

Green synthesis of silver nanoparticles using tea leaf extract and evaluation of their stability and antibacterial activity



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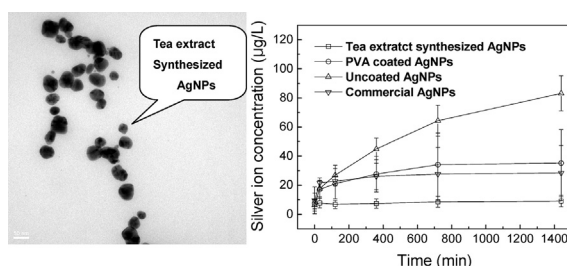
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HIGHLIGHTS

- A simple and green way was developed to synthesize AgNPs using tea extract.
- The synthesized AgNPs was characterized by TEM, XRD, FT-IR, and ICP-MS.
- Ag⁺ release from the synthesized AgNPs was lower indicating the high stability.
- The synthesized AgNPs showed slight antibacterial activity against *E. coli*.

GRAPHICAL ABSTRACT



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ABSTRACT

A simple, environmentally friendly and cost-effective method has been developed to synthesize silver nanoparticles (AgNPs) using tea leaf extract. We have studied the effects of the tea extract dosage, reaction time and reaction temperature on the formation of AgNPs. The AgNPs were synthesized using silver nitrate and tea extract, and the reaction was carried out for 2 h at room temperature. The synthesized AgNPs were characterized using transmission electron microscopy (TEM), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), thermogravimetric analyzer, and zeta potential analyzer. The synthesized AgNPs were nearly spherical, with the sizes ranging from 20 to 90 nm. FT-IR spectral analysis indicated the tea extract acted as the reducing and capping agents on the surface of AgNPs. Furthermore, the study of silver ion release from the tea extract synthesized AgNPs showed a good stability in terms of time-dependent release of silver ions. In addition, the antibacterial activity of AgNPs was determined by monitoring the growth curve and also by the Kirby-Bauer disk diffusion method. Due to the larger size and less silver ion release, the AgNPs synthesized by tea extract showed low antibacterial activity against *Escherichia coli*.

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1. Introduction

In recent years, silver nanoparticles (AgNPs) have been widely used in many consumer goods, such as medical devices, cleaning agents, and clothing, due to its unique antimicrobial properties. Generally, the method for the AgNP preparation involves the reduction of silver ions in the solution or in high temperature in gaseous

environments [1]. However, the reducing reagents, such as sodium borohydride, may increase the environmental toxicity or biological hazards [1,2]. Moreover, the capping agents like polyvinyl alcohol (PVA) or gelatin, have to be used to protect the AgNPs from aggregation. On the other hand, the high temperature may also increase the cost. Hence, the development of a green synthesis of AgNP by using environment-friendly solvents and nontoxic reagents is of great interest.

Huang et al. described the AgNP synthesis using a leaf extract of *Cinnamomum camphora*, while the reduction was considered due to the phenolics, terpenoids, polysaccharides and flavonoids present

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in the extract [3]. Moreover, the extracts of various plants, including *Eucalyptus hybrid* [4], *Syzygium cumini* [5], *Sesuvium portulacastrum* [6], *Boswellia ovalifoliolata* [7], *Calotropis procera* [8], *Musa paradisiacal* [9], *Acalypha indica* [10] were successfully used for AgNP synthesis. In addition, tea leaf extract was used for the AgNP synthesis. Begum et al. reported the AgNP synthesized by the ethyl acetate extract of tea leaves [11]. Nadagouda et al. showed the synthesized AgNP with the size range of 20–60 nm [12]. However, the reaction conditions, including the temperature or tea extract dosage, the synthesis mechanism, the AgNP stability, and the antibacterial activity have not been fully investigated.

Previous studies showed that AgNPs would likely release silver ions after entering the aquatic environment [13,14], which would reduce the stability of AgNPs. In addition, silver ions exhibited different physicochemical properties and biological toxicity from AgNPs [13,15,16]. Therefore, understanding of the silver ion release from AgNPs is necessary. Liu et al. reported more than 10% (w/w) silver ions were released from citrate coated AgNPs (2 mg/L) in the air-saturated (8.3 mg/L) water at pH 5.6 after 24 h [14]. Lee's study indicated that the silver ion release kinetics followed first-order kinetics [15]. In addition, the release rates of silver ions were mainly dependent on the particle sizes, the environmental factors (e.g., dissolved oxygen, pH, temperature) [13,17], and the capping agents [18]. However, quantitative data on the silver ion release from the green synthesized AgNPs are limited.

The present study attempts to fill the knowledge gap by investigating the synthesis, stability, and antimicrobial ability of AgNPs synthesized by tea extract. Tea extract solution was used as a reducing and capping reagent for the AgNP synthesis, and distilled water served as the reaction medium. The reaction conditions on the synthesis of AgNPs were studied. The obtained particles were analyzed by transmission electron microscopy (TEM), X-ray diffraction (XRD), Fourier transform infrared (FT-IR) spectroscopy, thermogravimetric analyzer, and zeta potential analyzer to understand the morphology and capping of AgNPs. The AgNP stability was evaluated via the time-dependent release of silver ions from the tea extract synthesized AgNPs. In addition, the antibacterial activity by tea extract synthesized AgNPs was also investigated.

2. Materials and methods

2.1. Synthesis of AgNPs by tea extract

Tea leaves extract was used as a reducing agent for the AgNP synthesis. 16 g of dried green tea leaves (Richun Tea Company, Fujian) was added to 100 mL ultrapure water in 250 mL Erlenmeyer flask. The mixer was boiled (5 min), cooled, filtered, and the filtrate was stored at 4 °C as the stock solution and was used within 1 week. The total organic carbon (TOC) content of tea extract analyzed by TOC analyzer (TOC-VCPH, Shimadzu, Japan), was approximately 20 g/L.

The stock solution of tea extract was diluted to 1%, 5%, 10%, 25%, 50% and 100% (v/v) as reducing and capping solution. 750 μ L silver nitrate (10 mM) was injected at the rate of one drop per second to 14.25 mL tea extract working solution with vigorously stirring. The working solution was stirred (700 rpm) for 120 min at 25, 40 and 55 °C, respectively. AgNPs were concentrated and purified by centrifugal ultrafiltration (Millipore, Amicon Ultra-15 3k, USA), and rinsed with Milli-Q water (Millipore, 18.2 M Ω cm, USA).

2.2. Characterization of AgNPs

The morphology of AgNPs was determined by TEM at 100 kV (Hitachi H-7600, Japan). Samples were prepared by placing a drop of fresh suspension on the TEM copper grids, followed by solvent evaporation at room temperature overnight. The configuration

of AgNPs was determined by XRD (PANalytical, X' Pert Pro, Netherlands), operated at a voltage of 40 kV and a current of 30 mA with Cu K α radiation. Thermogravimetric analysis (TGA) of the tea extract synthesized AgNPs was conducted in nitrogen atmosphere on a thermogravimetric analyzer (TG 209 F3 Tarsus, Germany Netzsch Instruments, Inc.) in the temperature range of 40–1000 °C at a scanning rate of 10 °C/min. Sample was prepared by adding 2 mL of tea extract synthesized AgNPs into petri dish and drying for 72 h in the freeze dryer. The zeta-potential (Malvern Instruments, ZetaPALS, UK) of AgNPs produced by tea extract was analyzed in order to recognize the surface charge of AgNPs. In addition, the hydrate particle size was also determined by Zetasizer (Malvern Instruments, ZetaPALS, UK).

The quantification of AgNP stock suspensions was analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES) (PerkinElmer Optima 7000 DV, USA) after nitric acid digestion. Silver concentrations in the solution were analyzed by inductively coupled plasma mass spectrometry (Agilent 7500cx, USA). The dissolved silver ion was isolated by removing AgNPs using centrifugal ultrafilter devices (Millipore Amicon Ultra-4 3K, USA), subjected to centrifugation for 30 min at 4000 rpm [14], whereas the total silver concentration was analyzed after nitric acid digestion. The AgNP concentration was calculated by deducting dissolved silver ion from total silver.

FT-IR spectroscopy measurements were carried out to identify the functional groups which are bound distinctively on the AgNP surface and involved in the synthesis of AgNPs. Samples for the FT-IR analysis were prepared by drying the tea extract taken before and after synthesis of AgNPs. Samples for FT-IR measurement were prepared by mixing 1% (w/w) specimens with 100 mg of potassium bromide powder and pressing the mixture into a sheer slice. Hand-ground samples were measured by a FT-IR spectrometer (FT-IR Nicolet 5700, Thermo Corp. USA). The average of 9 scans was collected for each measurement using a resolution of 2 cm⁻¹.

2.3. Silver ion release test

The dissolution kinetics of AgNPs synthesized by the tea extract in air-saturated (8.2 mg O₂/L) deionized water was investigated. PVA-coated, uncoated, and commercial AgNPs were also applied for comparison, for which the preparation processes are described in supplementary information (SI). The AgNP stock suspension was diluted with deionized water to 1.0 mg/L. The initial pH values of tea extract AgNPs, PVA-coated AgNPs, uncoated AgNPs, and commercial AgNPs were 6.9, 6.8, 6.9, and 7.2, respectively. Silver ion release experiments were carried out in triplicate on dark shaker (120 rpm) at 28 °C.

2.4. Antibacterial susceptibility test

The antibacterial test was carried out via a growth inhibition assay. *Escherichia coli* K12 strain MPAO1 (Coli Genetic Stock Center [CGSC; Yale University]) was grown on Luria-Bertani (LB) medium at 37 °C for overnight. The cultures were diluted in fresh LB medium to get an initial 0.05 absorbance at OD₆₀₀. 150 μ L of AgNPs solution under target concentrations were pipetted into eight parallel wells of a 96-well microplate (8 replicates), and 150 μ L of *E. coli* cells were inoculated in each well. The final concentrations of AgNPs were 50.0, 25.0, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.195 mg/L, respectively. The absorbance was measured at OD₆₀₀ through a 96-well microplate with a SpectraMax M5 Multi-detection Microplate Reader (Molecular Devices Inc., USA) at predetermined time intervals.

In addition, the antimicrobial susceptibility test was also performed according to a modified Kirby-Bauer disk diffusion method

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