

FACTORS AFFECTING SYNTHESIS OF SILVER-NANOPARTICLES AND ANTIMICROBIAL APPLICATIONS

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Abstract. Silver nanoparticles were synthesized from silver sulfate by using the chemical reduction method with dextran as both a reducing agent and a protective agent. The influence of reaction temperature, time, and initial pH on the synthesis was investigated. The formation of Ag nano-particles (AgNPs) and their morphology were characterized with UV-Vis spectroscopy, X-ray diffraction, scanning electron microscopy, energy dispersive X-ray analysis, and Fourier transform-infrared spectroscopy. The antifungal and antibacterial effects of AgNPs/dextran on *Xanthomonas oryzae* and *Pyricularia oryzae* were tested.

Keywords: silver nanoparticle, antimicrobial, reducing reaction, *Pyricularia oryzae*, *Xanthomonas oryzae*

1 Introduction

Nowadays, metal nanoparticles have attracted tremendous attention from both domestic and international scientists because of their outstanding properties, such as high-performance catalysis and unique electronic and optical properties [1]. Nanoparticles with noble metals such as Au, Ag, and Pt have widely been studied and applied to various fields, such as environment, catalysis, and nanomedicine. Among them, silver nanoparticles (AgNPs) with special, physical, and chemical properties and relatively low cost [2] have extensively been studied and applied in numerous fields. Under normal conditions, silver nanoparticles are not usually stable in solution. To avoid agglomeration of silver nanoparticles, several polymers, such as polyethylene glycol, ethylenediaminetetraacetic acid, polyvinyl

pyrrolidone, and polyvinyl alcohol, are used as stabilizing agents [3]. Among these polymers, dextran has received much scientific attention due to its great properties, such as biocompatibility, low toxicity, and slow degradation in the human body in comparison with other polymers [4]. Dextran is a biocompatible polysaccharide composed of D-glucose units and a substantial number of consecutive α -(1→6) glycosidic linkages in the main chain and α -(1→2), α -(1→3) or α -(1→4) branch glycosidic linkages. Dextran is an environmental-friendly biodegradable polymer and has applications in food and medicine as an emulsifier, a carrier, and a stabilizer [5, 6]. In this work, we have propose a simple, fast, and effective chemical reduction method to synthesize silver nanoparticles by using dextran as a reducing and protecting agent.

Xanthomonas oryzae (*X. oryzae*) causes bacterial blight disease, and *Pyricularia oryzae* (*P. oryzae*) fungus causes blast disease. These are the most important diseases of rice in most rice-producing countries [7, 8]. Silver nanoparticles exhibit antimicrobial activities against fungi and bacteria. However, the antimicrobial mechanism and characteristics of *X. oryzae* and *P. oryzae* of AgNPs/dextran have not been studied systematically.

In this study, AgNPs are synthesized with the chemical reduction method. Ag⁺ is reduced by dextran. The size and shape of particles are studied by varying the temperature, time, and initial pH of the reaction. The antimicrobial activities of AgNPs/dextran are tested against *X. oryzae* and *P. oryzae*.

2 Experimental

2.1 Materials

Analytical grade dextran ($H(C_6H_{10}O_5)_xOH$, 99%), silver sulfate pentahydrate ($Ag_2SO_4 \cdot 5H_2O$, 98%), ammonium hydrate ($NH_3 \cdot H_2O$, 25–28%), and ethanol (C_2H_5OH , 98%) are purchased from Sigma-Aldrich. All chemicals are used without further purification. Other chemicals are agar (Vietnam), peptone, and meat extract (Angle, Korea).

2.2 Preparation of silver nanoparticles

In a typical process, the glassware was cleaned in a bath of freshly prepared aqua regia solution (HCl/HNO_3 , 3:1, v/v) and rinsed first thoroughly with double distilled water and then acetone before use. A stock solution of 5% dextran was prepared by dissolving 5 g of dextran in 100 mL of distilled water. 100 mL of silver ammonium sulfate solution (1 mM) was prepared from a silver sulfate solution (20 mM) and an ammonia solution (5%, w/w). The silver ammonium sulfate solution was mixed with the dextran solution under stirring for

20 minutes. This suspension was finally precipitated overnight with ethanol 95% (v/v), followed by centrifugation (4000 rpm, 20 min, 25 °C). The resulting pellets were collected and calcinated at 350 °C for 4 h [5].

2.3 Characterisation

X-ray diffraction patterns were recorded on a Bruker D8 Advance X-Ray diffractometer. UV-Vis spectra measurements were carried out with a Jasco V-550 UV-vis spectrophotometer, within the range of 300–650 nm. Scanning electron microscopy (SEM), elemental analysis and energy dispersive X-ray spectroscopy were analyzed by using FESEM HITACHI S-4800 instrument. Transmission electron microscopy (TEM) images were acquired by using a JEOL JEM-2100F. Fourier-transform infrared (FTIR) spectrograms were measured on a Nicolet-6700 FTIR spectrometer with a wave-number range of 4000–500 cm^{-1} .

2.4 Antifungal test

The inhibition activity of AgNPs on dextran (AgNPs/dextran) against *X. oryzae* and *P. oryzae* was studied. For the antibacterial test, the modified Wakimoto medium was previously prepared as follows: a mixture containing 300 g of potato infusion, 5.0 g of peptone, 2.0 g of disodium phosphate, 0.5 g of calcium nitrate, 15.0 g of sucrose, and 17.0 g of agar was dissolved in distilled water to form 1 L of suspension and then sterilized in an autoclave at 125 °C for 15 min. The obtained medium was used to cultivate *X. oryzae* bacteria. Each Petri dish contains 10 mL of the Wakimoto medium and 0.1 mL of the colloidal AgNPs solution. After that, 1 mL (approximately 10^6 CFU ml^{-1}) of the bacterial suspension was spread onto these Petri dishes. Then, the dishes were incubated at 28 °C for 72 h. A Petri dish

without AgNPs was used as a reference. Visible colonies were quantified after incubation.

The antifungal action of AgNPs/dextran was evaluated against *P. oryzae* fungi in the potato dextrose agar (PDA) medium. To prepare PDA, a mixture composed of 200 g of potato infusion, 20 g of glucose, and 20 g of agar was dissolved and made up to 1 L with distilled water and then sterilized in an autoclave at 125 °C for 15 min. The fresh PDA medium was taken in 10 mL for each Petri dish and mixed with 0.1 mL of the colloidal AgNPs solution. After that, 1 mL of *P. oryzae* fungus strains (approximately 10⁶ CFU/ml) was inoculated in these Petri dishes. The inoculated plates were incubated at 28 °C for 5 days. The percentage inhibition of growth was calculated by using the Vincent equation [9]

$$I = \frac{C - T}{C} \times 100$$

where *C* is the diameter of the fungal colony on the control plate, and *T* is the diameter of the fungal colony on the treated plate. Each test for antimicrobial activity of these samples was repeated three times to guarantee uniformity.

3 Results and discussion

3.1 Effect of temperature

Temperature is one of the factors influencing the synthesis of AgNPs. This is confirmed by studying the UV-Vis spectra of the AgNPs synthesized at 90, 95, 100, and 105 °C. The results show that by increasing the synthesis temperature, the maximum absorption wavelength of the nanosilver solution shifts to a higher value (Fig. 1).

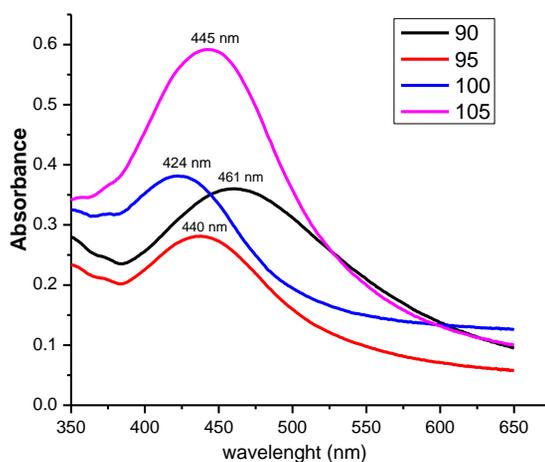


Fig. 1. UV-vis spectra of AgNPs synthesized at different temperatures

Table 1. Maximum absorbance of samples at different storage times

Temperature (°C)	Beginning	1 month	2 months	4 months
90	0.281	0.264	0.241	coagulated
95	0.379	0.343	0.293	coagulated
100	0.592	0.570	0.560	0.560
105	0.360	0.350	0.315	0.314

To investigate the effect of reducing temperature on the stability of AgNPs, the silver nano solutions were retained, and the maximum absorbance after different storage times was measured. The maximum absorbance of these solutions decreases with the storage time, and AgNPs coagulate after 4 months (Table 1).

The AgNPs sample synthesized at 100 °C is more stable than those synthesized at 90 and 95 °C. This might be explained by the fact that, at higher temperatures, not only the reaction rate and the movement of atoms in the solution increase but also many initial nuclei are created, which leads to smaller particle size and narrower dispersion. Furthermore, there is better interaction between dextran and the surface of AgNPs, making the system more stable. However, when the temperature continues to increase, the maximum absorbance decreases significantly (0.314), compared with the initially maximum absorbance although, at 105 °C, AgNPs do not coagulate after 4 months. The reason might be that, at high temperatures, dextran degrades, thus providing less protection ability. This leads to the conclusion that AgNPs are the most stable when synthesized at 100 °C.

3.2 Effect of reaction time

The UV-Vis spectra show that the maximum absorbance increases with reaction time (Fig. 2). However, the maximum absorbance increases rapidly during the first stage of the reaction, but, in the next 15 minutes, it increases slightly, and, after 30 minutes, no further increase is observed. At this time, the reduction reaction is almost completed, and this is entirely consistent with the law of reaction rate. Besides, the UV-Vis spectrum of the 30-minute sample is sharp, and this proves that the particles are relatively more uniform. Therefore, 30 minutes is a suitable reaction time for this reduction process.

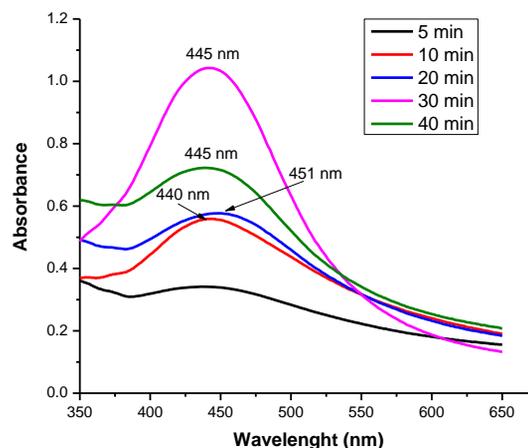


Fig. 2. UV-vis spectra of AgNPs at different reducing times

3.3 Effect of initial pH

Fig. 3 shows the UV-Vis spectra of AgNPs at different initial pHs. At pH 8, the reaction proceeds very slowly after 30 minutes. Increasing the initial pH to 9 enables the maximum absorbance to increase gradually and shifts the maximum absorption to a higher wavelength. This result is consistent with that in other reports [10, 11]. The reaction is as follows:

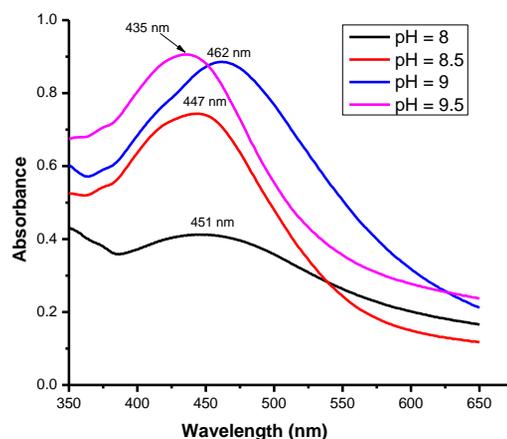


Fig. 3. UV-vis spectra of AgNPs at different initial pHs

3.4 Characterizations of AgNPs

The X-Ray diffraction (XRD) pattern was used to analyse the crystalline nature and identify the phases present in the as-prepared samples. The XRD pattern of AgNPs/dextran in Fig. 4 exhibits typical diffraction peaks at 38.2, 46.3, 64.9, 77.8, and 85.7°, corresponding to the (1 1 1), (2 0 0), (2 2 0), (3 1 1), and (2 2 2) planes of face-centered cubic silver (JCPDS card No. 04-0783) [5].

The morphology and particle size of AgNPs were analyzed from SEM and TEM images. The SEM image (Fig. 5a) depicts that AgNPs adhere to the surface of dextran and distribute uniformly. Besides, the TEM image (Fig. 5b) shows that the nanoparticles are spherical. The particle size of the silver nanoparticles on dextran is in the range of 3–18.40 nm, with an average particle diameter of around 8.7 nm (Fig. 5c).

The energy dispersive X-ray analysis (EDX) of AgNPs shows a strong signal in the silver region and thus confirms the formation of silver nanoparticles (Fig. 6). Besides, the EDX spectrum shows the elemental composition of dextran (carbon, chlorine). An EDX spectrum is ineffective with light elements such as H, so it does not appear in the spectrum. Therefore, the AgNPs synthesized in this study are pure.

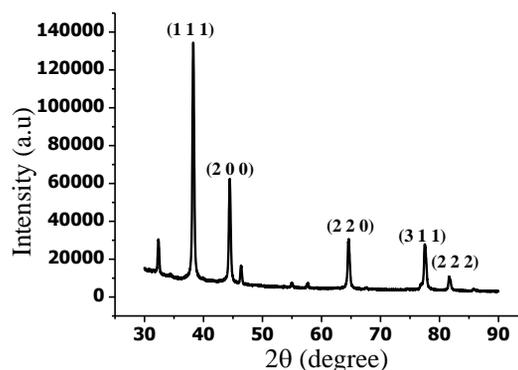


Fig. 4. XRD pattern of AgNPs

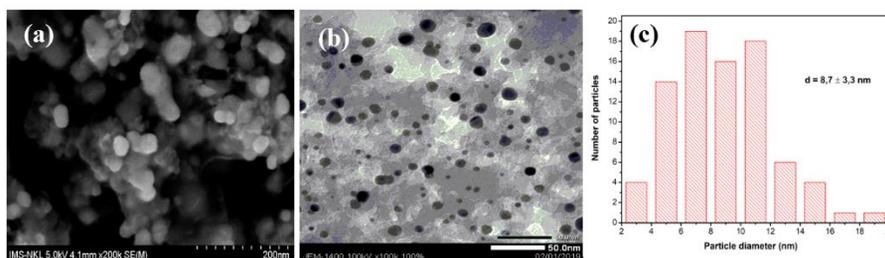


Fig. 5. (a) SEM image of AgNPs; (b) TEM image of AgNPs; (c) Particle diameter distribution of AgNPs

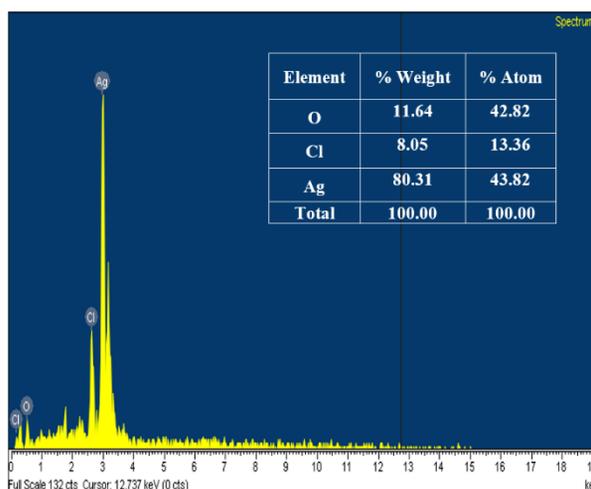


Fig. 6. EDX spectrum of AgNPs

The FTIR spectrum of pure dextran and AgNPs/dextran are shown in Fig. 7. In the spectrum of pure dextran, the region of 3417 cm^{-1} is assigned to the stretching vibration of the hydroxyl group [12]. The peaks at 2929 cm^{-1} and 1647 cm^{-1} are attributed to C–H bonds and carboxyl groups, respectively. The peaks at 1159 cm^{-1} and 1112 cm^{-1} are assigned to the stretching vibration of C–O–C and C–O bonds at the C-4 position of glucose, respectively [13]. Besides, the peak at 916 cm^{-1} is related to the α -glycosidic bond. When it comes to the spectrum of AgNPs/dextran, a similarity pattern is found. However, the intensity of the peak at 2929 cm^{-1} decreases considerably compared with that of dextran, and the peak at $1355\text{--}1345\text{ cm}^{-1}$ corresponding to the C–OH groups also shifts, indicating that some hydroxyl groups are oxidized to aldehyde, leading to the reduction of Ag(I) to Ag(0) [2].

3.5 Antibacterial and antifungal results

The antibacterial properties of AgNPs/dextran are tested against *X. oryzae*. The optical images of *X. oryzae* colonies incubated for the reference sample and AgNPs/dextran sample for 72 h are depicted in Fig. 8a, b. It is obvious that there are almost no colonies in Fig. 8b, indicating the inhibitory effect of AgNPs/dextran on the growth of *X. oryzae*. Similarly, the antifungal properties of AgNPs are tested against *P. oryzae* by determining the diameter of the fungal colonies after five days. The optical images of colonies of the fungal growth in the media with and without AgNPs are shown in Fig. 8c, d. The results indicate that AgNPs/dextran significantly inhibits the development of *P. oryzae* (its inhibition efficiency of 69.72% after five days). The evidence suggests that AgNPs/dextran is an effective antimicrobial material against the growth of *X. oryzae* and *P. oryzae*.

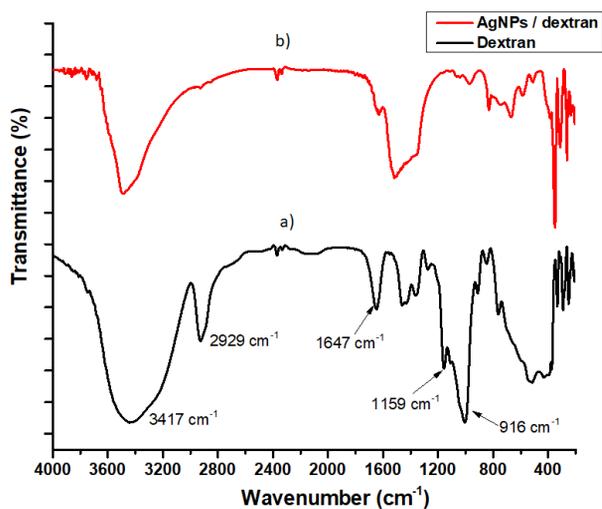


Fig. 7. FT-IR spectrum of dextran (a) and AgNPs/dextran (b)

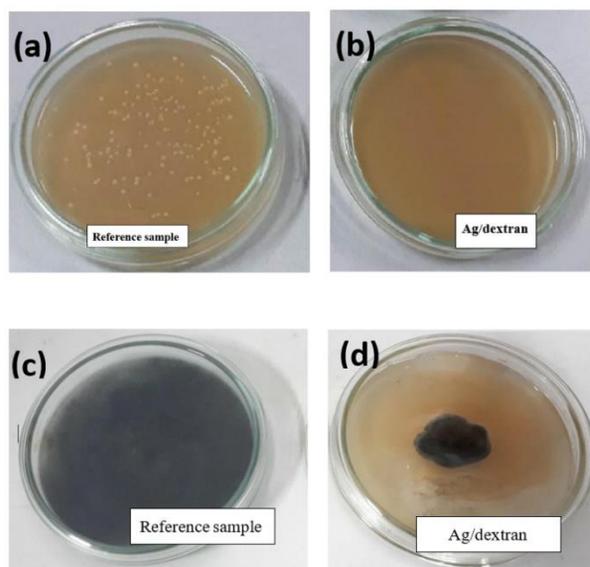


Fig. 8. Optical images of *X. oryzae* colonies incubated on AgNPs/dextran (b), reference sample (a); *P. oryzae* colonies incubated on AgNPs/dextran (d), reference sample (c)

4 Conclusion

In this study, the silver nanoparticles were successfully synthesized by using dextran as a reducing and protecting agent, with an average particle diameter of around 8.7 nm. The reaction parameters are as follows: silver sulfate solution 0.1 mM, dextran solution 0.5%, w/v, reaction temperature $100\text{ }^{\circ}\text{C}$, initial pH 9, and reaction time

30 minutes. The obtained AgNP/dextran product exhibits a high inhibitory effect on *P. oryzae* fungi causing rice blast after 5 days and on *X. oryzae* bacteria causing blight disease after 72 hours.

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