver-chitosan nanocomposite film and silver-chitosan-glycerol nanocomposite film.

4. Conclusion
It is concluded that addition of glycerol effects on structural properties of chitosan and their corresponding silver nanocomposite films. The glycerol is significant increasing on the mechanical properties of films. Elongation at break and tensile strength of the films were enhanced at high concentration of glycerol of 0.8 to 1.0 mL in 60 mL 1% chitosan solution or 160 to 200% (w/w) (glycerol/chitosan). Similarly, the swelling capacity, WVP, and crystallinity of the films also increase by increasing the concentration of glycerol. However, as the consequence, the thermal resistance of the plasticized film is lower than that of unplasticized films due to the weak intermolecular forces among chitosan structures.
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References
[1] Park J K, Chung M J, Choi H N and Park Y I 2011 Int.J.Mol.Sci. 266-277
[2] Kim M K, Son, J H and Kim S K 2006 J. Food Sci. E: Food Eng. Phys. Prop. 71(3) 119–124
[3] Fernandez-Saiz P, Lagaron J M, Hernandez-Munoz P and Ocho M J 2008 Int. J. Food Microbiol. 124 13-20
[4] Islam M D, Masum S M D and Mahbub K R 2011 J. Bangladesh Chem. Soc. 24(2)185-190
[5] Susilowati E, Maryani and Ashadi 2015 Indonesian J. Pharm. 26(1) 71(3) 119–124
[6] Rodriguez 2003 Cultivos Tropicales 24(2) 85-88
[7] Rinaudo M 2006 Prog.Polym.Sci. 31 603-632
[8] Rhim J W, Weller C L and Ham K S 1998 Food Sci. Biotechnol. 7 263–268.
[9] Rhim J W, Hong S-I, Park H M and Ng P K W 2006 J.Agric. Food Chem. 54 5814- 5822
[10] Ragi el A, Irusta S, Kyziol A and Arruebo M 2013 Nanotechnology 241-13
[11] Manikandan, A and Muthukrishnan S M 2015 J.Nanomed. Nanotechnol. 61
[12] Kavitha A L, Prabu G H and Babu S A 2013 Int.J.Polym.Mater 62(1) 45-49
[13] Liu B S and Huang T B 2008 Macromol. Biosci. 8 932–941.
[14] Bourtoom T 2008 Songklanakarin J. Sci. Technol. 30 (1) 149-165
[15] Ziani K, Oses J, Coma V and Mate J I 2008 Food Sci. Technol. 41(10) 2159-2165.
[16] Adila S N, Suyatma N E, FirlieyantiA S and Bujang A 2013 Adv.Mat. Res 748 155-159
[17] Park S I, Daeschel M A and Hao Y Z 2004 J. Food Sci. 69(8) 215-221
[18] Varaprasad K, Vimala K, Ravindra S, Reddy, N, Reddy G V S and Raju K M 2011 J Mater Sci: Mater. Med. 22 1863–1872
[19] Bertuzzi M A, Vidaurre E F C, Armada M and Gottifredi J C 2007 J. Food Eng. 80 972-978
[20] Guzmán M G, Dille J and Godet S 2009 Int. J. Chem. Biol. Eng. 2(3) 104-111
[21] Šileikaitė A, Puišo J, Prosyčėvas I and Tamulevičius S 2009 Mater. Sci., (Medžiagotyra) 15(1) 21-27
[22] Vimala K, Sivudu K S, Mohan Y M, Sreedhar B and Raju K M 2009 Carbohyd Polym 75 47–463.
[23] Siripatrawan U and Harte B R 2010 Food Hydrocolloids (24) 770-775
[24] Sharma P, Sanpui P, Chattopadhyay A and Ghosh S 2012 RSC Adv 2 5837–5843
[25] Sathishkumar M, Sneha K and Yun Y S 2010 Bioresource Technology 101 7958–7965
[26] Martinez-Camacho A P, Rocha M O C, Brauer J M E, Verdugo A Z G, Felix F R, Ortega M C, Gomez M S Y and Jatomea M P 2010 Carbohydr. Polym. 82 305-515