Materials Letters ■ (■■■■) ■■■–■■■

Contents lists available at ScienceDirect

Materials Letters

journal homepage: www.elsevier.com/locate/matlet

Effect of reducing agent concentrations and temperature on characteristics and antimicrobial activity of silver nanoparticles

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ARTICLE INFO

Article history:
Received 19 June 2014
Accepted 16 August 2014

Keywords:
Biomaterials
Nanoparticles
FTIR
Green synthesis
Rhodomyrtus tomentosa
Antibacterial activity

ABSTRACT

In this study, the fabrication of colloidal silver nanoparticles (AgNPs) by cost-effective and environmental friendly method has been demonstrated. *Rhodomyrtus tomentosa* acetone extract (RAE) was used for the first as combined reducing and capping reagent time for AgNPs synthesis. The AgNPs were characterized by UV–visible spectroscopy, FTIR, XRD, zeta potential and DLS. AgNPs demonstrated profound antibacterial activity against *Staphylococcus aureus* with MIC and MBC ranging between 3.1–6.2 and 6.2–50 μg/ml, respectively. The outcomes of this study indicated that the synthesized AgNPs could be applied as an effective antimicrobial agent.

1. Introduction

Metal nanoparticles have received considerable attention in recent years due to their wide range of applications in the biomedical field [1–3]. Silver nanoparticles (AgNPs) have drawn very high research interest due to its antimicrobial properties [4]. Green method of AgNPs synthesis has advantages over chemical reduction and physical processes in being environment friendly and cost effective [5]. Several plants have been explored as promising candidates for the synthesis of AgNPs including medicinal plants [1–5].

Downy rose myrtle, *Rhodomyrtus tomentosa* (Aiton) Hassk., is an evergreen shrub native to Southeast Asia including Thailand. *R. tomentosa* has been reported to be rich in diverse phytochemicals like terpenoids, steroids, tannins, and flavonoids, [6–8] which could act as strong reducing agents. A pure compound, Rhodomyrtone from *R. tomentosa* possesses significant antibacterial activity against Gram-positive bacteria as well as antioxidant activity [9,10]. In view of the above properties, *R. tomentosa* acetone extract (RAE) was used as combined reducing and capping agents for AgNPs synthesis for the first time.

2. Materials and methods

Chemicals: All chemicals were procured from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise stated. *Staphylococcus aureus* ATCC 25923 was used for antibacterial study. *S. aureus* was cultured on Mueller-Hinton agar (MHA) (Difco, France) at 35 °C for 24 h. *R. tomentosa* acetone extract (RAE) was prepared by the method described [10], dissolved in 100% DMSO and served as stock solution.

Synthesis of silver nanoparticles: For synthesis of AgNPs, 0.01, 0.05 and 0.1% (w/v) stock solution of RAE was dissolved in 20 ml of MiliQ water. The aqueous solution of silver nitrate ($AgNO_3$) was added drop-wise to above solution to make a final concentration of silver 1 mM and placed the flasks in a rotatory shaker at 28 and 50 °C, in dark (to minimize the photo activation of $AgNO_3$) at 150 rpm. After 48 h of reaction, the samples were centrifuged at 14,000 rpm for 30 min and washed the pellets with MiliQ water. The pellets of AgNPs were resuspended in MiliQ water and stored at 4 °C till further analysis. AgNPs synthesized using 0.01, 0.05 and 0.1% (w/v) RAE at 28 °C were designated as Ag28A, Ag28B, and Ag28C, respectively, however, AgNPs synthesized using 0.01, 0.05 and 0.1% (w/v) RAE at 50 °C were designated as Ag50A, Ag50B, and Ag50C, respectively throughout the manuscript.

Time dependent synthesis of AgNPs was monitored by reacting 1 mM solution of $AgNO_3$ with 0.01% (w/v) RAE at 28 °C on rotatory shaker. 1 ml samples were taken out at different time interval, diluted 5 times with MiliQ water and UV–visible spectra were recorded.

Characterization of nanoparticles: The AgNPs were digested with 1% (v/v) HNO_3 and concentrations of RAE and silver in AgNPs were determined by UV–visible spectrphotometer (by taking absorbance at 670 nm) and atomic absorption spectrophotometer, respectively.

UV–visible absorption spectra were measured using a UV/vis spectrophotometer (Perkins Elmer LAMBDA 25 UV/Vis

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http://dx.doi.org/10.1016/j.matlet.2014.08.100
0167-577X/

spectrophotometer) in the in the wavelength of 200–800 nm. In order to determine the functional groups involved in the synthesis and capping of AgNPs, Fourier transform infrared (FTIR) analysis was carried out. Fourier transform infrared (FT-IR) analysis was carried out at a resolution of 4 cm^{-1} in transmission mode at frequency ranged 4000–400 cm^{-1} with EQUINOX 55 spectrophotometer (Bruker, Germany) using a KBr pellet method.

Shape and size of AgNPs were determined by transmission electron microscopy (TEM). Thin films of AgNPs were prepared on a carbon coated copper grid by dropping 10 μl of the sample on the grid. The extra solution was removed from the grid using a blotting paper and then the film on the TEM grid were allowed to air dry. The image analysis of AgNPs was done using JEOL-1010 instrument operated at an accelerating voltage of 120 kV. The HR-TEM (high resolution transmission electron microscope) images were taken in JEOL-2010 instrument operated at an accelerating voltage of 200 kV. Zeta potential and particle size were measured by Zeta PALS-zeta potential analyzer (Brookenhaven Instruments Corporation) by the DLS method with respect to the refractive index of deionised water. Crystalline nature of metallic AgNPs was examined by X-ray diffraction (XRD). The film of AgNPs was prepared on glass slide by drop-coating with nanoparticle solution and air dried. The films on glass slide was then subjected to X-ray diffraction, which were performed in a transmission mode on a Philips PW 1830 instrument operated at 40 kV and a current of 30 mA with Cu *Kα* radiation.

Antimicrobial assay: Antimicrobial assay of the synthesized nanoparticles against *S. aureus* was performed using a modified broth microdilution method recommended by Clinical Laboratory Standardization Institute (CLSI) guideline [11].

For application of AgNP as dressing materials, the cotton gauze was cut in pieces of 1 cm × 1 cm, dipped into 2 MIC of Ag50C and RAE solution (stock solution of RAE diluted with sterile double distilled water) and air dried. An overnight grown culture of *S. aureus* in MHB was spread on petri plates containing MHA medium. The cotton gauze was placed at the center of the plate and incubated for 12 h at 37 °C. After 12 h the cotton gauze was removed and plates were re-incubated at 37 °C till 24 h.

3. Results and discussion

Characterization of nanoparticles: The reaction of RAE with aqueous $AgNO_3$ led to the change in the color of the reaction mixture from light yellow to brownish yellow depended on the time of reaction, the concentrations of RAE, and the reaction temperature (Fig. 1a and b). UV–visible spectra of the solution showed a strong absorption band centered around 420 nm, which is the characteristic surface plasmon resonance absorption of spherical AgNPs [12,13]. This clearly showed that RAE extract was able to reduce the silver ions to form AgNPs. The intensity of

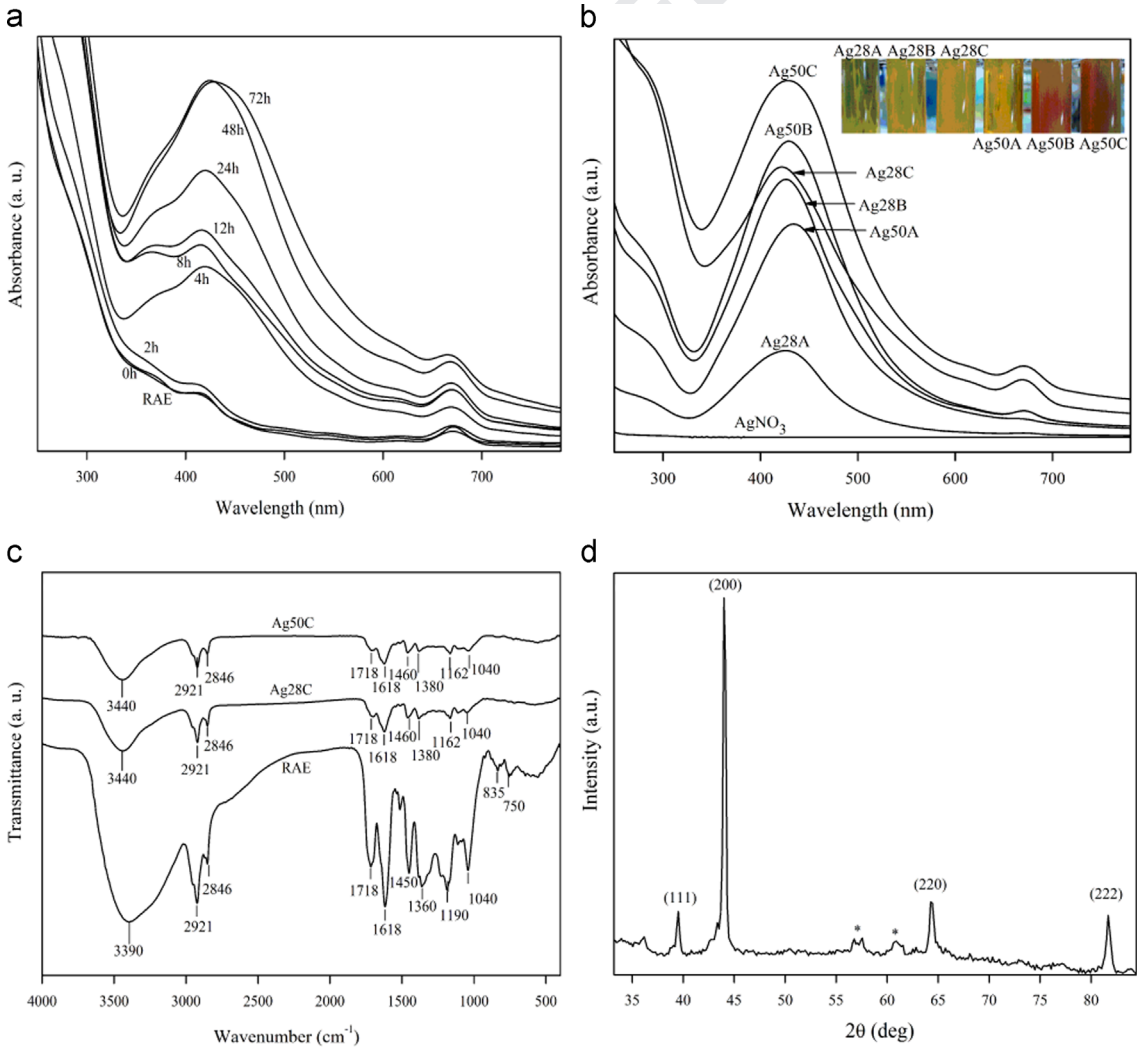

Fig. 1. UV–visible absorption spectra of AgNPs (a) with reference to time, (b) after 48 h with different RAE concentrations at 28 and 50 °C. (c) FT-IR spectra of RAE, Ag28C and Ag50C, and (d) XRD spectra of Ag50C.

Please cite this article as: Shankar S, et al. Effect of reducing agent concentrations and temperature on characteristics and antimicrobial activity of silver nanoparticles. Mater Lett (2014), http://dx.doi.org/10.1016/j.matlet.2014.08.100