

GREEN SYNTHESIS OF NANOSILVER PARTICLES FROM EXTRACT OF *EUCALYPTUS HYBRIDA* (SAFEDA) LEAF

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Biosynthesis of nanoparticles by plant extracts is currently under exploitation. Plant extracts are very cost effective and eco-friendly and thus can be an economic and efficient alternative for the large-scale synthesis of nanoparticles. We report on the use of methanolic extract of *Eucalyptus hybrida* leaf in the extracellular biosynthesis of silver nanoparticles. Bioactive silver nanoparticle synthesis by reacting the methanolic biomass of *Eucalyptus hybrida* leaf with aqueous solutions of silver nitrate (AgNO_3) at ambient temperature. Formation of silver nanoparticles was confirmed by UV-visible spectroscopy, X-ray diffraction patterns, Scherrer's formula and scanning electron microscopy (SEM) with Energy dispersive X-ray (EDX) patterns. The biomass of plants produces their nanomaterials by a process called biomineralisation. Biological methods are a good competitor for the chemical procedures, which are environment friendly and convenient.

(Received September 1, 2009; accepted September 29, 2009)

Keywords: Green nanoscience, Biological synthesis, *Eucalyptus hybrida*, Silver nanoparticle

1. Introduction

An important aspect of nanotechnology concerns the development of experimental processes for the synthesis of nanoparticles of different sizes, shapes and controlled dispersity. With the development of new chemical or physical methods, the concern for environmental contaminations are also heightened as the chemical procedures involved in the synthesis of nanomaterials generate a large amount of hazardous byproducts. Thus, there is a need for 'green chemistry' that includes a clean, nontoxic and environment-friendly method of nanoparticle synthesis [1]. As a result, researchers in the field of nanoparticle synthesis and assembly have turned to biological systems for inspiration. Many biological organisms, both unicellular and multicellular, are known to produce inorganic materials either intra- or extra-cellularly [2] often of nanoscale dimensions and of exquisite morphology and hierarchical assembly. Some well known examples of microorganisms synthesizing inorganic materials include magnetotactic bacteria for magnetite nanoparticles [3, 4]. S-layer bacteria for gypsum and calcium carbonate layers [5] and silver mine-inhabiting *Pseudomonas* sp. that reduces silver ions to form silver nanoparticles [6]. Nanocrystals of gold, silver and their alloys have been synthesized within the cells of lactic acid bacteria [7]. Eukaryotic organisms such as fungi may be used to grow nanoparticles of different chemical compositions and sizes. A number of different genera of fungi have been investigated in this effort and it has been shown that fungi are extremely good candidates in the synthesis of silver [1,8,9]. Several attempts of synthesis of metal nanoparticles have been made by researchers. This method can be very efficient in decontaminating soil polluted with heavy metal ions [10].

Biosynthesis of nanoparticles by plant extracts is currently under exploitation. Sastry et al. aforementioned attained the biosynthesis of metal nanoparticles by plant leaf extracts and their potential applications. They studied bioreduction of chloro-aurate ions or silver ions by a broth of geranium leaf [11] or Neem leaf [12]. Further, they had explored the formation mechanism of gold nanotriangles by lemongrass extracts [13]. Very recently, they have demonstrated synthesis of gold nanotriangles and silver nanoparticles using Aloe vera plant extracts [14]. Nevertheless, the bioreduction of silver ions proceeded merely in the presence of ammonia. Most of the above research on the synthesis of silver or gold nanoparticles utilizing plant extracts employed broths resulting from boiling fresh plant leaves. The green synthesis of silver nanoparticles using *Capsicum annum* L. extract has been reported [15]. According to previous reports, the polyol components and the water-soluble heterocyclic components are mainly responsible for the reduction of silver ions and the stabilization of the nanoparticles, respectively [16].

In this paper, we report on the biosynthesis of pure metallic nanoparticles of silver by the reduction of aqueous Ag^+ ions with the Methanolic Extract of *Eucalyptus hybrida* (Safeda) leaves.

2. Experimental details

2.1. Preparation of dried biomass

Crude plant extract was prepared by Soxhlet extraction method [17]. About 100 g of powder material was uniformly packed in to a thimble and run in Soxhlet extractor. It was exhaustible extracted with methanol for the period of about 48 hour or 22 cycles or till the solvent in the siphon tube of an extractor become color less. After that extracts were filtered with the help of filter paper and solvent was evaporated from extract in Rotary evaporator to get the syrupy consistency. Then extract was kept in refrigerator at 4 °C for future experiments.

2.2. Phytochemical analysis of dried biomass

Identification of active constituents was done by chemical test. Tests for alkaloids, flavonoids, sterols, triterpenes, protein, saponins and glycosides were carried out according to the methods of Harborne [18].

2.3. Biosynthesis of silver nanoparticles

Silver nitrate (AgNO_3) was purchased from Hi Media Laboratories Pvt. Mumbai, India and was used as received. In a typical synthesis for silver nanoparticles using Methanolic Extract of *Eucalyptus hybrida* leaves, the carefully weight biomass was added to 50 ml of 1 mM aqueous AgNO_3 solution, in conical flasks of 100 ml content at room temperature in the dark.

2.3. UV-vis spectra analysis

The bioreduction of Ag^+ in aqueous solution was monitored by periodic sampling of aliquots (0.2 ml) of the suspension, then diluting the samples with 2 ml deionized water and subsequently measuring UV-vis spectra of the resulting diluents. UV-vis spectroscopy analyses of silver nanoparticles produced were carried out as a function of bioreduction time at room temperature on ELICO UV spectrophotometers at a resolution of 1 nm.

2.4. XRD analysis

X-ray diffraction (XRD) analysis of drop-coated films of silver nanoparticles in sample was prepared for the determination of the formation of Ag nanoparticle by an X'Pert Pro X-ray diffractometer (X'Pert High Score Plus program) operated at a voltage of 40 kV and a current of 30 mA with $\text{Cu K}\alpha$ radiation.

2.5. Observation of silver particle size

XRD patterns were analyzed to determine peak intensity, position and width. Fullwidth at half-maximum (FWHM) data was used with the Scherrer's formula to determine mean particle size. Scherrer's equation is given by

$$d = \frac{0.9\lambda}{\beta \cos\theta}$$

where d is the mean diameter of the nanoparticles, λ is wavelength of X-ray radiation source, β is the angular FWHM of the XRD peak at the diffraction angle θ [19].

2.6. SEM observation of silver nanoparticles

The plant extract biomass after reaction spontaneously precipitated at the bottom of the tubes. After the precipitation, the suspension above the precipitate was sampled for SEM observation. SEM samples of the aqueous suspension of silver nanoparticles were fabricated by dropping the suspension onto clean electric Stubs and allowing water to completely evaporate. SEM observations were carried out on a ZEISS EVO 40 EP Electron microscope.

2.7. EDX observation of silver nanoparticles

Energy dispersive X-ray spectrometers take advantage of the photon nature of light. In the X-ray range the energy of a single photon is just sufficient to produce a measurable voltage pulse X-ray, the output of an ultra low noise preamplifier connected to the low noise are a statistical measure of the corresponding quantum energy. By digitally recording and counting a great number of such pulses with in a so called Multi Channel Analyzer, a complete image of the X-ray spectrum is building up almost simultaneously. This digital quantum counting technique makes the energy dispersive spectrometry exceedingly reliable. A semiconductor material is used to detect the x-rays together with processing electronics to analyses the spectrum. EDX observations were carried out by a Bruker LN₂ free X-Flash 4010 SDD Detector and analytical Software was QUANTAX 200.

3. Results and discussion

After methanolic extraction of *Eucalyptus hybrida* leaf the extracts were tested for the presence of chemical constituents such as alkaloids, saponins, flavinoids, protein, carbohydrates, triterpenoids, tannin and glycosides. *Eucalyptus hybrida* leaf extract was positive for alkaloids, tannins, flavinoids, triterpenoids and saponins. The flavanoid and terpenoid constituents of the leaf extract are believed to be the surface active molecules stabilizing the nanoparticles [11].

Formation of the silver nanoparticles by reduction of the aqueous Ag⁺ during exposure to the methanolic extract of *Eucalyptus hybrida* leaves were followed by UV-vis spectroscopy. It is well known that silver nanoparticles exhibit reddish-brown in water [20]. Fig. 2 shows the color changes before (a), during (b) and (c) after the process of reduction of Ag⁺ to Ag nanoparticle. After 3 h of the conversion process silver nanoparticle showed reddish-brown color, suggested the formation of silver nanoparticles in solution. These colors arise due to excitation of surface plasmon vibrations in the silver metal nanoparticles [21].

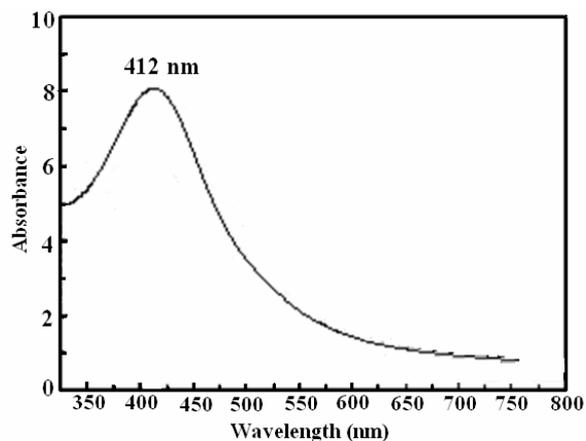


Fig 1. UV-visible absorption spectra of reduction of silver ions to silver nanoparticles

Fig.1 shows the UV-vis spectra recorded from the aqueous silver nitrate and *Eucalyptus hybrida* leaf extract. It is observed that the silver surface plasmon resonance band occurs at 412 nm and steadily increases in intensity as a function of time of reaction without any shift in the peak wavelength. The frequency and width of the surface plasmon absorption depends on the size and shape of the metal nanoparticles as well as on the dielectric constant of the metal itself and the surrounding medium [22, 23]. It is generally recognized that UV-vis spectroscopy could be used to examine size- and shape-controlled nanoparticles in aqueous suspensions [24].

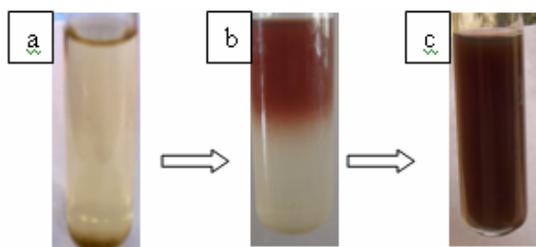


Fig 2. The pictures show the color changes before (a), during (b) and after the process of reduction of Ag^+ to Ag nanoparticles (c).

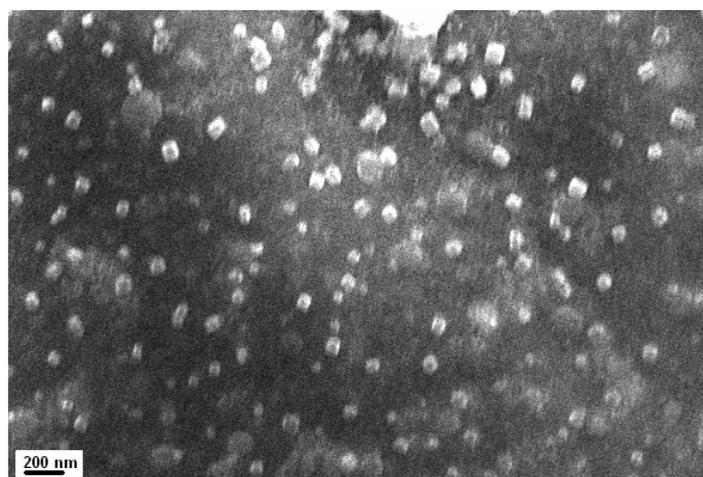


Fig. 3, SEM micrograph of Silver Nanoparticles from *Eucalyptus hybrida* leaf extract at 96.62 KX magnifications.

Fig. 3 shows representative SEM images recorded from drop-coated films of the silver nanoparticles synthesized by treating silver nitrate solution with *Eucalyptus hybrida* leaf extract. The silver nanoparticles formed were predominantly cubical with uniform shape. It is known that the shape of metal nanoparticles considerably change their optical and electronic properties [25].

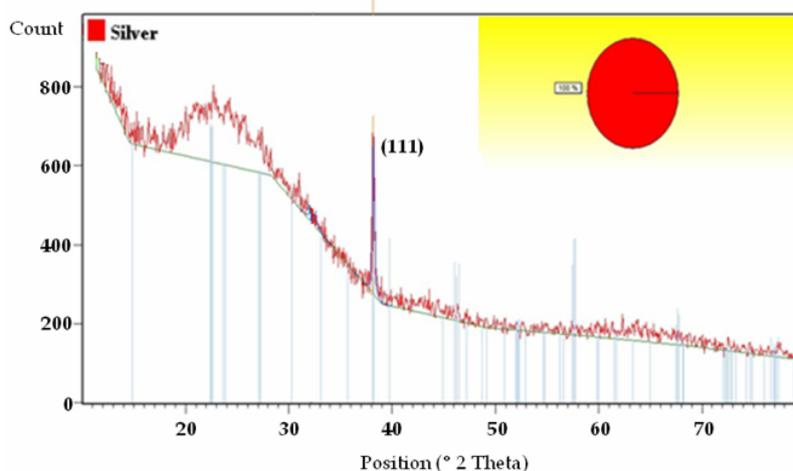


Fig. 4, XRD patterns recorded from drop-coated films on glass substrate of silver nanoparticles synthesized by treating *Eucalyptus hybrida* leaf extract with AgNO_3 aqueous solutions. The Bragg reflections are indexed on the basis of the fcc silver structure.

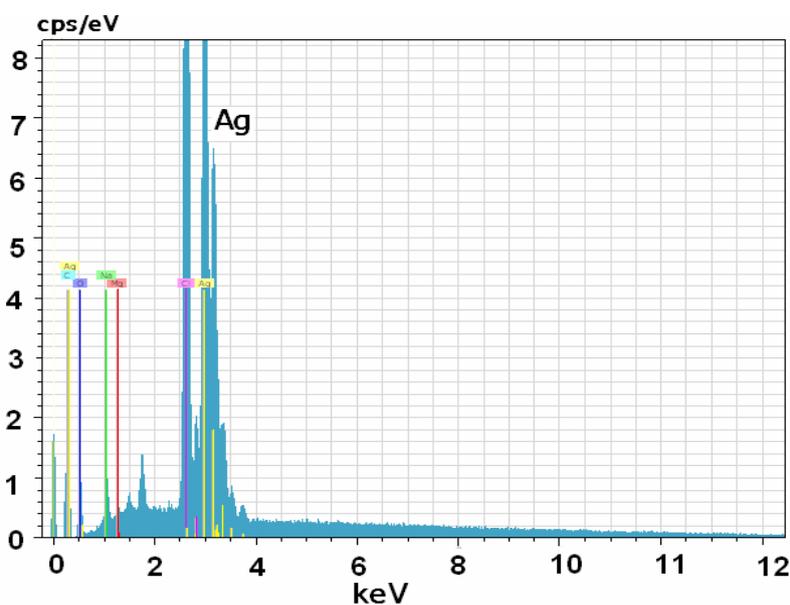


Fig. 5. EDX spectrum recorded from a film, after formation of silver nanoparticles. Different X-ray emission peaks are labeled.

XRD patterns obtained for silver nanoparticles synthesized using *Eucalyptus hybrida* leaf extract in figure 4 show characteristic peaks (at $2\theta = 30.8^\circ$), marked with (111). A number of Bragg reflections corresponding to the (111) sets of lattice planes are observed which may be indexed based on the face-centred cubic structure of silver. The XRD pattern thus clearly shows that the silver nanoparticles are crystalline in nature. The XRD pattern of pure silver ions is known to display peaks at $2\theta = 7.9^\circ, 11.4^\circ, 17.8^\circ, 30^\circ, 38^\circ, \text{ and } 44^\circ$ [26]. The value of the pure silver lattice constant has been estimated to be $a = 4.081$, a value that is consistent with $a = 4.0862 \text{ \AA}$ reported by the JCPDS file n°4-0783. This estimation confirmed the hypothesis of particle monocrystallinity.

The sharpening of the peaks clearly indicates that the particles are in the nanoregime. The size of the silver nanocrystallites as estimated from the FWHM of the (111) peak of silver using the Scherrer formula is 50 - 150 nm.

Analysis through Energy dispersive X-ray (EDX) spectrometers confirmed the presence of elemental silver signal of the silver nanoparticles (Fig. 5). The vertical axis displays the number of x-ray counts whilst the horizontal axis displays energy in KeV. Identification lines for the major emission energies for silver (Ag) are displayed and these correspond with peaks in the spectrum, thus giving confidence that silver has been correctly identified.

4. Conclusion

In conclusion, it has been demonstrated that the extract of *Eucalyptus hybrida* leaf are capable of producing silver nanoparticles extracellularly and the silver nanoparticles are quite stable in solution. The flavanoid and terpenoid constituents which present in *Eucalyptus hybrida* leaf extract are the surface active molecules stabilizing the nanoparticles. Achievement of such rapid time scales for synthesis of metallic nanoparticles contributes to an increase in the efficiency of synthetic procedures using environmentally benign natural resources as an alternative to chemical synthesis protocols and low cost candidate as reductant for synthesizing silver nanoparticles.

Acknowledgment

The authors are sincerely thankful to Dr. N.K. Saini, Dr. N.K. Juyal & Dr. Samay Singh from Wadia Institute of Himalayan Geology, Dehradun, for providing SEM, EDX and XRD facilities and to the Principal R.B.S. College, Agra for providing research facilities.

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