

Antibacterial activity of silver nanoparticles prepared by a chemical reduction method

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Abstract—Silver nanoparticles were obtained by chemical reduction of silver nitrate in water with sodium borohydride (NaBH₄) in the presence of SDS (sodium dodecyl sulfate) as a stabilizer. The synthesized silver nanoparticles were characterized by UV-vis spectroscopy (UV-vis) and transmission electron microscopy (TEM). The formation of silver nanoparticles was confirmed from the appearance of surface plasmon absorption maxima at 400 nm by UV-vis. TEM showed the spherical nanoparticles with size in 10-20 nm. The antibacterial activity of silver nanoparticles was tested by using Gram-positive *Staphylococcus aureus* (*S. aureus*) and Gram-negative *Escherichia coli* (*E. coli*). The silver nanoparticles, whose bacterial activity was dependent on the aggregation degree between particles, exhibited bacterial activity against *S. aureus* and *E. coli*.

Key words: Silver Nanoparticles, Antibacterial Activity, Chemical Reduction, *S. aureus*, *E. coli*

INTRODUCTION

Silver nanoparticles have been known to have bactericidal effects [1] and used in many fields including biomedical [2], food packaging [3] and waste water treatment [4]. The antibacterial activity of silver nanoparticles was recognized to relate to their shapes and sizes [5]. Many attempts have been made to synthesize the silver nanoparticles with controllable shape, size and size distribution [6].

Many methods, a chemical reduction method [7], a polyol method [8], and a radiolytic process [9] have been developed for the synthesis of silver nanoparticles. Among the methods, chemical reduction was widely studied, due to its advantages of yielding nanoparticles without aggregation, high yield and low preparation cost [10,11].

The chemical reduction method involves the reduction of AgNO₃ in aqueous solution by a reducing agent in the presence of a suitable stabilizer, which is necessary in protecting the growth of silver particles through aggregation. In the formation of silver nanoparticles by the chemical reduction method, the particle size and aggregation state of silver nanoparticles are affected by various parameters, such as initial AgNO₃ concentrations, reducing agent/AgNO₃ molar ratios, and stabilizer concentrations [12].

In this study, silver nanoparticles were prepared by reducing silver nitrate dissolved in water with sodium borohydride in the presence of SDS as stabilizing agent. The formation of silver nanoparticles was confirmed by UV-vis spectroscopy and transmission electron microscopy. The effect of NaBH₄/AgNO₃ molar ratios and SDS/AgNO₃ weight ratios on the particle size and aggregation state of silver nanoparticles was also investigated. In addition, the bacterial activity of the silver nanoparticles was studied against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*.

EXPERIMENTAL

1. Preparation of Silver Nanoparticles

Silver nitrate (AgNO₃, 99.5%) purchased from Junsei Chemicals was used as precursor to prepare silver nanoparticles. Sodium borohydride (NaBH₄, 98%) and sodium dodecyl sulfate (SDS, 98%) purchased from Aldrich Chemicals were used as the reducing agent and stabilizer for the silver nanoparticles, respectively. AgNO₃ (30.8 mg, 0.2 mmol) was dissolved in deionized water (100 mL). Separately, the NaBH₄ solution was prepared by dissolving the required amount of NaBH₄ and SDS in 50 mL distilled water for half an hour. Silver nanoparticles were obtained by dropping the AgNO₃ solution into the NaBH₄ solution containing SDS slowly. After all solutions were added, the mixed solutions were stirred for 1 hour more.

2. Characterization

The aggregation state between particles was observed by UV-vis spectroscopy (UV-2450, Shimadzu, Japan). The particle size and shape were further measured by using transmission electron micrographs (TEM, Jeol 2000-FXII, Japan) at 80 kV accelerating voltage.

3. Antibacterial Activity Testing

The antibacterial activity of silver nanoparticles was investigated by using an inhibition zone method. Two different bacteria, Gram-positive *Staphylococcus aureus* (ATCC 6538P, *S. aureus*) and Gram-negative *Escherichia coli* (ATCC 8739, *E. coli*), were used for testing the antibacterial activity of silver nanoparticles. A nutrition agar containing ca. 10⁵ colony-forming units (CFU)/mL of bacteria was poured in a Petri dish and then solidified for 1 hour at 5 °C. Three wells (diameter 8 mm) were made onto each bacteria-inoculated agar plate, and then the different sample solutions (100 μL) were dropped into the corresponding wells. The bactericidal activity showed a clear inhibition zone around the sample-loaded well after incubating at 37 °C for 24 hours. The inhibition zone, where no visible bacterial colonies formed, was measured by subtracting the well diameter

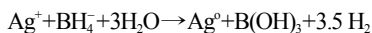
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from the total inhibition zone diameter.

RESULTS AND DISCUSSION

1. Effect of NaBH₄ Concentrations

To prepare stable colloidal silver nanoparticles via chemical reduction method, it is important to choose an appropriate stabilizer and reducing agent. In this work, a water soluble surfactant SDS and a strong reductant NaBH₄ were used as stabilizer and reducing agent, respectively. Silver colloids were reduced by using sodium borohydride in the presence of stabilizer, according to the following equation [10].



UV-visible (UV-vis) absorption spectroscopy is one of the most widely used, simple and sensitive techniques for the characterization of nanoparticles [14]. Fig. 1 shows the UV-vis spectra of colloidal silver nanoparticles prepared with different NaBH₄/AgNO₃ molar

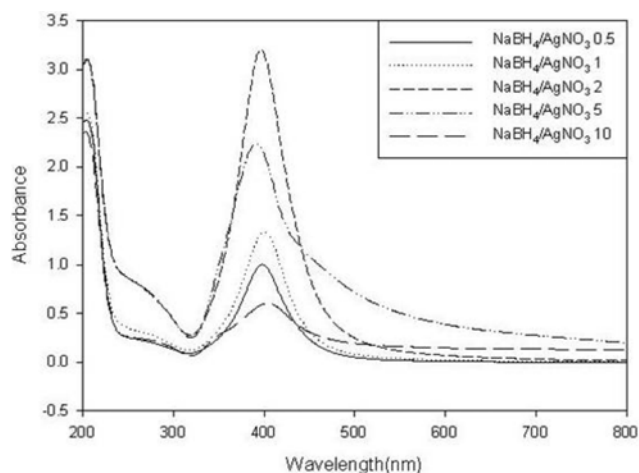


Fig. 1. UV-vis absorption spectra of the silver nanoparticles prepared with different NaBH₄/AgNO₃ molar ratios.

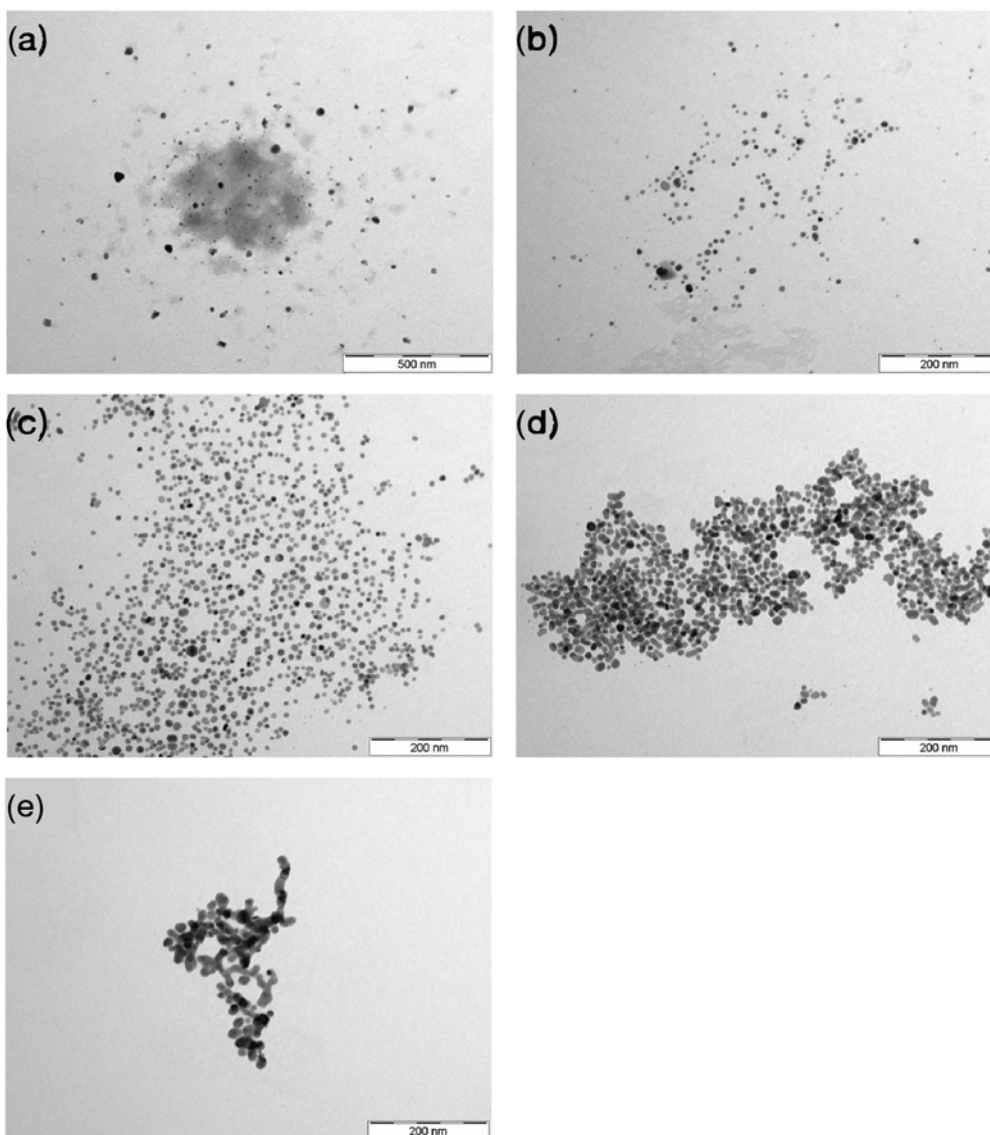


Fig. 2. TEM images of silver nanoparticles prepared with different NaBH₄/AgNO₃ molar ratios. (a) 0.5, (b) 1, (c) 2, (d) 5 and (e) 10.

ratios ranging from 0.5 to 10. The nanoparticles were synthesized at the conditions of initial AgNO_3 concentration of 0.002 M and SDS/ AgNO_3 weight ratio of 10. The color of the obtained solutions depended on the $\text{NaBH}_4/\text{AgNO}_3$ molar ratios. When the molar ratios of $\text{NaBH}_4/\text{AgNO}_3$ were low (0.5 and 1), the solutions showed a light yellow color. As the molar ratio of $\text{NaBH}_4/\text{AgNO}_3$ increased to 2, the color of the solution gradually changed to dark yellow. At high $\text{NaBH}_4/\text{AgNO}_3$ ratios (5 and 10) the solutions exhibited dark color. The absorption peak at around 400 nm in Fig. 1 was attributed to the surface plasmon excitation of silver nanospheres, indicating the formation of silver nanoparticles [15]. At low $\text{NaBH}_4/\text{AgNO}_3$ ratios (0.5 and 1) a weak absorption maximum of surface plasmon peaks was observed at 400 nm, indicating that silver nanoparticles were produced at a relatively low concentration due to insufficient reduction reaction, which resulted in light yellow color of solutions. With increasing the $\text{NaBH}_4/\text{AgNO}_3$ molar ratio to 2, the intensity of the maximum plasmon peak increased, suggesting that higher concentrations of silver nanoparticles were produced, which resulted in dark yellow color in solutions.

It is well known that the UV-vis absorption peak can give information on the aggregation degree between silver nanoparticles in solutions [12]. The narrower the absorption peak, the better dis-

persed the silver nanoparticles in solutions. When low molar ratios of $\text{NaBH}_4/\text{AgNO}_3$ were used (0.5, 1, 2), narrow absorption peaks were obtained, suggesting that silver nanoparticles were well dispersed in solutions. However, at high molar ratios of $\text{NaBH}_4/\text{AgNO}_3$ (5, 10), the absorption peaks became broad, indicating the profound aggregation between silver nanoparticles, which resulted in dark color in solutions.

Fig. 2 shows TEM images of the silver nanoparticles prepared at different $\text{NaBH}_4/\text{AgNO}_3$ molar ratios. Most particles were spherical with an average diameter of 10-20 nm. The particle size of obtained silver nanoparticles showed no obvious change, irrespective of the increase of NaBH_4 concentrations. It can be also seen that the aggregation degree between particles distinctly depend on $\text{NaBH}_4/\text{AgNO}_3$ molar ratios. When low molar ratios of $\text{NaBH}_4/\text{AgNO}_3$ were used (0.5, 1 and 2), the silver nanoparticles formed were highly stable and well dispersed. However, severe aggregation between particles was observed at high $\text{NaBH}_4/\text{AgNO}_3$ molar ratios (5, 10). These TEM results confirmed the UV-vis absorption spectra in Fig. 1.

Fig. 3 shows photographic images of bacterial inhibition zones against *S. aureus*, produced by the silver nanoparticles prepared with different $\text{NaBH}_4/\text{AgNO}_3$ molar ratios. The average diameter of the bacterial inhibition zone was correlated to antibacterial activity of

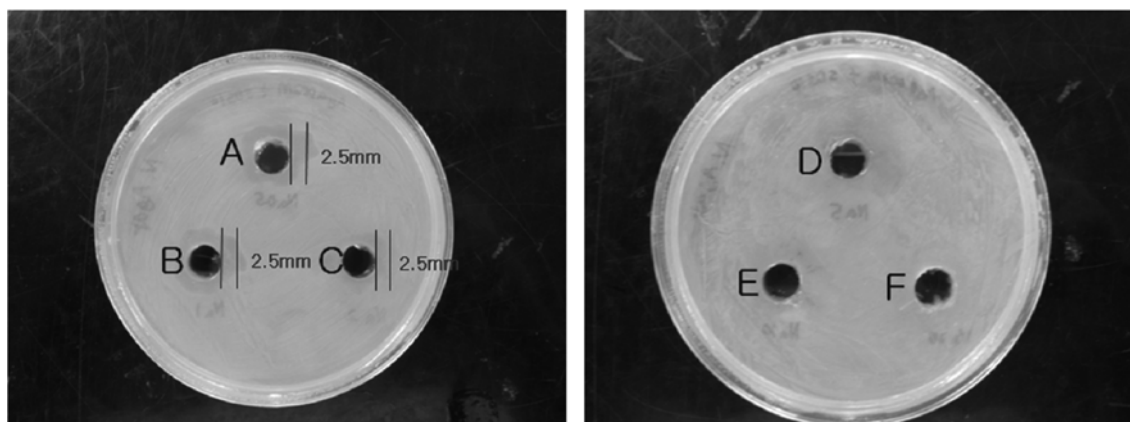


Fig. 3. Photographic images of bacterial inhibition zones against *S. aureus*, produced by the silver nanoparticles, prepared with different $\text{NaBH}_4/\text{AgNO}_3$ molar ratios. (a) 0.5, (b) 1, (c) 2, (d) 5, (e) 10 and (f) 20.

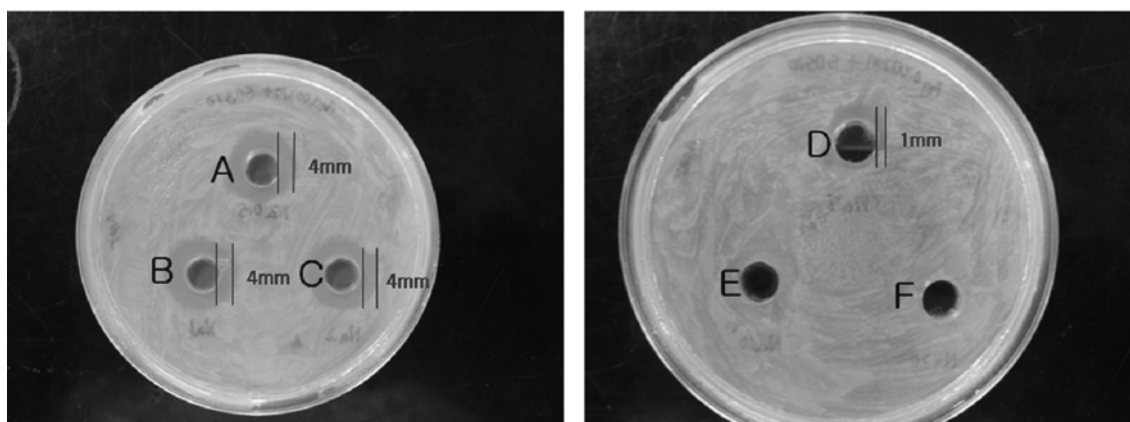


Fig. 4. Photographic images of bacterial inhibition zones against *E. coli*, produced by the silver nanoparticles, prepared with different $\text{NaBH}_4/\text{AgNO}_3$ molar ratios. (a) 0.5, (b) 1, (c) 2, (d) 5, (e) 10 and (f) 20.

the silver nanoparticles, i.e., the larger the clear area around the well, the higher the inhibitory efficiency. The nanoparticles, prepared at low $\text{NaBH}_4/\text{AgNO}_3$ molar ratios (0.5, 1 and 2), showed a good antibacterial activity with an average diameter of 2.5 mm of inhibition zone. However, the silver nanoparticles, prepared at high $\text{NaBH}_4/\text{AgNO}_3$ molar ratios (5, 10 and 20), did not produce any inhibition zones.

Fig. 4 shows photographic images of bacterial inhibition zones against *E. coli*, produced by the silver nanoparticles prepared with different $\text{NaBH}_4/\text{AgNO}_3$ molar ratios. These results are well in agreement with those in Fig. 3. The silver nanoparticles, prepared at low $\text{NaBH}_4/\text{AgNO}_3$ molar ratios (0.5, 1 and 2), showed a good antibacterial activity with an average diameter of the inhibition zones of 4 mm, while the silver nanoparticles, prepared at high $\text{NaBH}_4/\text{AgNO}_3$ molar ratios (5, 10 and 20), exhibited a small inhibition zone of 1 mm ($\text{NaBH}_4/\text{AgNO}_3$ molar ratio of 5) or no inhibition zones ($\text{NaBH}_4/\text{AgNO}_3$ molar ratios of 10 and 20).

These results indicate that the antibacterial efficacy of silver nanoparticles may depend on the aggregation degree between nanoparticles. The silver nanoparticles, which are well dispersed in solutions, exhibited much higher bactericidal activity compared to silver nanoparticles with severe aggregation. The mechanism of inhibition of silver nanoparticles on bacterial growth is partially known. Sondi

and Salopek-Sondi [16] studied the antimicrobial activity of silver nanoparticles against *E. coli*, and observed that *E. coli* cells treated with silver nanoparticles were damaged by formation of "pits" in

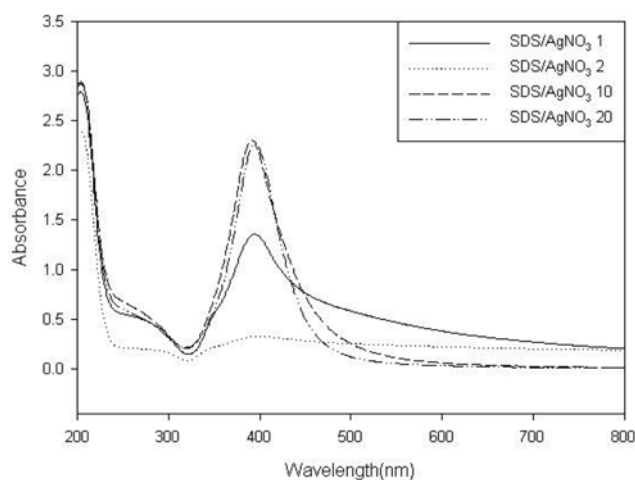


Fig. 5. UV-vis absorption spectra of the silver nanoparticles prepared with different SDS/AgNO_3 weight ratios.

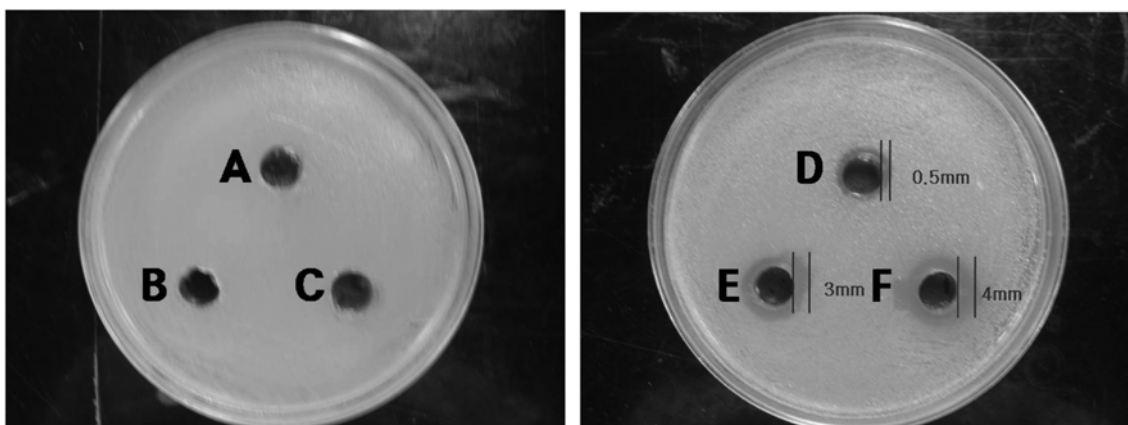


Fig. 6. Photographic images of bacterial inhibition zones against *S. aureus*, produced by the silver nanoparticles, prepared with different SDS/AgNO_3 weight ratios. (a) 0.5, (b) 1, (c) 2, (d) 5, (e) 10 and (f) 20.

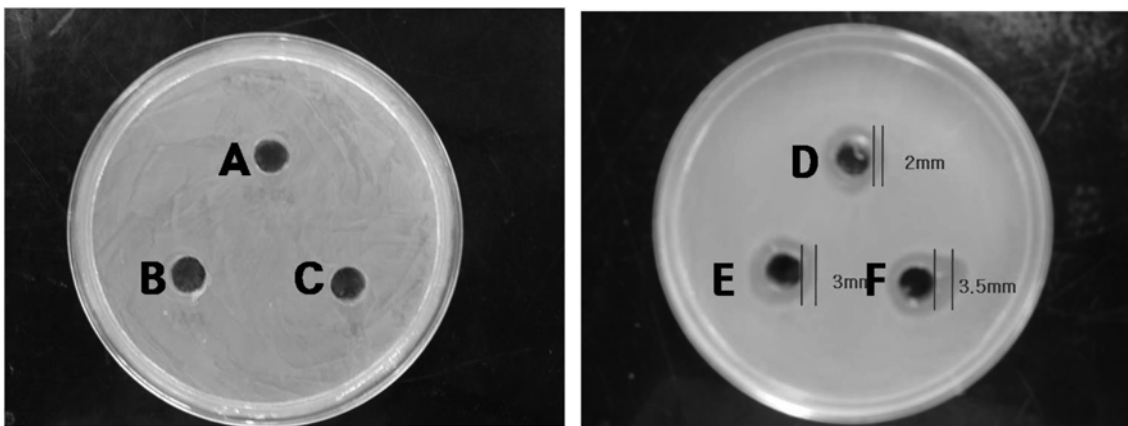


Fig. 7. Photographic images of bacterial inhibition zones against *E. coli*, produced by the silver nanoparticles, prepared with different SDS/AgNO_3 weight ratios. (a) 0.5, (b) 1, (c) 2, (d) 5, (e) 10 and (f) 20.

the cell wall of the bacteria, leading to cell death. In this study, the enhanced bactericidal activity of well dispersed silver nanoparticles may be attributed to more efficient transport of nanoparticles in solutions to the cell wall of bacteria compared to the aggregated silver nanoparticles.

2. Effect of SDS Concentrations

SDS was used to prevent the growth and aggregation of particles, and give stable colloidal silver nanoparticles with a narrow size distribution. Fig. 5 shows the UV-vis spectra of colloidal silver nanoparticles with different SDS/AgNO₃ weight ratios (1, 2, 10 and 20). The nanoparticles were synthesized at the conditions of initial AgNO₃ concentration of 0.002 M and NaBH₄/AgNO₃ molar ratio of 1. When the SDS/AgNO₃ weight ratios were low (1, 2), the obtained solutions showed dark color, and the absorption peaks exhibited a broad distribution, indicating that silver nanoparticles were aggregated. However, at high SDS/AgNO₃ weight ratios (10, 20), the solutions were colored yellow, and the absorption peaks became narrow, suggesting that the silver nanoparticles were well dispersed.

Figs. 6 and 7 show photographic images of bacterial inhibition zones against *S. aureus* and *E. coli*, respectively, produced by the silver nanoparticles prepared with different SDS/AgNO₃ weight ratios. It can be seen that the nanoparticles prepared at low SDS/AgNO₃ weight ratios (0.5, 1 and 2) did not produce any inhibition zones, indicating no antibacterial activity. However, the nanoparticles prepared at high SDS/AgNO₃ weight ratios (5, 10 and 20) showed inhibition zones of 0.5–4 mm and 2–3.5 mm for *S. aureus* and *E. coli*, respectively, exhibiting a good antibacterial activity. These results are well in agreement with the antibacterial results in Figs. 3 and 4. It can be also inferred that the well dispersed particles in solutions show higher antibacterial activity than the aggregated particles in solutions, which are due to much more efficient transport of well dispersed nanoparticles to the cell wall of bacteria.

CONCLUSIONS

Silver nanoparticles were successfully prepared by reducing AgNO₃ aqueous solution with sodium borohydride in the presence of SDS as stabilizer. The obtained particles were spherical with an average size of 10–20 nm. The diameter of the particles showed no obvious change with increasing NaBH₄/AgNO₃ molar ratios. TEM observation showed that when low molar ratios of NaBH₄/AgNO₃ were used (0.5, 1 and 2), the silver nanoparticles formed were highly stable and well dispersed in solutions. However, profound aggregation be-

tween nanoparticles was observed at high NaBH₄/AgNO₃ molar ratios (5, 10). The obtained silver nanoparticles inhibited the growth of the Gram-positive *S. aureus* and Gram-negative *E. coli*. Also, the silver nanoparticles, which were well dispersed in solutions, exhibited much higher bactericidal activity against *S. aureus* and *E. coli* compared to silver nanoparticles with severe aggregation.

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