FABRICATION AND EXTRACTION OF SILVER NANOPARTICLE USING *Acorus calamus*

A THESIS SUBMITTED for the PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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By

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CERTIFICATE

*This is to certify that the thesis entitled "**Fabrication and extraction of silver nanoparticle using Acorus calamus**" submitted by **Ms. Abhipsa Swain** (Roll No: 412LS2063) in partial fulfilment of the requirements for the award of **Master of Science in Life Science** to the National Institute of Technology, Rourkela, is an authentic and original record of research work carried out by her under my supervision and guidance.*

To the best of my knowledge, the work incorporated in this thesis has not been submitted elsewhere for the award of any degree.

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DECLARATION

To the best of my knowledge the research project report entitled "**FABRICATION AND EXTRACTION OF SILVER NANOPARTICLE USING *Acorus calamus***" reported here in its original and has been submitted to National Institute of Technology, Rourkela for partial fulfilment of the degree of Master of Science in Life Science is a bonafide record of the project work carried out by me under the supervision of Dr. Suman Jha, Assistant Professor, Department of Life Science, National Institute of Technology, Rourkela. This thesis is my own work and that, to the best of my knowledge and belief, the matter and results of this thesis has not been submitted by any other research persons or any students.

I do hope, this project work will satisfy our beloved teachers. I solicit kind and favourable appreciation from my teachers.

Abhipsa Swain

Dedicated to my parents....

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LISTS OF ABBREVIATIONS

Fe- Iron

Zn-Zinc

Cu-Copper

Mn- Manganese

Ag-Silver

Au-Gold

($AgNO_3$)-Silver nitrate

SEM-Scanning electron microscope

DLS- Dynamic Light Scattering

FTIR-Fourier transform infrared spectroscopy

PdI- Polydispers intensity

ABSTRACT

Nanotechnology is a rising field of science and technology that deals with the particles having a minimum of one dimension in 1 to 100 nm. Nanotechnology gives rise to nanomaterials as its product. Nanomateials can be nanoparticles, nanorods, nanospheres ,nanocylinders,etc. Ag nanoparticles has many properties like antifungal activity, antibacterial activity, optical properties, conductive properties,etc. Due to its demand of diversified use, we have fabricated Ag nanoparticle using rhizome extract from *Acorus calamus*. The fabrication is followed by characterisation of Ag nanoparticles using UV-vis spectroscope, FTIR, DLS, ZETA and SEM. The characterisation results in spherical shaped Ag nanoparticle approximately equal to the nano range. After fabrication the reduced Ag nanoparticle is extracted from the colloidal solution using Size exclusion chromatography. After extraction again characterisation is done,which shows that the size reduces after extraction and is more stable with a high zeta potential. *Acorus calamus* is such a biological enhancer that is capable of producing quality and effective Ag nanoparticles that can serve the mankind in various way and as well as give us a safer and healthier environment.

INTRODUCTION

With the advent of new parameters in scientific development, there is always a thirst for a newer, more advanced and précised form of technology that can enhance the limelight of science. Such a broadened field in this 21st century of science is Nanotechnology. Nanotechnology signifies the technology in developing molecules or objects in the nano range i.e, 1 to 100nm. Nanotechnology has been gaining importance and its use has been spread to number of areas including cosmetics, health care, food, mechanics, optics, environmental health, biomedical services, space industries, catalysis, single electron transistors, non linear optical devices, drug gene delivery and photoelectrochemical application.[1, 2]. There has been various outcomes of Nanotechnology in the forms of nanomaterials, which include nanoparticles, nanorods, ,nanocynlinders and many more. Nanotechnology has shown great importance for biological molecules. Using Nanotechnology, the biomolecules are assembled under high control for making them convenient for the synthesis of nanoparticles.[3] The synthesis of nanoparticles has been emerging as a great area of research interest for its wide range of properties, which can be used for potential applications to draw heights to scientific developments and technological advancements[4]. Till now various metal oxide nanoparticles such as Fe, Zn, Co, Mn, Ag, Au have been synthesized, which are generally inorganic. Majority of these nanoparticles possess magnetic property which draw the attention of the scientists for their application in electronics as wrist watches, vending machines, ignition systems, generators, magnetic sensors, medical implants, recording equipments, microwave absorbers, telecommunications etc.[5] Nanoparticles deviate from the normal particles by great differences in shape ,size ,morphology,distribution , which is comparatively more improved than earlier[6]. The surface area to volume ratio of nanoparticles is very high due to small size of nanoparticles . Along with these properties , the nanoparticle also have specific physiochemical properties such as

optical properties, catalic activity, antibacterial properties, and electronic properties[7-10]. Due to their functional versatility and superior material properties, inorganic nanoparticle i.e, silver nanoparticle is drawing a great interest in nanotechnology research. Because of the suitable properties, the nano-crystalline silver particles have potential applications in various fields such as diagnostics, high sensitivity biomolecular detection, antimicrobials, catalysis, therapeutics, etc. With the growing use of nanoparticles in various fields, the current situation demands an environmentally clean and economically viable way for the synthesis of nanoparticles[11]. Due to the need for nontoxic synthesis of nanoparticles , many biological approaches that are free from any toxicity, are used . This leads to the rise of green nanotechnology with huge demand [12] In the biological synthesis of nanoparticles , there is use of bacteria, fungi and plants . Among these the most widely accepted method is the use of plants for the synthesis of nanoparticles.

By using different composition of materials and surface modifications, nanoparticles of different shapes and sizes can be synthesized. [14, 15, 16]. These nanoparticles synthsized show size or shape dependent properties.[17-18] . The nanoparticles thus formed are prevail in monodisperse form and also are not identical to each other [19, 20] Thus as the nanoparticles remain in polydispersed form, it becomes very complex to self assemble the nanopaticles. And the polydispersity also affects the sizedependent properties of nanoparticles such as surface Plasmon resonance , magnetic susceptibility ,etc.[21,22] Therefore it is very essential to synthesize nanoparticles with highly ordered with well defined properties and functions, which can be achieved by lowering down the polydispersity of the components to maximize the stability of nanoparticles.[23,24] . There are various ways to reduce the polydispesity of nanopaticle, like thermal decomposition method, polymeric stabilizers, reverse micelles,etc. [25]

REVIEW OF LITERATURE

Nanoparticle

Nanotechnology is being an emerging field in science which deals with the synthesis and structural manipulation of molecules or particle of size ranging from 1 to 100 nm. Nanotechnology has its outcomes in forms of nanoparticles. Nanoparticles are broadly classified as organic and inorganic nanoparticles. Organic nanoparticles comprises of carbon nanoparticles such as fullerences , and inorganic nanoparticles consists of semi conductor nanoparticles, metal oxide nanoparticles and metal nanoparticle such as titanium oxide, zinc oxide, silver nanoparticles and etc.

Fabrication of nanoparticles

Basically there are three methods for fabrication of nanoparticles such as physical, chemical, and biological methods [26-29]. In physical approaches, the common methods used are laser ablation and evaporation –condensation [30]. In chemical synthesis, the nanoparticles are synthesized by chemical reduction through organic and inorganic reducing agents. [31]. Whereas biological method involves bioreduction of metal ions into metal nanoparticles using cellular extracts.[32,33]

Significance of green synthesis

The major approach for biological synthesis or green synthesis of silver nanoparticles is regarding biocompatibility and environmental toxicity [34,35]. The further advantage of this approach is as follows:

1) no formation of toxic substances so environmental friendly,
2) the antioxidant or reducing properties of biomolecules of the organism reduces the metal to form nanoparticles,
3) cost effective

4) no use of high pressure ,temperature,energy,toxic chemicals
5) can be easily scaled for large scale synthesis

Uses of Ag

Metallic silver is used in surgical prosthesis.It is also used in treating mental illness,nicotine addiction ,epilepsy, gastroenteritis ,etc.It can be used to treat infectious diseases such as syphilis and gonorrhoea[36]

Acorus calamus

Acorus calamus is an angiosperm monocot plant. It belongs to the family acoracea . It has various medicinal benefits .Its rhizome is used to treat gastrointestinal problems that include inflammation of stomach, flatulence, ulcers and anorexia .Other medicinal uses of *Acorus* are induction of sweating, treatment of stroke and treatment of rheumatoid arthritis. It is used as sedative in form of calming medicine. It acts as stimulant and also as hallucinogen.It is commonly used to treat skin diseases and is broadly used as spices.

Applications of Ag Nanoparticles

Silver nanoparticles have physical properties such as optical, conductive and antibacterial activity. The application of Ag nanoparticles are as follows-

1) Ag nanoparticles can act as biosensors and hence quantitative detection can be done using Ag nanoparticles as biological tags.

2) Due to its conductivity property it can be used to enhance electrical and thermal conductivity in composites, it has also a good optical property characterised by surface plasmon resonance (SPR) and metal enhanced florescence (MEF).

3) As Ag nanoparticle possess antibacterial properties , it can be used in paints , appliances, plastics,footwears, apparel and cosmetics.

MATERIALS AND METHODS

Preparation of rhizome extract from *Acorus calamus* rhizomes

In the experiment, silver nitrate ($AgNO_3$) of AR grade from Sigma-Aldrich was used. The rhizomes of *Acorus calamus* were collected from the local market of ROURKELA, ODISHA, INDIA. To remove the dust particles, the rhizomes were thoroughly washed. Then, to moisture the rhizomes were dried under the sun. And after drying those were grinded to fine powder. In 250 ml of deionised water, 1% of rhizome powder was added and was stirred for 15 mins. After proper mixing , it was then incubated for 30 mins at 25 dergree Celsius. Centrifugation of the solution was done at 5000rpm for 30 mis at 25 degree Celsius. Filtration was done by membrane filter using 2.5 microns filter paper with the help of a vaccum pump. Then the prepared rhizome extract solution was used to reduce silver ions to metal silver for the biosynthesis of nanoparticles.

Fabrication of Ag Nanoparticles

60:1 ratio of plant extract to silver nitrate solution was prepared. The Ag nanoparticle solution then formed was centrifuged at 12,000rpm for 15-20 min and then the pellet formed after centrifugation was diluted.

Characterisation of Ag nanoparticles

1) UV-Vis spectral analysis

After fabrication, the aqueous solutions of dissolved pellet and supernatant were taken to measure the absorbance in UV-Vis spectra. The absorbance of pellet showed the presence of Ag nanoparticle whereas the absorbance of supernatant didn’t show any presence of Ag nanoparticle.

2)DLS & Zeta-Potential analysis

After the separation of pellet from supernatant by centrifugation at 12,000rpm for 20 mins , the pellet was diluted 4-5 time. Then DLS of pellet was done to detect the size of nanoparticles and to study the distribution of nanoparticles in the solution. The Zeta potential analysis was done to detect the surface potential of the nanoparticles for the study of the stability of the nanoparticles.

3)FTIR spectral analysis

After the separation of pellet from supernatant by centrifugation at 12,000rpm for 20 mins , the pellet was collected and diluted. The diluted pellet was taken for characterization using ATR-FTIR (Bruker,Germany). The range of FTIR was taken to be 4000-500 cm^{-1}. The characterization using FTIR was done to determine the bond stretches present in the pellet.

4)SEM analysis

After the fabrication of nanoparticles, glass slide was prepared for SEM analysis. To prepare the slide, one drop of sample was taken on a glass slide and was dried in an incubator. After the slide was prepared, the sample was coated with gold for 3 mins to make it conductive.And then the SEM images were taken to study the size and morphology of the nanoparticle formed.

Extraction of silver nanoparticles

After centrifugation the diluted pellet was sonicated for 10-20 mins. The pellet was then filtered by membrane filter using 0.22 micron filter paper. After membrane filtration the very next step of extraction was to extract the silver nanoparticles from the colloidal solution by using size exclusion chromatography. In size exclusion chromatography there exist two phases, a mobile phase and a stationary phase. In this experiment SDS was the mobile phase

and Sephadex G 100 was taken as the stationary phase. The pore cut off size of Sephadex G 100 is 100KDa. According to the principle, smaller particles would move slowly through the column and the larger particles would be eluted out first from the void volume. In this process, the sample was loaded to the column and the eluents were collected. After completion of the extraction, the collected eluents were characterised using UV-Vis spectra,FTIR, DLS and Zeta potential.

RESULT AND DISCUSSION

After Fabrication

1)UV-VIS Spectral Analysis

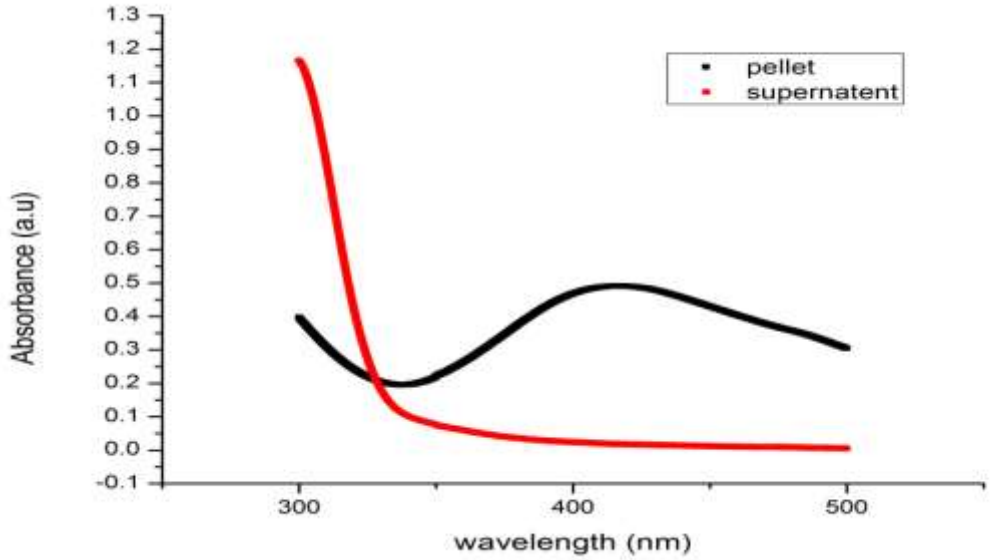

Fig 1:-UV –Vis Analysis of pellet and supernatent

Figure1 shows the absorbance is maximum with 0.483 OD at wavelength 414nm in pellet whereas the supernantent does not show any absorbance peak. Thus it can be concluded that the Ag nanoparticle is present in pellet but not in the supernatant.

2) ATR-FTIR Analysis

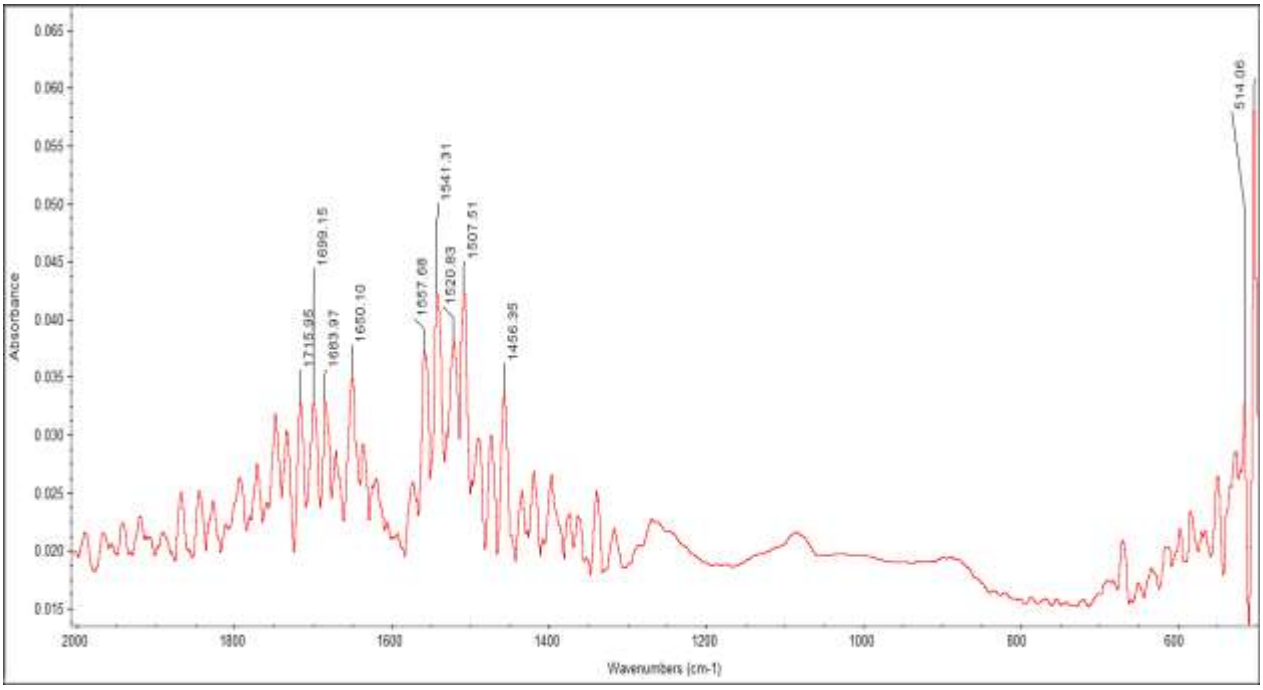

Fig 2:ATR-FTIR Analysis after fabrication

Figure 2 shows absorbance peaks in the range of 1400 to 1800nm and a sharp peak at 514.08 nm. Hence there is presence of nanoparticle along with proteins, flavonoids,tannins. As 514.08nm is for Ag nanoparticles and the proteins give peak in 1400 nm to 1800nm range

3)DLS Analysis

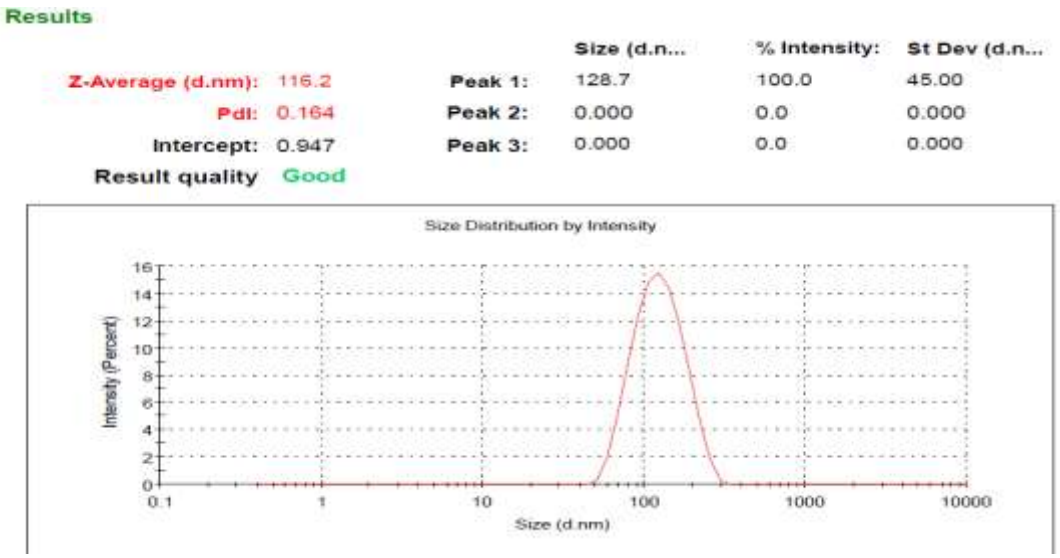

Fig 3:- DLS Analysis after fabrication

Figure3 shows that the particle size distribution is 128.7nm. The size of the nanoparticle is appropriate as it is close to 100 nm. The size of the synthesized particles are slightly bigger than usual range of 1-100 nm perhaps because of layering of plant proteins , which coats the nanoparticles and confers them stability by preventing from agglomeration .

4)SEM Analysis

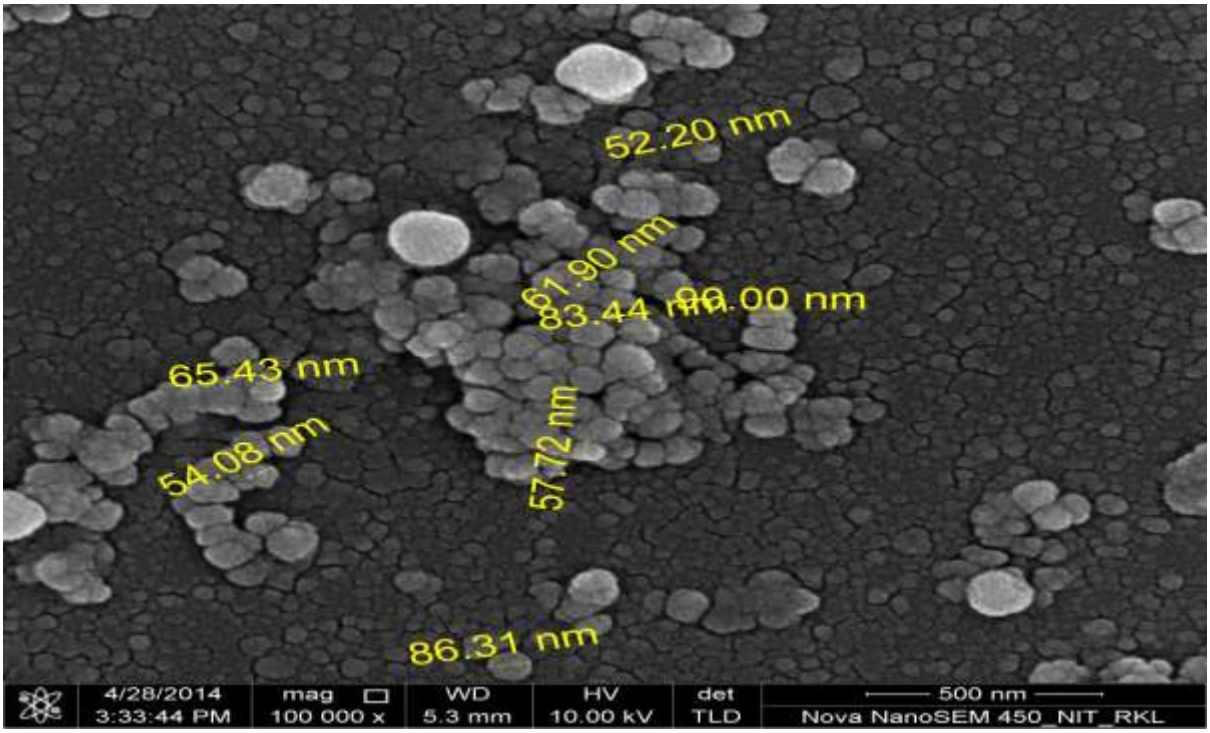

Fig 4: SEM Analysis showing nanoparticle morphology and size

Figure4 shows the nanoparticle size is within 100nm such as 86.31nm, 61.90nm, 65.43nm, etc. The nanoparticle exist in spherical shape The nanoparticles were well stabilized by capping agent (plant phytochemical) hence they were not in direct contact even within the aggregates as seen in sem image .

AFTER EXTRACTION

1)UV-Vis spectral Analysis

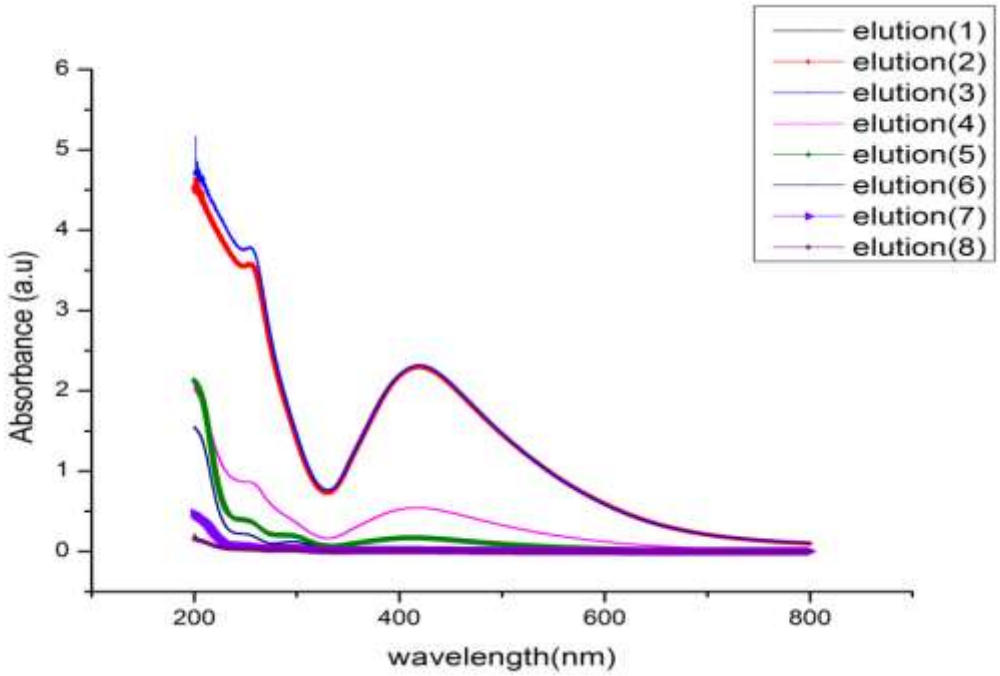

Fig 5: Uv vis Analysis of the elutions after extraction

Figure5 shows that the elution 2 and 3 give absorbance of 2.275 OD at 420.62nm, whereas the elution 4 gives 0.555OD at 415.85 nm, and gradually decreases further. This means that the intensity of peak sharply increases in eluent 2,3 and then decreases in eluent 4 while in eluent 5, 6 ,7 ,8 it absorbance is almost parallel to that of absorption of control. Sharp intensity increase maybe due to increase in conc. of nanoparticles in eluent. More the conc of nanoparticle in eluent, more the SPR.

2) ATR-FTIR ANALYSIS

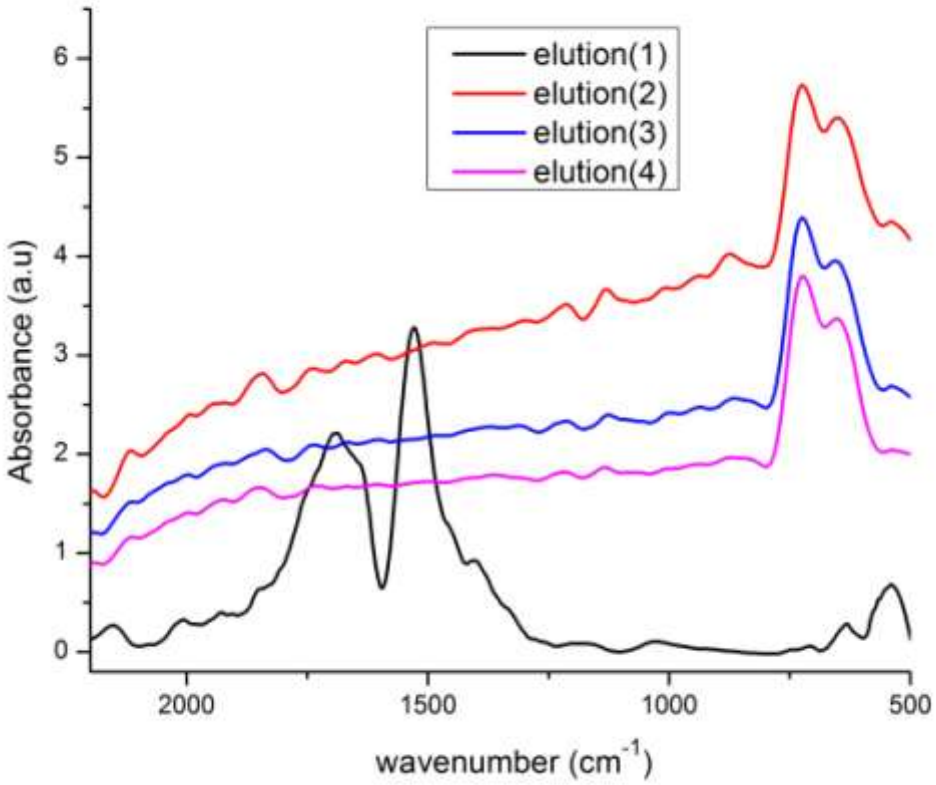

Fig6:FTIR Analysis after extraction

Figure6 shows elution 1 has two absorbance peaks at 1693.194nm and 1521.791nm specifying amide II and amide I bond vibration. And there is a small peak at 531.94nm showing the presence of less amount of nanoparticles. Thus in elution 1 both protein and a less amount of Ag nanoparticle is present. But in elution 2,3and 4 there are peaks at 7726.7nm, 723.21nm, 723.21nm respectively. Hence from the graph we can determine that elution 2,3and 4 has only Ag nanoparticles and no proteins. The concentration of nanoparticle is highest in elution 2 and gradually decreases.

3) DLS Analysis

Results

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	97.21	Peak 1:	116.4	98.5	37.56
PdI:	0.390	Peak 2:	5392	1.5	314.9
Intercept:	0.911	Peak 3:	0.000	0.0	0.000
Result quality	Good				

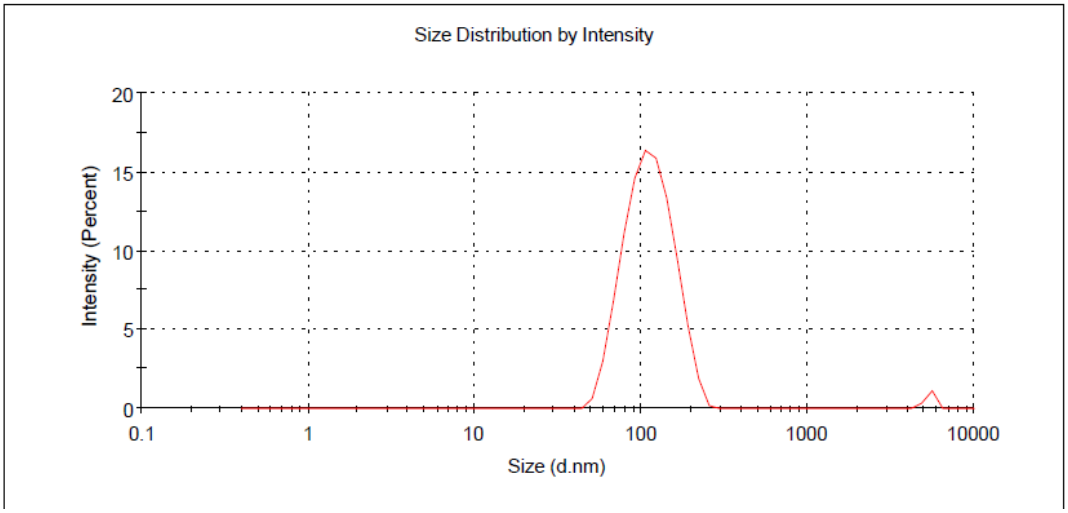

Fig7a: DLS Analysis of elution 2

Results

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	118.0	Peak 1:	99.76	100.0	23.24
PdI:	0.375	Peak 2:	0.000	0.0	0.000
Intercept:	0.937	Peak 3:	0.000	0.0	0.000
Result quality	Refer to quality report				

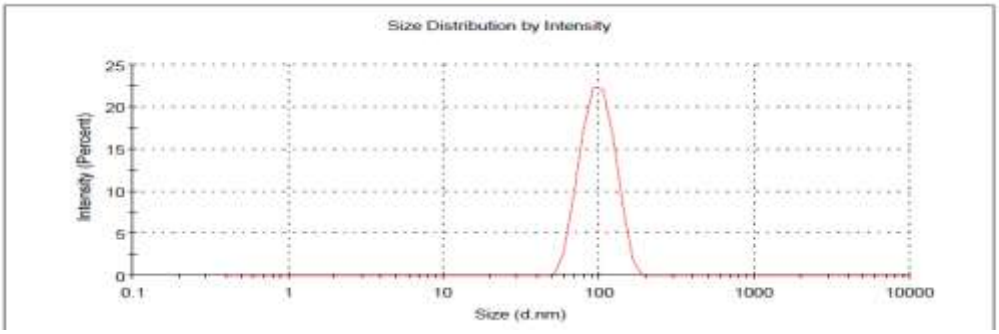

Fig7b:DLS Analysis of elution 3

Figure 7a and 7b shows that the size distribution of pure Ag nanoparticle is 116.4nm with a PdI of 0.390 and 99.67nm with a PdI of 0.375 respectively. There is a gradual decrease in

size after the extraction process. SEC gave fractions in which average NP size decreased with elution time. The largest sized nanoparticles were eluted first from the accessible volume or the void volume of the column this was followed by the smaller particles that meander freely and travel steadily down the column from the pores according to the retention time.

4) ZETA Potential

Results

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -36.1	Peak 1:	-37.4	37.9	4.36
Zeta Deviation (mV): 13.2	Peak 2:	-48.5	33.1	5.33
Conductivity (mS/cm): 0.131	Peak 3:	-24.5	19.6	4.25

Result quality See result quality report

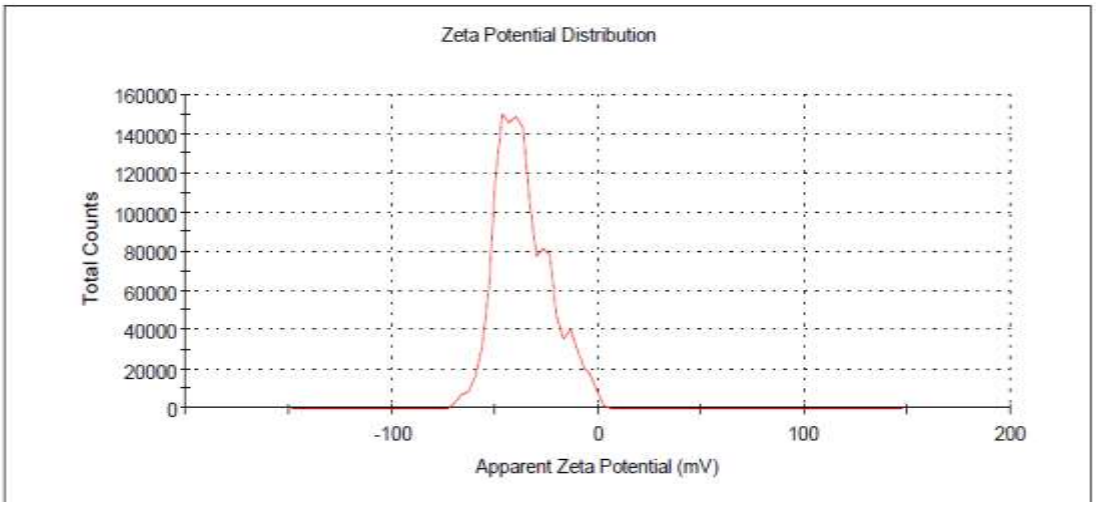

Fig 8a-ZETA potential of elution 2

Results

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -39.3	Peak 1:	-39.3	100.0	11.3
Zeta Deviation (mV): 11.3	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.173	Peak 3:	0.00	0.0	0.00

Result quality See result quality report

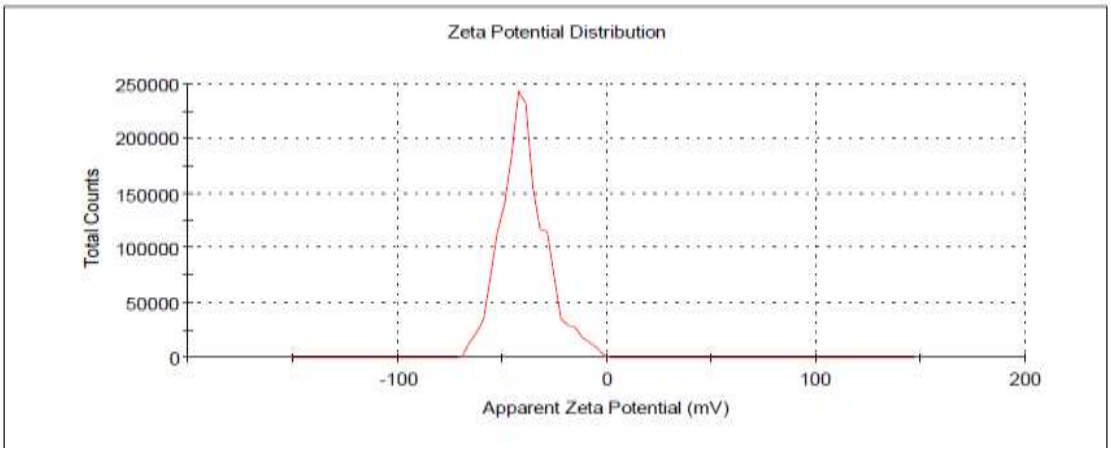

Fig 8b- ZETA Potential of elution 3

Figure 8a and 8b shows that the surface potential of Ag nanoparticle is -36.1 and -39.3 respectively .Hence the nanoparticles formed are stable. It can be seen that as the sdecrease the surface potential increase, that means smaller the size more stable is the nanoparticle.

CONCLUSION

The Ag nanoparticle were successfully fabricated from rhizome extract of *Acorus calamus* by biological method and extracted using Size exclusion chromatography with SDS as the mobile phase and Sephadex G 100 as the stationary phase. The size of nanoparticle is in the range of 99.76nm to 116.4nm with a PdI of 0.375 to 0.390.The shape of Ag nanoparticle formed is spherical. The Ag nanoparticles are highly stable with a surface potential in the range of -36.1to -39.3. It is also concluded that the nanoparticle size is further reduced when it is extracted from the colloidal solution after fabrication. This may have occurred due to the removal of capping agents after extraction. And also the size of nanoparticle is reduced whereas the stability or surface potential is increased with every step of chromatography.

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