Research Paper

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A Biological Approach of Silver (Ag) Nanoparticles Synthesis Using Leaf Extract of Adhatoda Vasica

Environment

KEYWORDS : Bioreduction, Adhatoda vasica, metal nanoparticles, UV-Vis, XRD, SEM

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ABSTRACT

The present study deals with the biosynthesis of silver nanoparticles using the leaf extract of medicinal plant Adhatoda vasica. The complete reduction of silver ions was observed after 48 h of reaction at 300 C under shaking condition. The colour changes in reaction mixture was observed during the incubation period, because of the formation of silver nanoparticles in the reaction mixture enables to produce particular colour due to their specific properties (Surface Plasmon Resonance). The XRD pattern thus clearly shows that the silver nanoparticles are crystalline in nature. The synthesized silver nanoparticles were predominately spherical in shape, polydispersed and average particles size of 42 nm.

Introduction

Nanoparticles are being viewed as fundamental building blocks of nanotechnology. They are the starting point for preparing many nanostructured materials and devices. These particles having at least one dimension of less than 100 nm. Synthesis of nanoparticles is an important component of rapidly growing research efforts. The nanoparticles of a wide range of materials can be prepared by numerous methods like chemical, physical and biological methods. The physical and chemical methods have been widely used to synthesize nanoparticles of varying size and shapes. The important criterion in these methods of synthesis was expensive and since it often requires extremes of temperature, pressure and energy. The chemical synthesis protocols employed previously have used toxic and/or flammable and chemicals, which can have undesirable side effects in some applications and also environmental concerns (Shankar *et al.,* 2003). Therefore presently, there is a growing need to synthesize the nanoparticles in a cost effective and safe way. The exploitation of biological systems emerged as a novel method for the synthesis of nanoparticles in this regard.

Biological resources like microorganisms and plants for synthesis of nanoparticles offer numerous benefits and compatibility for many applications. It provides advancement over chemical and physical methods as it is a cost effective and environment friendly and in this method there is no need to use high pressure, energy, temperature and toxic chemicals during the synthesis of nanoparticles (Parashar *et al.,* 2009). Among the use of living organisms for nanoparticles synthesis, plants have found application particularly in metal nanoparticles synthesis. Use of plants or their extract for synthesis of nanoparticles could be advantageous over other environmentally benign biological processes as this eliminates the elaborate process for maintaining the cell cultures. Plant use can also be suitably scaled up for large-scale synthesis of nanoparticles (Kumar & Yadav, 2008). Hence, in this present study we investigated the synthesis of silver nanoparticles through biological method using leaf extract of medicinal plant *Adhatoda vasica.*

Materials and Methods

The fresh and young leaf samples of *Adhatoda vasica* was washed thoroughly with double distilled water (to remove any dust particles that could interfere with formation of nanoparticles) and surface sterilized with 0.1 per cent $HgCl_2$ for 2 to 3 min under the hood of laminar air flow. Twenty gram of sterilized leaf samples were taken and cut into small pieces. The finely cut leaves were placed in a 500 ml Erlenmeyer flask containing 100 ml of sterile double distilled water. Then, the mixture was boiled for 5 min (process of boiling leads to rupture of the cell walls in leaves and thus, release of inter cellular material into solution). After boiling, the mixture was cooled and filtered.

Bioreduction of metal ions in solution was done through addition of leaf extract into the metal solution having known concentration. Silver nitrate ($AgNO_3$) was used as precursor for synthesizing the silver nanoparticles. Five ml of leaf extract was added to 100 ml of 1 mM $AgNO_3$ aqueous solution in conical flask of 250 ml content at room temperature. After that the flask was put into shaker (150 rpm) at 30^0 C and reaction was carried out for a period of 48 h.

Characterization of silver nanoparticle was done by using UV-Vis spectroscopy, X-Ray Diffraction (XRD) and Scanning Electron Microscopy (SEM). The colour changes of reaction mixtures (metal ion solution and leaf extract) was recorded through visual observation. The bioreduction of silver ions in aqueous solution was monitored by periodic sampling of aliquot (1 ml) of the aqueous component and subsequently measuring UV-Vis spectra of the solution. The aliquots were diluted to 10 times with double distilled water to avoid errors due to high optical density of the solution. UV-Vis spectra of these aliquots were monitored as a function of time of reaction on Elico UV-Vis spectrophotometer (model S3-159) operated at a resolution of 1 nm.

The aliquot was drop-coated onto aluminum plate by just dropping a small amount of aliquots on the plate frequently, allowed to dry and finally thick coat of aliquots on plate was prepared for XRD measurement. The XRD measurement was performed on a Shimazdu, model LabX-XRD-6000 instrument operated at a voltage of 20 to 30 keV and a current of 30 mA with Cu K α radiation with a wavelength of 1.5418 Å. The scanning was done in the region of 25^0 to 50^0 for 2θ at 5^0 min^{-1} and the time constant of 0.24 second in continuous scan mode.

The average crystallite size of silver was calculated from the width of the XRD peak using the Scherrer's formula,

$$D = k\lambda / \beta\cos\theta$$

Where,

D - Average crystallite domain size perpendicular to reflection planes

K - Constant

λ - X-ray Wavelength

β - Angular FWHM of the XRD peak at the diffraction angle

θ - Diffraction angle

The thin film of the aliquot was prepared on a small aluminum plate by just dropping a very small amount of the aliquot on the plate, extra solution were removed using a blotting paper and then the film on the plate was allowed to dry for overnight for SEM analysis. The SEM analysis was performed on a JEOL, model JSM-6390 instrument operated at an accelerating voltage

of 20 keV and counting time of 100 s.

Results and Discussion

Synthesis of silver nanoparticles occurred during the exposure of *Adhatoda vasica* leaf extract to 1 mM aqueous $AgNO_3$ solution. The complete reduction of silver ions was observed after 48 h of reaction at 30^0 C under shaking condition. The colour change in the reaction mixture was observed during the incubation period, because the formation of silver nanoparticles was able to produce the particular colour due to their specific properties. The appearance of dark yellowish-brown colour was a clear indication of the formation of silver nanoparticles in the reaction mixture (Fig.1). The colour exhibited by metallic nanoparticles is due to the coherent excitation of all the "free" electrons within the conduction band, leading to an inphase oscillation, which is known as Surface Plasmon Resonance (Akanna *et al.,* 2010). 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 (Dubey *et al.,* 2009).

UV-Vis spectroscopy analysis showed that the absorbance band of silver nanoparticles synthesized using *Adhatoda vasica* leaf extract centered at 442 nm and steadily increased in intensity as a function of time of reaction without any shift in the peak wavelength (Fig.2). The silver nanoparticles synthesized by the different plant leaf extracts shown the strong absorbance between 420 to 450 nm in UV-Vis spectral analysis (Roy & Barik, 2010).

The XRD pattern obtained for silver nanoparticles showed a characteristic peak near the 2θ value of 37.47^0 (Fig.3). Crystallite size of silver nanoparticles as estimated from the Full width at half maximum (FWHM) of the (111) peak using the Scherrer's formula exhibited an average particle size of around 42 nm (Table 1).

A Bragg reflection corresponding to the (111) sets of lattice planes was observed, which may be indexed based on the face-centered cubic (fcc) structure of silver (Dubey *et al.,* 2009). The XRD pattern thus clearly shows that the silver nanoparticles are crystalline in nature. The sharpening of the peaks clearly indicates that the particles are nanoregime. The size of the nanoparticles varied based on the FWHM of the peak. The FWHM values of peak get increased, the size of the nanoparticles get decreased. The line broadening of the X-ray diffraction peak is primarily due to the small particle size (Chudasama *et al.,* 2010). In addition to the Bragg peak representative of fcc silver nanocrystals, additional and yet unassigned peaks were also observed suggesting that the crystallization of bio-organic phase occurs on the surface of the silver nanoparticles (Sathyavathi *et al.,* 2009).

Table 1: Crystalline size of silver nanoparticles synthesized using *Adhatoda vasica* leaf extract

Leaf extract	θ value (degree)	d - pacing (Å)	FWHM (degree)	Intensity (CPS)	Average Particle size (nm)
Adhatoda vasica	18.74	2.397	0.370	23.0	41.35

SEM image showed individual silver particles as well as a number of aggregates. The morphology of the silver nanoparticles was predominately spherical and aggregated into larger irregular structure with no well-defined morphology observed in the micrograph (Fig.4). The SEM image nanoparticles were not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by a capping agent (proteins secreted by plant leaf extracts). The presence of secondary materials capping with the silver nanoparticles may be assigned to bio-organic compounds from leaf extract (Rajesh *et al.,* 2009).

Conclusion

Our investigation suggests that leaf extract of *Adhatoda vasica* capable of producing silver nanoparticles extracellularly and the synthesized nanoparticles are quite stable in solution. The synthetic methods based on naturally occurring biomaterials provide an alternative means for obtaining the nanoparticles. The use of plants in synthesis of nanoparticles is quite novel and this biological approach have many advantages such as, ease with which the process can be scaled up, economic viability and safe way to produce nanoparticles.

Fig. 1 (a) 1 mM AgNO3 solution (b) Leaf extract (c) Leaf extract + AgNO3 after 48 h of reaction (Inset of fig. Adhatoda vasica)

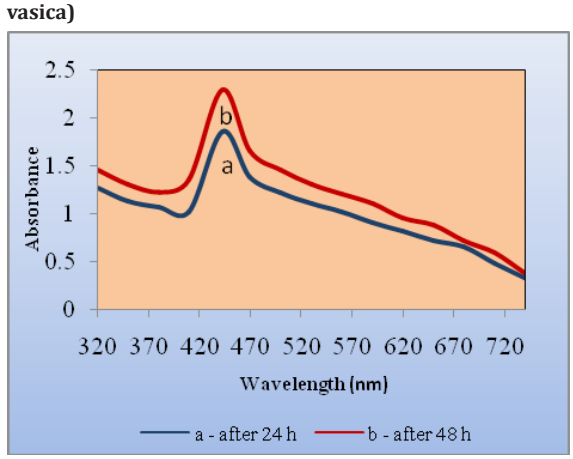

Fig. 2 UV-Vis spectrum of silver nanoparticles

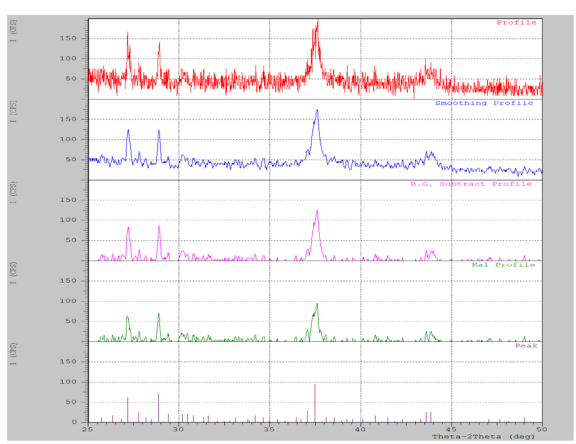

Fig. 3 XRD pattern of silver nanoparticles

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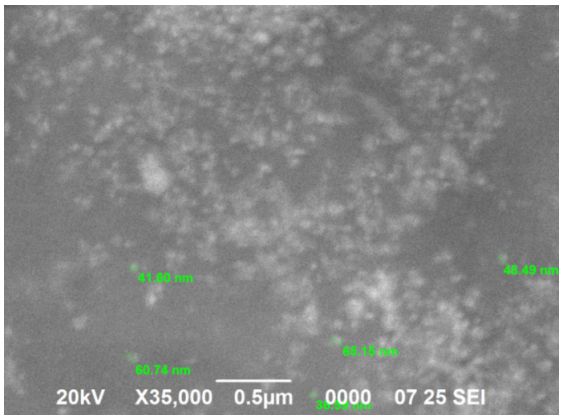

Fig. 4 SEM image of silver nanoparticles

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