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IN-VITRO BIO-FABRICATION OF MULTI-APPLICATIVE SILVER NANOPARTICLES USING NICOTIANA TABACUM LEAF EXTRACT

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ABSTRACT: The development of eco-friendly, economical green methods for synthesis of nanomaterials are day by day becoming popular among nanotechnologists. In the present study the authors have reported synthesis of silver nanoparticles at room temperature using tobacco leaf extract. The synthesized nanoparticles have been found to be stable for several months. UV-Visible spectroscopic analysis carried out ensured the formation of silver nanoparticles. Similarly, X-ray diffraction pattern confirmed the formation of silver nanoparticles in the reaction product. For morphological analysis of synthesized nanoparticles SEM and TEM imaging was done. TEM image reveals formation of spherical, non-uniform, poly-dispersed nanoparticles. The EDX of nanoparticles showed the presence of elemental silver. The synthesized nanoparticles are multi-applicative and showing potential activity against gram negative bacterium. Also it can be used as an efficient catalyst for organic transformation and dye degradation. In the near future, silver nanoparticles synthesized using green methods may be used in treatment of infections caused by the highly antibiotic resistant microorganisms.

KEYWORDS: Green-synthesis, Nano-catalyst, Anti-microbial activity, Synergetic effectiveness, Dye degradation

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1. INTRODUCTION

The index of human progress is valued by the use and development of materials and named after that such as Stone Age, copper age etc. Nanotechnology has opened the new doors for the development of entirely new kind of materials. Thus nanotechnology is emerging and interesting field of study. In recent years, the application of nanoparticles in various fields has expanded considerably. With the development of science and technology, revolutionary changes are taking place in the area of telecommunication, information generation and processing, memory devices, signal transmission, storage of energy and medicine etc which use specialized nanoparticles. Nanoparticles possess unique physicochemical characteristics such as high surface to volume ration, spatial confinement, reduced imperfections, high reactivity, and size in the range of nanometers. Nanoparticles have been successfully used in various fields such as in nano-chemistry for enhancing the activity of catalysts, in medicine and pharmacy for delievery of therapeutic in chronic disease diagnostics and also in sensors. Nanomaterials have a long list of applicability in improving human life and its environment. The development of nanoscience can be traced back to 5000 years. The ancient Indian system of medicine *Ayurveda* had some knowledge of nano-scale fabrication used for medicinal purposes. Thus it is clear that nanotechnology was in existence even before this term was coined. In the present age indeed nanotechnology the mania is sweeping through essentially all field of science and technology and public becoming aware of quote of Nobel laureate Richard Smalley " just wait, the next century is going to be incredible. We are about to be able to build things that work on smallest possible length atom by atom. These little nanotechnologies will revolutionize our industries and our life". A bulk material has constant physical properties regardless of its size, but at the nano-scale often this is not true. Several well characterized bulk materials have been found to possess astonishing properties at nano-scale. An interesting aspect about nanomaterials is that a number of factors can influence their physical, chemical, mechanical, optical, magnetic and electronic properties. These factors are size, shape, surface composition, dielectric environment of particle and the inter-particle interaction, dangling bonds, van der walls force etc. According to one of the theory, nanoparticles acquire novel properties because their dimensions are comparable to de Broglie wavelength of the charge carrier and they possess a very high aspect ratio i.e. surface to volume ratio. Nanoparticles are of great scientific interest as they bridge the gap between bulk materials and atomic and molecular structure. Metallic nanoparticles are intensely studied due to their unique optical, electrical and catalytic

properties. To utilize and optimize chemical and physical properties of nano sized metal particles, a large spectrum of research have been focused to control the size and shape which is crucial in tuning their properties [1-5]. With increasing importance and application of nanomaterials a number of physical and chemical routes of synthesis have been reported such as electro-chemical, sol-gel, co-precipitation, hydrothermal, chemical reduction, sono-chemical and microwave assisted process. But most of these strategies consist of utilization of high energy, hazardous chemicals and difficulty in purification or very sophisticated and costly instruments [6-7]. To switchover these technical hitches biological principles have attracted the attention of scientist and the same is implemented in our paper [8-10]. Bacterial cell surface, fungus, virus, DNA template, proteins, plants, small peptides and even pollen grains have been used for the synthesis of nanostructures with a variety of composition, size and shape. Table 1 shows the glimpses of nanomaterials synthesized by different biological routes.

Table.1. Synthesis of metallic nanoparticles by different biological routes

Medium	Type of nanoparticles	Location/ Shape	size range (in nm)	Reference
(A) Algae				
Sargassumwightii	Au	Extracullar	8-12	18
Chlorella vulgaris	Au	----------	9-20	19
(B) Bacteria				
Pseudomonas stutzeri	Ag	Intracellular	~200	20
Morginellasp	Ag	Extracellular	20-30	21
Lactobacillus srain	Ag and Au	Intracellular	----------	22
Plectomabaryanum	Ag	Intracellular	1-10	23
Escheria coli	CdS	Intracellular	2-5	24
Clostridium thermocetium	CdS	Intracellular & Extracellular	------	25
Actinobacter spp.	Magnetite	Extracellular	10-40	26
Shewanella algae	Au	Intracellular @ pH=7 & Extracellular @ pH=1	10-20 50-500	27
Rhodopseudomonas capsiculata	Au	Intracellular @ pH=4 &	50-400	28

		Extracellular @ pH=7	10-20	
Escheria coli DH 5 α	Au	Intracellular	25-33	29
Thermomonosporasp	Au	Extracellular	8	30
Rhodococcussp	Au	Intracellular	5-15	31
Klebsiella pneumonie	Ag	Extracellular	5-32	32
Pseudomonas aeruginosa	Au	Extracellular	15-30	33
Shewanellaaneidensis	Uranium IV	Extracellular	------	34
Bacillus sp.	Ag	Periplasmic space	5-15	35
Lactobacillus sp.	Ag, Au	Hexagonal/counter	20-50nm	36
*S. oneidensis*MR1	Pd	Periplasmic space	------	37
Aqua spirillummagnetotacticum	Fe_2O_3	Octahedral prism	40-50nm	38
Magnetotactic bacterium MV1	Fe_3O_4	Cell inside/Parallele piped	40-60nm	39
Magnetotactic bacterium	Fe_3S_4,FeS_2	Octahedral/ Cubo-octahedral	7.5nm	40
Sulfate reducing bacterium	FeS	Cell Surface	2nm	41
Magnetospirillummagnetotacticum	Fe_3O_4	Membrane bound	47.1	42
Magnetospirillummagnetotacticum MS1	Fe_3O_4	Inside cell	50nm	43
M. gryphiiswaldense	Magnetite	Hexagonal prismatic	35-120nm	44
Desulfosporosinus sp.	UO_2	Cell surface	1.5-2.5nm	45

Clostridiumthermoaceticum	CdS	Cell surface	------	46
Klebsiella pneumoniae	CdS	Cell surface	5-200nm	47
Escherichia coli	CdS	Spherical, elliptical	2-5nm	48
Desulfobacteriaceae	ZnS	Spherical	2-5nm	49
B. megatherium DO1	Au	Spherical	1.9nm	50
*Aeromonas sp.*SH10	Ag	----------	6.4	51
Enterobactor cloacae	Ag		52.5nm	52
Acetobacterxylinum	Ag	Cellulose fiber	50nm	53
Morgonella sp.	Ag	Spherical	20nm	54
Sulfospirillumbarnesaii	Se	Nanosphere	300nm	55
B. selenitireducer	Te	Nanorods	10nm	56
Sulfurous pirilliumbarnesii	Te	Irregular shape	50nm	57
Lactobacillus sp.	Ti	Spherical	40-60nm	58
P. boryanus UTEX 485	Pt	Spherical	30nm	59
*Geobactermetallireducens*GS15	Magnetite	---	10-50nm	60
Thermophilic bacteria TOR 39	Co, Cr, Ni	Octahedral	-----	61
Sulfospirilliumbarnesaii	Te	Irregular nanosphere	10nm	62
Sulphur reducing bacreia	Au	Cell envelop	10nm	63
Sulphur reducing bacreia	FeS	Cell surface	2nm	64
Tetrathiobacterkashmir	Se	---		65

ensis				
(C) Plant and Plant Extracts				
Geranium plant leaf extract	Ag	----------	16-40	66
Lemongrass plant extract	Au	----------	200-500	67
Avena sativa	Au, Ag	Extracellular	5-85	68
Cinnamon camphora	Au & Ag	Extracellular	55-80	69
Azadirachta indica	Au & Ag Bimetallic		50-100	70
Artocarpusheterophylus seed	Ag	----------	50	71
Jatropacurcas	Ag	Extracellular	10-20	72
(D) Fungi				
*Phoma sp.*3.2883	Ag	Extracellular	71.06-74.46	73
Fusarium oxysporium	Au	Extracellular	20-40	74
Verticillium	Ag	Intracellular	25	75
Aspergillus fumigatus	Ag	Extracellular	5-25	76
Trichodermaasperellum	Ag	Extracellular	13-18	77
Phaenerochaetechysosporium	Ag	Extracellular	50-200	78
Fusarium oxysporum	Magnetite	Extracellular	20-50	79
T. viride	Ag	Spherical	5-40nm	80
F. oxysporangium	Ag	ND	5-50nm	81
Phaenerochaetecrysosporium	Ag	Pyramidal	5-200nm	82

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*F. solani*USM 3799	Ag	Spherical	16-23nm	83
F. semitectum	Ag	Spherical	10-60nm	84
F. acuminatum	Ag	Spherical	5-40nm	84
A. fumigatus	Ag	Spherical	5-25nm	84
Coriolusversicolor	Ag	Spherical	25-75nm	84
A. nigre	Ag	Spherical	20nm	84
P. glomerata	Ag	Spherical	60-80nm	84
Penicillinumbre vicompactum	Ag	Spherical	58nm	84
Cladosporiumcl odasporiodes	Ag	Spherical	10-100nm	84
Penicillinumfell utanum	Ag	Spherical	5-25nm	84
F. oxysporum	Ag	--	8-14nm	84
Valvuriellavolva cea	Ag	Spherical	20-150nm	84
F. oxysporum	Si	Quasi-spherical	5-15nm	84
F. oxysporum	Ti	Spherical	6-13nm	84
F. oxysporum	Zr	Quasi-spherical	3-11nm	84
F.oxysporum	Pt	Trigonal	10-50nm	84
F. oxysporum	Magnetite	Quasi-spherical	20-50nm	84
Verticillumsp	Magnetite	Cubo-octahedral	100-400nm	84

F. oxysporum	CdSe	Spherical	9-15nm	84
F. oxysporum	$SrCO_3$	Needle shaped	----	84
F. oxysporum	$BaTiO_3$	Quasi-spherical	4nm	84
F. oxysporum	Bi_2O_3	Quasi-spherical	5-8nm	84
(E) Yeast				
Candida glabrate	CdS	Intracellular	200	85
MKY3	Ag	Extracellular	2-5	85

While micro-organisms continue to be investigated for bio-mineralization and metal nanoparticles synthesis, the use of plant extracts in similar nanoparticles biosynthesis methodologies is an exciting possibility and is relatively unexplored and under-exploited[11].Plants have been known to bio-mineralize calcium carbonate, silica and even magnetite internally. Similar to micro-organisms, plants have also been used for purification of heavy metal ions from contaminated soil and water. Certain plants are known to hyper-accumulate these heavy metals or can even be induced to hyper-accumulate them within different parts of plants. The internal accumulation of metal in plants can occur both via complex formation of the metal ion with a suitable bio-ligand in its native oxidation state or via its reduction to a lower oxidation state. The possibility of reduction of metal ions by plants and the presence of metal complex forming agents in them inspired material scientists to use plants for the goal of synthesizing nanoparticles and controlling their size and shape [12]. The possibility of synthesizing nanoparticles of different compositions using plants would offer a benign alternative to the existing potentially toxic chemical and costly physical methods of synthesis. While earlier literatures demonstrated the possibility of synthesizing metal nanoparticles using plants, they suffered with the inherent complication of being intracellular, making the isolation of particles an additional difficult job. Extract from well known commercial plant like *Coffeaarabica*, and *Cymbopogon citrus* were used as green reagents in AgNPs synthesis. Thus by reducing silver nitrate in solution of tea extract or epicatechin of varying concentrations, spherical silver nanoparticles were formed that had controllable size distribution depending upon the concentration of tea extract and epicatechin in the samples[13].Recently there has been considerable interest in the developments of the techniques for the controlled synthesis of metal nanoparticles of well defined size, shape and composition as they find applications in biomedical field and area such as optics and electronics. Solution temperature, concentration of metal salt and nature of reducing agent, pH of reaction medium, atmosphere i.e. inert or oxygen, rate of addition of reactant and reaction time all influence the shape and size of particle.

Amongst metal nanoparticles silver nanoparticles exhibits tremendous applications in various areas such as biomedical field, spectral selective coatings for solar energy absorption, optical receptors, bio-labeling, intercalation materials for electrical batteries, filters, textile coatings, antimicrobial agents and sensors etc[14]. This process leads to green chemistry route which is more advantageous because it does not require elaborate process such as intra cellular synthesis, multiple purification steps, and expensive maintenance of microbial cell culture. Here the reaction is normally carried out at room temperature although in some processes a little heating below 100^{o}C may be required. Thus this extracellular green synthesis route is an easy, economical and eco-friendly method of synthesis. In this method, instead of toxic chemicals, poly-phenolic compounds in the plant extract are generally supposed to influence the reduction process and stabilize nanoparticles preventing agglomeration. These nanoparticles are studied for their antibacterial properties and future work can be extended to study their antioxidant, anti-spasmodic, and anti-neoplastic properties [15]. It is well known that few medicinal plants exhibit anti-oxidant property. Thus they can act as biological source of reducing agent. On this belief choice of plants for this purpose were those having high medicinal and aromatic importance. Pioneer work in this area was done by S. Shiv Shankar who has reported synthesis of silver and gold nanoparticles using decoction solution of *Azadirachata indica*. However, the nanoparticles synthesized by him are in the range of 50- 100nm and are not well separated from each other and tend to form agglomerated structures [16]. Tobacco, i.e. *Nicotiana tabacum* is widely cultivated plant in tropical country like India. In the current research work *Nicotiana tabacum* leaf extract have been used as a reducing agent for AgNPs biosynthesis. The same extract also acts as a capping agent. This plant is described to possess medicinal properties and suggested for treatment of teeth pain in *Ayurveda*. The leaves are antispasmodic, discutient, diuretic, emetic, expectorant, irritant, narcotic, sedative and also acts as sialagogue. They are used externally in the treatment of rheumatic swelling, skin disease and scorpion stings. When taken internally it is an additive narcotic. The active ingredients of this plant can also be absorbed through the skin. They are also supposed to cure piles. However, the excessive use of this plant leaves are harmful and can lead to cancer. It has been reported by Lin et al. that the carbonyl groups of proteins found in some plant species have a strong affinity to bind with metals and thus prevent agglomeration and thus form nanoparticles. The chemical composition of the plant includes poly-phenols, sugars, chlorogenic acid isomers and alkaloids [17].

1.1 Silver nanoparticles

Silver nanoparticles find wide application in uncountable and diverse applications. So our research work was concentrated for the synthesis of AgNPs. They are widely used as a catalyst for organic transformation reactions. Due to the development of antibiotic resistance in pathogenic bacteria the pharmaceutical companies and researchers are searching for new antibacterial agents and the AgNPs

are promising candidate for the same. They are also useful substrate for Surface Enhanced Raman Spectroscopy (SERS) since it requires partially electrically conducting surface. The biological method leads to the formation of colloidal AgNPs which are separated by high speed centrifugation. When the colloidal particles are much smaller than the wavelength of visible light, the solution possess characteristic yellow colour with an intense band in the 400-480nm range in the UV-visible absorption spectrum.

2. MATERIALS AND METHODS

Nanoparticles of Ag were prepared by using N. tabacum leaves extract. N. tabacum leaves have been purchased from local market of Jaysingpur, India. About 50gm of dry leaves have been taken for preparation of extract. The obtained leaves were sieved and thoroughly washed in running water and finally rinsed with deionized water until no foreign material remained behind. After complete washing the leaves were kept in 100ml of deionized water over night and macerated to soften the plant leaves. Then it was finely crushed in grinder mixer and filtered by using cotton wool and collected in clean stopperd glass bottle. Then the extract was diluted to 250ml using deionized water and the solution was kept in refrigerator at 4°C temperature. The AgNO3 used in reaction was analytical grade chemical obtained from Sigma Aldrich and used without further purification. The further laboratory work is described as follows. Now 0.1M AgNO3 solution was prepared and 100ml of the same was taken in 500ml beaker. Then 50ml of diluted N.tabacum extract was taken in burette and drop wise added in beaker at room temperature with constant stirring. The reaction between AgNO3 and N.tabacum takes place and therefore as soon as the extract was added dark brown coloured precipitate appeared in the solution. After complete addition of 50ml of extract a sufficient amount of the precipitate was formed and it was separated by high speed centrifugation at about 8000 rpm. The separated solid mass was washed with alcohol for few times to remove organic impurities soluble in alcohol. After complete washing the solid mass was kept in hot air oven over night for drying. The complete drying of this solid mass resulted in a black coloured material which was powdered in mortar and sampled for characterization purpose.

3. RESULTS AND DISCUSSION

Biosynthesis of AgNPs was primarily indicated by visual change to dark brown which had taken place during the course of reaction. The intensity of colour depends upon degree of bio-reduction of silver particles. Formation of AgNPs and their morphology was confirmed by using different advanced characterization techniques described as follows.

3.1 UV-Visible Spectroscopic graph showing absorbance peak for silver nanoparticles

The blackish brown coloured sample powder was dissolved in deionized water and sonicated. Then this solution was taken in cuvette and exposed to UV-visible radiation and the absorbance of the solution was recorded. Because of the surface plasmon resonance phenomena resonant peak occurs

at different wavelength for different nanoparticles solution and as per the theory of resonance maximum wavelength is absorbed at resonant wavelength. It is reported in the literature that typical AgNPs show the characteristic SPR at the wavelength in the range of 400-480nm. Fig. 1 shows SPR for the sample solution to occur at the wavelength of 425nm which confirms the presence of Silver nanoparticles in the prepared solution. The SPR absorbance is sensitive to the nature, size and shape of particles present in the solution and also it depends upon their inter particle distance and the surrounding media. The MATLAB analysis of this UV image shows that the image format is JPEG with bit-depth of 24 bits and image resolution is found to be 492 x 492 and the file-size is 13.7 KB. Also it is seen that the image class is uint8. The UV characterization has been done at Department of Chemistry, Shivaji University, Kolhapur, India.

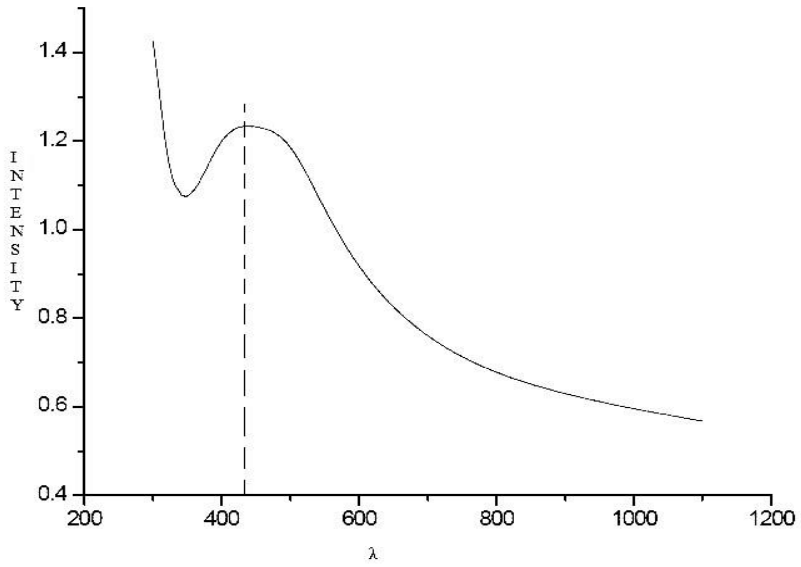

Fig. 1 UV-Visible Spectra showing absorbance of silver nanoparticles

3.2 XRD pattern showing silver nanoparticles

XRD patterns of synthesized AgNPs are shown in Fig. 2 where four major peaks appeared. The peak position explains about the translational symmetry namely size and shape of the unit cell whereas the peak intensities give details about the electron density inside the unit cell. Thus the pattern confirms the crystalline nature of the synthesized nanoparticles. These peaks are an indication of cubic structure of synthesized nanomaterials and in agreement with the JCPDS file No. 00-004-0783. The JCPDS data indicates the melting point of the sample to be 960.6°C. The XRD analysis has been done at Department of Nanoscience and Biotechnology, Shivaji University, Kolhapur, India.

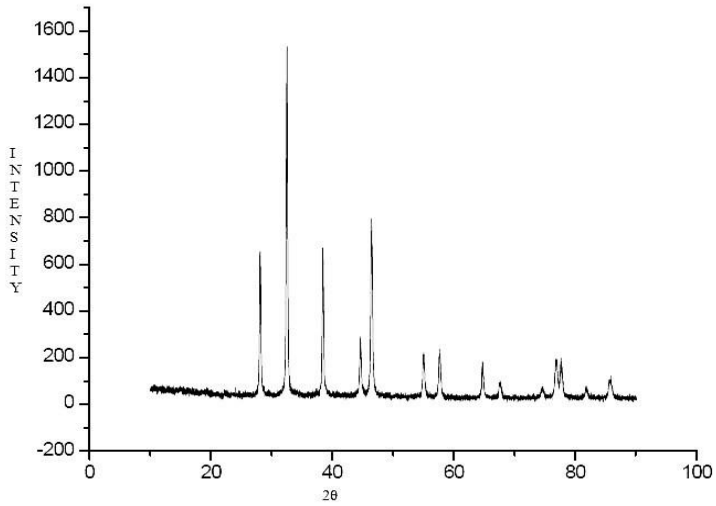

Fig. 2 XRD pattern showing formation of silver nanoparticles

3.3 Dynamic light Scattering

Particle size distribution histogram in Fig. 3 indicates that 10.07% particles size is around 113nm with a standard deviation of 16.48% size while the remaining particles are around 633.5nm with a standard deviation of 20.58%. The difference in size as observed from TEM and PDS may be due to presence of bio active molecules of N. tabacum on AgNPs surface. Prathna et al. found the different particle size of AgNPs by changing the characterization techniques and the particle size order is reported to be DLS> AFM>TEM>XRD and our results are in accordance with this finding. The MATLAB analysis of this Particle size distribution image shows the bit-depth of 24 bits and image resolution of 1024 x 768. Also the image file-size was 124.9KB and image format JPEG with image class as uint8. The particle size distribution histogram is taken from Dr D Y Patil Medical University, Kolhapur, India.

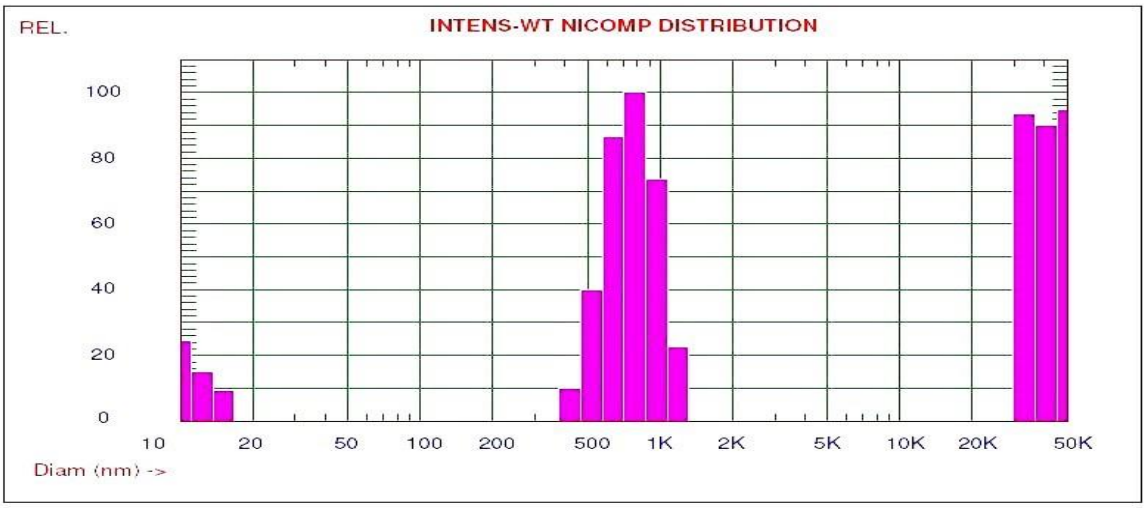

Fig.1 DLS analysis of the synthesized sample

3.4 SEM Image of Silver nanoparticles

Scanning electron microscopy represents a powerful tool for imaging of synthesized nanomaterials with nanoscale spatial resolution. During such measurements the conductive sample is scanned by focused electron beam to study its surface topography. The SEM image from Fig. 4 reveals the formation of cluster of spherical beadlike structure of silver nanoparticles with non-uniform distribution. The MATLAB analysis gives the pixel depth of the image equal to 8 bits and the image resolution of 1280 x 960. Also the image file-size was found to be 1.23MB and the image format as TIFF. The SEM image has been taken with JSM-6360 instrument which uses accelerating voltage of 20KV. The SEM imaging has been done at Department of Physics, Shivaji University, Kolhapur, India.

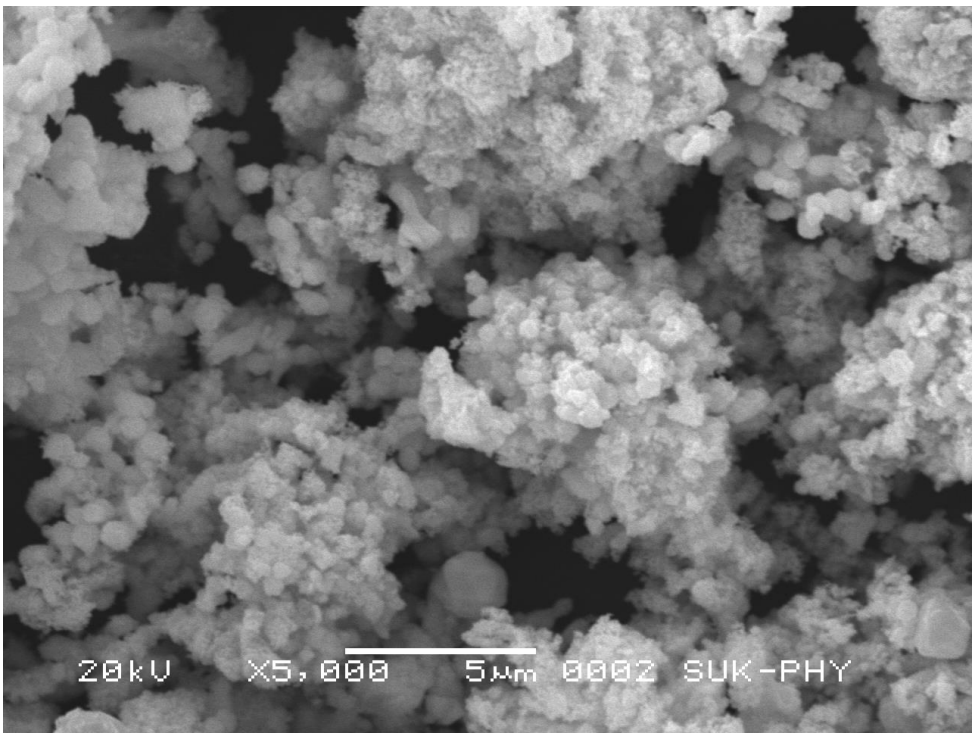

Fig. 4 SEM imaging of synthesized sample

3.5 TEM Imaging of Silver nanoparticles

Transmission electron microscopy (TEM) has highest magnification and resolving power and even atomic level resolution can be achieved. TEM imaging is currently considered as the most accurate method for characterizing the size and shape of nanoparticles. TEM Image from Fig. 5 reveals that there was poly-disperse spherical particles with non-uniform distribution of nanoparticles in the prepared sample. For TEM measurements, a drop of solution containing the particle was deposited on a copper grid covered with amorphous carbon. After allowing the film to stand for two minutes the excess solution was removed by means of blotting paper and the grid allowed drying before the measurement. It was observed that the nanoparticles formed were of different sizes and particle size was found to be 4.74nm, 8.17nm, 14.23nm and 18.98 and the mean size of about 11.5nm which lies in the nano range. The TEM measurement was done with JEOL model 1200Ex instrument operated at an accelerating voltage of 80kV. MATLAB analysis gives the pixel depth of the image equal to 24bits and the image format as JPEG. The TEM Images have been taken from National Chemical Laboratory, Pune, India.

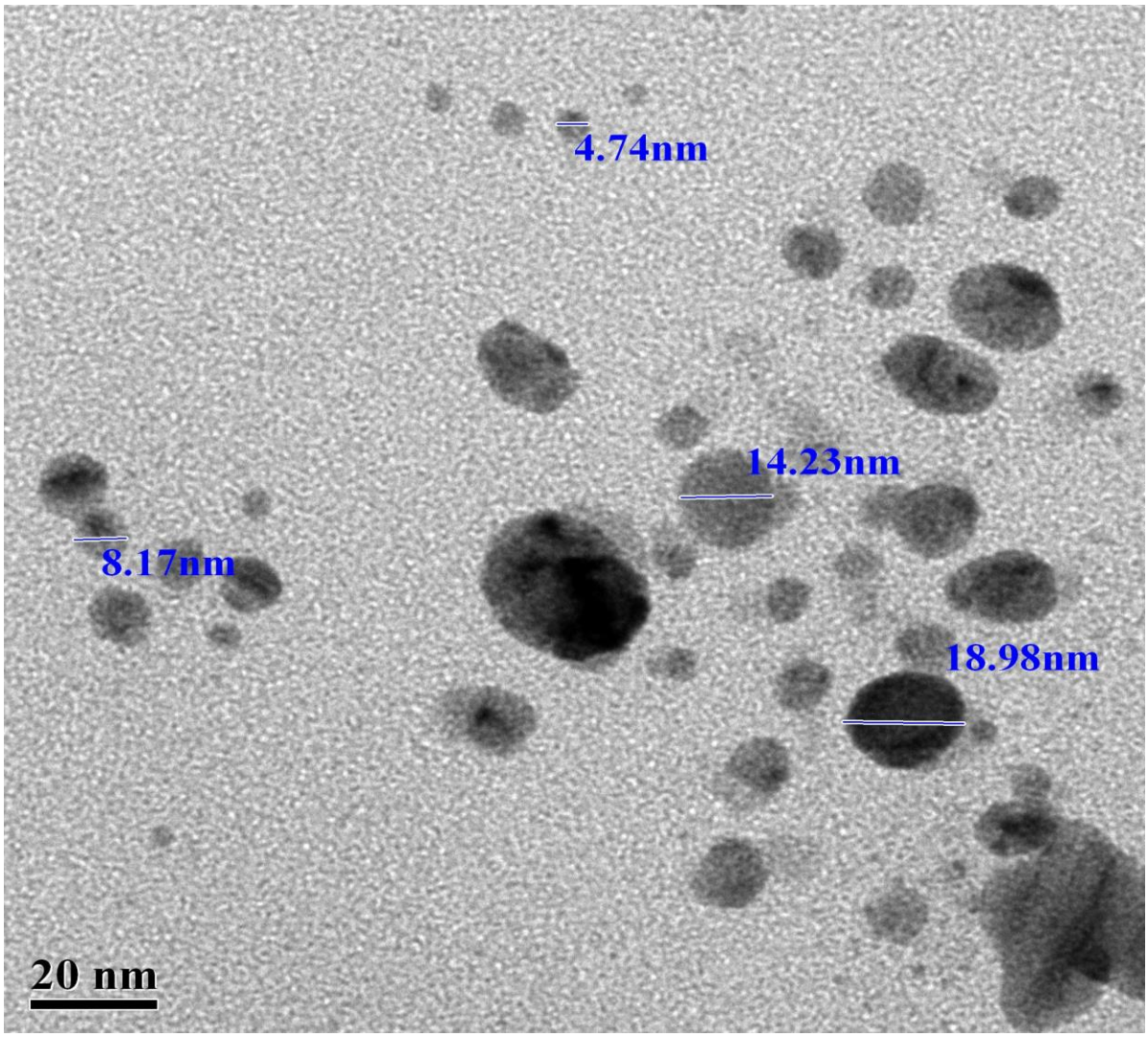

Fig. 5 TEM imaging of synthesized sample showing size of nanoparticles

3.6 EDX Image of Silver nanoparticles

Several types of signals are generated due to excitation of Surface Plasmon Resonance and interaction between the electron beam and the silver nanoparticles which provides both topographical and chemical information about the sample. EDX image of silver nanoparticles shown in Fig. 6 reveal strong signals in silver region of 3KeV which confirms the formation of nano-silver and its elemental nature. EDX analysis have been carried out at Indian Institute of Technology, Kharagpur, India

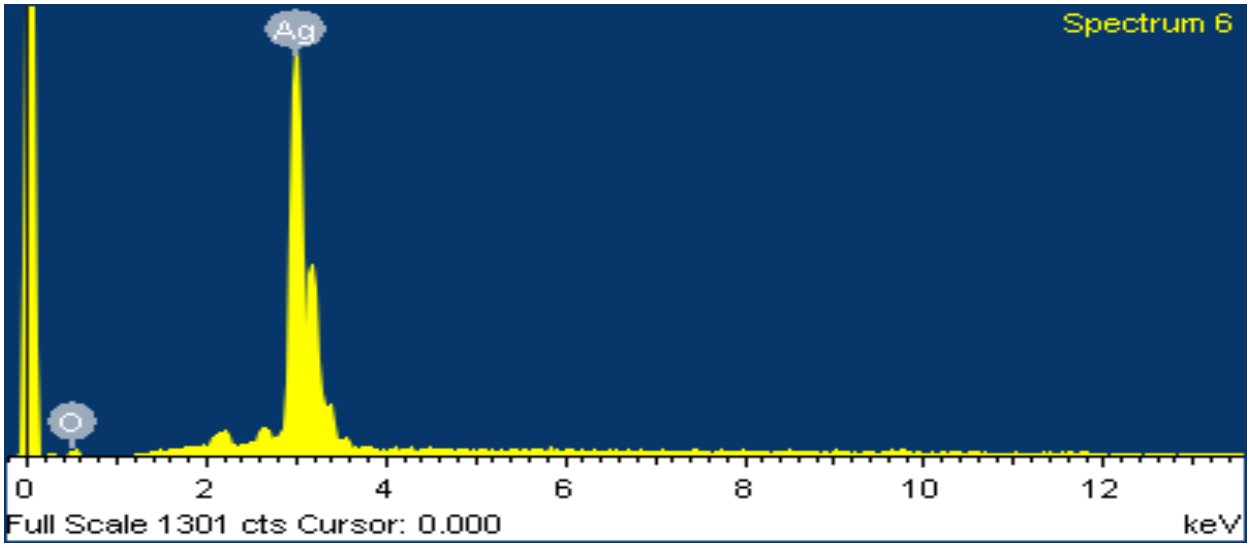

Fig: 6 EDAX analysis of synthesized silver nanoparticles

4. Applications of synthesized AgNPs: The synthesized silver nanoparticles are found to be multi-applicative. The nanoparticles are used for different applications as under

4.1 Antimicrobial Activity of Silver nanoparticles

The antimicrobial activity has been tested against gram-positive and gram-negative bacteria. A Gram-negative bacterium used for experimentation was Salmonella typhimurium. The antimicrobial activity in Fig. 7 showed the well size to be 8mm and zone of inhibition of about 10mm. Also the antimicrobial activity of common narrow spectrum drug Penicilline is observed and is found to be 26mm, but the synergetic study with 1:1 mixture of AgNPs and Penicilline shows a significant increase in antimicrobial activity and the zone of inhibition is found to be 35mm. Thus it can be claimed that these AgNPs are exhibiting excellent anti-microbial property against Salmonella typhimurium also the antimicrobial activity of Penicilline has increased in presence of AgNPs. The image was captured by Sony Cybershot camera and shown in the picture. The MATLAB analysis of this antimicrobial culture image shows that the image format is JPEG with bit-depth of 24 bits, image class is uint8 and image resolution 4000 x 3000 and the file-size is 1.55MB.

4.1.1 Mechanism of Anti-microbial Action:

The exact mechanism of the antibacterial effect of silver ions is not clearly understood. Literature survey reveals that positive charge on silver ion may be responsible for its antimicrobial activity. It is possible that the antibacterial activity is derived through the electrostatic attraction between negatively charged cell membrane of micro-organism and positively charged nanoparticles. It is possible that silver nanoparticles may attach to cell membrane disturbing permeability and respiration function of the cell. Smaller AgNPs have larger surface area available for interaction and would give more bactericidal effect than bigger one. It is also possible that silver nanoparticles may penetrate inside the cell. In the present scenario, AgNPs as antimicrobial agent have come up as promising

candidate in the medical field. There are several theories which explain the action of silver nanoparticles on microbes. Silver nanoparticles have the ability to anchor to the bacterial cell wall and subsequently penetrate it, thereby causing structural changes in the cell membrane like the permeability of cell membrane and death of cell. There is formation of 'pits' on the cell surface followed by accumulation of nanomaterials on the surface of cell. According to another postulate free radicals are formed by silver nano-materials there have been electron resonance spectroscopy studies that suggested that there is formation of free radicals by the silver nano-particle when in contact with bacteria, and these free radicals have the ability to damage the cell membrane and make it porous which will lead cell death. According to another hypothesis, there can be release of silver ions by nanoparticles and these ions can interact with thiol groups of many vital enzymes and inactivate them. The bacterial cells in contact with silver take in silver ions, which inhibit several functions in the cell and damage the cell. Then, there is generation of reactive oxygen species, which are produced possibly through the inhibition of respiratory enzymes by silver ions and attack the cell itself. Silver is a soft acid. There is the natural tendency of acid to react with base. Thus according to Pearson's Rule soft acid silver will preferably react with soft base. The bacterial cells are majorly made up of sulphur and phosphorus which acts as soft bases.

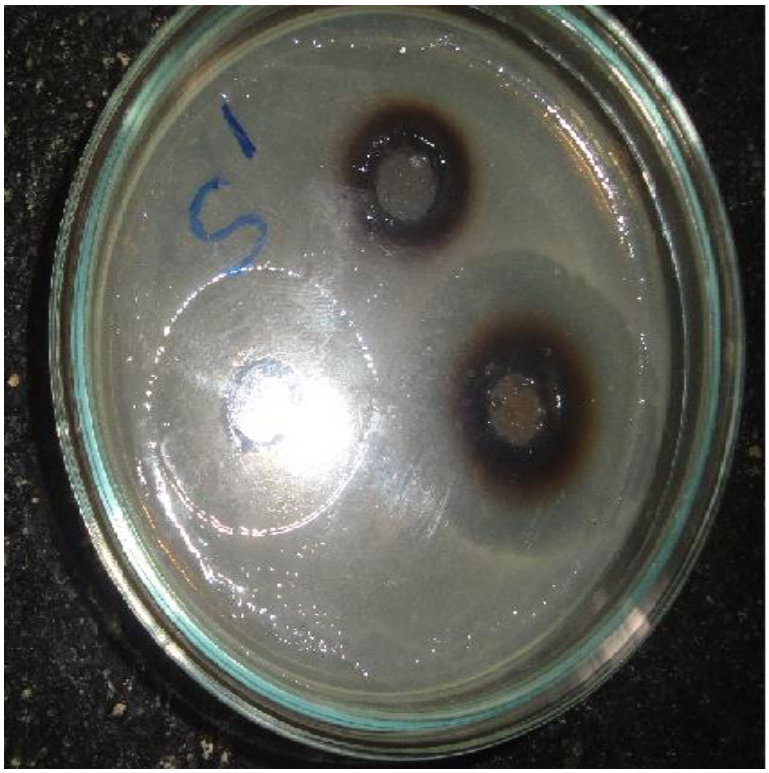

Fig. 7 Antimicrobial activity of Ag nanoparticles

4.2 Photo-catalytic degradation of an azo dye

The usual degradation of dye takes place by photolytic activity but it is a very slow reaction. This

reaction can be speeded-up by using AgNPs which act as Photo-catalyst thereby speeding-up the degeneration of dyes. In the present study, degradation of methylene blue dye was carried out in the presence of silver nanoparticles and the extent of degeneration was observed at different times by measuring the intensity of absorbance peak. The absorption spectrum showed the continuous decrease in intensity of peaks. As observed in Fig 8, the intensity of absorption peak for methylene blue dye at 660nm decreased continuously with progress of time and disappears almost completely within 120 minutes. This indicates that methylene-blue dye can be completely degraded by using the AgNPs as a catalyst.

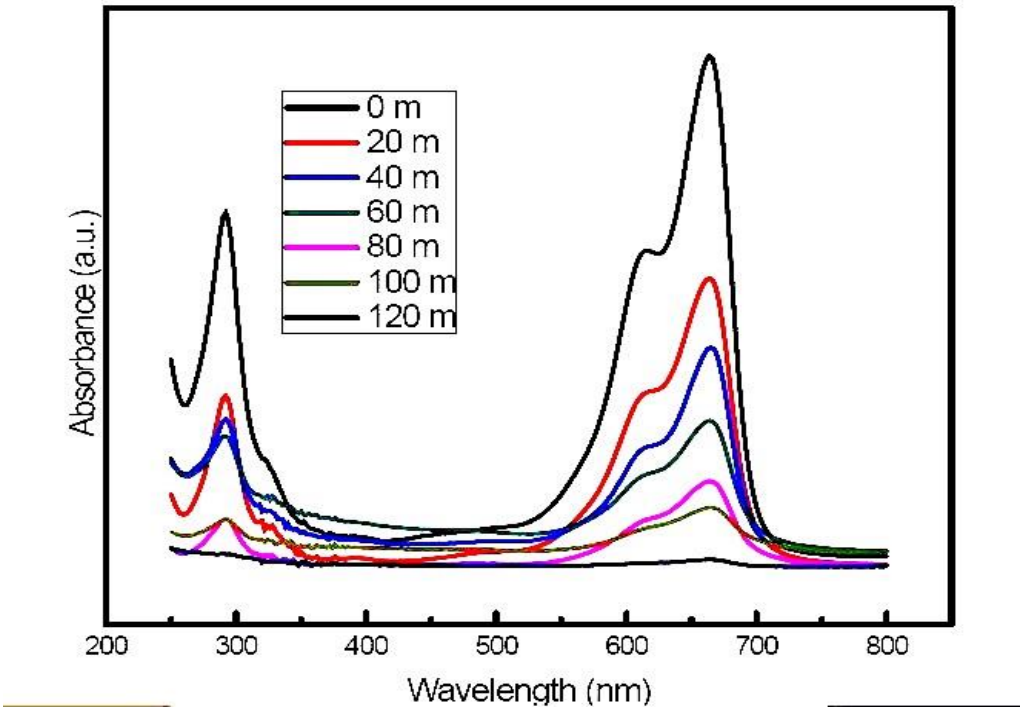

Fig. 8 UV-vis absorbance spectra for Ag catalyzed methylene blue dye degradation

4.3 Organic Synthesis

The silver nanoparticles find a wide range of applications in organic synthesis reactions. Traditionally Pyridines with 2,4,6-triaryl substitution pattern have been synthesized through reaction of N-phenacylpyridinium salts with α, β-unsaturated ketones in the presence of ammonium acetate. A multi-component reaction for the synthesis of organic compound 4-(4-nitrophenyl)-2,6diphenyl pyridine has been catalyzed by silver nanoparticles. This organic synthesis in un-catalyzed organic reaction takes place at higher temperature, but in presence of silver nanoparticles this reaction is possible at room temperature. The use of this heterogeneous catalyst in liquid phase over homogeneous one offers several advantages such as easy recovery, recycling with adequate efficiency, and enhanced stability. The reaction is taking place in alcoholic medium. The synthesized organic compound acts as a bactericide.

The NMR spectra of the synthesized organic compound as shown in fig. 9 confirms the formation of 4-(4-nitrophenyl)-2,6diphenyl pyridine.

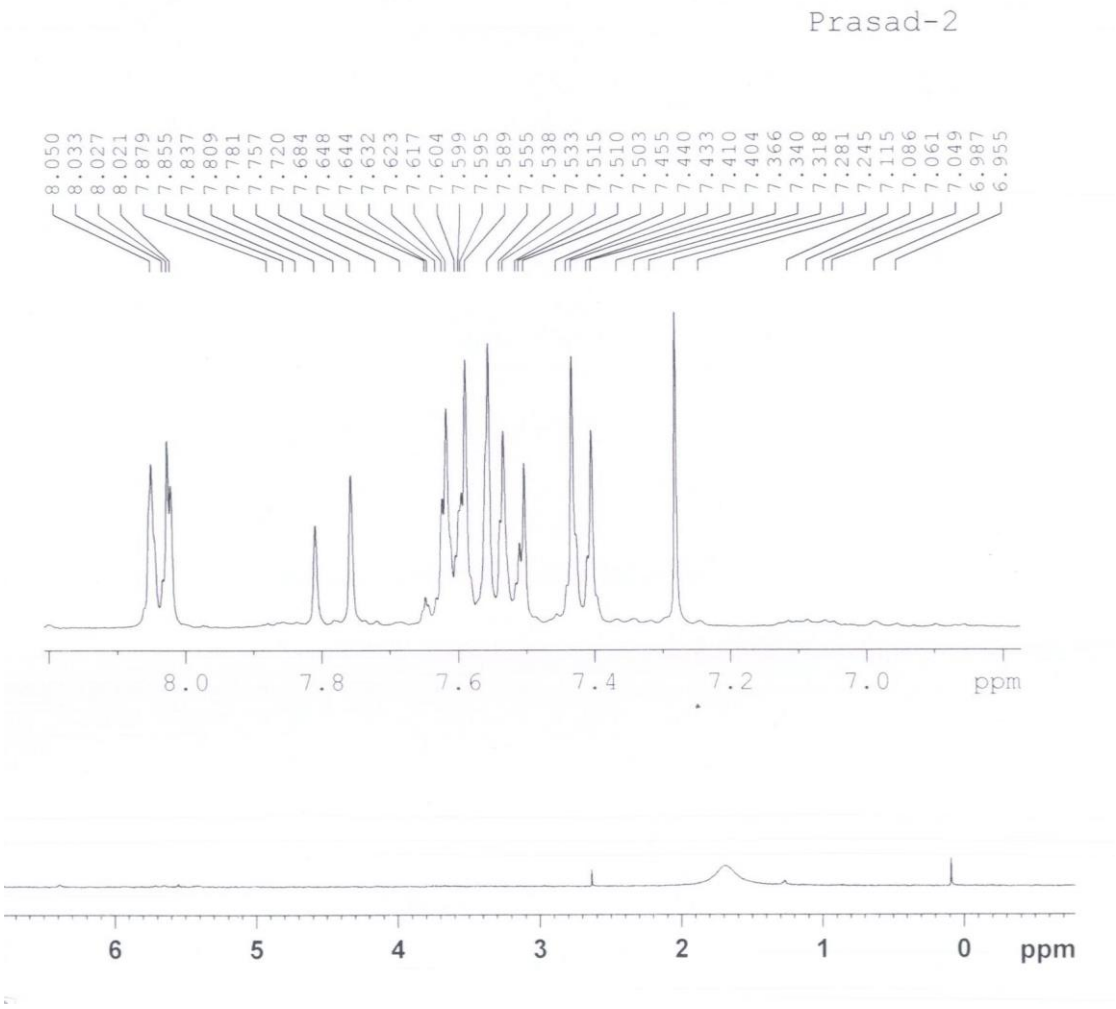

Fig.9 NMR spectra of synthesized organic compound

4. CONCLUSION

The use of natural products for green synthesis of nanoparticles is an emerging and exciting area of nanotechnology. The green synthetic method reported here is easy, economical and eco-friendly way to synthesize metallic nanoparticles at room temperature. Moreover, this plant assistedbio-synthesis represents a considerable improvement over other routes of synthesis such as reduced reaction time, no need of capping agent, and better control over size and shape. The rate of production of nanoparticles, and their characteristics depends upon several factors such as the nature of plant extract, its concentration, concentration of metal salt, pH, temperature, contact time etc. The synthesized AgNPs exhibited significant antimicrobial activity against gram-negative bacterium *Salmonella*

typhimurium. The synthesized nanoparticles alongwith routinely used antibiotics showed improved antimicrobial activity, which claims its synergistic effectiveness. Hence AgNPs have great potential in the preparation of antibacterial drugs. The synthesized AgNPs were successfully used to catalyze organic synthesis reaction. Similarly, faster dye degradation was found to take place under catalytic presence of AgNPs.

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