Biogenetic Synthesis of Silver Nanoparticles from Extract of Lupinus Arboreus and Its Antimicrobial Properties

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Abstract:

Ag nanoparticles were synthesized successfully by green synthesis methods (From Lupinus arboreus extract), respectively. Using UV-Vis spectroscopy, particle size analysis, scanning electron microscopy (SEM), dynamic light scattering (DLS) particle size, and SEM image analysis, the nanoparticles were thoroughly characterized.

The average particle size was determined to be in the range of less than 1000 nm Ag. Antibacterial potential of Ag nanoparticles as a function of nanoparticles concentration was tested against four different bacteria like Escherichia coli and S. aureus. The test was performed by both Disc diffusion assay and colony forming unit (CFU) estimation method. From the study, both types of nanoparticles were observed to have strong antimicrobial potential.

The growth study of Escherichia coli and S. aureus was carried out in presence of different concentration of both nanoparticles to observe the effect on the growth of the bacteria in liquid media.

It was observed that both the nanoparticles strongly affected the specific growth rate of E.coli and S. aureus. It was also observed that the growth rate was strongly inhibited by the presence of small concentration of nanoparticles. Stability Study of silver nanoparticle formulation (F3) for a period of 3 months at accelerated stability conditions (250C±2 0C and 60 ± 5% RH) and (400C±2 0C and 70 ±5% RH). Physicochemical parameters, including color, order, appearance, and particle size were not altered significantly.

Key words: Ag nanoparticles, *Lupinus arboreus*, Anti-microbial activity.

1. Introduction

"Nanotechnology" is the newest and one of the most promising and active areas of modern research. The technology deals with the design, synthesis, and manipulation of particles size ranging from 1–1000 nm [1]. Many fields, including optics, mechanics, chemical and space industries, electronics, energy science, single-electron transistors, light emitters, nonlinear optical devices, photo-electrochemistry, catalysis, biomedical, cosmetics, drug and gene delivery, food and feed, and photo-electrochemicals, have seen an increase in the use of nanotechnology [2].

Silver nanoparticles (AgNPs) are particularly important because of their diverse and surprising applications in a variety of fields, including medical and pharmaceutical fields, drug delivery, photothermal therapy, sensing, catalysis, energy conversion, solar cells, optoelectronics, and ecological applications [3].

The various types of nanoparticles are used such as metallicnanoparticles, quantum dots, carbon nanotubes, magnetic nanoparticles, fullerenes, liposomes and dendrimers [4].

AgNPs have also been reported in recent research to be used in the detection and treatment of cancer as well as as active or passive medication carriers [5]

2. Material and methods

2.1 Soxhlet extraction

Dried and powered flower of *Lupinus arboreus.* Successively defatted with petroleum ether and then placed in a thimble of Soxhlet apparatus. The extraction was carried out using 30% methanol (hydroalcoholic)

solvent system at 40-60^{O}C temperature of the heating mantle for 8-10 hours. After the extraction process, the extract of sample was filtered and concentrated to dryness. Extracts were collected in air tight container [6]. Extraction yield of all extracts were calculated using the following equationbelow:

Formula of Yield= Actual yield X 100 / Theoritical yield

2.2 Qualitative Phytochemical Estimation ofExtracts

Detailed phytochemical testing was performed to identify presence or absence of different phyto constituents in extracts of *Lupinus arboreus* using standard procedures [7]. The extracts were subjected to following tests:

2.3 Organoleptic Properties

Organoleptic properties were performed by human sensory organs. The organoleptic studies of *Lupinus arboreus* like general appearance like appearance, color, odor, state etc. were observed/ performed.

2.4 Solubility study

Qualitative solubility of *Lupinus arboreus* different solvents was determined according to USP NF, 2007 and Indian pharmacopoeia. Approximately 1 mg of *Lupinus arboreus* was weighed and transferred into a 10 ml test tube; then, it was dissolved in the respective solvents (1 ml each of Pet. Ether, Methanol, Ethanol, Water, and Dimethylsulfoxide).

2.5 Fourier transmission Infra-Red Spectroscopy(FTIR)

FT-IR spectrum of extract was recorded over the range of 4000 to 400 cm−1 by KBr pellet method using a FT-IR spectrophotometer. The KBr disc was prepared using 1 mg of extract and 100 mg of spectroscopic grade KBr which has been dried using IR lamp. Both KBr and drug was mixed and subjected to hydraulic pressure to form disc. This disc was placed in FT- IR chamber. Infrared spectrum was recorded in the 4000 - 400 cm-1 region [8].

2.6 Formulation of Silver nanoparticle Preparation of 1mM $AgNo_3$ solution

For preparation of 1mM $AgNo_3$ solution we have to take 0.016gm $AgNo_3$ and dilute it with 100ml of distilled water with continues stirring.50 ml (1mM) aqueous solution of silver nitrate was prepared in conical flask with continuously stirring for 15 minute. Then five

dilutions of *Lupinus arboreus* extract will be prepared in water (100mg/ml, 75mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml) About 1 ml of each filtrate will be taken into a beaker and 9 ml of 1mM AgNo3 added and continuously stirring for 15 minutes. The solution was kept in dark chamber until solution color changes to dark yellow to brown color. After, 15 min, the solution turns dark yellow to Brown color it indicates the formation of silver nanoparticles. The bio reduction of silver ions was monitored by periodic sampling by the UV visible spectrophotometer.

Table 1: Composition of silver nanoparticle formulation

S. no.	*Lupinus arboreus* (mg/ml) (Each 1 ml)	Silver nitrate solution (ml)	Stirring (time)
1	100	9.0	15
2	75	9.0	15
3	50	9.0	15
4	25	9.0	15
5	12.5	9.0	15

2.7 Characterization of Silver nanoparticle:

2.7.1 Color change

Color change in the preparation of nanoparticle section will be monitored at different interval of 30 min, 60 min, and 120 min.

2.7.2 UV-Visible spectrophotometric analysis

The primary characterization of the synthesized nanoparticles will be performed using UV- visible spectroscopy by measuring the UV-visible spectrum of the reaction mixture at 200– 800 nm wavelength by sampling the aliquots withdrawn from the reaction mixture at different time intervals of 30 min, 60 min, and 120 min (as mentioned above) [9].

Observations:

o Surface Plasmon resonance at 300 to 800 nm will represent nanoparticle synthesis.

o Analysis will help to identify the time of nanoparticle synthesis initiation and progressive increase in intensity of peak will help to ascertain the extent of nanoparticles formed.

2.7.3 Particlesize

The particle size is one of the most important parameter for the characterization of nanoparticle. The size of nanoparticle was measured using Malvern Zeta sizer (Malvern Instruments). The dispersions were diluted with Millipore filtered water to an appropriate scattering intensity at 25°C and sample was placed in disposable sizing cuvette. The size data is documented in Table 11 [10].

2.7.4 Zeta potential

The zeta potential was measured for the determination of the movement velocity of the particles in an electric field and the particle charge. In the present work, the nanoparticle was diluted 10 times with distilled water and analyzed by Zetasizer Malvern instruments. All samples were sonicated for 5-15 minutes before zeta potential measurements. The zeta potential data is documented in Table 12. [11]

2.7.5 Scanning Electron Microscopic(SEM)

The optimized nanoparticle's morphological traits were obtained using a scanning electron microscopes electron beam. A vacuum-sputter coater was then used to coat the nanoparticle with a thin layer (2–20 nm) of gold, palladium, or platinum. Following the preparation, the specimen was exposed to an electron beam,

which caused secondary electrons known as auger electrons to develop. From this interaction between the electron beam and the specimen's atoms, only the electrons scattered at 90° were selected and further processed based on Rutherford and Kramer's Law for acquiring the images of surface topography [12].

2.7.6 Antibacterial activity of Ag nanoparticles by Well diffusion assay

2.7.7 Preparation of Nutrient Agar Media

28 g of Nutrient Media was dissolved in 1 litre of distilled water. pH of media was checked before sterilization. Media was sterilized in autoclave at 121^{o}C at 15 lbs pressure for 15 minutes. Nutrient media was poured into plates and placed in the laminar air flow until the agar was get solidified.

2.7.8 Well Diffusion Assay

The bacterial suspension of *E. coli* and *S. aureus* was standardized to 10^8 CFU/ml of bacteria and kept into the shaker. Then, 100µl of the inoculums from the broth (containing 10^8 CFU/ml) was taken with a micropipette and then transferred to fresh and sterile solidified Agar Media Plate [12].

The agar plate was inoculated by spreading the inoculums with a sterile spreader, over the entire sterile agar surface. Three wells of 6 mm were bored in the inoculated media with the help of sterile cork-borer. The wells were then formed for the inoculation of the AgNo3, AgNPs and extract (1mg/ml) solution. 100 µl of the sample was loaded.

It was allowed to diffuse for about 30 minutes at room temperature and incubated for 18-24 hours at 37^{o} C. Following incubation, plates were checked to see if a clear zone formed around the well, indicating that the chemicals under test had antimicrobial activity. A measurement of the zone of inhibition (ZOI) in millimeters was made.

Zones were measured to a nearest millimeter using a ruler, which was held on the back of the inverted Petri plate. A black, non-reflective background was held a few inches above the Petri plate. The diameters of the zone of complete inhibition, as determined by the unassisted eye, were measured, encompassing the well's diameter [13].

2.8 Stability study

The silver nanoparticle formulation was packed and were placed in the stability test chamber and subjected to stability studies at accelerated testing (25^0 C±2^0 C and 60 ± 5% RH) and (40^0 C±2^0 C and 70 ±5% RH) for 3 months.

RESULTS ANDDISCUSSION

3.1 Plant Collection

Table 2: Plant collection

S. No.	Plant name	Plant part used	Weight
1.	*Lupinus arboreus*	Flower	250

3.2 Percentage yield

Table 3: Percentage yield of extracts

S. No	Plant name	Solvent	Colour of extract	Theoretical weight (gm)	Yield (gm)	% Yield
1.	*Lupinus arboreus*	Pet. Ether	Yellow	250	0.529	0.215
2.		Methanol	Brown	236.65	8.37	3.52

3.2 Qualitative Phytochemical Analysis of different extracts

Table 4: Phytochemical analysis of *Lupinus arboreus* Extract

S. No.	Experiment	Results	
		Petroleum ether	Methanolic
Test for Carbohydrates			
1.	Molisch's Test	-	+
2.	Fehling's Test	-	+
3.	Benedict's Test	-	+
4.	Bareford's Test	-	+
Test for Alkaloids			
1.	Mayer's Test	-	-
2.	Hager's Test	-	-
3.	Wagner's Test	-	-
4.	Dragendroff's Test	-	-
Test for Terpenoids			
1.	Salkowski Test	-	+
2.	Libermann-Burchard's Test	-	+
Test for Flavonoids			
1.	Lead Acetate Test	-	+
2.	Alkaline ReagentT	-	+
3.	Shinoda Test	-	+
Test for Tannins and Phenolic Compounds			
1.	FeCl3 Test	+	+
2.	Lead Acetate Test	+	+
3.	Gelatine Test	+	+

4.	Dilute Iodine Solu Test	+	+
Test for Saponins			
1.	Froth Test	+	+
Test for Protein and Amino acids			
1.	Ninhydrin Test	-	+
2.	Biuret's Test	-	+
3.	Million's Test	-	+
Test for Glycosides			
1.	Legal's Test	-	-
2.	KellerKillani Test	-	-
3.	Borntrager's Test	-	-

3.3 Organoleptic properties

Table 5: Organoleptic properties of *Lupinus arboreus*

S. no.	*Lupinus arboreus*	Study (Observed)
1	Colour	Green to reddish
2	Odour	Offensive
3	Appearance	Dark reddish (Solid)

Discussion

An evaluation of the plant extract organoleptic qualities, including colour, odour, and appearance was conducted. Extract was discovered to have a Green to reddish-colored to it when tested. Plant extract has an Offensive odour and has a solid state according to research conducted on it.

Plant extract exhibited the same Colour, odour, and appearance as the requirement.

3.4 Solubility study

Table 6: Solubility study of *Lupinus arboreus*

Drug	Solvents	Observation/Inference
Lupinus arboreus	Methanol	Freely soluble
	Distilled water	Soluble
	Dimethyl sulfoxide	Freely soluble
	Ethyl acetate	Sparingly soluble

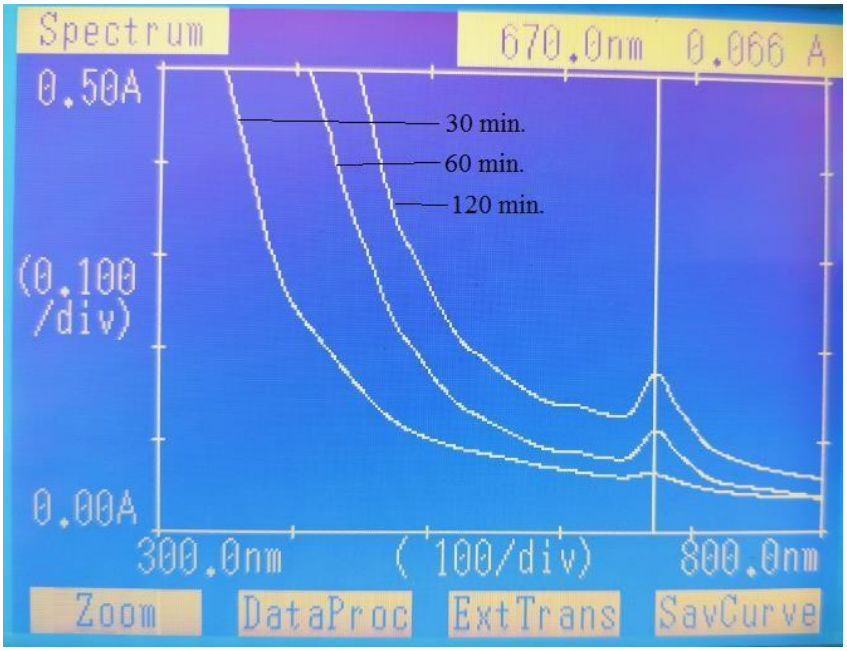
	Acetone	Slightly soluble

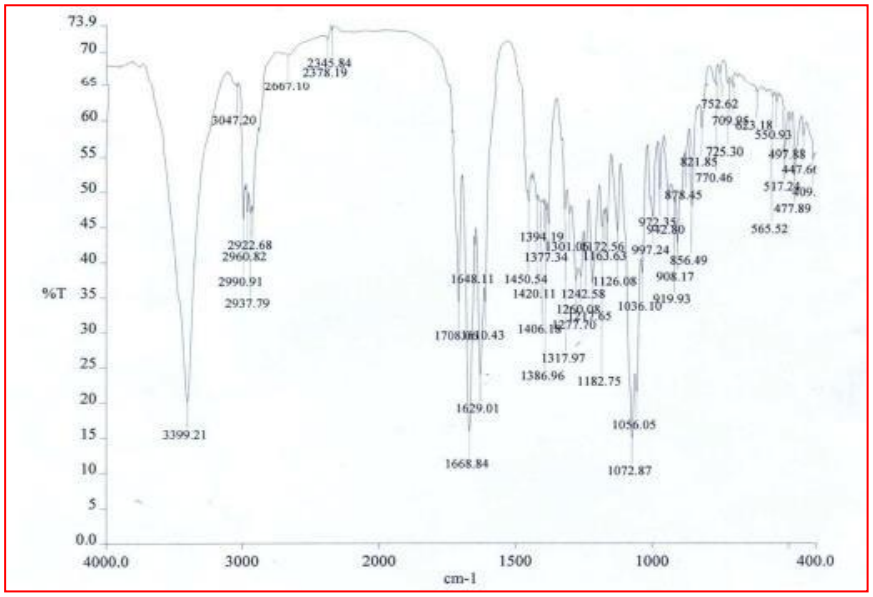
Discussion

The solubility of *Lupinus arboreus* extract was determined in various non-volatile or volatile liquid vehicles such as methanol, Acetone, Dimethyl sulfoxide, Ethyl acetate and water. From the results, it was observed that the drug is freely soluble in methanol and Dimethyl sulfoxide, sparingly soluble in Ethyl acetate and Soluble in water.

3.5 Fourier transmission Infra-RedSpectroscopy

Figure 9: FTIR of extract





3.5.1 UV-Visible spectrophotometricanalysis

Figure 2: UV Peak detection after 30, 60 and 120 min. (F3) Table 7: UV peak detection

S. No	Silver nanoparticle Formulations (After 30 min., 60 min. and 120 min. Show formulation of SNPs	Peak detection
1	SNPs (F3)	670.0 nm

Discussion

The UV- Vis spectra of silver nanoparticle after 30 min., 60 min. and 120 min. of reaction were documented, indicating the formation of silver nanoparticle due to excitation of surface Plasmon vibration in silver nanoparticles.

The synthesized SNPs showed the following absorption spectrum at the wavelength range of 300-800 nm. The surface Plasmon resonance peak at range 300 to 800 nm will confirm the formation of silver nanoparticle as shown in above Figure UV analysis of silver nanoparticle. Surface Plasmon resonance at 670 nm (F3) will represent best nanoparticle synthesis. Analysis will help to identify the time of nanoparticle synthesis initiation and progressive increase in intensity of peak will help to ascertain the extent of nanoparticles formed.

The increase in intensity could be due to increasing number of nanoparticles formed as a result of reduction of silver ions presented in the aqueous solution

3.5.2 Particle size determination

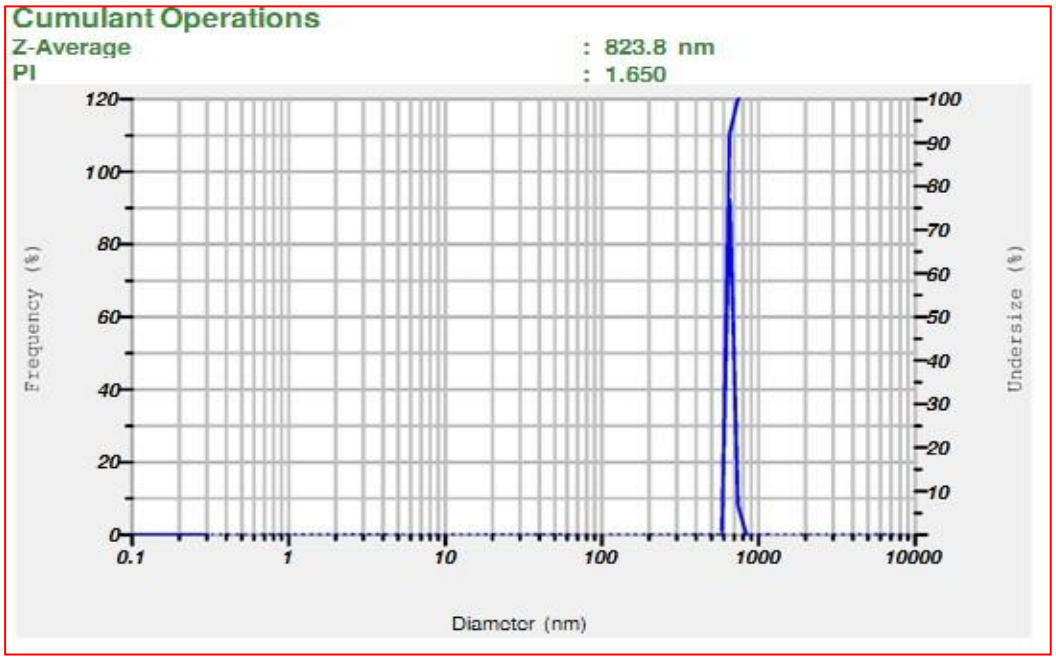


Figure 3: Particle size (F1)

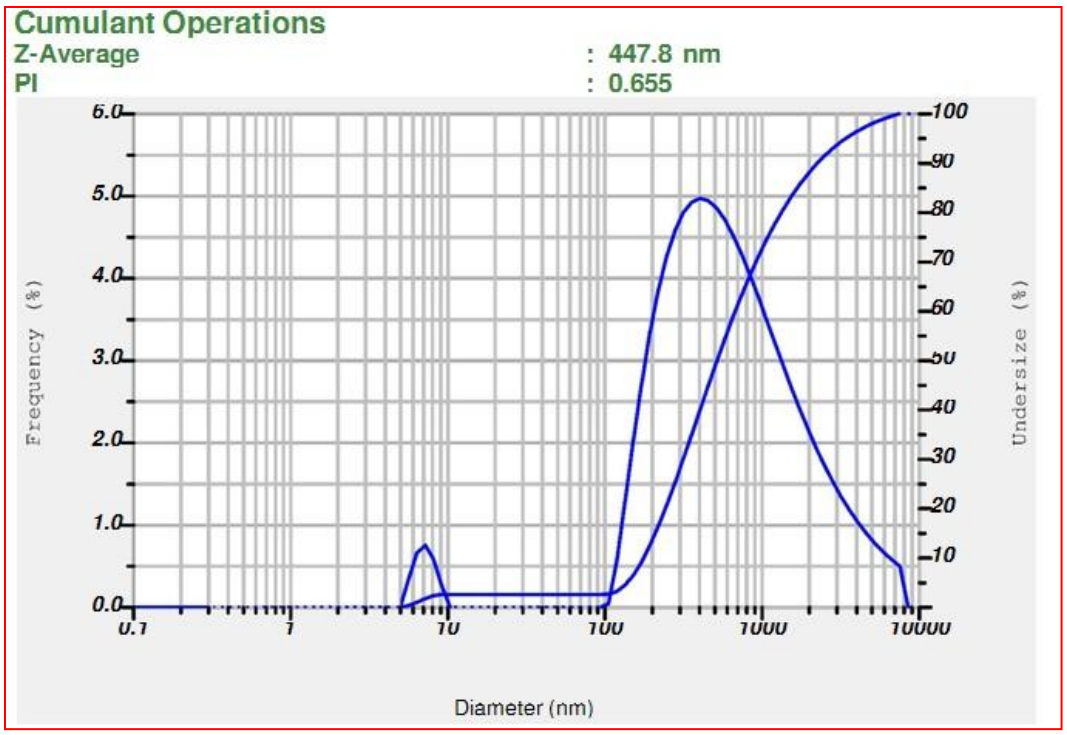


Figure 4: Particle size (F2)

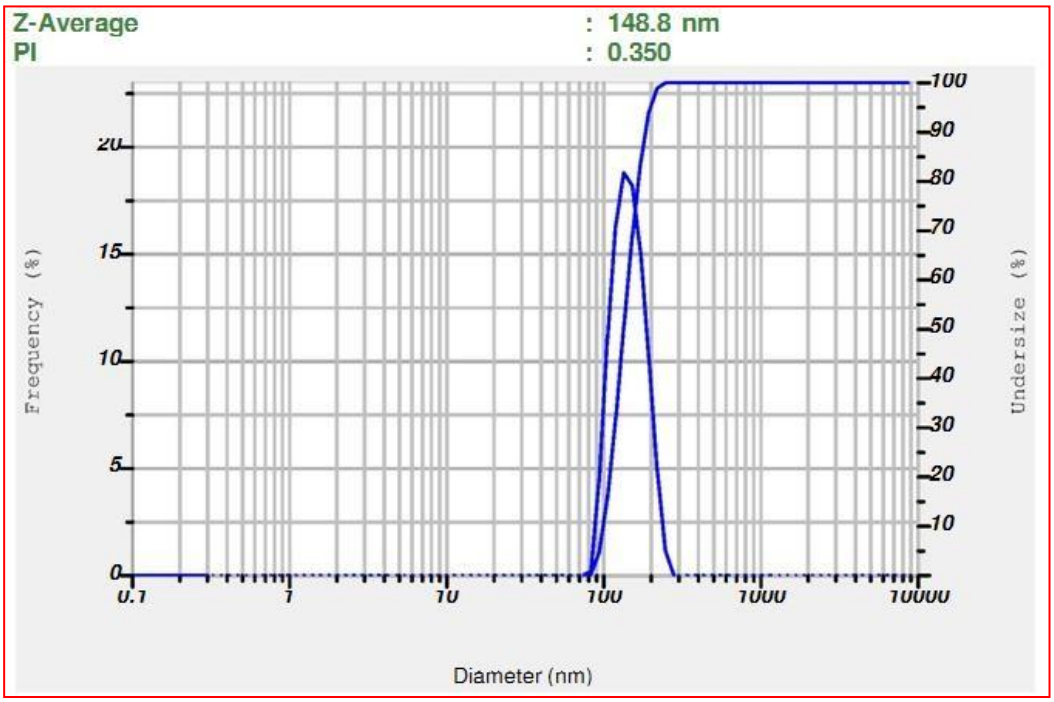


Figure 5: Particle size (F3)

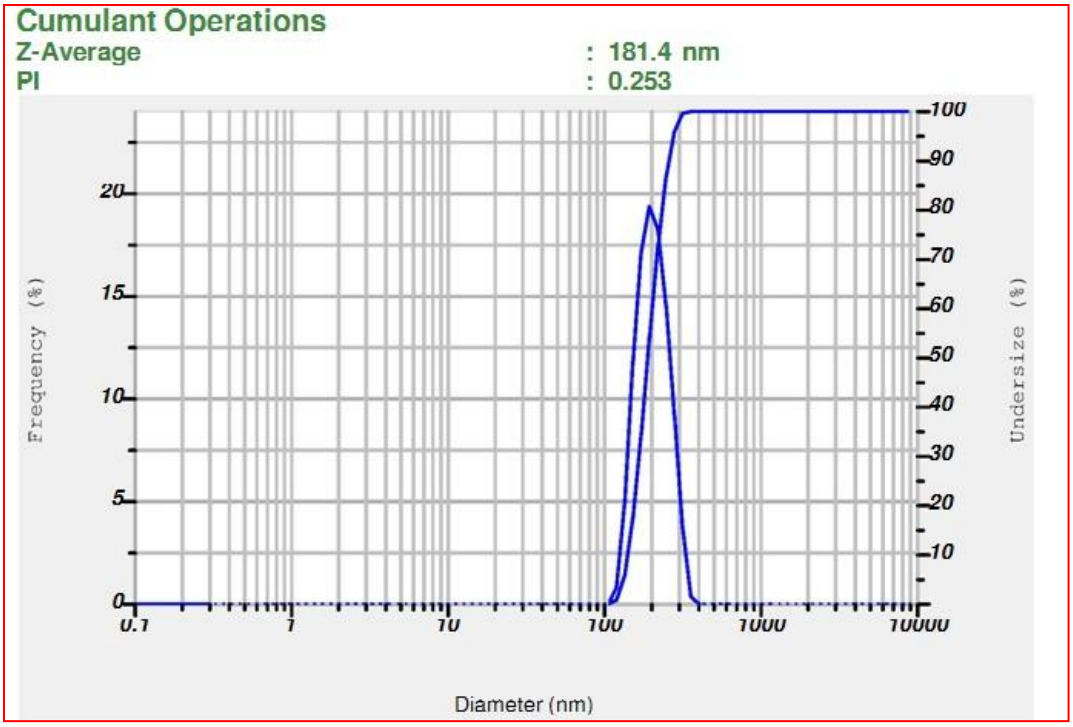


Figure 6: Particle size (F4)

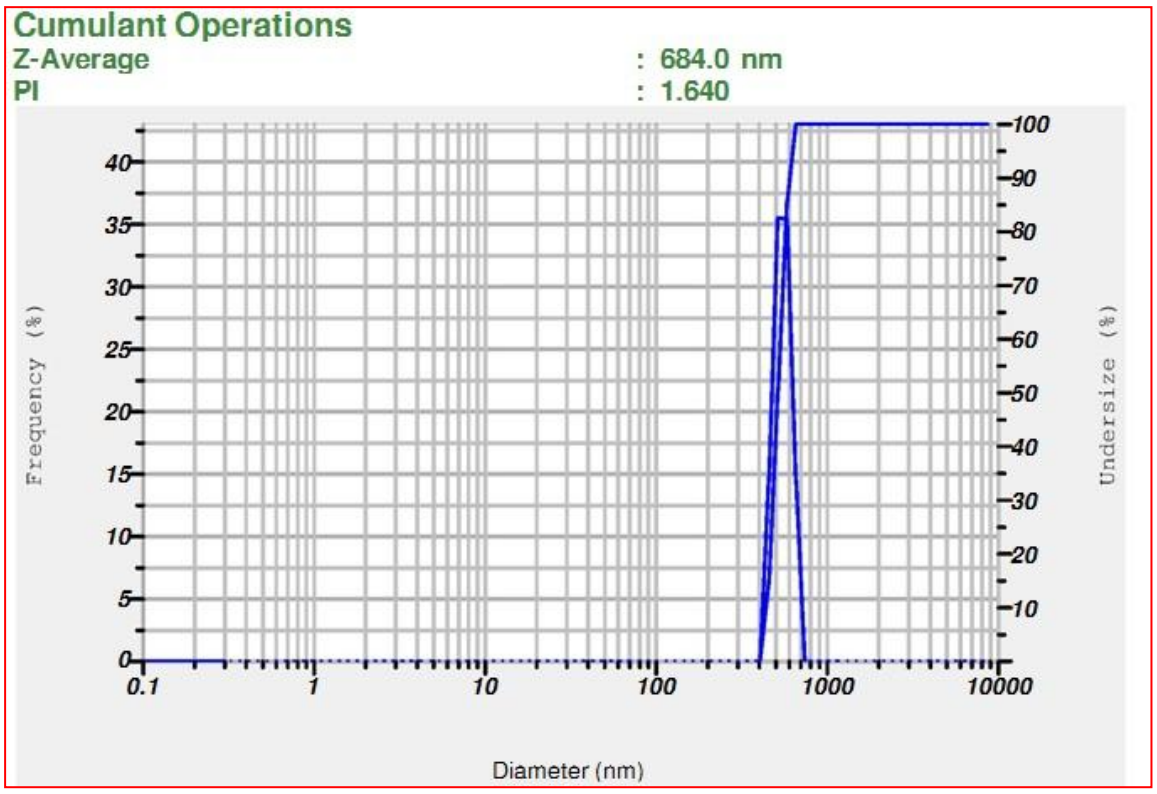


Figure 7: Particle size (F5)

Table 8: Particle size of Silver nanoparticle

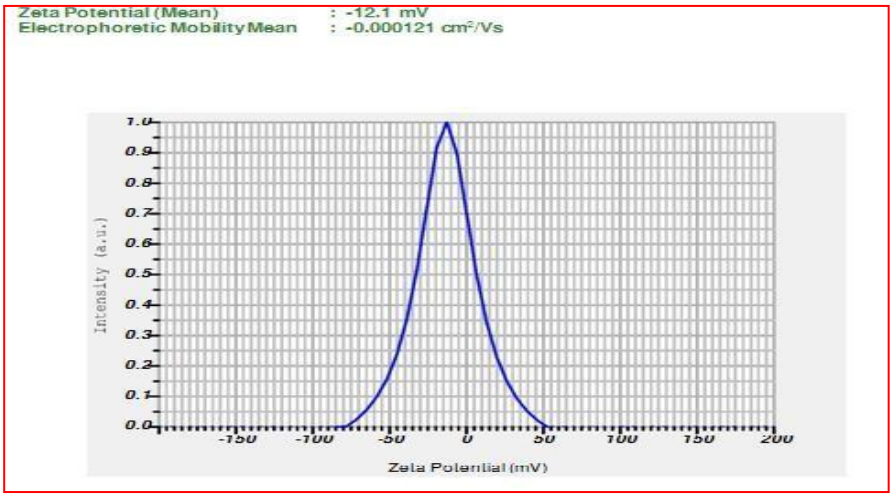
S. No	Formulation	Particle size	PI value
1	SNPs (F1)	823.8 nm	1.650
2	SNPs (F2)	447.8 nm	0.655

3	**SNPs (F3)**	**148.8 nm**	**0.350**
4	SNPs (F4)	181.4 nm	0.253
5	SNPs (F5)	684.0 nm	1.640

DISCUSSION

The particle size is one of the most important parameter for the characterization of nanoparticles. The average particle sizes of the prepared silver nanoparticle formulation were measured using Malvern zeta sizer. Particle size analysis showed that the average particle size of nanoparticles was found to be range between 148.8 to 823.8 nm. These particle size values indicate that the all formulated nanoparticle is under the range (Below 1000 nm) of nanoparticle and F3 is the lowest particle size of all formulation shown in above.

3.5.3 Zeta potential



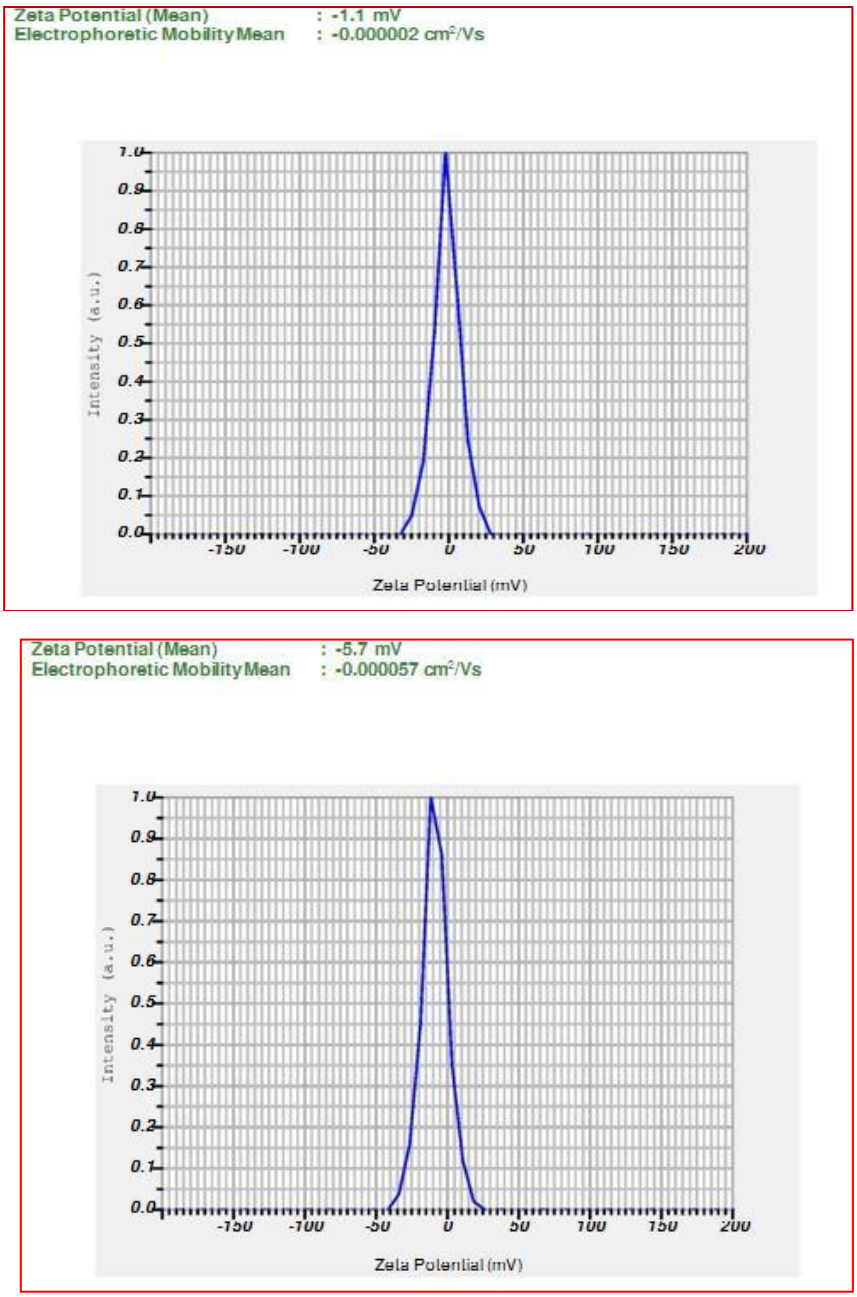


Figure 8: Zeta potential l (F3)

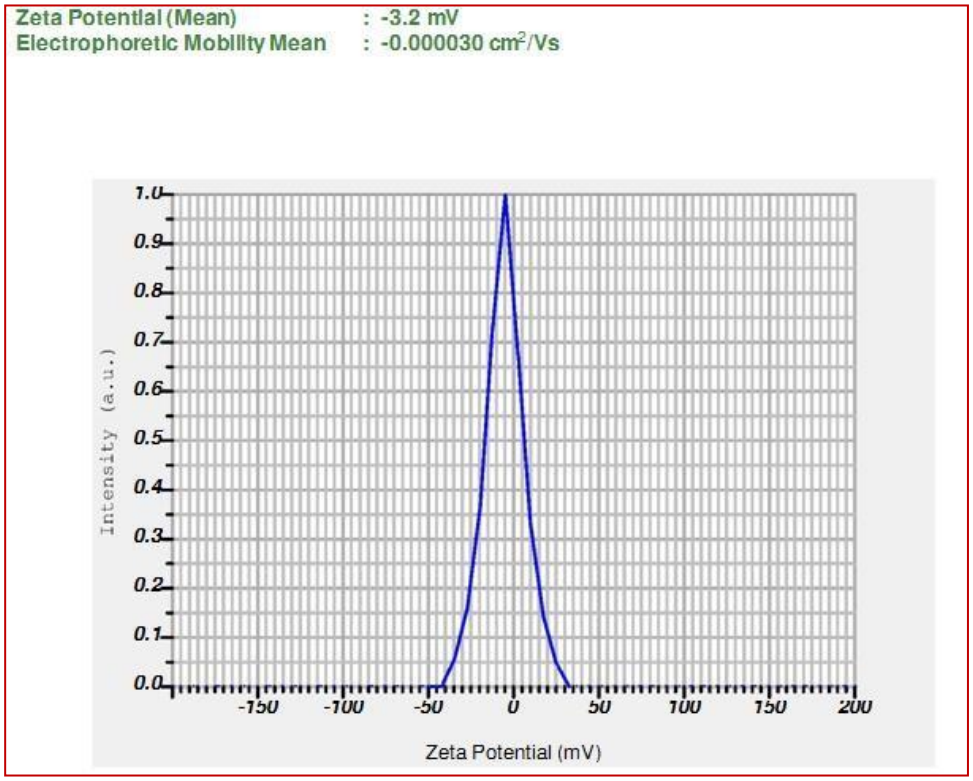


Figure 9: Zeta potential (F4)

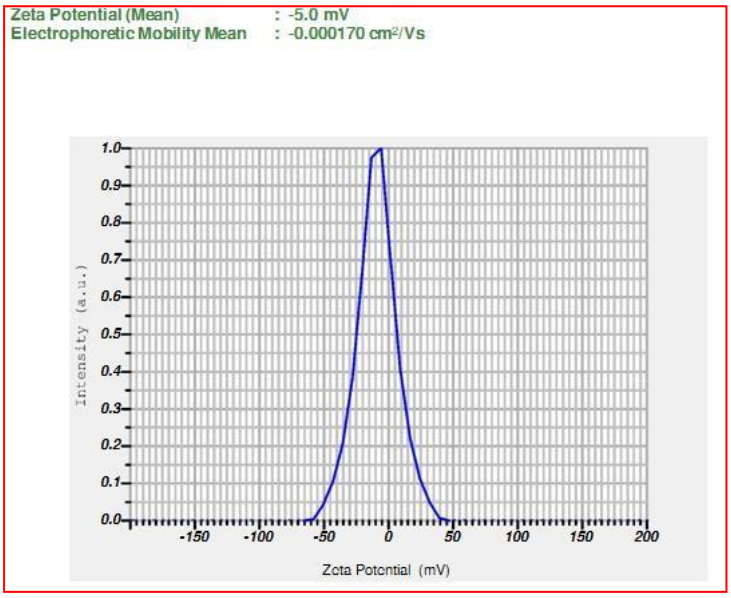


Figure 10: Zeta potential (F5)

Table 9: Zeta potential

S. No	Formulation	Zeta potential (mV)
1	Nanoparticle (F1)	-12.1 mV
2	Nanoparticle (F2)	-5.7 mV
3	Nanoparticle (F3)	-1.1 mV
4	Nanoparticle (F4)	-3.2 mV

5	Nanoparticle (F5)	-5.0 mV

DISCUSSION

Zeta potential analysis is carried out to find the surface charge of the particles. The magnitude of zeta potential is predictive of the colloidal stability. Zeta potential was foundto
be all formulation range -1.1 to -12.1 mV with peak area of 100% intensity. These values indicate that the all formulated nanoparticle is stable.

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