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# GREEN SYNTHESIS, CHARACTERIZATION OF SILVER NANOPARTICLES USING METHANOLIC EXTRACTS OF *GALINSOGA PARVIFLORA* LEAVES

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## ABSTRACT

Drugs from plant source are safe, effective with no adverse effects.Plants are the better candidates for nanoparticle synthesis because they are free from toxic chemicals and provide natural capping agents. The nanoparticles produced from plant parts are more stable and the rate of synthesis is rapid. Leaf extracts of *Galinsoga parviflora* has pharmacological properties such as antibacterial, antifungal, antioxidant, anti-inflammatory and antitumor, and also used in the treatment of eczema and lichens. The characterization of biosynthesized GPAgNPs was carried out by using analytical parameters such as UV-Visible spectral analysis, SEM with EDS imaging, FTIR analysis, and Zeta potential Particle size analyzer. The average sizes of nanoparticles were found to be 55nm by SEM and the obtained nanoparticles are of good stability.

**KEYWORDS:** *Galinsoga parviflora*, Methanolic extract, silver nanoparticles.

## INTRODUCTION

Medicinal plants have been discovered since ancient times and used in traditional medicinal practices. Plants synthesize many phytochemicals which have potent biological activity. The chemical compounds also include functions like defense against disease, insects and fungi. However, since a single plant includes a wide variety of phytochemicals, the results of the medicinal applications of an entire plant remain unknown. Furthermore, extensive scientific analysis is carried out to establish efficacy and protection.[1] Medicinal plants are primarily used by non-industrialized cultures, because they are easily available and cheaper than modern drugs.[2] In every part of the country traditional usage of herbal drugs exists and some of the major areas are Indian, Chinese, and European medicinal systems. These medicinal systems have some resemblance in their philosophies but widely they differ from allopathic medicinal systems. In view of advancement of allopathic medicine rather than synthetic drugs herbal drugs have to fulfill international standards on safety, efficacy and quality. Special advantage of herbal drugs is they are easily available in the geographic area of particular traditional medicine.[3] Every country has its own traditional systems, which includes ancient civilization of India, Egypt, and China. Thus, the Indian medicinal system ayurveda came into existence usually plant sources in the form of crude drug such as extracts in their dried herbal powder form or their extracts or mixtures were used in ayurvedic medicinal systems. Usually, Plants are the raw materials source in ayurveda.[4] Plants are the better candidates for nanoparticle synthesis because they are free from toxic chemicals and provide natural capping agents. Usage of plant extracts is cost effective than micro-organisms isolation and culture media improving the cost-effective viability over nanoparticles synthesis by microorganisms.[5]

## NANOPARTICLES

Nanoparticles are usually defined as particles with any shape that ranges between 1-100 nm (nano meters) in diameter and $10^{-9}$ to $10^{-7}$ m of dimension.[6,7] Nanoparticles exhibit unique property of larger surface area to volume ratio that helps in better drug delivery as compared to bulk drugs. Preparation of nanoparticles by physical and chemical methods need more energy and requires toxic chemicals for synthesis. The biological method of synthesis involves the use of plants, algae, fungus and microorganisms. For synthesis of nanoparticles plants are reported to be the better candidates. The nanoparticles produced from plant parts are more stable and the rate of synthesis is rapid. In present scenarios researchers are actively involved in the green synthesis of nanoparticles using noble metals such as zinc, silver, gold,

platinum, and palladium because of their wide applications in medical and pharmaceutical products.[8] Silver nanoparticles have drawn the attention of Researchers in the last two decades thanks to their extensive applications related fields. The surface resonance of the plasmon and the large efficacy scattering cross section of individual silver nanoparticles make. They are ideal candidates for molecular marking where phenomena occur Such as Raman Scattering enhanced surface (SERS) can be used.[9]

Green synthesis of nanoparticles using plant material is most effective method, and they are worked to boundless extent due to its wide distribution, easy availability, safe handling, and compatibility for its pharmaceutical and biomedical application. They are non-toxic as toxic chemicals are not used in synthesis.[10]

Nanoparticle biosynthesis are inspired because they are advantageous over physical and chemical methods as it has simplified step, environmentally friendly, economical and to prepare nanoparticles by biological method there is no requirement of high energy or pressure, temperature and hazardous chemical.[11,12,13]

It is well known fact that silver-based substances were used in numerous of antimicrobial applications. This character of silver makes silver as an outstanding choice to perform numerous of roles in medicinal field. Silver is generally used in nitrate form to produce anti-microbial effect, but when nanoparticles are synthesized there will be huge increase in the surface area for the microorganism to be exposed.[14]

*Galinsoga* genus belongs to Asteraceae family. It is located in Central and South America, Europe, the West Indies, Mexico, Australia, Africa and Asia.[15] The physicist Mariano Martinez de Galinsoga and Spanish botanist gave the plant name as Galinsoga parviflora.[16] Parviflora (parvo = small, and Flor = flower), is derived from a Latin word which gives its meaning as small flower, it is a reference for its small size of flowers.[17] Crude extracts and pure substances derived from the herb have Potent pharmacological properties such as antibacterial, antifungal, antioxidant, anti-inflammatory and antitumor. In ancient medicinal preparations Galinsoga parviflora is used to treat dermatological problems such as eczema and lichens generally, aerial parts are used for its anti-inflammatory drug preparations.[18] Due to high levels of vitamin c this plant has been used to treat scurvy.[19]

**Fig. 1: *Galinsoga parviflora* whole plant.**

**Fig. 2: *Galinsoga parviflora* leaf.**

## MATERIALS AND METHODS

### Collection of plant material

The leaves of *Galinsoga parviflora* used for the present studies was collected from Hassan district of Karnataka. The plant was identified, confirmed and authenticated by Botanist Dr.S.P. Purushotham, Associate professor, PG department of botany. The leaves were shade dried. The dried material was then pulverized separately into coarse powder by a mechanical grinder. The resulting powder was then used for extraction.

### Preparation of Methanolic Extract

*Galinsoga parviflora's* coarsely powdered leaves were extracted with 500ml of methanol to exhaustion in a 50$^{o}$C Soxhlet apparatus. The extract was concentrated and dried using Rotary flash evaporator. It was kept in desiccator until used.

### Preparation of stock solution 0.2g/ml

1g of the methanolic extract was weighed and diluted to 5mL with methanol.

**Fig. 3: Stock solution 0.2g/ml.**

**Fig. 4: Aqueous solution of 1mM $AgNo_3$.**

**Preparation of 1mM silver nitrate aqueous solution ($AgNO_3$)**

An accurately weighed 0.017g of silver nitrate was dissolved with 100mL of double distilled water and stored in amber colour bottle until further use.

**Preparation of silver nanoparticles**

5 ml of methanol leaf extract (0.2g/ml) was added drop wise to 95ml of 1mM aqueous silver nitrate solution was placed separately on magnetic stirrer with constant stirring 120 rpm and 30-95$^o$c (hot plate). The blend was gradually heated at varying temperature (30, 45, 60, 90$^o$c). The colour change was checked periodically. The colour change of the medium from colourless to brown after 5h was observed which indicated the formation of silver nanoparticles. It showed that aqueous silver ions could be reduced by the methanolic extract of *Galinsoga paarviflora* generate extremely stable silver nanoparticles.

To make certain of better separation of the particles, the hydrosol was centrifuged at 10,000 rpm for 20 min at 4$^o$C thrice and the final pellet was collected and stored at 4$^o$C for further analysis.

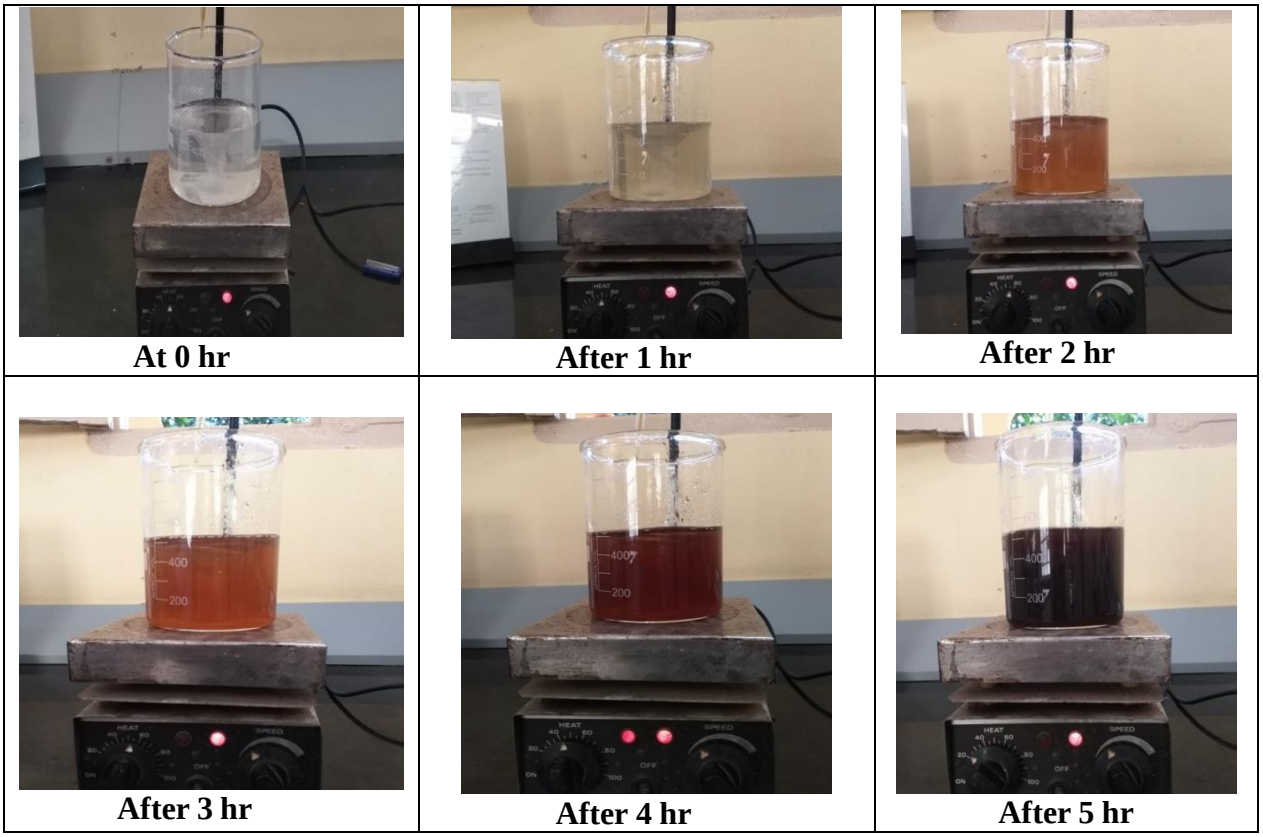


**Fig. 5: Showing the colour change of the medium from colourless to brown colour after 5hrs.**

**Synthesized GPAgNPs**

**Fig. 6: Synthesis of silver nanoparticles of Methanolic Leaf extract of *Galinsoga parviflora* (GPAgNPs).**

There was a visible color change after the substrate was added to the plant extract. Initially the plant extract was colourless. Upon adding the silver salt, it turned brown. Increased concentrations of silver nitrate resulted in a brown solution of nano silver indicating the completion of reaction. Reduction of silver ions into silver nanoparticles using methanolic leaf extract of *Galinsoga parviflora* was evidenced by visual change of colour from colourless to brown colour which indicated the formation of silver nanoparticles.

**Characterization of synthesized GPAgNPs**

The characterization of synthesized GPAgNPs was carried out by using the following analytical parameters

UV-Visible spectal analysis

SEM with EDS imaging

FTIR analysis

Zeta potential

Particle size analyzer

**UV-Visible spectroscopy**

The formation and completion of silver nanoparticles was characterized by UV-Visible spectroscopy by using Shimadzu UV- Visible spectrophotometer, Model 1800. The bio-reduction of the $Ag^{+}$ ions in solution was monitored by periodical sampling of aliquots and the UV-Visible spectra of these aliquots were monitored as a function of time of reaction in 200-600nm range operated at a resolution of 1nm. Distilled water was used as a blank.[20]

**Morphological studies of synthesized GPAgNPs by using Scanning Electron Microscopy (SEM) with EDS**

Morphological evaluation of the GPAgNPs was carried out by using scanning electron microscope (SEM) XL 30 ESEM with EDAX: Resolution up to 2; Acc. Voltage:30kv; Magnification: up to 2,50,000x. SEM gave high-resolution images on the surface of the sample. EDS imaging was conducted with same instrument to confirm the elemental composition of the sample.

**FTIR analysis**

To identify the biomolecules, present with in GPAgNPs after synthesis of silver nanoparticles. FTIR spectra GPAgNPs were analyzed by FTIR spectroscopy ((FTIR Shimadzu 8400S, Japan). The FTIR analysis was performed with KBR pellets. The FTIR was recorded in the range of 400–4000 $cm^{-1}$. The various modes of vibrations were identified and assigned to determine the different functional groups present in the GPAgNPs.

**Determination of Particle size and Zeta potential**

The mean particle size (z-average), polydispersity index (PI) and zeta potential of GPAgNPs were determined by dynamic light scattering technique using a zeta size analyzer (Nano ZS 90, Malvern Instruments Ltd., UK).

**RESULTS AND DISCUSSION**

**UV-Visible Spectroscopy**

The UV-Vis Spectroscopy was the preliminary technique for the characterization of the silver nanoparticles. When silver nitrate was mixed with the leaf extract, color change to brown was visually observed among all the hydrosols at 30˚C, 60˚C, 90˚C, 95˚C with the hydrosol at 90˚C indicating an eminent color change following the dilution of a small aliquot of the sample in distilled water. The UV-Vis spectral analysis was conducted using Shimadzu UV-Vis spectrophotometer, Model 1800 range between 200 and 800 nm. The UV–vis spectrum showed well observable peaks at 60˚C (423 nm), 90˚C (428 nm) and 95˚C (425nm). The reduction of silver ions in the aqueous solution of nanoparticles in the solution could be correlated with the respective UV-Vis Spectra of the colloidal solution which exhibited a strong absorption at 420nm shown in Fig. The absorption peaks around 390–420 nm can be attributed to AgNPs in size range of 25–50 nm.[21]

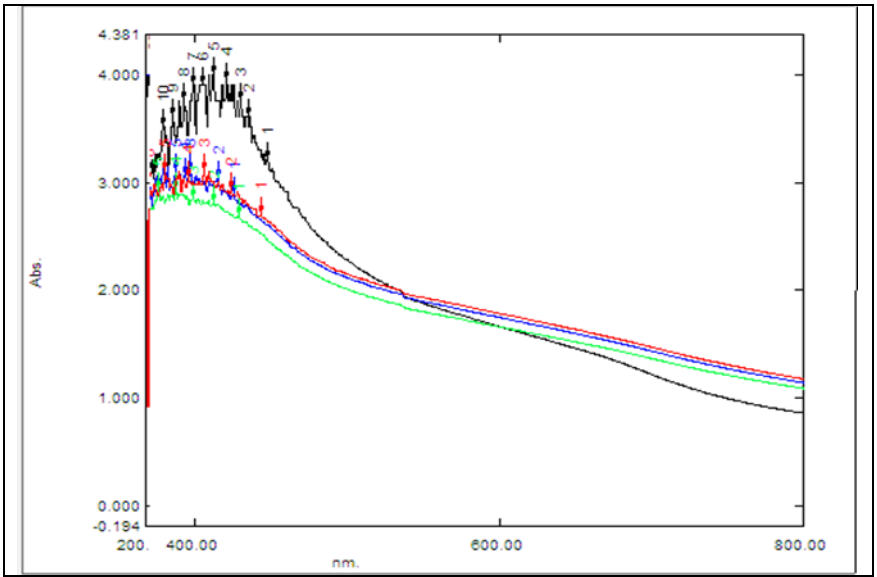


**Fig.7: UV-Vis spectra of GPAgNPs.**

**Morphological studies of silver nanoparticles by using Scanning Electron Microscopy (SEM) with EDS imaging**

Morphological studies of silver nanoparticles by using Scanning Electron Microscopy (SEM). A SEM employed to analyze the morphology and size details of the silver nanoparticles that were formed. From (Fig) it was showed that the silver nanoparticles formed were spherical in shape, with an average size of around 55nm and uniformly distributed silver nanoparticles on the surface was observed.

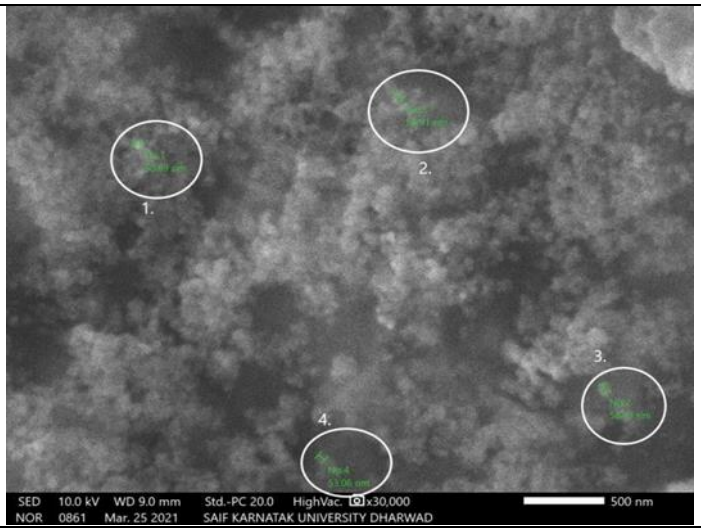


**Fig.8: SEM images of GPAgNPs with nanosize [1] 50.89nm, [2] 56.91nm, [3]58.43nm, [4]53.06nm.**

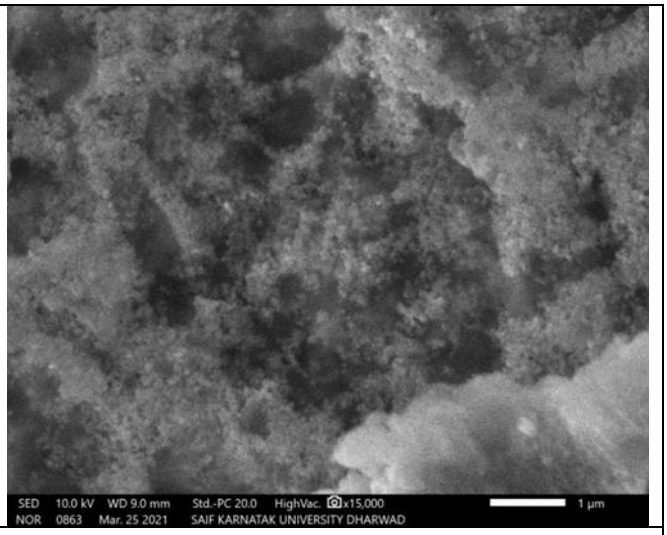


**Fig. 9: SEM images of GPAgNPs.**

The elemental constituents and relative abundance of the biosynthesized GPAgNPs were obtained from Energy Dispersive X-ray spectroscopy (EDS) (Fig). The EDS spectrum reveals the purity and the complete chemical composition of GPAgNPs. The percentage of Ag metal

found in occurrence with other chemical elements was found to be appreciable. The reduced silver nanoparticles were subjected to EDS analysis with an optical absorption characteristic peak at 3 keV. The EDS analysis showed percentage relative composition of elements such as Oxygen (O) 23%, Carbon (C) 29%, Aluminum (Al) 0.42%, Phosphorus (P) 0.63%, Calcium (Ca) 0.74% and Silver (Ag) 40.98%. The other elements served as capping organic agents bound to the surface of the silver nanoparticles.[22]

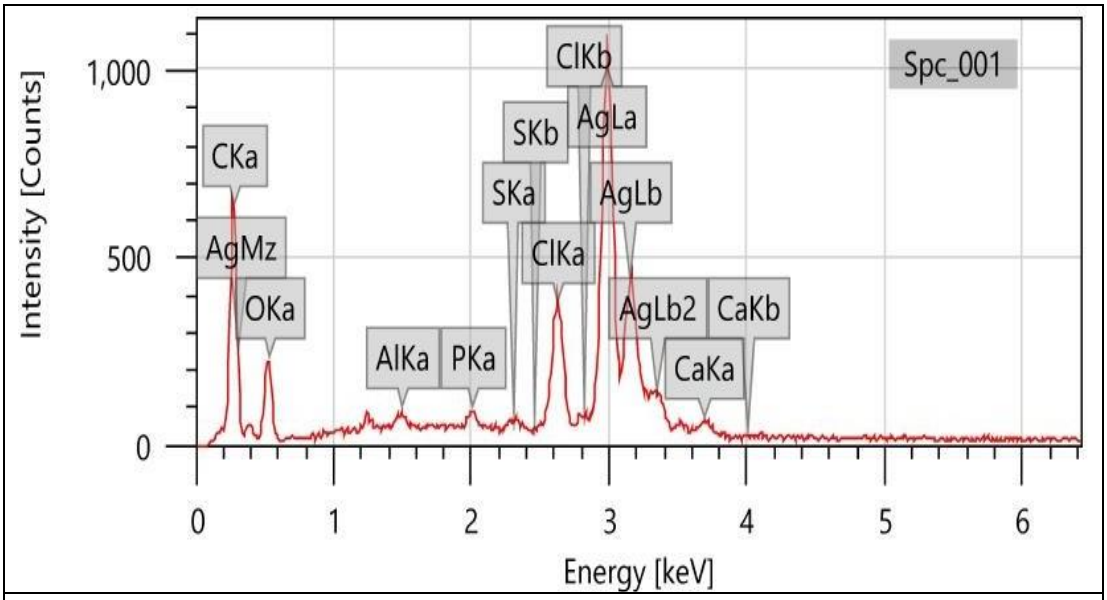


**Fig.10: EDS SPECTRUM OF GPAgNp's.**

**FTIR**

The IR spectra provided information about organic molecules and their environment on the surface of nanoparticle. In the preset work, FTIR spectral measurements were carried out to identify the biomolecules in *Galinsoga parviflora* leaf extract which is responsible as reducing and capping agents for bio reduced silver nanoparticles. FTIR measurements were carried out to identify the possible biomolecules responsible for capping and stabilization of silver metal nanoparticles synthesized by *Galinsoga parviflora* leaf extract. The results of FTIR analysis of this study (Fig) showed different stretches of bonds shown at different peaks: 635.81cm$^{-1}$ – C-Br stretch-, 806.77 cm$^{-1}$ -C-Cl stretch - alkyl halides, 1087.43 cm$^{-1}$, 1138.72 cm$^{-1}$ -C-N stetch-aliphatic amines, 1186.15 cm$^{-1}$ represents-C-H alkyl halides, 1521.49 cm$^{-1}$ N-O asymmetric stretch-nitro compounds, 2979.38 cm$^{-1}$ represents C-H stretch alkanes, 3291.12 cm$^{-1}$ frequency corresponds to N-H stretch with functional group primary or secondary amines, the FTIR spectra of silver nanoparticles exhibited outstanding peak at 2979.38 cm$^{-1}$,1391.56 cm$^{-1}$.[23]

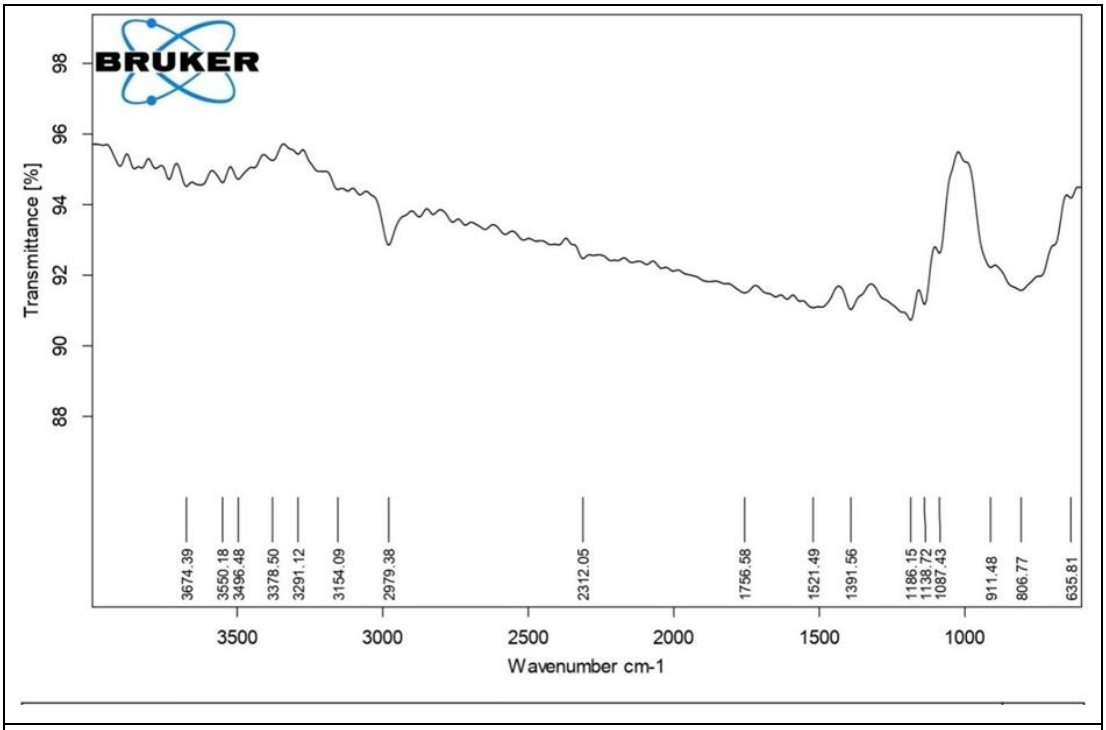


**Fig. 11: FTIR spectra of GPAgNp's.**

**Determination of Particle size and Zeta potential**

Particle size, size distribution and zeta potential were important characterizations of the silver nanoparticles because they govern the other characterizations, such as saturation solubility and dissolution velocity, physical stability, or even biological performances.[24]

**Particle size measurements**

Mean particle size diameter and polydispersity indices were all measured in solutions directly after synthesis, using photon correlation spectroscopy (PCS). The size of the colloidal silver nanoparticles, their granulometric distribution has been recorded, expressed against the particles number and their occupied volume.[25]

The average particle size (z-average) is found to be 271.8 nm. Particle size analysis showed the presence of nanoparticles with polydispersity indices PDI value 0.461. It is presented in the Fig.

| Peak No. | S.P.Area Ratio | Mean | S. D. | Mode |
|---|---|---|---|---|
| 1 | 1.00 | 271.8 nm | 111.5 nm | 231.6 nm |
| 2 | --- | --- nm | --- nm | --- nm |
| 3 | --- | --- nm | --- nm | --- nm |
| Total | 1.00 | 271.8 nm | 111.5 nm | 231.6 nm |

Cumulant Operations

Z-Average : 306.4 nm

PI : 0.461

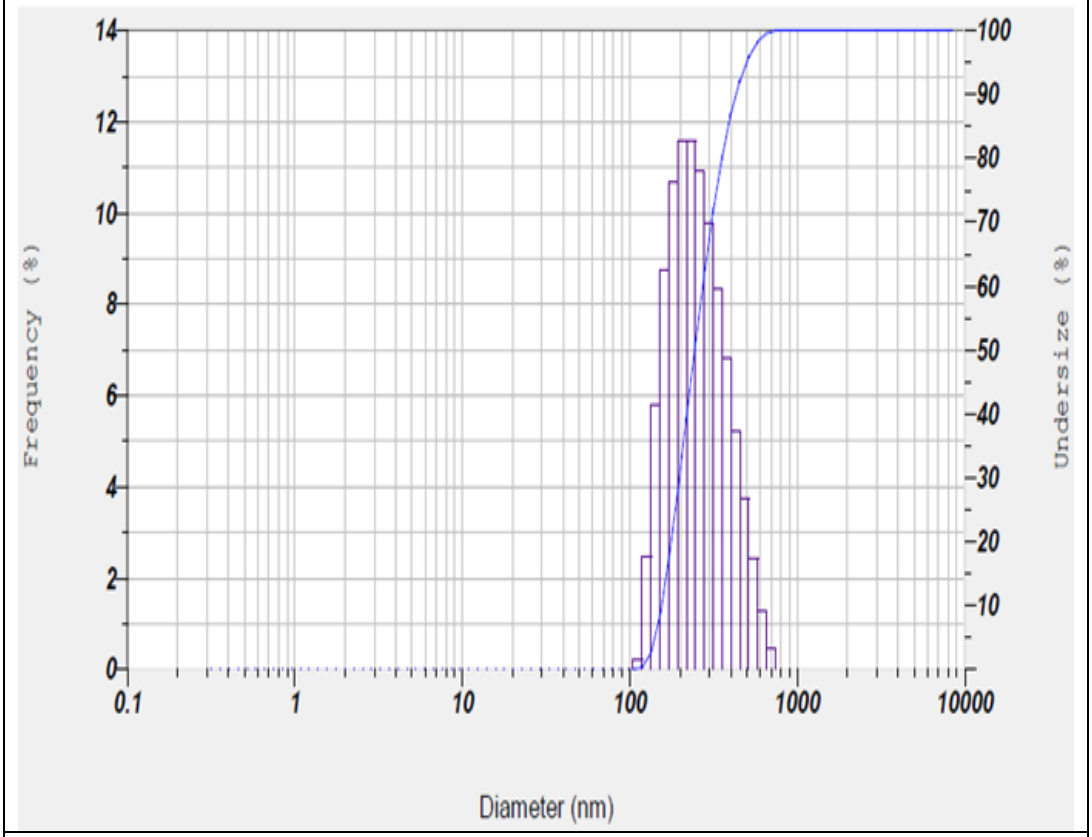


**Fig. 12: Percentage intensity of particle size distribution of GPAgNPs.**

**Zeta Potential measurement**

A zeta potential was used to determine the surface potential of the silver nanoparticles. Zeta potential is an essential characterization of stability in silver nanoparticles. A minimum of +30mV zeta potential is required for the indication of stable silver nanoparticles. For the obtained nanoparticles, zeta values were measured and found to be -33.4mV with a peak area of 100% intensity. These values provide full stabilization of the nanoparticles, which may be the main reason in producing particle sizes with a narrow size distribution index.

| Peak No. | Zeta Potential | Electrophoretic Mobility |
|---|---|---|
| 1 | -33.4 mV | -0.000258 cm2/Vs |
| 2 | --- mV | --- cm2/Vs |
| 3 | --- mV | --- cm2/Vs |

Zeta Potential (Mean) : -33.4 mV

Electrophoretic Mobility Mean : -0.000258 $cm^2/Vs$

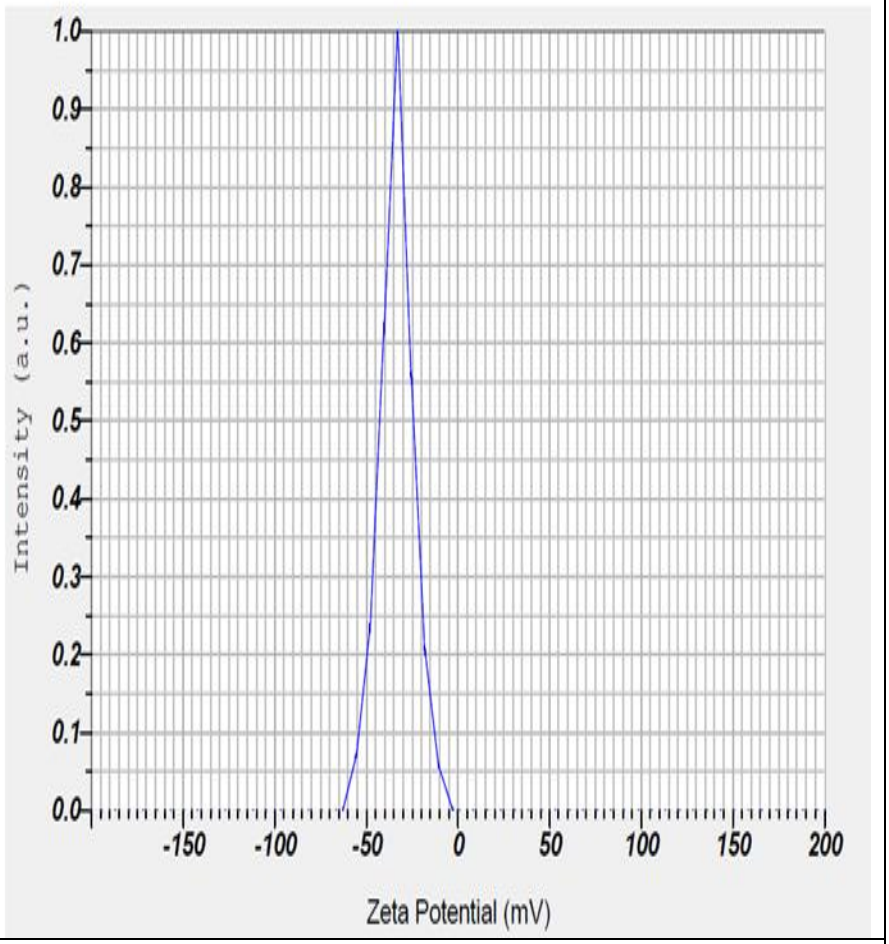


**Fig.13: Zeta potential distribution of GPAgNPs.**

## CONCLUSION

Synthesized silver nanoparticles were characterized by UV, FTIR, SEM, Zeta potential and particle size analyser. The average size of nanoparticles were found to be 55nm by SEM and the obtained nanoparticles are of good stability.

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