

Rapid and Green Synthesis of Monodisperse Silver Nanoparticles Using Mulberry Leaf Extract

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Abstract: A simple, rapid and eco-friendly technique for the preparation of small-sized silver nanoparticles with narrow distribution from 5 nm to 15 nm was developed. Using silver nitrate as silver precursor, and mulberry leaves extract as both capping and reducing agent, silver nanoparticles were obtained at room temperature, without employing any other reducing and capping agents. The formation of silver nanoparticles was observed by the change of color from pale yellow to brown and the UV-vis absorption spectroscopy. The effects of reaction time, temperature, silver nitrate concentration and mulberry leaves extract amount were studied. The silver nanoparticles were characterized by transmission electron microscopy (TEM), UV-visible spectroscopy (UV-vis), X-ray diffraction (XRD) and Fourier-transform infrared spectroscopy (FT-IR). The results demonstrate that the obtained metallic nanoparticles are highly crystalline silver nanoparticles with a spherical shape and capped with extract. A possible formation mechanism was proposed. The method in this paper may be extended to synthesize other noble metal nanoparticles using renewable materials as capping and reducing agents.

Key words: silver nanoparticles; green synthesis; mulberry leaf extract

Over the past decade, metal nanoparticles, such as nickel, copper, silver and gold nanoparticles, have attracted significant research interest in engineering technology and nanoscale science due to their unique catalytic, magnetic and optical properties, which lead to potential applications in industrial fields involving catalysis, antimicrobial agents, information storage, optoelectronics, electronic devices and biological sensors^[1-6]. Hence, the formation and preparation of metallic nanoparticles have become a momentous theme in nano science and technology. Among metallic nanoparticles, silver nanoparticles have received extensively attention due to their relatively cheapness, excellent non-oxidizing properties and high conductivity^[7,8].

A variety of physical and chemical methods have been employed to prepare silver nanoparticles, such as wet chemical reduction, electron-chemical, reverse micelles, alcohol reduction, and laser ablation^[9-11]. Among these

methods, the wet chemical method is the most common technique to large scale preparation of silver nanoparticles with tunable size and shape by choosing optimum synthesis conditions^[12,13]. The wet chemical method is mostly achieved by reduction of silver salt using reducing agents in the presence of capping agents^[14,15]. However, most reducing agents used in the wet chemical method, such as sodium borohydride, hydrazine and N, N-dimethylformamide, are toxic chemicals, which usually associate with environment pollution^[16-18]. Usually, the toxic chemicals will be absorbed on the surface of the as-prepared silver nanoparticles, making them unsuitable for biological and biomedical applications^[19].

To address this problem, there is a growing attraction on the subject of preparing silver nanoparticles using environmentally benign, such as glucose, maltose, ascorbic acid, amino acid and protein, to avoid or reduce the use and generation of harmful substances^[19-21]. However, most of these

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environmentally benign need a long reaction time and high reaction temperature^[19]. As a result, the development of eco-friendly, energy saving, convenient, facile and rapid method to preparation of silver nanoparticles without using harmful and toxic chemicals, has gained tremendous research interest.

Several promising synthetic techniques relying on the above rapid and green method has developed for biosynthesis of silver nanoparticles using a wide range of microorganisms and plants extract^[22-24]. Compared with microorganism, biological synthesis of silver nanoparticles using plants extract is relatively low-cost, simple and time-saving. As a consequence, researchers switched over their attention to synthesis of silver nanoparticles using plants extract^[25]. Various researches prepared pure and high-crystalline silver nanoparticles by reduction of silver salts using different plants extract at ambient temperature, such as piper nigrum^[22], hibiscus sabdariffa^[26], semen cassiae^[27], rosmarinus officinalis L.^[28], vitex negundo L.^[29], and calendula officinalis^[30]. In the biosynthesis process, the plant compounds such as vitamins, amino acids, polysaccharides, citrates and proteins served as reducing and capping agents by reducing silver ion to silver nanoparticles and preventing them from aggregation^[31]. However, most of the reported methods need a long reaction time. Hence, a rapider and simpler method for preparation of silver nanoparticles is desired.

In the present paper, we report the biosynthesis of silver nanoparticles using mulberry leaves extract as the both reducing and capping agents in 2 h. Mulberry, a multipurpose agro-forestry plant that belongs to the family of Moraceae, is usually used as silkworm diet^[32]. It is extensively cultivated in China, India, Japan and Korea, and grows well all over the year^[33]. Due to its significant bioactivities, mulberry and its parts is widely used to produce various functional foods, such as mulberry leaf-carbonated beverage and healthy beverage^[34]. Furthermore, it is also served as a traditional herbal medicine and an alternative tea in China and Japan because of its low toxicity and good therapeutic performance^[35]. To the best of our knowledge, there have seldom been any reported studies on the use of mulberry leaves extract as reducing as well as capping agents for the biosynthesis of metal nanoparticles. The prepared silver nanoparticles were further characterized by UV-vis spectroscopy, Fourier-transform infrared spectroscopy, X-ray diffraction and transmission electron microscopy.

1 Experiment

Mulberry leaves and silver nitrate were used as the starting materials for biosynthesis of silver nanoparticles. Fresh mulberry leaves were collected in Hubei Polytechnic University, Huangshi. Silver nitrate (99.9%, analytical grade) purchased from Sinopharm Chemical Reagent Co. Ltd. China, was acted as the precursor in the generation of silver nanoparticles. Water used in all the experiments was doubly

distilled water. All the raw materials were of analytical grade and were used as received without further purification.

The freshly collected mulberry leaves was washed with doubly distilled water several times to remove other impurities. Washed samples were shade dried for 10 d and stored for further studies. The leaves extract was prepared in a conical flask by taking 10 g dry leaves and 200 mL distilled water. It was boiled at 100 °C for 30 min and cooled down to room temperature. The leaves extract was prepared after filtration with Whatman No.1 filter paper. The extract volume was adjusted to 200 mL by adding distilled water. The leaves extract was stored at 4 °C and was used for further studies within 3 d. The filtrate extract was used as capping and reducing agent.

The synthesis of silver nanoparticles was simply attained by the reduction of silver nitrate using mulberry leaves extract as reducing as well as stabilizing agents in aqueous solution. In a typical synthesis process, a certain volume (5 mL) of mulberry leaves extract was added to 50 mL of 11.8 mmol/L silver nitrate solution (silver nitrate: 0.1 g) in a flask, and the volume was adjusted to 60 mL with double distilled water. The solution was stirred by a magnetic stirrer at room temperature and allowed to react for 2 h, resulting in a brown-yellow or pink-red solution revealing the formation of silver nanoparticles. Finally, the silver nanoparticles were collected and then separated from the mulberry leaves extract by centrifugation, washed with distilled water and ethanol in sequence, and further dried in vacuum at 40 °C for 12 h.

Synthesis of silver nanoparticles by reducing silver ion solution with mulberry leaves extract can be easily observed by UV-vis spectroscopy. The absorption spectra of reaction solutions at different mulberry leaves extract quantities and silver nitrate concentrations were recorded at wavelengths ranging from 200 nm to 750 nm at a resolution of 0.5 nm using a Specord S 600 UV-vis spectrophotometer. Doubly distilled water was used as a blank solution.

Transmission electron microscope (TEM) was applied to analyze the shape and size of nanoparticles. TEM measurements were operated using Tecnai G2 F20 microscope at an accelerating voltage of 200 kV. HRTEM observations were done on a JEM2100 transmission electron microscope instrument operated at an accelerating voltage of 200 kV.

Crystalline metallic pattern of silver nanoparticles was analyzed using an X'Pert PPO X-ray diffractometer with Cu K α radiation of wavelength of $\lambda=0.15406$ nm in the 2θ range of 20° to 90° with a scanning rate of 0.05°/s at room temperature.

The Fourier-transform infrared (FT-IR) analysis was carried out on a Tensor 27 spectrophotometer to characterize the surface structure of silver nanoparticles in the wavelength range of 4000~400 cm⁻¹. The synthesized silver nanoparticles were blended with KBr powder and pressed into a pellet for measurement.

2 Results and Discussion

2.1 Characterization of silver nanoparticles

To synthesize nano silver colloid, the mulberry leaves extract was used as both capping and reducing agent. To confirm the formation of silver nanoparticles, the as-prepared nano-sol was studied by UV-vis spectroscopy, which has proven to be a useful spectroscopic tool for the detection of metallic nanoparticles. The typical UV-visible absorption spectrum of the silver nano-colloid is shown in Fig.1a. A peak at 433 nm is observed, which is the characteristic absorption band of spherical silver nanoparticles. The absorption band is the surface plasmon absorption of silver nano colloids, which relates to the size and the shape of silver nanoparticles. The strong surface plasmon band of 433 nm indicates that silver nanoparticles can be synthesized using mulberry leaves extract.

The typical XRD pattern of the as-synthesized silver nanoparticles is shown Fig.1b, confirming the crystalline structure of silver nanoparticles. The XRD pattern shows five intense peaks in the whole spectrum of 2θ values from 20° to 90° , which is consistent with metallic silver. The intense peak values are located at 37.90° , 43.93° , 64.27° , 77.23° and 81.47° , which correspond to the (111), (200), (220), (311) and (222) lattice planes of a face-centered cubic (fcc) crystal structure of silver nanoparticles (JSPDS No.04-0783). No other peak of possible impurities, such as silver oxide, can be detected, which suggests that the mulberry leaves extract can protect silver nanoparticles very well.

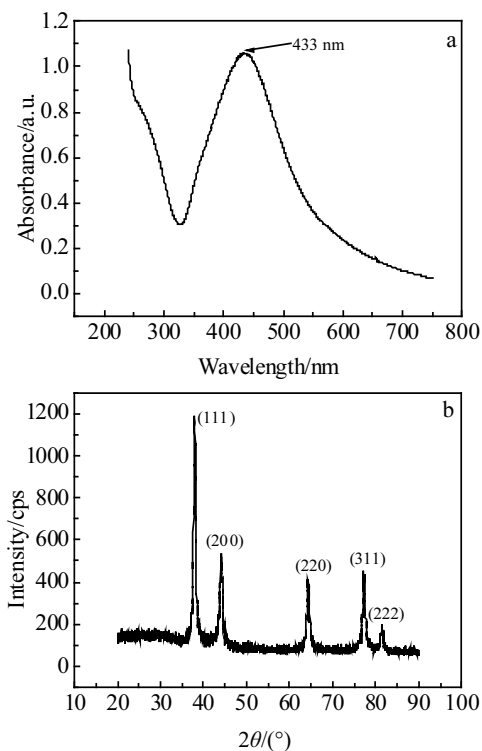


Fig.1 Typical UV-vis spectrum (a) and XRD pattern (b) of silver nanoparticles

FT-IR measurement was conducted to analyze the possible biomolecules present in mulberry leaves extract, which is used as capping and reducing agent for the preparation of silver nanoparticles, and absorbed on the surface of the resulting particles. The FT-IR spectra of crude mulberry leaves extract is shown in Fig.2. The peak at 3430 cm^{-1} indicates the O-H stretching vibration, the band at 2930 cm^{-1} illustrates the C-H stretching vibration, and the peak at 2380 cm^{-1} displays the C-H transiting angle in the leaves extract. All these data agree with the published value^[32-35], confirming the main constituents of the mulberry leaves extract are polysaccharides. The FT-IR spectra of as-prepared silver nanoparticles is also shown in Fig.2, suggesting the polysaccharides are absorbed on the surface of the particles.

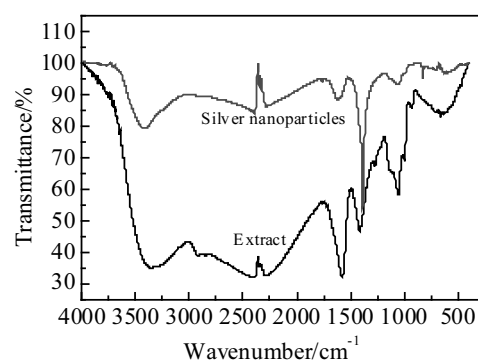


Fig.2 FT-IR spectra of leaves extract and synthesized silver nanoparticles using mulberry leaves extract

The typical TEM images of the as-prepared silver nanoparticles are displayed in Fig.3. The low magnification TEM images indicate that the morphology of the particles are relatively small and well dispersed. The silver nanoparticles are spherical in shape with a narrow distribution from 5 nm to 15 nm, which suggests that the mulberry leaves extract can serve as capping agent to prepared nano-size silver very well. For a more comprehensive study of the as-synthesized silver nanoparticles, HRTEM observation was carried out. The HRTEM images of the typical silver nanoparticles are shown in Fig.4a. The clear and uniform d -spacing of the lattice can be easily seen, which reveals that the prepared silver nanoparticles are highly crystalline. From the HRTEM, a d -spacing of about 0.23 nm is found by measuring the distance between the planes (as shown in Fig.4b). This distance is consistent with (111) planes of the fcc phase of metallic silver, which is 0.23 nm.

2.2 Effect of the amount of extract

In this reaction, mulberry leaves extract was used as capping and reducing agents because of their nice biodegradability and biocompatibility. Silver nanoparticles were obtained by reduction of silver nitrate with extract in water solution. As we all know, the initial concentration of reactant is one of the most important factors in the preparation of silver

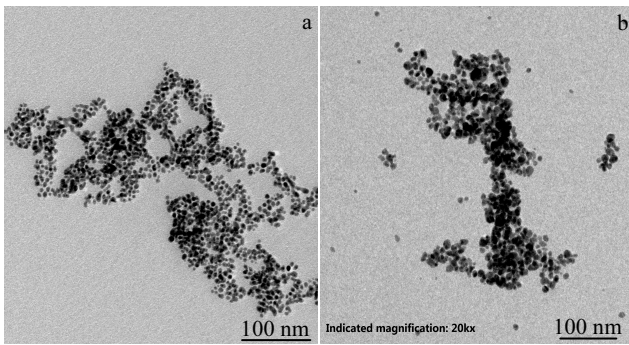


Fig.3 Typical TEM images of the as-prepared silver nanoparticles

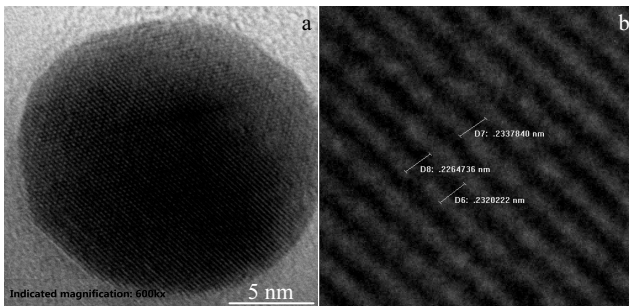


Fig.4 HRTEM image of the prepared silver nanoparticles (a) and the corresponding lattice fringes (b)

nanoparticles. To investigate the effect of extract concentration on silver nanoparticles formation, different amounts of extract were used to react with some concentration of silver nitrate. The UV-vis absorbance spectra of silver nanoparticles prepared with different amounts of extract when the silver nitrate concentration was kept constant at 10 mmol/L are shown in Fig.5a. Silver colloid synthesized at the lowest amount of extract is pale red, showing a SPR peak at 422.5 nm. As the amount of extract increases to 10 mL, the color of the silver colloids gradually change to wine red, brown, revealing a red shift of the SPR peak from 422.5 nm to 435 nm with increase in its intensity. The red shift and the increase in intensity indicates that the higher the amounts of extract, the higher yields and bigger size the silver nanoparticles have.

2.3 Effect of silver nitrate concentrations

The UV-vis absorption spectroscopy of the prepared silver colloids using various concentrations of silver nitrate, where the amount of extract was kept constant at 5 mL are displayed in Fig.5b. As the concentration of silver nitrate is varied from 2 mmol/L to 10 mmol/L, the color of the obtained colloids are all dark red, but the SPR peak of the prepared silver nanoparticles blue shifts from 440 nm to 431.5 nm, which indicates that the higher concentration of silver nitrate, the smaller size of silver nanoparticles gained.

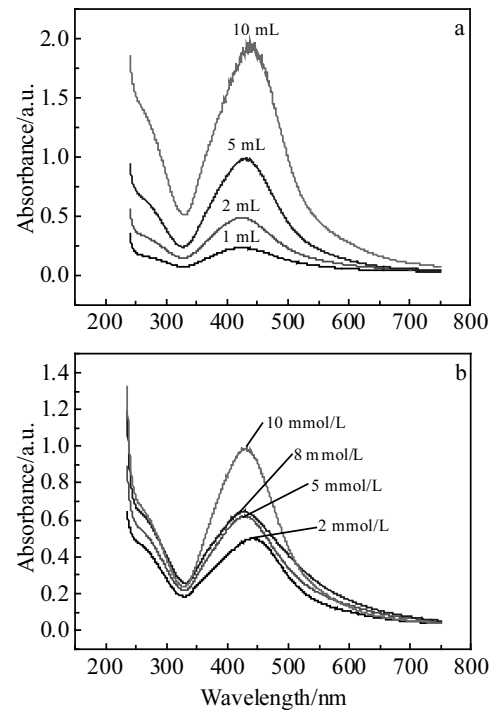


Fig.5 UV-vis spectra of as-synthesized nano-silver sols with different mulberry leaves extract amounts (a) and silver nitrate concentrations (b)

2.4 Effect of reaction time

As we know, time is a crucial parameter in the production of metallic nanoparticles during the synthesis process. In order to study the effect of reaction time on the formation of product, the gradual evolution of silver nanoparticles was detected using UV-vis spectroscopy at different reaction periods in the typical synthesis. During the incubation procedure of silver nitrate with leaves extract, the color of reaction mixture changes from pale yellow to dark yellow, red and wine red (not shown here), which demonstrates the synthesis of silver nanoparticles with different sizes and shapes. As the leaves extract is mixed with silver nitrate solution, no SPR peak pertaining to the silver nanoparticles is observed (Fig.6a), suggesting that no silver nanoparticles are synthesized at the beginning. After 1 min reaction, a SPR peak at approximately 430 nm is discovered, revealing the formation of silver nanoparticles. However, the reaction rate is quickly; after 8 min incubation, the intensity of UV-vis spectroscopy is too high to be measured (not shown in the picture). Hence, the later UV-vis spectroscopy was taken by diluting to the same amount, as shown in Fig.6b. As the incubation time increases from 10 min to 100 min, the corresponding SPR peak intensity increases with concomitant red shifts from 430 nm to 434 nm. The improvement in intensity and the red shift implies the increase of the concentration and the particle size of the silver nanoparticles. Similar results have been published in our

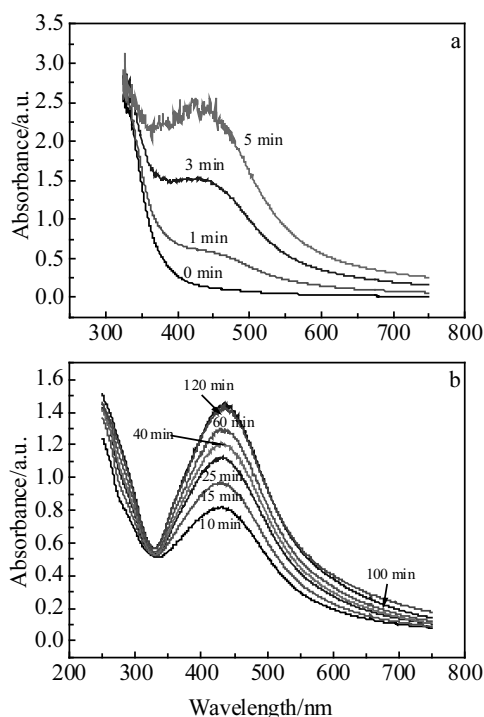


Fig.6 UV-vis spectra of silver nanoparticles synthesized at different time intervals: (a) undiluted and (b) diluted

previous research, using Semen cassia extract as capping agent and reducing agent to prepare silver nanoparticles^[27]. When the incubation time changes from 1 h to 24 h, the SPR peak intensity of the obtained silver nanoparticles is enhanced, while the position is red shift from 415 nm to 426 nm, indicating the increase of the concentration and the size of the silver nanoparticles. Zargar et al reported that there is a red shift from 423 nm to 432 nm as the reaction time changes from 1 h to 48 h, when the vitex negundo L. extract was used as reducing agent and capping agent to synthesize silver nanoparticles^[29]. This phenomenon can be described as follows: at the initial stage of the reaction, a large number of silver nuclei are generated, and then precipitated to certain size silver nanoparticles as the nuclei concentration exceeds the critical saturation concentration. Prolonging the reaction time, more and more small particles are formed and the particle size increases. After 100 min incubation, the intensity and position of SPR peak is unchanged, as shown in Fig.6b, indicating the end of the reaction.

2.5 Effect of reaction temperature

The reaction temperature plays an important role in the synthesis of metallic nanoparticles. The effect of temperature on the preparation of silver nanoparticles was studied by carrying out reactions at different temperatures using 5 mL mulberry leaves extract and 10 mmol/L silver nitrate solution. Fig.7 shows the UV-vis spectra of silver nanoparticles at three different temperatures 30, 60 and 90 °C. As can be seen,

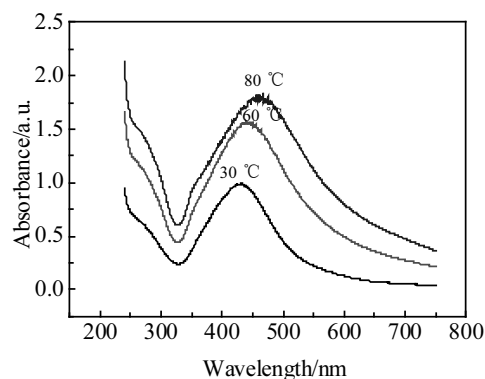


Fig.7 UV-vis spectra of as-synthesized nano-silver sols at different reaction temperatures

increasing the temperature, the SPR peak intensities of the obtained silver nanoparticles are enhanced, with concomitant red shift from 431.5 nm to 456 nm. The results suggest that the higher the reaction temperature, the higher yields and bigger size the silver nanoparticles have. Increase in the reaction temperature raises the reduction rate. At a relatively higher reduction rate, the number of silver nuclei is quickly increased since plenty of silver atoms are formed and exceed the critical saturation concentration during the nucleation step. The obtained silver nuclei then grow to silver nanoparticles by particle growth. Hence the yield of obtained silver nanoparticles are higher. On the other hand, at a relatively higher reduction rate, the reduction rate of silver ions exceeds the consumption rate of silver clusters, which may lead to an extended nucleation and fast growth of nuclei^[36]. Rapid nucleation and fast growth of nuclei is attributed to the formation of larger particles. Furthermore, the biomolecules capped on the nanoparticles may be destroyed at high temperature, which results in agglomeration of silver nanoparticles and generation of larger particles^[37].

2.6 Possible mechanism

It has been reported that mulberry leaves contain a large number of bioactive compounds, such as polysaccharides, moracin, flavonol glycosides, anthocyanins and chlorogenic acid^[33-35]. Hence, in our research, mulberry leaves extract was used as capping agent and reducing agent to prepare silver nanoparticles. The diverse functional group, such as phenols, proteins, flavonoids and terpenoids has reacted with silver ion and reduced their size into nano range^[31]. At the same time, the long chain group in the extract have also capped around the formed silver particles, making it dispersible in the aqueous solution by preventing them from agglomeration. It is confirmed by the HRTEM image as shown in Fig.4a. The particle is isolated and surrounded by a layer of organic matrix which may be some polymer containing hydroxyl or amino group from the mulberry leaves extract. The formation mechanism is displayed in Fig.8.

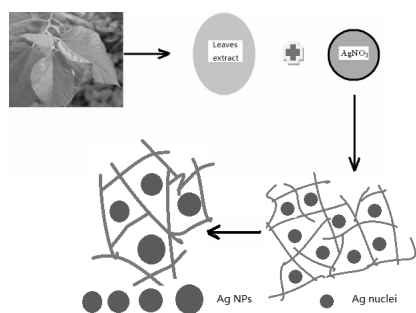


Fig.8 Schematic sketch of the formation mechanism of silver nanoparticles

3 Conclusions

1) A simple, rapid and eco-friendly route using mulberry leaves extract as reducing agent and capping agent, is demonstrated to prepare silver nanoparticles. It is the first time to use mulberry leaves extract to synthesize metallic nanoparticles.

2) The proposed protocol requires 120 min to prepare silver nanoparticles at room temperature when the silver nitrate concentration is 10 mmol/L, and the amount of extract is 5 mL.

3) The as-prepared silver nanoparticles are small, well-dispersed, spherical in shape, and with a narrow distribution from 5 nm to 15 nm.

4) The amount of extract, silver nitrate concentration, temperature, and time play important roles in the synthesis of small size particles.

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桑叶提取液快速绿色合成单分散银纳米粒子

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摘要: 报道了一种在水溶液中的简单、快速、绿色制备 5~15 nm 纳米银溶胶的方法。以硝酸银为银源, 桑叶提取液为还原剂和保护剂, 没有利用其它的还原剂和保护剂, 在常温下制备纳米银。纳米溶胶的颜色从浅黄色变到棕色, 表明生成了纳米银粒子。利用紫外可见光谱 (UV-vis), 透射型电子显微镜 (TEM), 红外光谱 (FT-IR) 和 X 射线衍射 (XRD) 对样品进行了分析。结果表明所得到的银粒子为分散性较好、粒径较小、结晶度很高、被植物提取液包覆的球状纳米银颗粒。通过 UV 光谱对反应温度、反应时间、银离子浓度及提取液用量对粒径的影响进行了研究, 提出了一种可能的反应机理。该方法可以扩展到用其它可再生的物质来制备贵金属纳米粒子。

关键词: 纳米银粒子; 绿色合成; 桑叶提取液

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