

Cold plasma-assisted synthesis of silver nanoparticles using a sand-bath reactor and their antibacterial activity against *E. coli*

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ABSTRACT

Silver nanoparticles (AgNPs) are widely studied because of their strong optical response and antibacterial activity. In this work, AgNPs were synthesized by a cold atmospheric-pressure plasma-assisted method using AgNO₃, NaBH₄, and sodium citrate in a sand-bath reactor. The reaction was performed in a 25 mL glass beaker under Ar plasma treatment at 100 sccm and 60–70 °C. The plasma jet operated stably and produced OH, N₂, and Ar emissions, indicating active plasma–liquid interaction. The effects of plasma irradiation time, citrate amount, and NaBH₄ amount were investigated by UV-Vis spectroscopy. The optimized condition produced a yellow-orange AgNP colloid with a clear LSPR peak at 395–400 nm. After centrifugation at 9000 rpm for 5 min, the FWHM decreased from 82 to 70 nm. The optimized AgNPs had a hydrodynamic size of about 14 nm, a zeta potential of –24 mV, and a TEM size of 12.28 ± 2.04 nm. HR-TEM showed a lattice spacing of 0.23 nm. The plasma-synthesized AgNPs showed stronger inhibition against *E. coli* than commercial citrate-stabilized AgNPs. These results show that sand-bath plasma-assisted synthesis is a simple and effective route to prepare small AgNPs for antibacterial applications.

Keywords: Silver nanoparticles; Cold atmospheric-pressure plasma; Sodium citrate; Antibacterial activity; *E. coli*.

1. INTRODUCTION

Silver nanoparticles (AgNPs) are important metal nanomaterials because of their localized surface plasmon resonance, high surface activity, and broad antibacterial performance [1-3]. Their properties depend strongly on particle size, morphology, surface chemistry, and colloidal stability [4]. Small and well-dispersed AgNPs usually show strong visible absorption and high interaction efficiency with bacterial cells [5]. Therefore, simple synthesis routes that can control particle formation and surface properties remain important for antibacterial applications.

Chemical reduction is a common method for AgNP synthesis [4]. In this approach, Ag⁺ ions are reduced to Ag⁰ atoms by reducing agents such as NaBH₄, citrate, or ascorbate, followed by nucleation and growth. Sodium citrate is often used as a weak reducing agent and stabilizer. However, conventional chemical reduction requires careful control of reagent concentration, temperature, and reaction time [6]. Excess chemicals or dense surface capping may also reduce the accessible active surface of AgNPs [7, 8].

Cold atmospheric-pressure plasma has recently been used for liquid-phase nanomaterial synthesis [9]. Plasma–liquid interaction can generate energetic electrons, UV photons, ions, and reactive oxygen and nitrogen species at the liquid surface [10]. These components can support

metal-ion reduction and promote nanoparticle nucleation [11]. In our preliminary tests, plasma-only treatment, previously applied to synthesize stabilizer-free AuNPs [9], was not sufficient to form AgNPs in AgNO_3 solution. In citrate/ NaBH_4 -assisted synthesis, AgNP formation usually requires heating at about 75 - 80 °C for 1 - 2 h to obtain stable colloids. Therefore, a sand-bath reactor was used here to provide indirect and uniform heating, reduce temperature gradients, and assist controlled AgNP formation.

Several studies have reported plasma-based AgNP synthesis. Habib et al. used a non-thermal atmospheric-pressure plasma jet with AgNO_3 and trisodium citrate to rapidly form AgNPs [12], while Kondeti et al. showed that plasma-delivered electrons, radicals, reactive species, and UV/VUV photons can assist Ag^+ reduction at the plasma-liquid interface [13]. Based on these studies, the present work combines plasma treatment with indirect sand-bath heating to improve thermal control under low chemical-reductant conditions.

In this study, AgNPs were synthesized by cold plasma-assisted chemical reduction in a sand-bath reactor. AgNO_3 , NaBH_4 , and sodium citrate were used as the silver precursor, reducing agent, and stabilizer, respectively. The plasma jet was characterized by electrical measurement and optical emission spectroscopy. The effects of plasma irradiation time, NaBH_4 amount, and citrate amount were investigated by UV-Vis spectroscopy. The optimized AgNPs were characterized by DLS, zeta potential, SEM, TEM, and HR-TEM. Their antibacterial activity against *E. coli* was then evaluated and compared with commercial citrate-stabilized AgNPs.

2. EXPERIMENTAL

2.1. Chemicals and precursor solutions

AgNO_3 , NaBH_4 , sodium citrate, and deionized water were used for AgNP synthesis. AgNO_3 and sodium citrate stock solutions were prepared at 1% w/w, corresponding to 58.86 and 38.75 mM, respectively. NaBH_4 was prepared at 0.1% w/w, corresponding to 26.43 mM. Deionized water with a resistivity of 15 M Ω .cm was used. Commercial citrate-stabilized AgNPs from Sigma-Aldrich/Merck (product No. 730785, nominal size: 10 nm, 0.02 mg/mL in aqueous buffer) were used as the reference sample and denoted as AgNP-c.

2.2. Plasma-assisted synthesis setup

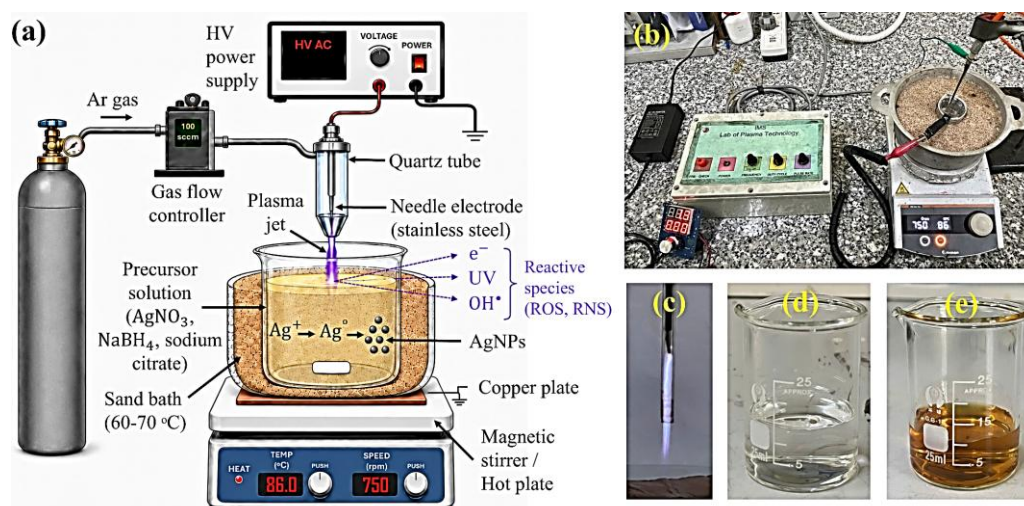


Figure 1. Plasma-assisted setup for AgNP synthesis. (a) Schematic of the cold atmospheric-pressure plasma system coupled with a sand-bath reactor. (b) Photograph of the experimental setup. (c) Plasma plume generated at the quartz-tube outlet. Photographs of the solution (d) before and (e) after plasma treatment.

The plasma-assisted synthesis setup is shown in Figure 1. The plasma jet was generated using Ar gas and a tunable high-voltage power supply of 2–6 kV and 20–100 kHz. The discharge was formed inside a quartz tube with a stainless-steel needle electrode and directed toward the precursor solution. The reaction was carried out in a 25 mL glass beaker placed in a sand bath for indirect heating, with stirring at 750 rpm.

Before plasma treatment, the precursor solution containing AgNO₃, NaBH₄, and sodium citrate was heated to about 80 °C. The beaker was covered with aluminum foil to reduce evaporation, with a small hole for the quartz plasma tube. Ar gas was introduced at 100 sccm, and the solution was treated for 5 min at about 60–70 °C. No noticeable volume loss was observed, and the temperature decrease was mainly due to the cooling effect of the Ar gas flow. After treatment, the solution was further stirred for 10 min and stored at room temperature.

NaBH₄ acted as the main reducing agent, while sodium citrate stabilized the AgNPs. Thus, the process is considered plasma-assisted chemical reduction rather than plasma-only reduction. Without plasma, AgNP formation was very slow at room temperature, whereas plasma treatment produced a yellow-orange colloid within a few minutes, likely due to the activated plasma–liquid interface that supports Ag⁺ reduction, nucleation, and early particle growth.

2.3. Plasma characterization

The plasma jet was characterized by voltage-current waveform measurement and optical emission spectroscopy (OES). The electrical waveform was used to determine discharge stability, frequency, and pulsed operation. The OES spectrum was recorded under the same synthesis condition to identify OH, N₂, and Ar emissions near the plasma-liquid interface.

2.4. Optimization of synthesis conditions

The effects of plasma irradiation time and precursor composition were evaluated by UV-Vis spectroscopy. Plasma treatment times of 1, 3, and 5 min were tested. Sodium citrate volumes of 0.5, 1.0, and 1.5 mL and NaBH₄ volumes of 0.05, 0.10, and 0.15 mL were examined. AgNP quality was assessed from LSPR peak position, absorbance intensity, FWHM, and long-wavelength tail. Selected samples were centrifuged at 9000 rpm for 5 min.

2.5. Material characterization

UV-Vis spectroscopy was used to evaluate AgNP formation and optical properties. Samples were diluted 10 times before measurement. DLS and zeta potential were used to determine hydrodynamic size and surface charge. SEM, TEM, and HR-TEM were used to analyze morphology, particle size, and lattice structure.

2.6. Antibacterial test

The antibacterial activity was tested against *E. coli* by the agar well diffusion method. AgNP-p was tested at 0.1, 1, 10, and 20 µg/mL, while AgNP-c was tested at 1, 10, and 20 µg/mL. Deionized water was used as the negative control, and cephalexin at 100 and 2000 µg/mL was used as the positive control. After sample loading, the plates were kept for 20–30 min for diffusion and incubated at 37 °C for 24 h. The inhibition-zone diameter was then measured. All inhibition-zone diameters are presented as mean ± standard deviation from three repeated measurements.

3. RESULTS AND DISCUSSION

3.1. Electrical and optical characterization of the plasma jet

Figure 2 shows that the plasma jet operated as a stable periodic discharge. The peak-to-peak voltage was about 4.2 kV, with a period of 24 µs, corresponding to a frequency of about 41 kHz. The discharge worked in a pulsed mode of about 0.7 s on and 0.5 s off, giving a duty cycle of about 58%. This mode was controlled by the electronic unit of the plasma power system and helped maintain a stable plume while reducing excessive heating. The OES spectrum shows OH emission

near 309 nm, N_2 bands in the 330–390 nm region, and strong Ar lines mainly at 700–850 nm. These emissions confirm the formation of reactive species at the plasma–liquid interface, which, together with energetic electrons and UV emission, can assist Ag^+ reduction and AgNP nucleation.

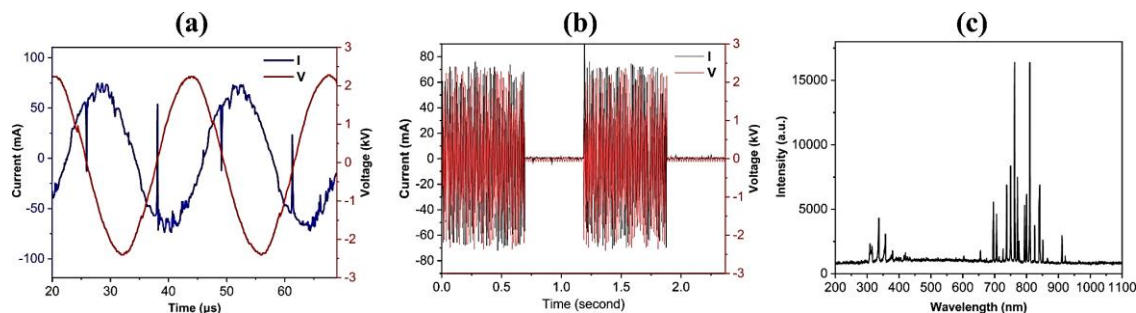


Figure 2. Electrical and optical characteristics of the plasma jet. (a) Voltage-current waveform of the discharge. (b) Pulsed operating mode of the plasma source. (c) Optical emission spectrum showing OH, N_2 , and Ar emission bands/lines under the AgNP synthesis conditions.

3.2. Effect of plasma irradiation time on AgNP formation

Figure 3 shows the effect of plasma irradiation time on AgNP formation. All samples showed an absorption band at 395–400 nm, confirming AgNP formation. After 1 min, the LSPR peak was weak, with a maximum absorbance of about 0.36, indicating limited Ag^+ reduction. Increasing the irradiation time to 3 min increased the absorbance to about 0.52, showing more efficient AgNP formation. At 5 min, the absorbance slightly decreased to about 0.48, and the absorption tail at 450–550 nm became more visible. This suggests that prolonged exposure may promote secondary growth or partial aggregation. Therefore, 3 min gave the strongest LSPR response, while the 3–5 min range was suitable for AgNP formation.

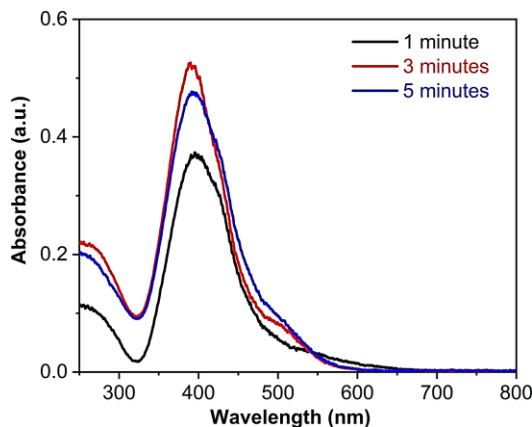


Figure 3. UV-Vis absorption spectra of AgNPs synthesized with different plasma irradiation times of 1, 3, and 5 min.

3.3. Effect of $NaBH_4$ and sodium citrate concentration

The effects of citrate and $NaBH_4$ amounts were evaluated by UV-Vis spectroscopy (Figure 4). With 0.5–1.5 mL citrate, all samples showed a main LSPR band at 400–405 nm. The 0.5 mL citrate sample had a stronger long-wavelength tail above 470 nm, suggesting insufficient stabilization, while 1.0 mL citrate reduced this tail and 1.5 mL gave no further improvement. Thus, 1.0 mL citrate was selected. $NaBH_4$ strongly affected Ag^+ reduction. Increasing $NaBH_4$ from 0.05 to 0.10 mL raised the maximum absorbance from about 0.27 to 0.67 at 395–400 nm, while 0.15 mL broadened the spectrum and lowered the peak intensity. Therefore, 1.0 mL citrate and 0.10 mL $NaBH_4$ were selected as the optimized precursor condition.

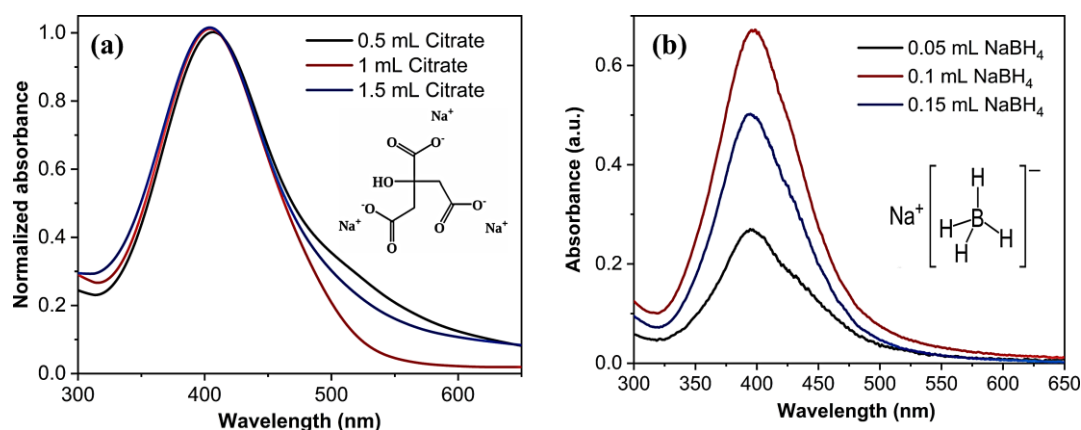


Figure 4. Effect of precursor composition on AgNP formation. UV-Vis absorption spectra of AgNPs synthesized with different volumes of (a) sodium citrate and (b) NaBH₄.

The insets show the molecular structures of sodium citrate and NaBH₄.

3.4. Effect of centrifugation and colloidal properties

Centrifugation improved the optical quality of the AgNP colloid (Figure 5a). Before centrifugation, the LSPR peak appeared at 395–400 nm with a FWHM of about 82 nm. After centrifugation at 9000 rpm for 5 min, the LSPR position remained almost unchanged, while the FWHM decreased to about 70 nm and the long-wavelength tail above 450 nm was reduced, indicating partial removal of larger particles or weak aggregates. The purified AgNPs showed a main hydrodynamic size of about 14 nm (Figure 5b), consistent with the near-400 nm LSPR band. The zeta potential was about –24 mV (Figure 5c), indicating citrate-assisted negative surface charge and colloidal stabilization.

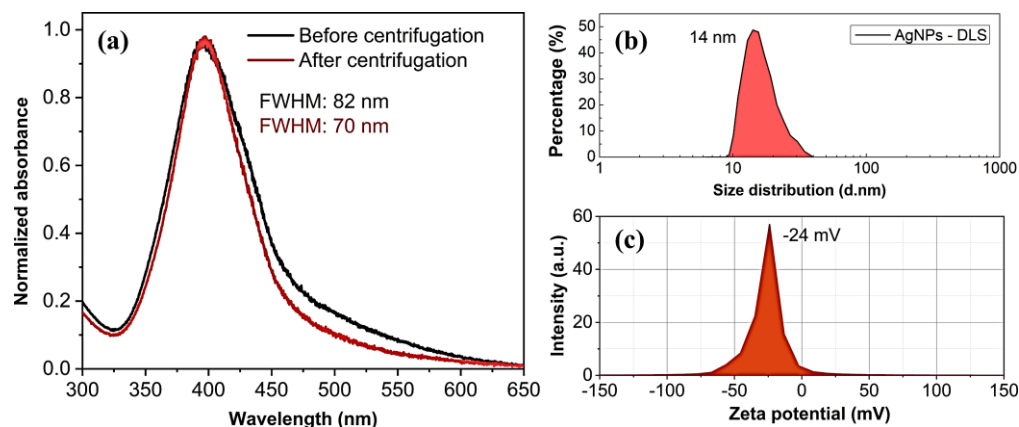


Figure 5. Optical and colloidal properties of optimized AgNPs.

(a) Normalized UV-Vis spectra before and after centrifugation. (b) DLS size distribution and (c) zeta potential distribution of AgNPs after centrifugation.

3.5. Morphology and crystal structure

The morphology of the optimized AgNPs is shown in Figure 6. SEM and TEM images confirm that the particles were mainly spherical or nearly spherical, with limited large aggregation after centrifugation. Some local clustering was observed after drying on the TEM grid, but most particles remained separated. The TEM size distribution gave an average diameter of 12.28 ± 2.04 nm, close to the DLS result. HR-TEM showed clear lattice fringes with an interplanar spacing of about 0.23 nm, which can be assigned to the (111) plane of face-centered cubic Ag. These results confirm the formation of small and crystalline AgNPs under the optimized plasma-assisted condition.

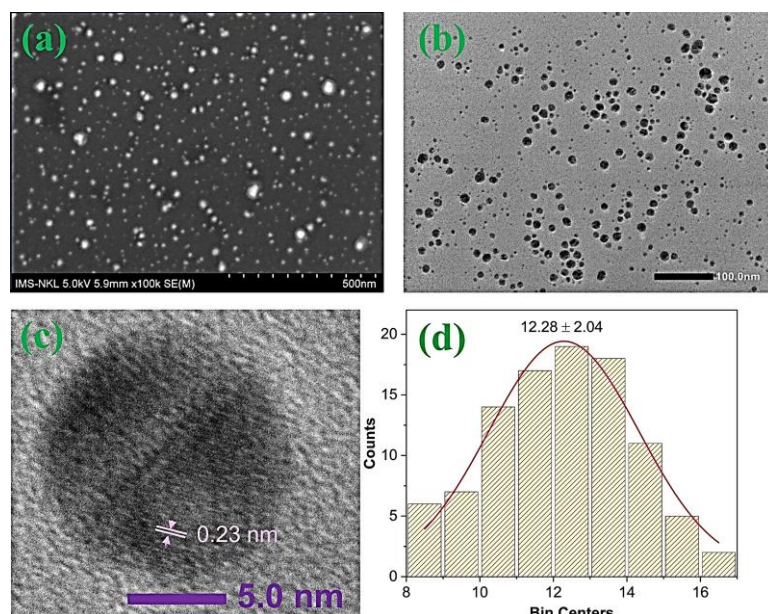


Figure 6. Morphology and size distribution of the optimized AgNPs. (a) SEM image, (b) TEM image, (c) HR-TEM image showing lattice fringes with a spacing of 0.23 nm, and (d) particle-size distribution obtained from TEM analysis of ~100 particles.

The particle size is presented as mean $\pm$ standard deviation.

3.6. Antibacterial activity against *E. coli*

The antibacterial activity of AgNPs against *E. coli* was evaluated by the agar well diffusion method (Figure 7). Deionized water showed no inhibition zone, while cephalaxin produced clear zones of about 6 mm at 100 $\mu\text{g/mL}$ and 20 mm at 2000 $\mu\text{g/mL}$. For plasma-synthesized AgNPs (AgNP-p), the inhibition-zone diameter increased from ~0.1 mm at 0.1 $\mu\text{g/mL}$ to about 2, 10, and 11 mm at 1, 10, and 20 $\mu\text{g/mL}$, respectively, confirming concentration-dependent antibacterial activity.

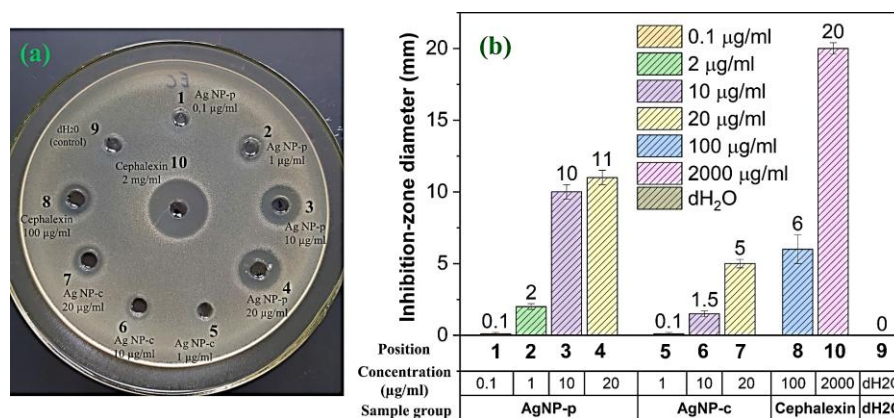


Figure 7. Antibacterial activity of AgNPs against *E. coli*.

(a) Agar well diffusion test showing inhibition zones of plasma-synthesized AgNPs (AgNP-p), commercial citrate-stabilized AgNPs (AgNP-c), cephalaxin, and dH₂O control.

(b) Inhibition-zone diameters measured from different sample positions and concentrations.

The numbers shown in the agar plate images indicate sample positions.

Commercial AgNPs (AgNP-c) showed weaker inhibition, with zones of about 0.1, 1.5, and 5 mm at 1, 10, and 20 $\mu\text{g/mL}$, respectively. The antibacterial effect of AgNPs is mainly related to Ag⁺ release, interaction with the bacterial membrane, membrane damage, and oxidative stress

generated during AgNP–cell interaction [14]. Since the AgNP colloid was washed and centrifuged before testing, short-lived plasma-generated ROS are not expected to directly contribute to bacterial killing. Thus, the ROS discussed here refer to oxidative stress generated during AgNP–bacteria interaction, not residual plasma species. The stronger inhibition of AgNP-p may be related to its small particle size and surface condition, which can favor bacterial contact and Ag⁺ release.

4. CONCLUSIONS

AgNPs were successfully synthesized by cold atmospheric-pressure plasma-assisted chemical reduction using AgNO₃, NaBH₄, and sodium citrate in a sand-bath reactor. The plasma jet operated stably and generated OH, N₂, and Ar emissions, confirming a reactive plasma–liquid environment. UV-Vis results showed that plasma irradiation time and precursor composition affected AgNP formation, with 1.0 mL citrate and 0.1 mL NaBH₄ selected as the optimized condition.

The optimized AgNPs showed a clear LSPR peak at 395–400 nm, and centrifugation reduced the FWHM from 82 to 70 nm, indicating improved optical quality. The particles had a hydrodynamic size of about 14 nm, a zeta potential of –24 mV, and a TEM size of 12.28 ± 2.04 nm. HR-TEM showed a lattice spacing of 0.23 nm. The plasma-synthesized AgNPs exhibited concentration-dependent antibacterial activity against *E. coli* and stronger inhibition than commercial citrate-stabilized AgNPs at comparable concentrations.

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TÓM TẮT

Nghiên cứu tổng hợp hạt nano bạc bằng phương pháp plasma lạnh kết hợp gia nhiệt bề cát và đánh giá hoạt tính kháng khuẩn đối với *E. coli*

Trong nghiên cứu này, hạt nano bạc (AgNPs) được tổng hợp bằng phương pháp plasma lạnh áp suất khí quyển trong hệ phản ứng nối cát gia nhiệt gián tiếp. Dung dịch AgNO₃, NaBH₄ và natri citrate được xử lý bằng plasma Ar ở 100 sccm và 60–70 °C. Đặc trưng điện và phổ phát xạ cho thấy plasma hoạt động ổn định, tạo ra các phát xạ OH, N₂ và Ar, hỗ trợ quá trình khử Ag⁺ và hình thành AgNPs. Ảnh hưởng của thời gian chiếu plasma, lượng NaBH₄ và natri citrate được khảo sát bằng phổ UV-Vis. Điều kiện tối ưu tạo ra dung dịch AgNPs màu vàng với đỉnh LSPR khoảng 395–400 nm. Sau ly tâm ở 9000 vòng/phút trong 5 phút, độ rộng bán cực đại giảm từ 82 xuống 70 nm. AgNPs thu được có kích thước thủy động học khoảng 14 nm, thế zeta –24 mV và kích thước trung bình theo TEM là 12.28 ± 2.04 nm. AgNPs tổng hợp bằng plasma thể hiện khả năng ức chế *E. coli* phụ thuộc nồng độ và hiệu quả hơn mẫu AgNPs thương mại.

Từ khóa: Hạt nano bạc; Plasma lạnh áp suất khí quyển; Natri citrate; Kháng khuẩn; *E. coli*.