

SILVER NANOPARTICLES: GREEN SYNTHESIS AND ANTIBACTERIAL EFFICIENCY[#]

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Abstract. In this study, silver nanoparticles were synthesized from aqueous silver nitrate through a simple and eco-friendly route using a combination of two reducing agents: sodium citrate and tannic acid. By this method, the obtained Ag nanoparticles (NPs) were stable within the studied period of six months. Besides, both TEM images and UV-Vis results showed that the size of silver NPs could be controlled by changing the concentration of tannic acid. The antibacterial ability of Ag NPs with different sizes was also examined. In detail, the smaller the Ag NPs were, the more efficient their antibacterial activity was.

Keywords: green synthesis, Ag nanoparticles, antibacterial.

Classification numbers: 1.3.3, 2.5.1.

1. INTRODUCTION

The field of nanotechnology has rapidly grown over the past few years and has even ventured into the field of clinical medicine [1 - 3]. Among types of metallic nanoparticles, silver nanoparticles (AgNPs) seem to have attracted the most interest in terms of their potential applications. Ionic or metallic silver features low toxicity to human cells, high thermal stability and low volatility [2 - 4], such properties can be exploited in medicine for burn treatment and dental materials, and in the manufacturing industry as coatings for stainless steel materials, textile fabrics, water treatment, sunscreen lotions, etc [5, 6].

For the production of metallic silver from different salts, a variety of different reduction methods have been proposed [6 - 16] and can be divided into two ways. The first route to synthesize nanoparticles is using naturally occurring reagents such as biodegradable polymers (chitosan, polysaccharides, etc.) [17 - 21], plant extracts [22 - 24], and microorganisms [25] as reductants and capping agents. In recent times, this approach has received a lot of attention for green synthesis, however, the collected nanoparticles were not high-qualified, polydisperse and

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difficult to control the stable quality. The second one is a chemical approach in which the metal ions in solution are reduced by various organic and inorganic reducing agents. The advantages of this method is that a substantial amount of NPs can be synthesized in a short period of time and requires only basic equipment.

In the aqueous medium, there are several reductants commonly being used for the synthesis of the AgNPs such as sodium borohydride, citrate, ascorbate and elemental hydrogen. Among them, sodium borohydride and citrate reduction of noble metal salts remain the most popular approach to quickly generate noble metal colloids. However, sodium borohydride is so strong reducing agent that the reduction reaction takes place very quickly with an explosion of nucleation, leading to non-uniform size of nanoparticles.

Recent studies have shown that the mono-dispersed spherical AgNPs were synthesized via a one-step reduction method using sodium citrate and varying quantities of tannic acid (TA). Dadosh obtained AgNPs in the 18 - 30 nm diameter range with a standard deviation of less than 15 % [26]. Bastus and co-workers synthesized monodisperse sodium citrate-coated spherical AgNPs and AuNPs with controlled sizes ranging from 10 to 200 nm by 2-step seed-regrowth method [27]. Very recently, La Spina found that the final size and dispersity of AgNPs can be further tailored by controlling the amount of TA and reaction time at 60 °C [28]. Additionally, TA is a typical hydrolysable tannin derived from plants, thus eco-friendly, and has significant effect on dispersion and mobility of nanomaterials in aqueous medium [29, 30].

In this work, the size of AgNPs can be controlled from 16 to 29 nm by adding a different amount of tannic acid. Moreover, the Ag nanoparticles also showed stability for a period of 6 months. The antibacterial activity (of differently sized AgNPs) against *E. coli* bacterial was examined.

2. MATERIALS AND METHODS

2.1. Chemicals

Silver nitrate (AgNO_3), trisodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$), and tannic acid ($\text{C}_{76}\text{H}_{52}\text{O}_{46}$) were purchased from Sigma-Aldrich. All chemicals were used as received without further purification. Distilled water was used in all experiments. All glassware was first rinsed with acetone and then with distilled water before use.

2.2. Synthesis of Ag nanoparticles

A 100 mL of an aqueous solution containing sodium citrate (SC) (5 mM) and TA was prepared and heated with a heating mantle in a 250 ml glass beaker for 15 min under vigorous stir. When the solution started boiling, 1 mL of AgNO_3 (25 mM) was injected into this solution. The solution turned dark yellow immediately. The size of the AgNPs was controlled by adjusting the TA concentration from 0.2 mM to 5 mM, corresponding to the ratios TA/SC varied from 0.04/1 to 1/1. The resultant AgNPs were purified by centrifugation (at 11000 rpm - 13000 rpm for 30 min, depending on their size) in order to remove the excess TA (or SC), then further redispersed in distilled water before the sample characterization.

2.3. Measurement tools

Transmission electron microscope (TEM) images were recorded with a JEM 1010 (Japan) equipment. The UV-Vis absorption spectra of samples were acquired on a Jasco V-670 (Japan) spectrophotometer. The size distribution of AgNPs was collected by using ImageJ software.

2.4. Antibacterial testing

The bacteriostasis of the samples was also evaluated by disk diffusion, a visualized and semi-quantitative method. The so-called disk diffusion method involves multiple steps. Firstly, 100 mL of bacteria suspensions were placed uniformly on the surface of the agar Petri dish and solidified. Secondly, the sterile paper disks with a diameter of 6 mm were placed on the Petri dish and marked. Thirdly, each Ag sample (fabricated by different amount of TA) was diluted to different concentration and dropped into separated regions of the Petri dish. The as-separated paper disks were dried at ambient conditions for 15 min. Finally, the agar Petri dishes were kept in the constant temperature incubator at 37 °C for 22 h. Upon completion of measurements, the bacteriostasis effect was evaluated by determining the diameter of the visible transparent inhibitory zone.

3. RESULTS AND DISCUSSION

3.1. Effect of tannic acid on the morphology of AgNPs

Figure 1 shows the TEM images and particles size distribution of the obtained AgNPs at different TA/SC mole ratios varied from the 0.04/1 to 1/1. It can be seen that Ag nanoparticles were well separated from each other with an apparently uniform interparticle distance. The spherical and monodisperse nanoparticles were produced and their size increased from 14.5 ± 5.5 nm to 29 ± 4.9 nm when the concentration of TA increased from 0.2 to 5 mM. This result is also consistent with the Ag nanoparticles obtained by Dadosh [26]. The change of particle size can be explained by the following mechanism: In the first stage of the reaction, the citric acid (CA)-TA complex is formed [31]. In the next stage, the Ag(I) precursor is reduced to form Ag (0) atoms by CA-TA complex. At low concentrations of TA, the Ag nanoparticles is form by both SC and CA-TA complex. At high concentrations of TA, the Ag nanoparticles are mainly produced by CA-TA complex. The adduct shows better reducing properties than either SC or TA alone. When the concentration of Ag (0) atoms steadily increases to a certain level greater than the point of supersaturation, they will aggregate into small clusters or nuclei *via* homogeneous nucleation. These nuclei then grow into larger nanocrystals with a relatively broad size distribution [32].

3.2. The UV-Vis absorption spectra of AgNPs.

3.2.1. Effect of tannic acid on UV-Vis spectra of AgNPs

UV - Vis spectra of the as-synthesized AgNPs from different concentrations of TA and the constant concentration of SC are shown in Figure 2. UV-Vis spectra showed strong surface plasmon resonance (SPR) band around 410 nm, indicating the formation of silver nanoparticles. Besides, UV-Vis spectral measurement illustrates a shift of SPR peak from 403 to 420 nm when

the concentration of TA varies from 0.2 to 5 mM. It is clear that the increase in particle size induces the shift of the SPR band.

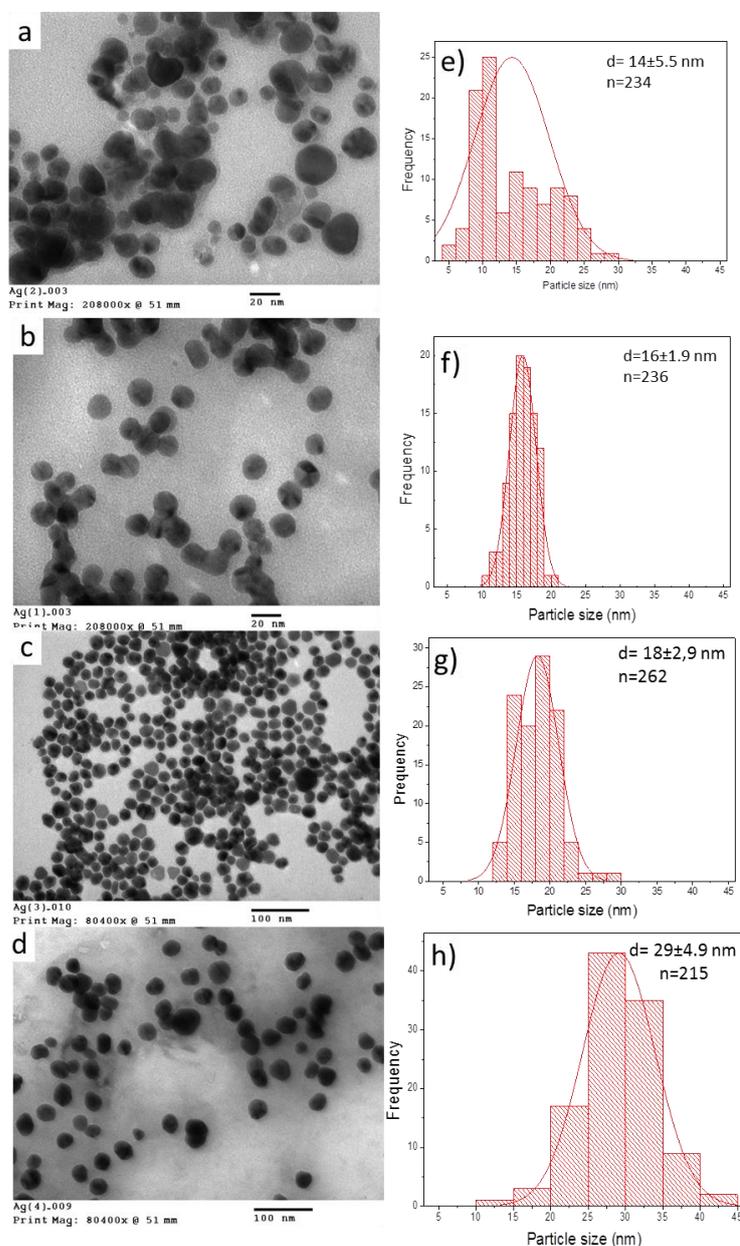


Figure 1. The TEM images (a,b,c,d) and histograms of the particles size distribution (e, f, g, h), respectively, of Ag nanoparticles with different concentrations of TA (0.2, 0.5, 2 and 5 mM).

3.2.2. The stability of silver NPs

UV–Vis technique was used to track the changes in the AgNP solutions. The UV–Vis spectra recorded for the samples fabricated within TA concentrations 0.2 and 5 mM during different storage time (for 3 and 6 months) of this study are shown in Figure 3.

According to Yin *et al.* [33], when the oxidation degree of the NPs increases, the plasmon absorption peak decreases in intensity, and a redshift of the SPR peak is observed. As shown in Fig. 3b, the UV–Vis spectra of AgNPs synthesized with a larger amount of TA do not show any obvious change even after 6 months, implying negligible oxidation. However, for the sample obtained by a smaller amount of TA (Fig. 3a), the absorbance spectra indicate an increase in absorbance of AgNP solutions and a small redshift of SPR peak during the investigated time period of 6 months.

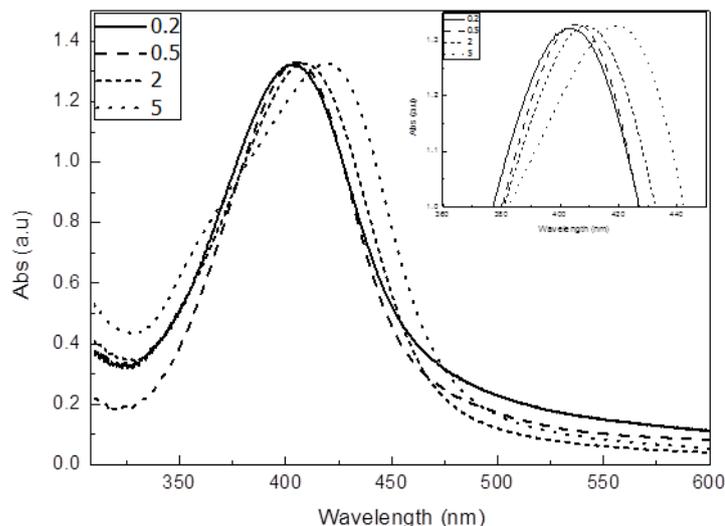


Figure 2. UV – Vis spectra of AgNPs fabricated with different concentration of TA (0.2, 0.5, 2 and 5 mM).

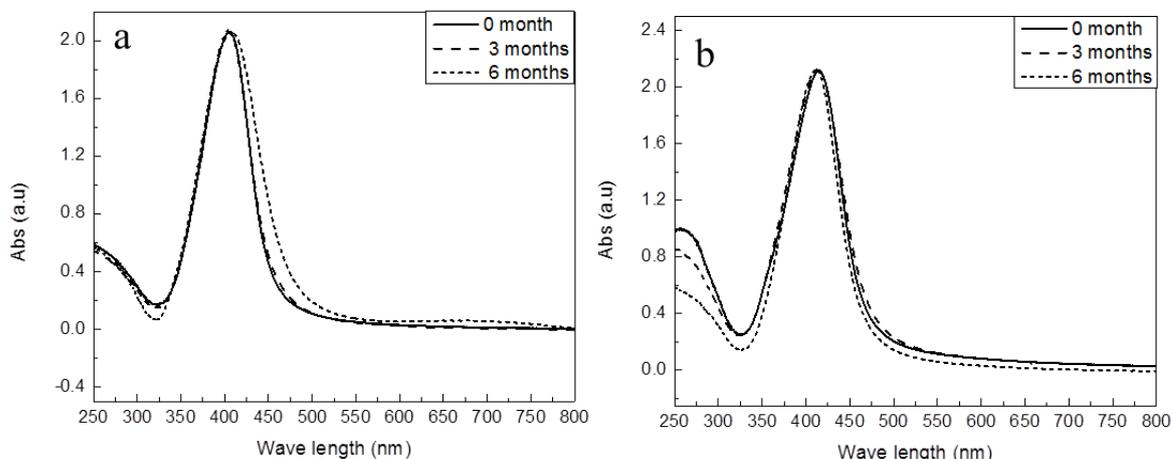


Figure 3. UV–Vis absorption spectra measured during time for the AgNP solutions prepared with concentration of TA 0.2 mM (a) and 5 mM (b).

3.3. Antibacterial activities of AgNPs

The antibacterial effect of silver nanoparticles is closely related to their size. Figure 4 shows the diameters of inhibition zones for the obtained samples with 0.2, 2 and 5 mM tannic acid (denoted as sample 1, sample 2, and sample 3, respectively) at different concentrations (4, 1 and 0.25 mg/mL). The whole visual results of the disk diffusion test, the inhibition zone photographs of samples 1 - 3 against bacteria *E. coli*, are shown in Fig. 5. The positions of (1 - 3) in dishes

correspond to the samples with concentrations of 4, 1, and 0.25 mg/mL. It is seen from Fig. 5 that there is no obvious inhibition zone for the control test of Ag-NPs. However, all samples of AgNPs with different sizes exhibited distinct inhibition zone, implying that the as-prepared AgNPs had good antibacterial properties. Furthermore, at the same concentration of AgNPs, the sample fabricated with lower concentration of tannic acid induced clearer and larger inhibition zone than that prepared with higher quantity of tannic acid. It is suggested that the smaller AgNPs possess superior bacteriostasis. It also can be seen from Fig. 5 that for the same AgNPs sample, the diameter of inhibition zone enlarges with the increasing of concentration.

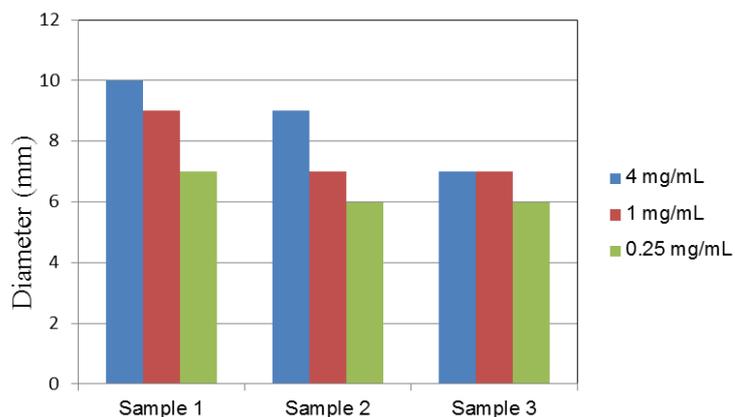


Figure 4. Histograms of diameters of inhibition zones of samples against *E. coli* at different concentration of TA (4, 1 and 0.25 mg/mL).

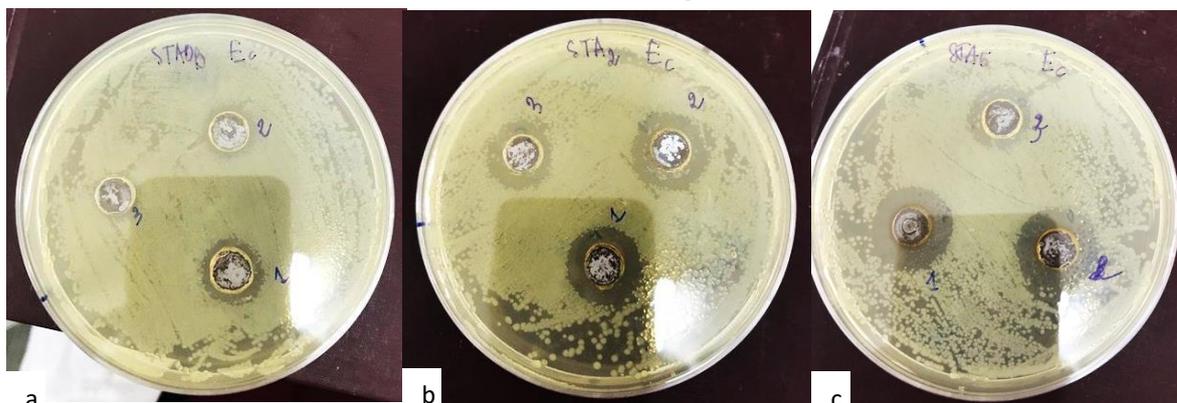


Figure 5. Inhibition zone photographs of samples fabricated with 0.2 (a), 2 (b) and 5 mM (c) TA against bacteria *E. coli* in plates are corresponded to the samples concentration of 4, 1, and 0.25 mg/mL.

4. CONCLUSIONS

In conclusion, the uniform and size-tunable AgNPs were successfully fabricated by using a mixture of two reducing agents including sodium citrate and tannic acid. The obtained results clearly indicate that the combined use of sodium citrate and tannic acid produces monodisperse spherical AgNPs, as it allows the control of nucleation, growth and stabilization of the synthesis process. The size of AgNPs can be adjusted by changing TA concentration. The obtained AgNPs by this method also showed the stability during a period of time. The antibacterial effect of the

as-prepared AgNPs with different particle sizes was assessed. It can be seen that the as-prepared AgNPs exhibit good antibacterial properties.

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Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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