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AN EFFECTIVE BIOGENIC PROTOCOL FOR ROOM TEMPERATURE ONE STEP SYNTHESIS OF DEFECTIVE NANOCRYSTALLINE SILVER NANOBUNS USING LEAF EXTRACT

BALAPRASAD ANKAMWAR[a*], GOPA MANDAL[b], UJJAL KUMAR SUR[c], TAPAN GANGULY[d]

[a]*Bio-inspired Materials Science Laboratory, Department of Chemistry, University of Pune, Ganeshkhind, Pune-411007, India*
[b]*Department of Physics, Gobardanga Hindu College, North 24 Parganas, Pin-743273, India*
[c]*Department of Chemistry, Behala College, Kolkata-700060, India*
[d]*Department of Spectroscopy, Indian Association for the Cultivation of Science, Jadavpur, Kolkata-700032, India*

The development of eco-friendly protocol for the synthesis of nanomaterials is an important aspect of nanotechnology. The green and cost-effective solution of fabrication of nanomaterials is key for biotechnology development. In this article, we report the room temperature one step bottom up synthesis of silver nanoparticles using *Murraya Koenigi* leaf extract as the natural source of reducing and stabilizing agent for the first time. The synthesized silver nanocrystals were characterized by UV-visible absorption spectroscopy, Fourier transform infrared spectroscopy, X-ray diffraction measurements as well as by high resolution electron microscopy. High-resolution transmission electron microscope reveals the presence of multiply twinning defects in the synthesized nanoparticles.

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1. Introduction

Metal nanoparticles have been extensively studied for many years due to their unique properties and potential applications in optics [1] catalysis [2], biodiagnostics [3], and surface-enhanced Raman scattering (SERS) [4,5]. The chemical and physical properties of metal nanoparticles depend not only on their size but also on their shape [6]. Noble metal nanoparticles like gold (Au) and silver (Ag) exhibit surface plasmon resonances in the UV-visible/UV-visible-NIR spectral range. The location and the intensity of the surface plasmon resonance (SPR) peak in the electromagnetic spectrum, and therefore the color of the nanoparticles, are dependent on the size and the shape of the particles. Various chemical and physical methods have been applied to synthesize anisotropic nanoparticles of variable shapes such as nanocubes [7], nanorods/nanowires [8,9], nanodisks [10], nanotapes/ nanobelts [11], and nanoteardrops/ nanoarrows/ nanotetrapods [12]. However, most of these methods are highly tedious, expensive and non eco-friendly, involving multiple steps.

The natural components of our environment such as plants, microorganisms could be exploited as a rich source of reducing and stabilizing agents for the synthesis of nanocrystals due to their special feature of biocompatibility and low cost. Silver nanoparticles have been successfully synthesized using microorganism such as fungus [13,14], bacteria [15] and yeast [16]. However, the plant extracts can be used to eliminate the elaborate process of maintaining cell cultures and large-scale synthesis of nanocrystals at lower cost. Gold and silver nanoparticles have

*Corresponding author: bankamwar@yahoo.com

been synthesized using both plant and fruit extract [17-22]. Still, there is much more space for the improvement in bio-based methods for metal nanoparticles synthesis of different size and shapes.

In this article, we have reported an eco-friendly protocol for the room temperature synthesis of silver nanoparticles by the reduction of aqueous silver ions using *Murraya koenigi* leaf extract for the first time. The synthesized nanocrystals were characterized by UV-visible absorption spectroscopy, X-ray diffraction (XRD) measurements as well as by High resolution electron microscopy.

2. Materials and method

2.1 Plant material and preparation of the extract

The aqueous broth used for the reduction of Ag^{+} ions to Ag^{0} was prepared by taking 10 gm of thoroughly washed *Murraya koenigi* leaves in a 500 ml Erlenmeyer flasks with 40 ml of sterile distilled water and boiled it for 15 min. On cooling to room temperature, it was filtered through four fold muslin cloth and used for the synthesis of silver nanoparticles.

2.2 Synthesis of Ag nanoparticles

In a typical experiment, 0.04 ml (0.625%) ammonia solution was added to 9 ml of 10^{-3} M aqueous silver nitrates solution followed by addition of 0.4 ml of broth. After shaking, it was kept for 6 hours at room temperature followed by centrifugation at 10000 rpm for 25 min. To remove any free biomass residue or compound that is not the capping agent of the nanoparticles, the nanoparticles were redispersed in millipore water and centrifuged again at 10000 rpm for 10 min and collected nanoparticles were dried in hot air oven at 65°C for 6 hrs and characterized for FTIR and XRD. Thus obtained dry powder was dissolved in water for UV-vis and TEM analysis.

2.4 Instrumentation

UV-visible spectroscopy studies were carried out on a Shimadzu dual-beam spectrophotometer (model 1650 PC) operated at a resolution of 1 nm. X-ray diffraction (XRD) measurement were carried out on a Bruker axs (model D8 Advance) instrument operating at a voltage of 40 kV and current of 40 mA with Cu Kα radiation. Fourier transform infrared (FTIR) spectroscopy measurements were performed on a Shimadzu (model FTIR 8400) FTIR Spectrum One spectrophotometer in the diffuse reflectance mode operating at a resolution of 4 cm^{-1}. The silver nanocrystals were dried and grinded with KBr pellets and analyzed in this instrument. The shape and size of the synthesized nanoparticles were studied using High Resolution Transmission Electron Microscope (HRTEM) (JEOL, model JEM-2010) instrument operated at an accelerating voltage at 120 kV. Samples for TEM analysis were prepared by solution-casting the Ag nanoparticle samples on a carbon-coated TEM grid.

3. Results and discussion

In the biosynthesis of silver nanoparticles in presence ammonia and MK broth together, we observed very good results (Fig. 1 A, curve 2). However in order to understand the role of ammonia and MK broth, controlled experiments were carried out using ammonia solution without MK broth and MK broth without ammonia, the other experimental conditions kept unchanged. Here, 0.04 ml aqueous solution of ammonia (0.625%) and 0.4 ml MK broth were mixed with 9 ml of aqueous silver nitrate solution (10^{-3} M) separately and reacted for 6 hrs. In Figure 2, the UV-vis spectra reveals neither ammonia (curve 1) nor MK broth (curve 2) has shown accountable effect on reduction of silver ions, separately. In order to check the pH effect, we tested pH of various solutions at 25ºC ($1x10^{-3}$ M 9 ml $AgNO_3$+ 0.04 ml NH_3 (0.625%) = 9.04, $1x10^{-3}$M 9 ml $AgNO_3$ + MK broth = 5.65 and $1x10^{-3}$ M 9 ml $AgNO_3$+ 0.04 ml NH_3 (0.625%) + MK broth = 9.4). Ankamwar et al. reported earlier [20] the significance of pH in silver nanoparticles synthesis and this observations also reveal that higher pH (9.4) must be providing necessary environment for reducing and capping agents of MK broth in reduction of silver ions and stabilization of Ag

nanoparticles respectively. Ammonia complexes with Ag^+ ion to form $Ag(NH_3)_2^+$ and it is a stable complex ion resulting from ammonia's strong affinity for Ag^+, therefore, the ammonia concentration and nature of the reductant must play a major role in controlling the Ag NP size [23]. Therefore, ammonia plays an important role in the bio-synthesis of silver nanoparticles.

The UV-vis spectra reveals the reduction of aqueous Ag^+ ions with the MK leaf extract. Fig. 1A shows the UV-vis absorption spectra recorded from the MK leaf extract (curve 1), as-prepared aqueous silver nanoparticle solution (curve 2) and the oven dried silver nanoparticles re-dissolved in sterile distilled water (curve 3). A strong resonance at ca. 424 nm is clearly seen in curve 2 and arises due to the excitation of surface plasmon oscillations in the silver nanoparticles. However, SPR band is blue shifted to 416 nm as shown in curve 3. This sharp peak is followed by a broad band between 500-600 nm. This feature is characteristic of either formation of spherical nanoparticles that aggregate with time [24], formation of anisotropic particles whose aspect ratio increases with time [10] or a combination of both processes[18,25]. The inset of Figure 1A exhibits the pale yellow color of MK broth (bottle 1), colorless silver nitrate (middle bottle) and dark brown silver nanoparticles solution (bottle 2).

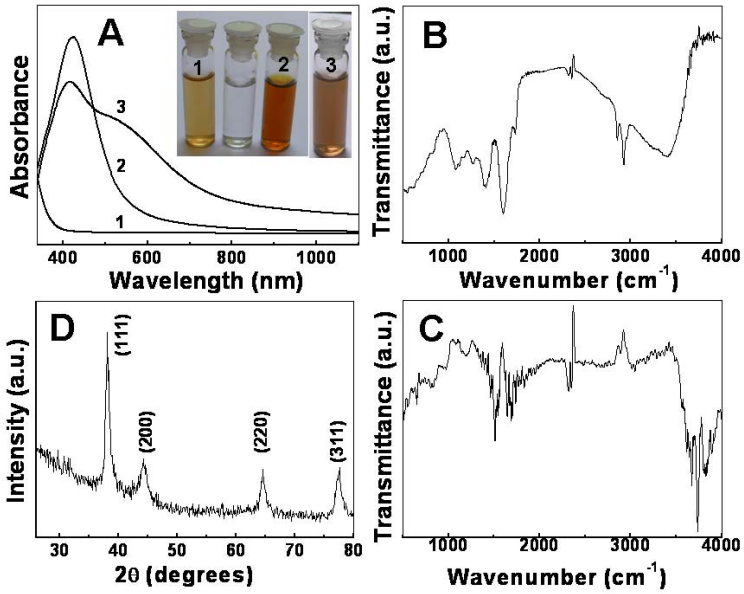

Fig. 1. (A) UV-vis absorption spectra recorded from MK leaf extract (1), MK leaf extract-reduced silver nanoparticles (2), pellet of MK leaf extract-reduced dried silver nanoparticles (3); FTIR spectra recorded (B) from pure MK leaf extract and (C) from MK extract-reduced silver. (D) XRD pattern of the MK leaf extract - reduced silver nanocrystals. The Bragg reflections are identified in the XRD pattern.

Fig. 1B represents the FTIR spectrum of MK leaf extract which shows prominent absorption bands at 1080 cm^{-1}, 1267 cm^{-1},1400 cm^{-1},1597 cm^{-1}, 1730 cm^{-1}, 2355 cm^{-1}, 2855 cm^{-1}, 2924 cm^{-1} and 3408 cm^{-1}. The shoulder at 1597 cm^{-1} is characteristic of CO_2^- stretching, while the stretch at 1400 arises due to the C-O stretching and O-H deformation possibly from the acid groups present in the MK leaf extract [26, 27]. The broad stretching at 3408 cm^{-1} arises due to N-H groups present in the amines. Figure 1C represents the FTIR spectrum of the MK leaf extract reduced silver nanoparticles with the absorption bands at 972 cm^{-1},1462 cm^{-1},1510 cm^{-1}, 1645 cm^{-1}, 1701 cm^{-1}, 2315 cm^{-1}, 2351 cm^{-1}, 3673 cm^{-1}, 3738 cm^{-1}, 3823 cm^{-1}, 3894 $cm^{-1}$and 3283 cm^{-1}. The shift in the CO_2^- stretching frequency (1597 cm^{-1}) to lower wavenumbers (1510 cm^{-1}) followed by the disappearance of the 1597 cm^{-1} resonance may be due to its binding with the silver nanoparticle surface. The shift in the C-O stretching and O-H deformation frequency (1400 cm^{-1}) to lower wavenumbers (1364 and 1395 cm^{-1}) followed by the disappearance of the 1400 cm^{-1} resonance indicate the facilitation of the binding of O-H group of acids with the silver nanoparticle surface. In addition to above supportive evidence the 3408 cm^{-1} feature shifts to 3283 cm^{-1} due to

the binding of the amine group with silver nanoparticle surface. The peak at 1267 cm^{-1} corresponds to C–N stretching vibrations of the primary amines and shifts to 1198 cm^{-1} due to binding with silver nanoparticles. This shows amino acids and proteins have the stronger ability to bind metal nanoparticles [28]. Obviously, biological molecules are performing dual functions of reduction and stabilization of silver nanoparticles in the aqueous medium [29].

The formation of silver nanocrystals formed here was further supported by X-ray diffraction (XRD) measurements (Figure 1D). The Bragg reflections corresponding to the (111), (200), (220) and (311) sets of lattice planes are observed that may be indexed on the basis of the face-centered cubic (fcc) structure of silver (PCPDF file no.- 030921 for Ag). The 2θ values of the standard 38.098°, 44.597°, 64.674°, 77.544° and observed Ag nanoparticles 38.1°, 44.2°, 64.6°, 77.5° correspond to the Bragg reflections (111), (200), (220) and (311) respectively. The (200), (220) and (311) Bragg reflections are weak and considerably broadened relative to the intense (111) reflection.

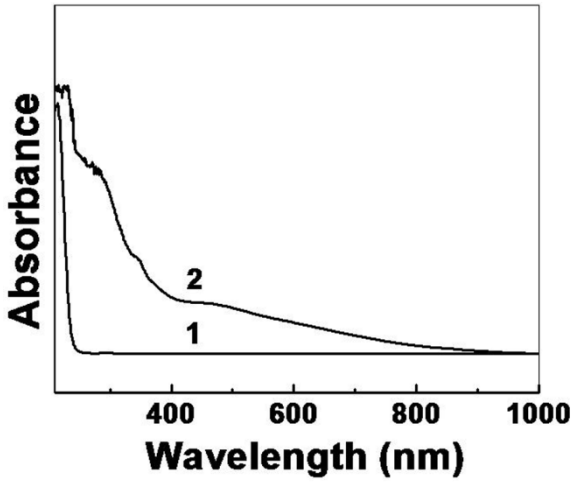

Fig. 2. UV-vis absorption spectra recorded from effect of ammonia (1) and MK broth (2) separately on reduction of silver ions.

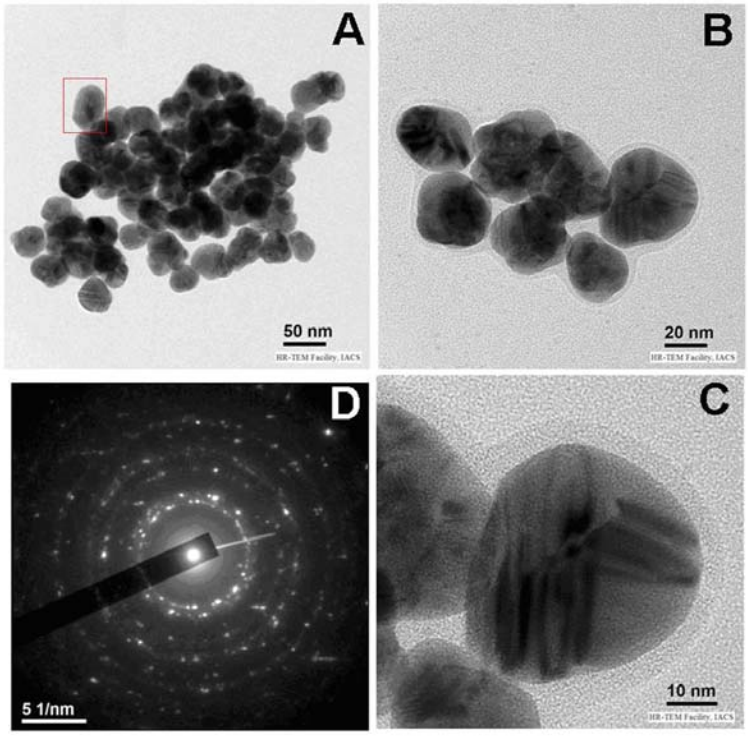

Fig. 3. Representative TEM images of MK leaf extract-reduced silver nanoparticles from lower to higher magnification (A, B, C), and SAED pattern of C (D).

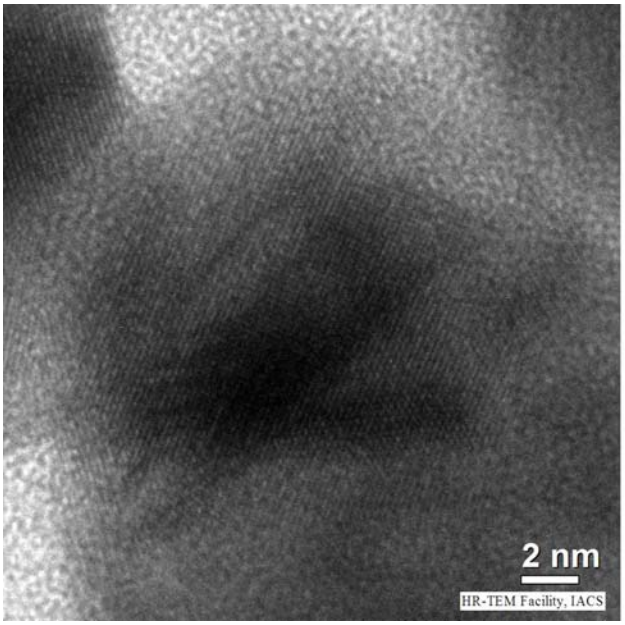

Fig. 4. TEM picture of a single Ag nanoparticle showing the multiply twinning defects

An effective biogenic protocol for room temperature one step synthesis of defective nanocrystalline silver nanobuns using leaf extract
Balaprasad Ankamwar • Gopa Mandal • Ujjal Kumar Sur • Tapan Ganguly

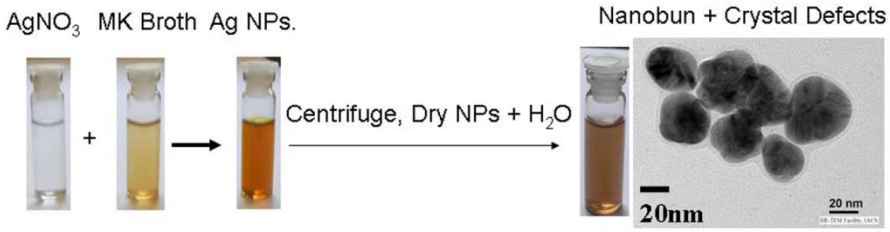

Fig.5. Mechanism of room temperature one step synthesis of defective nanocrystalline silver nanobuns using leaf extracts.

Fig. 5 shows an effective biogenic protocol for room temperature one step synthesis of defective nanocrystalline silver nanobuns using leaf extract. Fig. 3 shows the TEM image of the bio-synthesized Ag nanoparticles. TEM images of various magnification as shown in Figure 3 (A,B,C) clearly indicates the formation of various Ag nanostructures which include 'nanobuns' and also spherical nanoparticles with defects on the surface. Generally, twins are the most common defect in fcc metal nanocrystals [30]. The TEM image in Figure 3A shows the presence of various Ag nanostructures, where the nanostructure marked with a red box will be named as 'nanobun'. To get access to the internal structure, we carried out a detailed HRTEM analysis. The HRTEM image shown in Figure 3C clearly shows the presence of multiply twinned defects in the Ag nanoparticles. The selected-area diffraction pattern (SAED) of the synthesized Ag sample is shown in Figure 3 D. Figure 4 illustrates the TEM image of a single Ag nanoparticle. The TEM image shows that particle diameter varies in the range of 20-40 nm. The average diameter of silver nanoparticle is found to be ~ 30 nm. The TEM images in Figures 3B and C also show the presence of an ultrathin layer of 2-3 nm thickness, which may be due to formation of monolayer by the stabilizing agents on the surface of the nanoparticles. This kind of defective nanocrystals, especially Ag can play important role in catalysis. It is expected that the synthesis of such defective nanoparticles such as Ag and Pt to be of importance to both theoretical investigations and practical applications, such as electrocatalysis and SERS substrate. Further

experiments and theoretical calculation based on this subject are under investigation in our laboratory, which will be reported later.

4. Conclusions

Defective nanocrystalline silver nanobuns have been synthesized successfully in aqueous solution at room temperature using *Murraya Koenigi* leaf extract for the first time through an one-pot biogenic protocol. On treating aqueous silver nitrate solution with *Murraya Koenigi* leaf extract, reduction of the silver ions is observed leading to the formation of crystalline nanoparticles. This simple eco-friendly method can be further extended for different transition metals for preparing the nanocrystals of various shapes and sizes.

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