

## SYNTHESIS OF POLYETHYLENE GLYCOL-CHITOSAN-NANO Ag COMPOSITES AND THEIR ANTIBACTERIAL PROPERTIES

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This study showed the synthesis of polyethylene glycol-chitosan-nano Ag (PEG-Chi-Ag) composites to improve the homogeneous distribution of nano Ag and their antibacterial properties. In particular, various concentration ratios of nano Ag to PEG-Chi were adjusted to control the microstructure of the composites. Chitosan was used as a reducing and stabilizing agent. All the composites showed a rough microstructure with a homogeneous distribution of nano Ag on the matrix. In addition, the homogeneous distribution of nano Ag on the PEG-Chi-Ag composites resulted in antibacterial activities. These results indicated that PEG-Chi-Ag composites had great potential application in antibacterial treatment and nanomedicine.

**Keywords:** nanoparticle Ag, antibacterial, microstructure, composite.

## СИНТЕЗ КОМПОЗИТОВ ПОЛИЭТИЛЕНГЛИКОЛЬ-ХИТОЗАН-НАНОЧАСТИЦЫ СЕРЕБРА И ИХ АНТИБАКТЕРИАЛЬНЫЕ СВОЙСТВА

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Композиты полиэтиленгликоль-хитозан-наночастицы Ag (PEG-Chi-Ag) синтезированы для улучшения однородного распределения наночастиц Ag и их антибактериальных свойств. Различные соотношения концентраций наночастиц Ag к PEG-Chi скорректированы для управления микроструктурой композитов. В качестве восстановителя и стабилизирующего агента использован хитозан. Все композиты имели грубую микроструктуру с однородным распределением наночастиц Ag на матрице. Показано влияние однородного распределения наночастиц Ag в композитах PEG-Chi-Ag на антибактериальную активность.

**Ключевые слова:** наночастица Ag, антибактериальное средство, микроструктура, композит.

**Introduction.** Nano silver (Ag) has received considerable attention in nanomedicine in designing functional materials because of its plasmonic properties and highly antibacterial activities, depending on the particle size and shape [1, 2]. However, nano Ag may undergo agglomeration and surface oxidation over time [3, 4]. To achieve its potential application in nanomedicine, it is necessary for nano Ag to have a combination of various properties such as good dispersion, high stability, and spherical nanoparticles [5, 6]. Therefore, considerable effort has been made to functionalize nano Ag by compositing its inorganic materials [7, 8], surfactants [9, 10], and polymers [11–13]. Inorganic materials are considered as an effective matrix for decoration of well-dispersed nano Ag, but chemical instability may occur because inorganic materials could not fully cover the nanoparticles. Another potential approach is enhancing the performance of antibacterial activity using polymer materials such as chitosan (Chi) [14], ethylene glycol [15], and polyvinylpyrrolidone [16]. Although instability may not occur when using this approach, homogeneous distribution on the matrix may be of concern.

As a biopolymer, polyethylene glycol (PEG) has received considerable attention as a matrix for decoration of nanoparticles because of its important advantages such as biocompatibility, biodegradability, and hydrophilic property compared with available polymers [17, 18]. Nevertheless, few reports have been found on the synthesis of PEG-Chi-Ag composites for their potential application in antibacterial treatment and nanomedicine [19]. In particular, to our knowledge, no reports have been found on the effect of nano Ag concentration on the microstructure and antibacterial activities of PEG-Chi-Ag composites synthesized via a chemical method using chitosan as the reducing and stabilizing reagent coupled with PEG as the matrix. Therefore, in this study, PEG-Chi-Ag composites were synthesized using PEG and chitosan (Chi) as the reducing and stabilizing agents, respectively. In particular, the microstructures of the composites were characterized by field-emission scanning electron microscopy (FE-SEM) and high-resolution transmission electron microscopy (HRTEM). The disk diffusion test was used to characterize the antibacterial activities of the PEG-Chi-Ag composites.

**Experimental.** In the typical preparation of PEG-Chi-Ag composites, various concentrations of  $\text{AgNO}_3$  (VWR Chemicals), namely, 0.25, 0.5, 0.75, and 1%, were dissolved in 15 mL of distilled water at 80°C. A fixed amount of chitosan (Chi, Sigma-Aldrich, 25 mL of 0.5%) dissolved in 2% acetic acid (Sigma-Aldrich) was added and stirred for 30 min. Then, 20 mL of 1% PEG (Sigma-Aldrich) was poured into the aforementioned solution, and the mixture was stirred for 120 min. Finally, while stirring at room temperature, 1 mL of 2% glutaraldehyde (Sigma-Aldrich) was added as a cross-linker for the synthesis of PEG-Chi-Ag composites.

The microstructure of the PEG-Chi-Ag composites was determined by FE-SEM (JEOL, JSM-7600F, JEOL Techniques, Tokyo, Japan) and HRTEM (JEM 2100, JEOL Techniques, Tokyo, Japan). The optical absorption of the PEG-Chi-Ag composites was characterized by using a Cary 500 spectrophotometer. Gram-negative (*Enterobacter aerogenes* and *Escherichia coli*) and Gram-positive (*Listeria monocytogenes* and *Staphylococcus epidermidis*) bacterial strains were used for the antibacterial activity test. For the disk diffusion test, the agar medium mounting on a Petri dish was cut into circles with a 10-mm diameter, and the sample with different Ag concentrations was placed on the hole of the agar plate. The respective organism ( $1 \times 10^8$  CFU/mL) growing in nutrient broth was then cultured on a nutrient agar medium, and the plates were incubated at 37°C for 48 h. The antibacterial activity was observed as a zone of inhibition around the sample discs. The experiment was performed in triplicate.

**Results and discussion.** Figure 1 shows the UV-Vis spectra of PEG-Chi-Ag composites synthesized at different Ag concentrations. As shown in Fig. 1, the UV-Vis spectra of the PEG-Chi-Ag composites displayed similar UV-Vis absorption properties. The absorption peak at 420 nm corresponds to the formation of nano Ag [20]. The intensity of the 420-nm peak slightly enhances with the increase of nano Ag concentration, thereby increasing the formation of nano Ag. The UV-Vis absorption spectrum has no other absorption peaks in the visible area. This result demonstrated that no other forms of nano Ag were produced during the reduction of  $\text{AgNO}_3$  precursors.

The representative microstructure of the nano Ag decorated on the PEG-Chi composite was characterized by scanning electron microscope (SEM) (Fig. 2). All the specimens showed a rough microstructure, as is commonly the case with PEG-based composite synthesized by a chemical method [21]. The PEG-Chi matrix is composed of homogeneous distribution of Ag nanoparticles with a diameter of ~10–20 nm in the case of 0.5% Ag (Fig. 2a). When the amount of Ag increased to 1%, the PEG-Chi matrix still showed homogeneous distribution of Ag nanoparticles with a slight increase in the concentration of nano Ag decorated on the

matrix (Fig. 2b). This homogeneous distribution of Ag nanoparticles can increase the antibacterial properties of the materials.

The morphology of nano-Ag-decorated PEG-Chi composites was further characterized by transmission electron microscope (TEM). As shown in Fig. 3a, the black areas in the image were attributed to the presence of the PEG-Chi polymer matrix. In addition, the TEM image showed tiny round nanoparticles with homogeneous distribution, indicating the formation of nano Ag on the PEG-Chi matrix. The size distribution of Ag nanoparticles was narrow with a diameter of  $\sim 12$  nm (Fig. 3b).

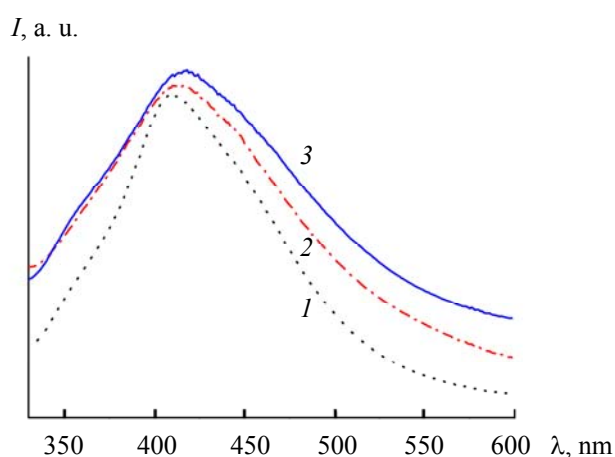


Fig. 1. UV-Vis spectra of PEG-Chi-Ag composites: 0.5% Ag (1), 0.75% Ag (2), and 1.0% Ag (3).

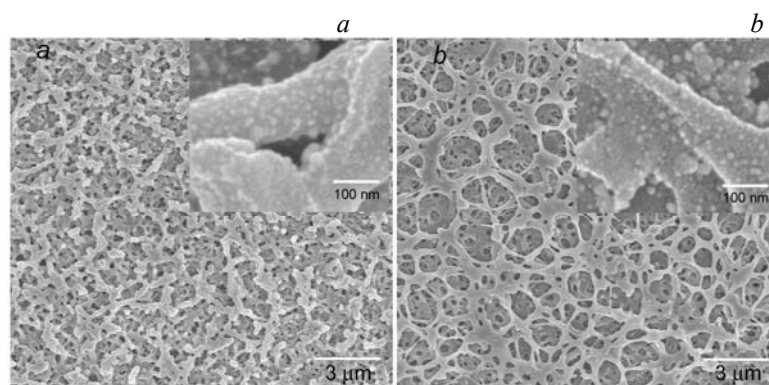


Fig. 2. SEM image of PEG-Chi-Ag composites with concentrations of nano Ag 0.5% (a) and 1% (b).

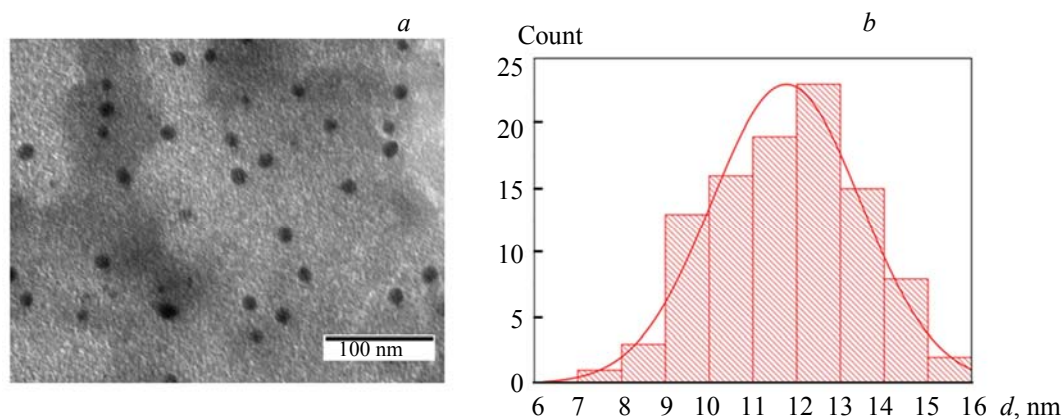


Fig. 3. TEM image (a) and particles size distribution (b) of nano Ag on the PEG-Chi-Ag composites.

Figure 4 shows representative images for the antibacterial properties of the PEG-Chi-Ag nanocomposites as a function of different nano Ag concentrations against *L. monocytogenes*, *S. epidermidis*, *E. aerogenes*, and *E. coli* strains after culturing for 48 h. All the specimens showed an inhibition zone, indicating good antibacterial properties. In particular, the growth inhibition ring size increased as the concentration of nano Ag increased from 0.25 to 1% for the entire bacterial strain test. These results could be explained on the basis of the high number of nano Ag, which is efficient for the antibacterial properties of the PEG-Chi-Ag composites. In addition, the inhibition zone showed a defined ring for Gram-positive bacteria, including *L. monocytogenes* and *S. epidermidis*, indicating that the PEG-Chi-Ag composites were specific antibacterial properties for the Gram-positive bacterial strain. The antibacterial properties of PEG-Chi-Ag composites may directly disrupt the bacterial cell envelope that causes damage [22]. These results implied that the interactions of nano Ag on the PEG-Chi-Ag composites with the bacterial cell membrane played an essential role in bacterial inactivation and subsequent destruction of the bacterial strain. Therefore, the homogeneous distribution of nano Ag on the PEG-Chi-Ag composites would increase the antibacterial activity.

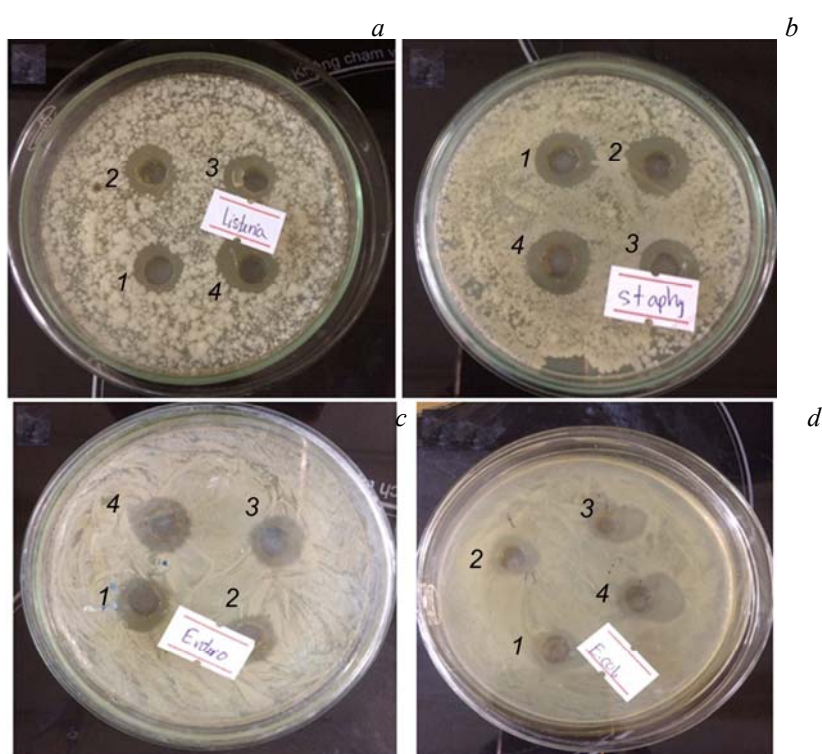


Fig. 4. Antibacterial properties of the PEG-Chi-Ag composites: a) *Listeria monocytogenes*; b) *Staphylococcus epidermidis*; c) *Enterobacter aerogenes*, and d) *Escherichia coli*: 0.25% Ag (1), 0.5% Ag (2), 0.75% Ag (3), and 1% Ag (4).

**Conclusions.** Various PEG-Chi-Ag composites with different Ag concentrations were synthesized using chitosan as reducing and stabilizing agents, and their microstructure and antibacterial properties were evaluated. The use of chitosan significantly enhanced the stability of nano Ag on the PEG-Chi matrix, whereas PEG polymer provided excellent homogeneous distribution of nano Ag on the PEG-Chi-Ag composites. The PEG-Chi-Ag composites showed good antibacterial properties, which were assessed by an in vitro bacterial cell test using *L. monocytogenes*, *S. epidermidis*, *E. aerogenes*, and *E. coli* bacterial strains.

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