

**MICROWAVE ASSISTED BIOSYNTHESIS OF SILVER NANOPARTICLES BY
AQUEOUS EXTRACTS OF LEAVES*****Vidya V. G. and Salma P. P.**

University College, M G Road, Palayam, Thiruvananthapuram- 695 034, Kerala, India.

***Corresponding Author: Vidya V. G.**

University College, M G Road, Palayam, Thiruvananthapuram- 695 034, Kerala, India.

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ABSTRACT

The green synthesis of silver nanoparticles have fascinated much consideration in modern time because of cost effective and environment friendly synthesis. The green synthesis of silver nanoparticles using aqueous extract of Njavara (*Plectranthus amboinicus*), Neem (*Azadirachta indica*), Moringa (*Moringa oleifera*) as capping and reducing agent by microwave irradiation from 3mM AgNO₃ solution has been done. On mixing leaf extract to silver salt solution in ratio 1:4 the colour changed from colourless to yellowish brown which partially confirmed the degradation of silver ions to silver nanoparticles. In present study the effect of microwave irradiation, interaction time on the morphology and size of silver nanoparticles is investigated. The synthesised nanoparticles were characterised by using IR, UV-visible spectroscopy and transmission electron microscopy (TEM). Nanoparticles range from 10-20nm in size with nearly spherical shape were produced. These silver nanoparticles have proven to be stable for more than 3 months.

KEYWORDS: Green synthesis, silver nanoparticles, microwave irradiation, *Plectranthus amboinicus*, *Azadirachta indica*, *Moringa oleifera*, TEM, XRD.

INTRODUCTION

Silver nanoparticles (SNPs) have received great attention due to their applications in wide areas such as biotechnology, packaging, electronics, medicine and coatings. Their strong antibacterial properties as well as low toxicity towards human cells has led to their applications in various areas such as wound dressing, medicinal imaging, protective clothing, therapeutic compounds, antibacterial surfaces, water treatment, preservation of food and cosmetics to kill living bacterial and disinfecting agents.^[1] The biomedical efficacy of silver nanoparticles (SNPs) depends only on the sensitivity of pathogen and size of nanoparticles. Smaller the size of the nanoparticle, greater will be the surface area and the better will be the antibacterial efficacy.^[2,3] Silver nanoparticles can be used against many infectious organisms such as *Escherichia coli*, *Bacillus subtilis*, *vibria cholera*, *Pseudomonas aeruginosa*, *Syphilis typhus* and *Staphylococcus aureus*.^[4]

There are various physical, chemical and biological methods to synthesize metal nanoparticles.^[5] But "Green chemistry methods have successively used for sustainable production and development of nanoparticles because these methods are eco-friendly, economical, can easily be utilized for mass-scale synthesis. Moreover, these methods do not require high temperature, high pressure, high energy and toxic chemicals unlike chemical methods. Also, the results obtained are

relatively reproducible.^[6] Plants, algae, fungi, bacteria and viruses have been utilized for the bio production of NPs in the last few years.^[7-15] Since the maintenance of cell culture is not easy, production of nanoparticles from microbes has been replaced by plant extract because plant extract acts as both reducing and capping agents during the synthesis. They reduce metal ions faster than fungi or bacteria and there is a no need to maintain a cell culture.^[16] Almost all parts of plants composed of antioxidants or sugars which are water soluble, and therefore can be utilized for NPs synthesis.^[17] The rate of production of nanoparticles their yield and various other characteristics are controlled by the nature of plant, concentration of metal salt as well as plant extract, pH, temperature and contact time.^[18] The need for an environmentally sustainable synthesis process has led to the development of some biomimetic approaches. Recently a number of inorganic nanomaterials have been synthesised within cells of lactic acid bacteria.^[19] But the plant mediated silver nanoparticle is a relatively newer concept. In this race of AgNP preparation utilizing plants/parts of plants could prove advantageous over other biological processes.

This work reports successful synthesis of silver nanoparticles through a green route where the reducing and capping agent selected was the latex obtained from *Plectranthus amboinicus*, *Azadirachta indica* and *Moringa oleifera*. Here we have developed a rapid eco-

friendly and convenient green method for the synthesis of AgNPs from silver nitrate using leaf extracts of Indian medicinal plant *Plectranthus amboinicus*, *Azadirachta indica* and *Moringa oleifera* by microwave irradiation method.

MATERIALS AND METHOD

Materials

Silver nitrate was procured from Merck, India and used as received. All reagents were of analytical grade. Domestic microwave was purchased from Electrolux, India. Fresh leaves of *Plectranthus amboinicus*, *Azadirachta indica* and *Moringa oleifera* were collected from home and University College campus, Trivandrum, Kerala, India.

Preparation of Leaf extracts

The fresh leaves were collected and washed several times. Twenty five grams of the collected leaves were grinded in mortar and pestle and added to 100 ml deionized water. It was heated on a water bath for 1 hr and then cooled to room temperature. The resulted mixture was filtered through Whatmann No.1 filter paper. The resulted extract was stored in refrigerator for further use.

Biosynthesis of silver nanoparticles

Silver nitrate solution was prepared as follows: 1mM of AgNO₃ was prepared. 15 ml of leaf extract was added to 85ml of 1mM solution of AgNO₃. The mixture was microwaved for 80 seconds in an Electrolux domestic Microwave Oven 1200 W, 230-240 volts. The change of colour from colourless to yellowish brown solution indicated the formation of silver nanoparticles.^[20]

Characterization of silver nanoparticles

The synthesised silver nanoparticles were characterized by UV-Visible spectra recorded on a Perkin Elmer Lambda 25 UV-Vis spectrophotometer in range 200 to 600nm using a quartz cuvette. The powder X-ray diffraction studies were done on Philips X-ray diffractometer (PW1710) using Cu K α ($\lambda=1.5405\text{\AA}$) radiation. The morphology and size of the synthesised nanoparticles were determined by transmission electron microscopy (TEM). The TEM was operated at an accelerating voltage of 2000kv, which can produce magnification detail upto 1000,000 x with resolution better than 10 \AA . The image can be resolved over fluorescent screen or a photographic film. Furthermore the analysis of the x-ray produced by the interaction between the accelerated electrons with the sample allows determining the elemental composition of the sample with high spatial resolution. It was then imaged using Philip CM200kV. FTIR measurements of silver nanoparticles were made with Perkin Elmer, Spectrum 2400-4000 IR spectrometer.

RESULT AND DISCUSSIONS

Silver nanoparticle formation by reduction of silver nitrate during exposure to different leaf extracts can be easily monitored from the colour change of the reaction mixture. When silver atoms aggregates into particles of nano size, they begin to absorb visible radiations due to the excitation of surface plasmon vibration (SPR). This imparts various colours to nanoparticles. As the nanoparticles size changes, colour of the solution also changes. So UV-Vis absorption spectrum is quite sensitive to the formation of silver nanoparticles and is used as a potential tool in characterising nanoparticles.

The change in colour of the reaction mixture was obtained after reluxing and on cooling within 2 to 3 minutes, from colourless to yellowish brown, which indicated the formation of AgNP. This formation indicates that silver ions in reaction medium have been converted to elemental silver having the size of nanometric range. Here, the silver nanoparticles formation was initially confirmed using surface plasmon resonance (SPR) phenomenon. For silver nanoparticles, λ_{max} values were reported in the visible range of 300-500 nm

Here synthesised nanoparticles from silver nitrate solution using three different leaf extracts have been subjected to UV-Vis study. The reduction of silver ions to silver nanoparticles is reflected in their electronic spectral data. The spectra recorded for the sample exhibited an absorption band in the range of 300-500 nm, a typical plasmon resonance band of silver nanoparticles. The nanoparticles synthesised respectively from Njavara, Neem and Moringa showed maximum absorption at 300 nm, 380 nm and 330 nm respectively. The variation in absorption maximum depends on their size in the three samples. A representative UV-Vis spectra is shown in Fig.1. The appearance of broad band can be due to the presence of components in leaf extract that are also being read in spectrophotometric range.^[21]

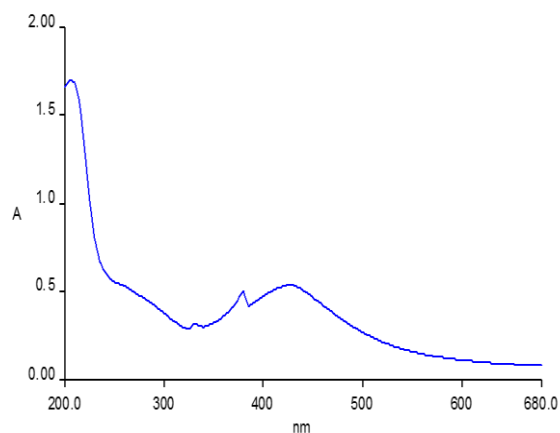


Fig. 1: UV-Vis spectrum of prepared silver nanoparticles.



Fig 2: Synthesised silver nanoparticles indicated by yellow and yellowish brown colour from *Plectranthus amboinicus*, *Azadirachta indica* and *Moringa oleifera*.

The silver nanoparticle synthesised from different leaf extracts have been subjected to TEM analysis. TEM studies were carried out to find out exact particle size and morphology of nanoparticles. It illustrates the aggregation of nanoparticles and particle are

predominantly spherical in shape and are almost uniform in size. The size of the particle ranged from 15-20 nm and around 80% of the particle size is to be less than 20nm. The TEM images of silver nanoparticles from different leaf extracts are shown in Fig. 3.

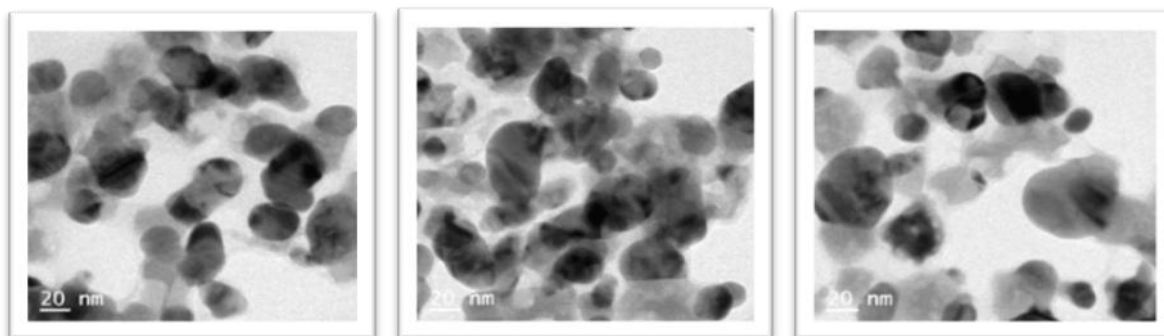


Fig. 3: TEM Images of the synthesised silver nanoparticle from *Plectranthus amboinicus*, *Azadirachta indica* and *Moringa oleifera*

The average size, crystalline nature of particles and quality of nanoparticles were determined by XRD analysis.^[22] There exists strong diffraction peaks at 38.04°, 44.13°, 64.38° and 77.35° corresponding to planes (111), (200), (220) and (311) of crystalline silver nanoparticles. The obtained data matched with the Joint Committee on Powder Diffraction Standards (JCPDS) File No. (04-0783). The size of the synthesized iron oxide nanoparticles using the Debye Scherrer equation, $D = k\lambda / \beta \cos\theta$ by determining the width of the Bragg's reflection, where k is the Scherrer constant (0.89), λ is the wavelength of X ray, β is the half width of the peak and θ is the Bragg angle. The average particle size is calculated from the high intensity peak at 38.04°. The average size was estimated to be 16 nm. A representative XRD pattern of silver nanoparticles is shown in Fig.4.

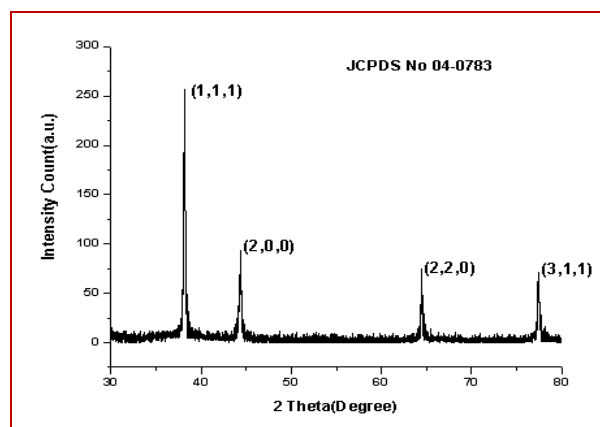


Fig. 4: XRD pattern of silver nanoparticles.

The crystallinity of the particles was evaluated through a comparison of crystallite size from XRD and TEM particle size determination by the following equation.

$$I_{cry} = \frac{D_p(TEM,SEM)}{D_{cry}(XRD)} \quad (I_{cry} \geq 1)$$

Where, I_{cry} is the crystallinity index D_p is the particle size (obtained from either TEM or SEM morphological analysis) D_{cry} is the particle size (calculated from equation). The biosynthesized silver nanoparticle has I_{cry} 1.25, indicating highly crystallite size, monocrystalline units and FCC phase structure.^[23]

The FTIR was done to determine the functional groups responsible for reduction and molecular interactions. The dual role of plant extracts as bioreductant and capping agent was confirmed from IR studies of silver nanoparticles. The high intensity peak at 3442 cm^{-1} corresponds to $-\text{OH}$ stretching vibrations of polyphenolic groups. The peak at 1391 cm^{-1} can be attributed to $-\text{NH}$ stretching vibration and at 1641 cm^{-1} can be of $-\text{C}=\text{C}$ - aromatic stretching.^[24] Analysis of plant spectra suggests the presence of flavonoids and polyphenols, apart from other phytochemicals which are mainly responsible for formation of silver nanoparticles.^[25] A representative IR spectra is given in Fig.5.

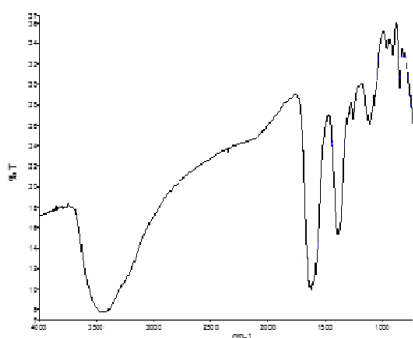


Fig. 5: IR Spectra of plant extract silver nanoparticles.

CONCLUSION

The major conclusion drawn from the above study were the following.

1. Formation of silver nanoparticles by reduction of silver nitrate during exposure to leaf extracts can be easily monitored from the change in colour of the reaction mixture. Silver nanoparticles bear a characteristic by brown colour due to the excitation of surface plasmon vibrations.
2. In a short interaction time of few minutes, highly monodisperse AgNPs are synthesised in a range 15-20 nm-size with nearly spherical shape was produced using microwave irradiation. The particles have shown to remain stable for over 3 months.
3. As observed in UV-Visible analysis, bio-organic components from the extracts acted as probable stabilizer for the silver nanoparticles.
4. The future scope of the work is on the mechanistic aspects of the process in characterising the reducing and

stabilizing contents. Since fine tuning of biological process parameters give quality products.

Conflict of interest

Conflict of interest declared none.

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