



## Biosynthesis of silver Nanoparticles from Glycyrrhiza Glabra root extract

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### ABSTRACT

Asingle-stepenvironmental friendly approach is employed to synthesize silver nanoparticles. The environmental friendly synthesis of nanoparticles process is a revolutionary step in the field of nanotechnology. In this study, the biosynthesis of silver nanoparticles was carried out using Glycyrrhiza Glabra root extract as reducing agent. UV-visible spectroscopy was used for quantification of silver nanoparticle synthesis. The synthesized silver nanoparticles were characterized with Scanning electron microscopy (SEM), Energy dispersive X ray analysis (EDX), X-ray Diffraction (XRD) and Fourier transform Infrared Spectroscopy (FTIR).

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### Introduction

Nature has made noble metals part of our daily life. Recently there has been considerable interest in the development of techniques for the biosynthesis of metal-nanoparticles of well-defined size, shape and composition, as they find applications in areas such as optics and electronics [1], [2], [3] and [4]. Among metal-nanoparticles, silver nanoparticles exhibit tremendous applications in spectrally selective coatings for solar energy absorption, optical receptors, bio-labeling, and intercalation materials for electrical batteries, filters, antimicrobial agents and sensors [5]-[7]. Silver nanoparticle-embedded antimicrobial paint [8] is a promising area of environmentally friendly applications. Hence, a variety of techniques including physical and chemical methods have been developed to synthesize silver nanoparticles. Therefore, there is a growing need to develop environmentally friendly nanoparticle synthesis processes that do not use toxic chemicals in the synthesis protocols [6] and [9].

Silver nanoparticles can be produced either intra- or extracellularly by using living organisms [10]. Over the past decade, a variety of microorganisms such as bacteria, fungi and yeast have been used to synthesize silver nanoparticles [11-13]. Green synthesis of silver nanoparticles and their antimicrobial activities have been reviewed by Sharma et al. [14]. Mohanpuria et al. [10] have reviewed the synthesis of silver nanoparticles using plants. Most of the reported biological synthesis methods using plants took more than 1 h for the formation of colloidal silver. Herein, a rapid biological synthesis of stable silver nanoparticles using aqueous extract of Glycyrrhiza Glabra root is reported. The root is used traditionally to treat various infections and has anti-diabetic and anti-hyperglycaemic effect. The root extract is known for its antioxidant and antibacterial activity.

### Experimental

Glycyrrhiza Glabra root was washed several times with de-

ionized water. 50 g of the root was finely cut and stirred with 150 mL de-ionized water at 300 K for 5 min and filtered to get the extract. The filtrate is used as reducing agent and stabilizer. AgNO<sub>3</sub> and NaOH were procured from Sigma-Aldrich. 7 mL extract is added to a vigorously stirred 100 mL aqueous solution of AgNO<sub>3</sub> ( $5 \times 10^{-4}$  M) and stirring continued for 10 min. Reduction takes place slowly at 300 K and is complete in 30 min as shown by stable redish brown colour of the solution giving colloid. It is found to be stable for more than 2 months, showing no precipitation or colour change.

### UV-VIS spectra analysis

The UV-vis spectra were recorded using, ELCO 150 UV-visible spectrophotometer with samples in quartz cuvette. X-ray diffraction pattern of dry nanoparticle powder was obtained using Siemens D5005 X-ray diffractometer with CuK $\alpha$  radiation ( $\lambda = 0.1542$  nm). The FTIR spectra were obtained on a Nicolet 5700 FTIR instrument with the sample as KBr pellets. The morphology of the nanoparticles was analysed using the high-resolution images obtained with a JEOL 3010 transmission electron microscope.

SEM (Scanning Electron Microscopic) and EDX (Energy dispersive X-ray spectrometer) analysis of silver nanoparticles

SEM analysis was done using Hitachi S-3500SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

### EDX

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 volt 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.

#### XRD analysis of silver nanoparticles

The silver nanoparticle solution thus obtained was purified by repeated centrifugation at 5000 rpm for 20 min followed by redispersion of the pellet of silver nanoparticles into 10 ml of deionized water. After freeze drying of the purified silver particles, the structure and composition were analyzed by XRD (Rigaku RINT 2100 series). The dried mixture of silver nanoparticles was collected for the determination of the formation of Ag nanoparticles by an X'Pert Pro x-ray diffractometer operated at a voltage of 40 kV and a current of 30 mA with Cu K $\alpha$  radiation in a  $\theta$ - 2  $\theta$  configurations. The crystallite domain size was calculated from the width of the XRD peaks, assuming that they are free from non-uniform strains, using the Scherrer formula.

$$D = 0.94 \lambda / \beta \cos \theta \rightarrow (1)$$

where D is the average crystallite domain size perpendicular to the reflecting planes,  $\lambda$  is the X-ray wavelength,  $\beta$  is the full width at half maximum (FWHM), and  $\theta$  is the diffraction angle. To eliminate additional instrumental broadening the FWHM was corrected, using the FWHM from a large grained Si sample.

$$\beta \text{ corrected} = (\text{FWHM}^2_{\text{sample}} - \text{FWHM}^2_{\text{Si}})^{1/2} \rightarrow (2)$$

This modified formula is valid only when the crystallite size is smaller than 100 nm [15]

#### Results and discussion

It is reported that aqueous extract of Glycyrrhiza Glabra root extract is composed of a variety of The herb contains glycyrrhizin, glycyrrhetic acid, flavonoids, asparagine, iso-flavonoids, and chalcones [16] and these may be responsible for reduction of metal ions and efficient stabilization of nanoparticles.

#### UV-vis, TEM and SEM analysis

UV-vis spectroscopy is an important technique to ascertain the formation and stability of metal nanoparticles in aqueous solution Fig. 1. Figure 1a shows the UV-vis spectra of the silver. Colour of silver colloid is attributed to surface plasmon resonance (SPR) arising due to the collective oscillation of free conduction electrons induced by an interacting electromagnetic field [17]. SPR bands of the colloids are centered at 440 nm. The bands are broad and the intensity increases indicating increase in production of nanoparticles. The SEM and TEM images of the colloids are shown in [Fig. 2 and 3].

Colloids consist mainly of large nanoparticles having nearly spherical shape particles of size 20–30 nm. It is clear from the images (Fig. 2 and 3) that the particles in colloid are well-dispersed with a more uniform size 20 nm. A similar TEM image was observed for polydispersed silver nanoparticles by Lee et al. [18] in chemical reduction and Huang et al. [19] in biosynthesis. It is evident from the TEM image that the shape and size of the silver nanoparticles could be controlled by adjusting the reaction mixture. The high resolution TEM images (Fig. 2) indicate good crystallinity of the nanoparticles.

#### EDAX analysis of silver nanoparticles

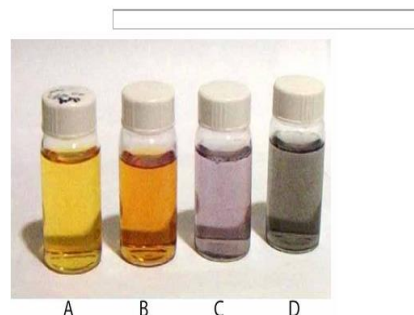
The energy dispersive X-ray analysis (EDX) reveals strong signal in the silver region and confirms the formation of silver nanoparticles (Fig 4). Metallic silver nanocrystals generally show typical optical absorption peak approximately at 3 keV due to surface plasmon resonance. This analysis revealed that the

nano-structures were formed solely of silver (Table 1).

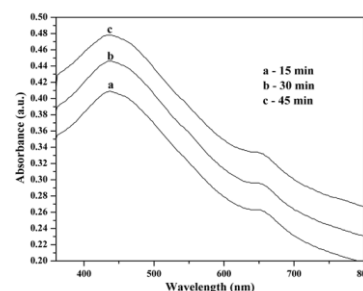
#### XRD and FTIR studies

The crystalline nature of Ag nanoparticles was confirmed from the analysis of the X-ray diffraction (XRD) pattern (Fig. 5). The five diffraction peaks [18] and [19] are indexed as (1 1 1), (2 0 0), (2 2 0), (3 1 1) and (2 2 2) planes of fcc silver (JCPDS, file no. 04-0783). The (2 0 0), (2 2 0), (3 1 1) and (2 2 2) Bragg reflections are weak and broadened relative to the intense (1 1 1) reflection. This feature indicates that the nanocrystals are highly anisotropic [20] and suggests that the particles are (1 1 1)-oriented as confirmed by high resolution TEM measurements. The mean size of nanoparticles is calculated using Debye–Scherrer's equation by determining the width of the (1 1 1) peak and found to be 19 nm which is fairly in agreement with the TEM measurement.

FTIR measurement was carried out to identify the possible biomolecules in Glycyrrhiza Glabra root responsible for capping leading to efficient stabilization of the silver nanoparticles. Prominent IR bands (Fig.6a and 7b) are observed at 2914, 2847, 1708, 1601, 1464, 1376, 1008, 880, 716 and 469  $\text{cm}^{-1}$ . Most of the IR bands are characteristic of flavonoids and terpenoids present in the root. The sharp bands at 2914 and 2847  $\text{cm}^{-1}$  arise from C–H stretching modes. The medium intense bands at 1708 and 1601  $\text{cm}^{-1}$  are assigned to the stretching vibrations of C=O; and C=C, respectively [21] and [22]. The absorption bands located at 1376 and 1008  $\text{cm}^{-1}$  may be attributed to –C–O and –C–O–C stretching modes. The medium intense band at 1464  $\text{cm}^{-1}$  arises from the C–N stretching mode of the aromatic amine group [23]. The vibrational bands corresponding to bonds such as –C=C, –C=O, –C–O, –C–O–C and –C–N are derived from the water soluble compounds such as flavonoids, terpenoids and thiamine present in Glycyrrhiza Glabra root. Hence, it may be assumed that these biomolecules are responsible for capping and efficient stabilization. The presence of reducing sugars in the extract could be responsible for the reduction of silver ions and formation of the nanoparticles [23, 24]. This rapid and environmentally benign method is a faster synthesis comparable to chemical reduction methods.



**Fig 1. Colloidal silver in various stages of aggregation, (A) clear yellow sol, (B) dark yellow sol, (C) violet sol, and (D) grayish sol, as aggregation proceeds.**



**Fig 1a. UV-vis spectra of Silver colloid**

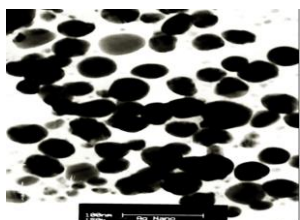


Fig 2. TEM of Silver nanoparticles

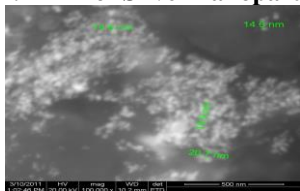


Fig 3. SEM of Silver nanoparticles

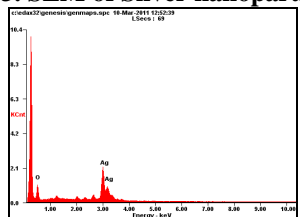


Fig 4. EDAX of Silver nanoparticles

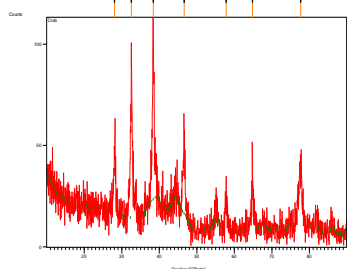


Fig 5. XRD pattern of silver nanoparticles.

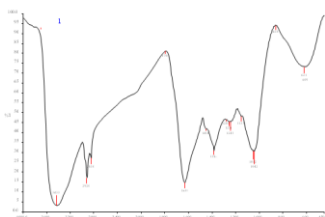


Fig 6. FTIR Spectra of Silver nanoparticles

### Conclusions

Silver nanoparticles are synthesized from silver precursor using aqueous extract of *Glycyrrhiza Glabra* leaf at room temperature. The reduction is found to be accelerated by the change reaction mixture. The colloid obtained by rapid reduction is found to consist of well-dispersed nearly spherical particles having size around 20 nm. The nanoparticles are found to be highly crystalline as evidenced by the peaks in the XRD pattern corresponding to Bragg reflections from the (1 1 1), (2 0 0), (2 2 0), (3 1 1) and (2 2 2) planes of the fcc structure, and clear lattice fringes in the high resolution TEM image. The biomolecules responsible for efficient stabilization of the silver colloid is suggested by studying the FTIR spectrum.

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