

## Green Synthesis and Characterization of Silver Nanoparticles Using Ginkgo Biloba Leaf Extract

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A facile, effective and green method using Ginkgo Biloba leaf extract was applied and optimized for the preparation of well dispersed silver nanoparticles. In the method, Ginkgo Biloba leaf extract was employed as both stabilizing and reducing agent without the addition of a toxic agent. 0.1 % silver nitrate solution (w/v) was used silver source. The synthesized silver nanoparticles were investigated and examined by UV-vis absorption spectroscopy (UV-vis), Scanning electron microscope (SEM), Transmission electron microscopy (TEM), powder X-ray diffraction (XRD) and Dynamic light scattering (DLS). The formation of silver nanoparticles was found by a change of color from light yellow to red, which was further proved by absorbance peak at 456 nm in UV-vis spectroscopy. The prepared nanoparticles are global in shape, highly crystalline in nature with a narrow distribution from 10 nm to 40 nm. The silver nanoparticles were capped with extracts, which prevented them from agglomeration and oxidation. Different parameters affecting the generation performance of silver nanoparticles, such as time, amount of silver nitrate and extract were investigated. The results demonstrate that these reaction parameters play important roles in the synthesis of silver nanoparticles.

*Keywords:* silver nanoparticles, green method, Ginkgo Biloba, powder technology.

### 1. INTRODUCTION

Over the last few decades, metal nanoparticles like copper, silver and gold nanoparticles have received much attention due to their special and considerable physical, chemical and biological properties, which may attribute to their small sizes and large specific surface area [1–12]. Due to their particular and unique properties, metal nanoparticles have been found used in many applications in different fields such as biomedical, food industries, catalysis, electronics, photonics and so on [13–21]. Nowadays, there are a lot of commercially available products containing metal nanoparticles [18, 22–24]. Among metal nanoparticles, silver nanoparticles have gained more and more focus because of their excellent applications in various fields [25–29]. Silver nanoparticles are one of the most important and applicable metal nanoparticles which were first synthesized a century ago. Until now, several methodologies have been used to prepare different types of silver nanoparticles, such as chemical reduction, thermal decomposition, photo-chemicals, laser ablation, biological and so on [28, 30–37]. However, some of the above-mentioned methods are subject to high energy consumption or suffer from difficulty in the purification of the last specimen. Furthermore, due to the use of noxious agents during the synthesized procedure, most of these reported ways created toxic silver nanoparticles which are potentially dangerous to the environment and human health [38–43]. Therefore, there is an increasing trend to evolve new

methods to prepare silver nanoparticles based on green chemistry using non-toxic, clean raw materials.

In recent years, green and eco-friendly technologies have remarkably been considered for the preparation of silver nanoparticles. In these methods, a lot of no-toxic, biodegradable raw materials, such as PVP, oleic acid, glucose, maltose and ascorbic acid were used as capping and reducing agents to the formation of silver nanoparticles [32, 38, 44–47]. It is a nice strategy to produce high quality silver nanoparticles. Very recently, the green synthesis of silver nanoparticles using all kinds of biological materials such as bacteria, fungi and plant extracts has attracted a large number of interests [40, 48–51]. Compare with the bacteria and fungi methods, the plant extracts methods can be more acceptable due to the wide range of natural resources with significant bioactive compounds, which served as the size-controlling agent [41, 52, 53]. In the synthesis process, the biochemical species in the extract can be reduced silver ion to silver nanoparticles and attach to the surface of silver nanoparticles, acting as reducing and capping agents. Various plants, such as black tea, *Rubia tinctorum* L., *Citrullus lanatus* and *Tamarix gallica* leaf have been used to prepare silver nanoparticles [54–56].

In our previous research, wolfberry fruit (*Lycium barbarum*), *Osmanthus fragrans* and *semen cassiae* extracts have been used as capping agents as well as reducing agent to prepare small size silver and gold nanoparticles [57–61]. In the present research, Ginkgo biloba leaf extract were exploited as a reducing and capping agent for the preparation of silver nanoparticles. Ginkgo biloba, has

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survived on the earth for 200 million years, as is widely planted in China, Korea, Japan, Europe and USA [62]. Ginkgo biloba leaf is an important medicinal herb in many countries. It has the effects of dredging collaterals and relieving pain, reducing lung qi and relieving asthma, activating blood circulation to remove blood stasis, and resolving turbidity and reducing blood fat. It was traditionally used for blood stasis obstructing collaterals, chest pain, apoplexy hemiplegia, lung deficiency, cough, asthma and hyperlipidemia [63, 64]. Ginkgo biloba leaf was composed of a variety of compounds such as Vitamin C, sterols, thiamin, niacin, polyphenol, flavonoids, terpene lactones protein, riboflavin, amino acid and carboxylic acids. These bioactive components can act as reducing or capping agent to prepare metallic nanoparticles [65]. Though, there were some reports on the synthesis of metal nanoparticles using ginkgo biloba leaf extract through heating [66, 67]. In the present study, we have adopted a method to prepare silver nanoparticles using ginkgo biloba leaf extract without heating, which may have a potential application in medicine.

## 2. EXPERIMENTAL METHODS

### 2.1. Reagents

Silver nitrate ( $\text{AgNO}_3$ ) was purchased from Sinopharm Chemical Reagent Co. Ltd. Silver nitrate used in the experiment acted as the precursor in the formation of Ag NPs without any renewed purification. The water used in the experiment was doubly distilled water. Fresh ginkgo biloba leaf was collected from Hubei Polytechnic University, Huangshi, Hubei, China. All chemicals and solvents were analytical grade, and used as received without further purification. Doubly distilled water was used in all experiments. To remove pollution, all glassware were cleaned with dilute nitric acid and rinsed with distilled water as well as dried in an oven.

### 2.2. Preparation of silver nitrate solution

To prepare 0.1 % silver nitrate solution (w/v), 0.5 gram of silver nitrate was dissolved in 500 mL of distilled water. The solution was kept in dark for further use.

### 2.3. Preparation of leaf extract

It is preferable that ginkgo biloba leaves extract can be used to reduce silver ions to silver atoms. For a typical process, the fresh leaves were collected from campus and washed thoroughly with distilled water to remove any pollution and dirt attached. The cleaned ginkgo biloba leaves were kept overnight for drying at room temperature. The aqueous extract was consequently prepared by mixing 30 grams of dried fresh leaves with 150 mL distilled water in a 250 mL flask. The prepared mixture was boiled at 100 °C for 30 min. The obtained ginkgo biloba leaves extracts were cooled to room temperature and then filtered through Whatman filter paper no. 1 in a sterilized bottle. At last, the total volume of extract was adjusted to 150 mL by adding distilled water and stored at 4 °C in a refrigerator. The extract was used for further studies within 3 days.

### 2.4. Synthesis of silver nanoparticles

In this research, ginkgo biloba leaves extract was utilized as a source of reducing and protecting agents for the

production of small silver nanoparticles in a simple and one-pot reaction. In a typical synthesis, silver nanoparticles were synthesized by mixing 8 mL ginkgo biloba leaves extract and 0.7 mL silver nitrate solution in a 100 mL conical flask. The volume of the mixture was adjusted to 25 mL by dropping the appropriate amount of distilled water. The mixing solution was continuously stirred using a magnetic stirrer at room temperature and allowed to react for 280 min. The colour of the reaction solution gradually varied from pale yellow to red, which exhibited the generation of silver nanoparticles. Finally, the silver nanoparticles were separated and purified from the solution by repeated centrifugation, and further dried in a vacuum at 40 °C for 12 h. The effect of various parameters, such as the amount of extract, the reaction temperature and the dosage of silver nitrate, was estimated by conducting several groups of experiments.

### 2.5. Characterization of the synthesized silver nanoparticles

After synthesis, the metal nanoparticles were measured by different analytical test technology, including transmission electron microscope (TEM), scanning electron microscope (SEM), X-ray powder diffraction (XRD) analysis, Fourier-transform infrared spectroscopy (FT-IR) nanoparticles and dynamic light scattering (DLS) analysis. The formation of silver nanoparticles by reduction of silver nitrate using leaf extract as a reducing agent can be easily monitored by UV-vis spectroscopy.

The reduction of silver ions to silver nanoparticles by the ginkgo biloba leaves extract was monitored at regular time intervals in the reaction medium by measuring the UV-vis absorption spectra at a resolution of 0.5 nm between 300–800 nm to prove the formation of silver nanoparticles. The UV-vis spectroscopy was operated at UV-2550 spectrophotometer.

The surface morphology of the silver nanoparticles was analyzed using TEM. TEM detection was carried out on a JEM2100 transmission electron microscope at an accelerating voltage of 200 kV. The TEM samples were acquired by dropping a small amount of nanosilver solution onto carbon-coated copper grids and evaporating the water at room temperature.

The shape and morphology of metal nanoparticles were also examined by SEM. SEM testing was operated on JSM-7600F SEM. Thin film of the sample was operated by simply dropping a very small amount of dried silver nanoparticles on the copper grid.

The composition and microstructure of silver powder were analyzed by using an X'Pert PPO X-ray diffractometer, operated at a voltage of 40 Kv. The data was collected using  $\text{CuK}\alpha$  radiation ( $\lambda = 1.5406 \text{ \AA}$ ) in the  $2\theta$  range of 30° to 85° at room temperature.

FT-IR analyses were conducted on Tensor 27 Fourier-transform infrared spectrometer to check the presence of functional groups of the surface chemistry of the prepared silver nanoparticles. The samples were prepared by using the KBr pellets technique. The data were collected at a spatial resolution of 1  $\text{cm}^{-1}$  in the transmission mode in the wavelength range of 4000–400  $\text{cm}^{-1}$ . The hydrodynamic diameter and size distribution of the obtained silver

nanoparticles were examined by dynamic light scattering (DLS) using a Malvern Zetasizer Nano ZS.

### 3. RESULTS AND DISCUSSION

#### 3.1. UV-visible absorption analysis

The UV-vis spectroscopy technique has been proven a useful tool to examine the size and shape of metal nanoparticles in metal colloids. Due to SPR, silver nanoparticles usually exhibit a UV-vis absorption maximum in the range of 400–500 nm. Generally, spherical NPs exhibit a single symmetric absorption peak, while anisotropic NPs show an asymmetric absorption peak. The UV-vis spectroscopy of the silver nanoparticles synthesized in a typical process was exhibited in Fig. 1. An intense absorption band was observed at 456 nm, indicating the formation of silver nanoparticles. As can be seen, the shape of the absorption peak is symmetric, demonstrating the presence of spherical particles with a narrow distribution. The shape is a little broad, which may be due to the presence of leaf extracts in the solution. Similar results were obtained in other studies that silver nanoparticles synthesized by plant extracts [4, 25, 35].

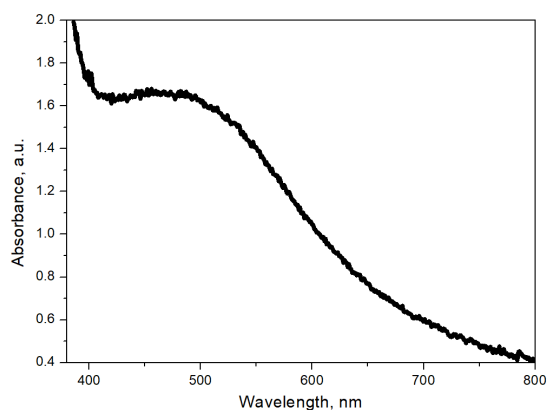


Fig. 1. The typical UV-Visible spectra of the nanosilver colloids

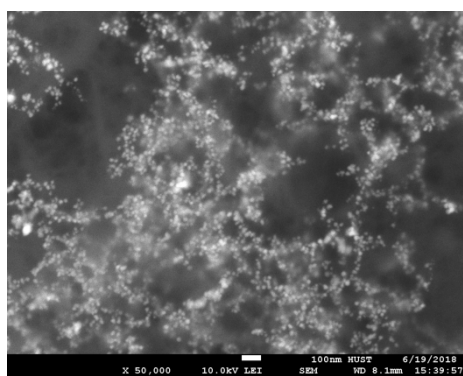


Fig. 2. The typical SEM image of silver nanoparticles

#### 3.2. SEM and TEM analysis

The size and surface morphology of the silver nanoparticles were analyzed by SEM and TEM. The typical SEM image of silver nanoparticles is illustrated in Fig. 2, which shows the morphological character of the silver nanoparticles synthesized by ginkgo biloba leaves extract. The SEM image reveal that prepared silver nanoparticles were spherical in shape with narrow distribution from 10 nm

to 30 nm. The particles sizes were obtained by measuring 300 silver particles. The TEM image of silver nanoparticles is exhibited in Fig. 3. Apart from some amorphous particles, silver nanoparticles were found to be of spherical or roughly spherical forms in most of the cases. The TEM image confirms that the synthesized silver nanoparticles are small in size with narrow distribution from 10 nm to 40 nm.

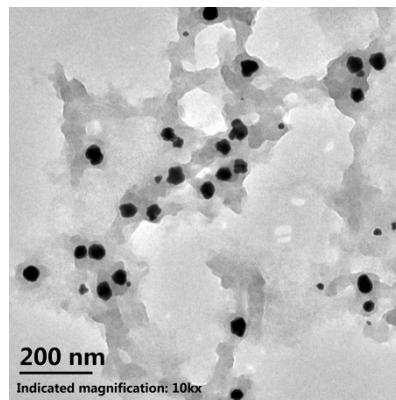


Fig. 3. The typical TEM image of silver nanoparticles

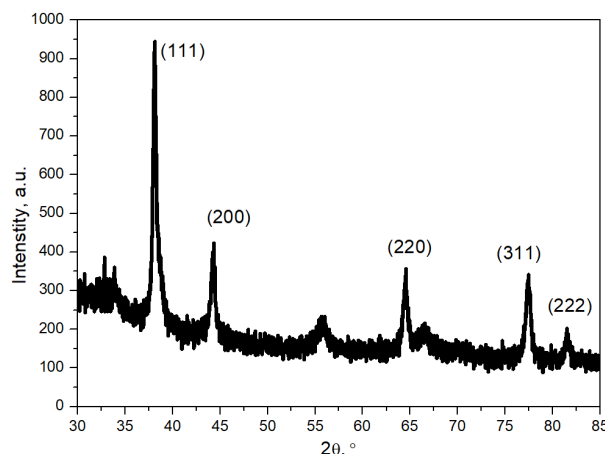


Fig. 4. XRD pattern of silver nanoparticles

#### 3.3. X-ray diffraction analysis

To confirm the UV-vis results, the sample of silver nanoparticles has been purified by ultra-centrifugation and checked by XRD analysis. The typical XRD pattern of Ginkgo Biloba leaf extract capped silver nanoparticles is shown in Fig. 4. From the XRD pattern, five obvious diffraction peaks are found at 38.2°, 44.5°, 64.7°, 77.5° and 81.8°. The five diffraction peaks are indexed as (111), (200), (220), (311) and (222) planes of FCC silver, which suggest that the samples are metallic silver nanoparticles with FCC crystal structure (JCPDS No. 87–0717). Intense and broadening peaks of silver nanoparticles owing to the nature of the crystalline and the small size of metallic nanoparticles. Compared with the strong (111) reflection, it is obviously discovered that the other Bragg reflections are relatively weak, which shows that the nanocrystals are highly anisotropic. The mean crystallite size of obtained silver nanoparticles is estimated by the Debye-Scherrer formula [68]:

$$D = K\lambda / \beta \cos\theta, \quad (1)$$

where  $D$  is the average diameter of particles;  $K$  is the Scherrer constant, which is related to the crystallite shape;  $\lambda$  is the radiation wavelength ( $\lambda=1.5406 \text{ \AA}$ );  $\beta$  is the full width at half maximum of the diffraction peak;  $\theta$  is the Bragg's angle. Usually, if the  $\beta$  is the full width at half maximum of the diffraction peak, the  $K$  equal to 0.89 [56, 66]. The most intense peak (111) was applied to Debye-Scherrer formula. The mean size of the silver nanoparticles obtained in the green synthesis process is about 20 nm, which is consistent with the formerly mentioned results of TEM and SEM.

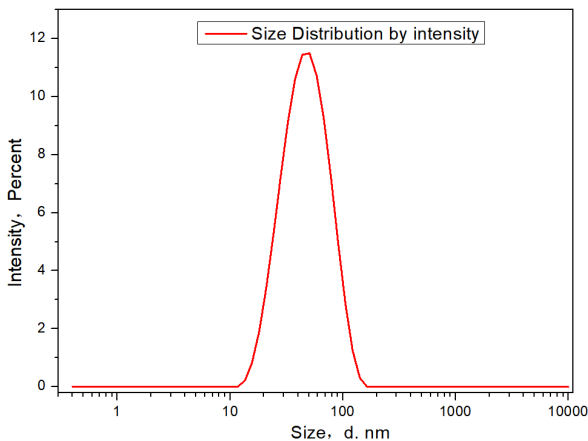


Fig. 5. Size distribution of silver nanoparticles

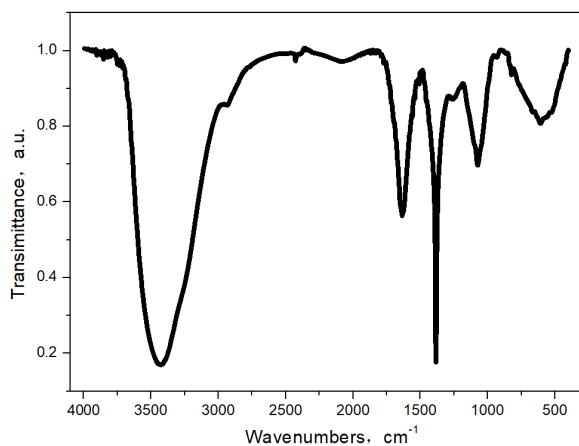


Fig. 6. FTIR spectra of the silver nanoparticles

### 3.4. DLS and FTIR analysis

The size distribution of noble metallic nanoparticles prepared in a typical process is also examined by DLS analysis, which is illustrated in Fig. 5. According to the result, the average size of the prepared silver nanoparticles appears to be higher as compared to TEM, SEM and XRD measurements. This is may owe to interference which caused by overlapping nanoparticles or an electrical double layer on charged particles.

Moreover, FTIR analysis was carried out to approve possible molecules in the surface of silver nanoparticles at the 4000–500  $\text{cm}^{-1}$  range. Fig. 6 shows many peaks related to functional groups of biloba leaf extract on the surface of silver nanoparticles. The 3420  $\text{cm}^{-1}$  broad absorption peak corresponds to the stretching vibrations of the hydroxyl group (-OH). The peak observed at 1640  $\text{cm}^{-1}$  is due to the propyl stretching vibration. The peak located at 1080  $\text{cm}^{-1}$

can be assigned to the stretching vibration of the C-OH bond. The peak found at 667  $\text{cm}^{-1}$  represented C-H stretching. The result of the FTIR analysis approved the capping agent in the extract play important role in the formation of silver nanoparticles.

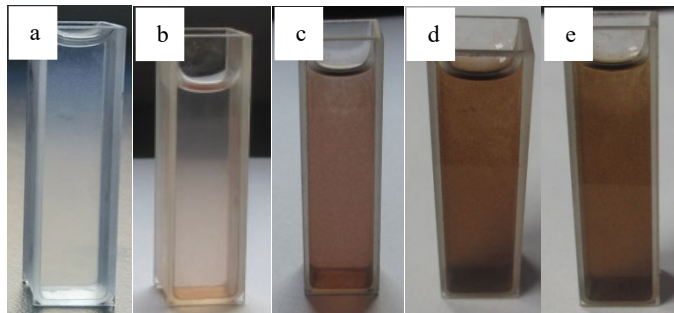


Fig. 7. Photos of silver colloids synthesized: a–25 min; b–70 min; c–130 min; d–200 min; e–280 min

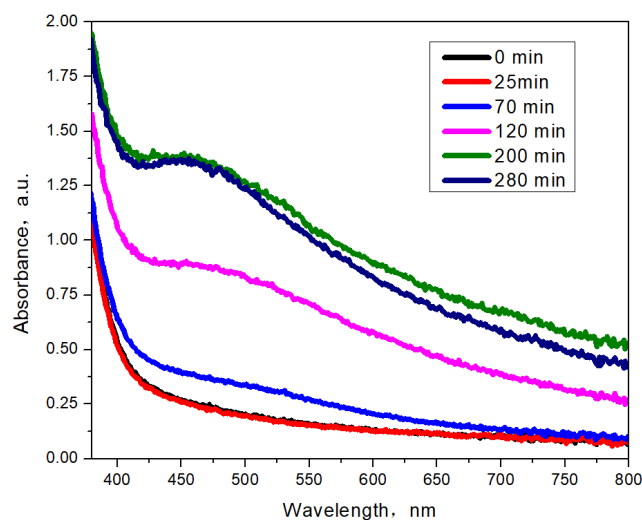


Fig. 8. UV-vis spectra of silver colloids synthesized at the different time intervals

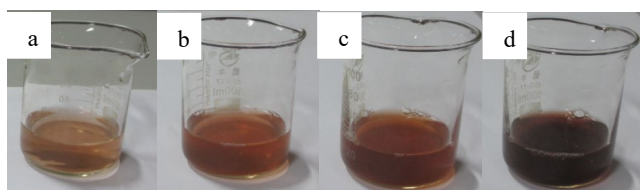
### 3.5. The effect of reaction time

In the present study, ginkgo biloba leaf extract was used as a reducing agent and stabilizing agent to prepare silver nanoparticles. The ginkgo biloba leaf extract appears to be a water soluble, bio-compatible and bio-degradable source which is found to be an efficient reducer and stabilizer. Silver nanoparticles were prepared in an aqueous solution by reduction of silver nitrate using ginkgo biloba leaf extract. After 70 minutes of adding the extract to the silver nitrate solution at room temperature, the colour of the colloidal solution started to change from pale to light yellow, which suggests the generation of silver nanoparticles. The colour of the colloid became darker with time and transfer to brown after 280 minutes as illustrated in Fig. 7. UV-vis spectrophotometer was conducted to check to the formation of silver nanoparticles in the aqueous colloidal solution. The UV-vis absorbance spectroscopy of silver nanoparticles synthesized at different time intervals is exhibited in Fig. 8. At the beginning, no peak pertaining to silver nanoparticles was observed. After 70 minutes of reaction, it is obvious from the figure that each absorption spectrum consists of a peak which is the characteristic

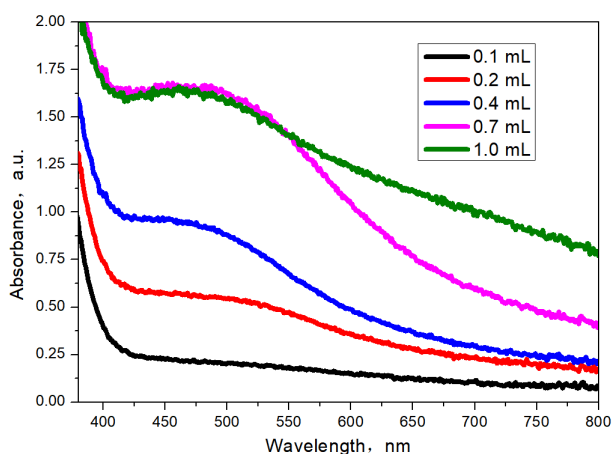
surface Plasmon peak of silver nanoparticles, indicating the formation of silver nanoparticles. The absorption peak prepared during 70 minutes is measured at 470 nm. However, the absorption peaks prepared during 200 minutes and 280 minutes are 460 nm and 456 nm respectively. Such a blue shift in the absorption peak might owe to a decrease in particle size.

### 3.6. The effect of amounts of silver nitrate

From the previous paper, the amounts of silver nitrate play a vital role in the synthesis of silver nanoparticles. Fig. 9 and Fig. 10 show the photos and UV-visible spectra of silver nanoparticles prepared at different amounts of silver nitrate where the other parameters are kept constant (8 mL ginkgo biloba leaf extracts). It can be seen that the color and absorption intensities increases with increasing silver nitrate amount. As the silver nitrate increased from 0.2 mL to 0.7 mL, the absorption peaks pertaining to silver nanoparticles were changed from 480 nm to 456 nm. The small blue shift demonstrates the decrease in particle size.



**Fig. 9.** Photos of silver colloids synthesized at different amount of silver nitrate: a–0.1 mL; b–0.2 mL min; c–0.4 mL; d–0.7 mL

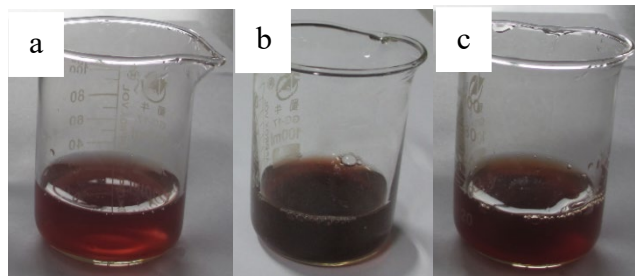


**Fig. 10.** UV-vis spectra of silver colloids synthesized at different amount of silver nitrate solution

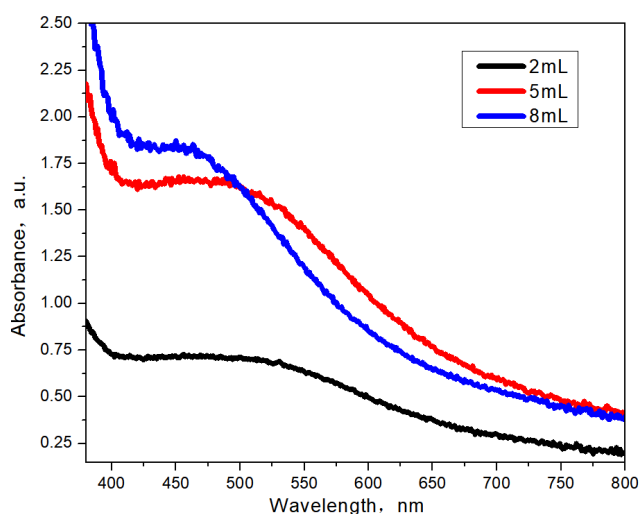
### 3.7. The effect of amounts of extracts

In this research, ginkgo biloba leaf extract was obtained by heating dried ginkgo leaves and water at 100 °C for 30 minutes. Compare with other methods, the process to gain extract in this study was simple. At 100 °C, long chain polymer may be obtained in the extract by some natural compounds reacting with each other, which can be served as capping agents. To investigate the effect of the amount of Ginkgo biloba leaf extracts, different amounts of extract were employed to prepare silver nanoparticles, while the other parameters were kept constant (0.7 mL silver nitrate solution). The photos and UV-visible spectra

of silver nanoparticles of the obtained silver nano colloids are illustrated in Fig. 11 and Fig. 12. As the amount of extracts varied from 2 mL to 8 mL, the color of the silver nanoparticles was changed from litter red to wine red, and the peaks position were a small blue shift from 475 nm to 456 nm, demonstrating the formation of different size silver particles.



**Fig. 11.** Photos of silver colloids synthesized at different amount of leaf extracts: a–2 mL; b–5 mL min; c–8 mL



**Fig. 12.** UV-vis spectra of silver colloids synthesized at different amount of leaf extract

## 4. CONCLUSIONS

In conclusion, silver nanoparticles were prepared via a facile and friendly method by using different volumes of ginkgo biloba leaf extract and silver nitrate solution. In this method, ginkgo biloba leaf extract served as a novel bio-reductant and capping agents to control the particle growth. Due to the use of nontoxic agents, the prepared silver nanoparticles have the characteristics of low toxicity and bio-compatibility. The synthesized silver nanoparticles were measured by many analytical techniques, such as TEM, SEM, DLS and XRD. The results show that the prepared silver nanoparticles were mostly spherical in shape, small with narrowly distribution from 10 nm to 40 nm, and highly crystalline in nature with a FCC structure. The reaction time amounts of extracts and silver nitrate solution play significant roles in the formation of small size silver nanoparticles.

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