



## Biosynthesis of Nanoparticles : A Review

Kapil Punjabi<sup>1</sup>, Pallavi Choudhary<sup>2\*</sup>, Lalit Samant<sup>2</sup>, Sandeepan Mukherjee<sup>3</sup>, Shashikant Vaidya<sup>1</sup>, Abhay Chowdhary<sup>3</sup>

<sup>1</sup>Department of Clinical Pathology, Haffkine Institute for Training, Research and Testing, Mumbai, Maharashtra, India.

<sup>2</sup>Department of Zoonosis, Haffkine Institute for Training, Research and Testing, Mumbai, Maharashtra, India.

<sup>3</sup>Department of Virology, Haffkine Institute for Training, Research and Testing, Acharya Donde Marg Parel, Mumbai, India.

\*Corresponding author's E-mail: [kurkurep@gmail.com](mailto:kurkurep@gmail.com)

Accepted on: 06-11-2014; Finalized on: 31-12-2014.

### ABSTRACT

Nanotechnology has now advanced into a phase where application of nanoparticles in various domains of science and technology is being cited. At the same time employment of biosynthetic methods for synthesizing nanoparticles, have drawn pronounced attention of researchers. And is now, being looked forward as a simple and viable alternative against conventional physical and chemical methods. Under the biological realm range of entities from unicellular microbes to multicellular plant structures are reported to synthesize nanoparticles. This review, attempts to consolidate the substantial data reported and to generalize the methodologies & mechanisms employed for the synthesis of nanoparticles using various microbes and plant extracts; and, highlight key factors that lead to maximum production of size and shape controlled nanoparticles with these bio-reduction processes.

**Keywords:** Nanoparticles, biosynthesis, plant extracts, methodology, mechanism

### INTRODUCTION

Nanotechnology is a field that is burgeoning, making an impact in all spheres of human life. A number of approaches are available for the synthesis of nanoparticles mainly chemical synthesis like reduction in solutions, chemical and photochemical reactions in reverse micelles, physical synthesis like thermal decomposition, radiation assisted, electrochemical, sono-chemical, microwave assisted process and recently via green chemistry route or biological synthesis using plants, bacteria, fungi etc. The use of environmentally benign materials like plant leaf extract, bacteria, fungi and enzymes for the synthesis of nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol.<sup>1</sup> Most of the chemical and physical methods of nanoparticle synthesis can control the size and shape of nanoparticles. Therefore, a biological process with the ability to do the same has been an exciting prospect.

In the current scenario, importance is being given to developing environmentally benign technologies in material synthesis for the fabrication of a range of nanomaterials. Due to their minute structure and characteristic crystallographic nature of nanoparticles its surface area increases, thereby increasing its reactivity. The importance of biological synthesis is being emphasized globally at present because chemical methods are capital intensive, toxic, non eco-friendly and have low productivity. Potential biological systems from plants or microbes are being used for biosynthesis of nanoparticles. The synthesis of inorganic materials may occur either extracellularly or intracellularly. Exposure to varying temperature, pH and substrate concentration

influences, directly or indirectly, the rate of nanoparticles fabrication. It is important to understand the biosynthetic mechanism involved in the fabrication of metal nanomaterials mediated by a biological system in order to gain better control of the process and products. So far, little is known about the interaction between biomolecules and nanoparticles, though several analyses have been made.<sup>2</sup>

It is a need of today's nanotechnology to develop reliable, non-toxic, clean and eco-friendly experimental protocols for the synthesis of metal nanoparticles of controlled size, shape and monodispersity, which is possible through ambient biological resources. It is further necessary to elaborate this technology in a consolidated way with an approach that provides an overview of the current trend of research on the biosynthesis of nanoparticles for their further applications.<sup>2</sup>

### Synthesis of Metal Nanoparticles by Microorganisms

Microorganisms like bacteria, fungi, yeast and algae most of them are capable of synthesizing nanoparticles. The fundamental principle of synthesis is reduction of metal ions to nanoparticles.<sup>3</sup> Various microbes have been reported for synthesizing silver, gold and other metal nanoparticles. Silver and gold have received considerably more attention compared to others. Nanoparticles of these metals have found applications in various fields and are biocompatible having low toxicity. This makes them excellent drug delivery systems and carrier material for sensors in diagnostic tools.<sup>4</sup>

The first report of bacteria synthesizing silver nanoparticles was back in 1984 when *Haefeli* reported *Pseudomonas stutzeri* AG259, a bacterial strain originally isolated from silver mine capable of synthesizing silver nanoparticles.<sup>5</sup> A comprehensive list of microorganism



reported to synthesize nanoparticles is compiled in Table - 1.

**Table 1:** List of microorganism reported for synthesis of nanoparticles

Bacteria				
Sr. No.	Microorganism	Nanoparticle	Size in nm	Reference
1	<i>Pseudomonas stutzeri</i> AG259	Silver	200	5
2	<i>Lactobacillus Strains</i>	Silver	500	5
3	<i>Bacillus megaterium</i>	Silver	46.9	5
4	<i>Klebsiella pneumonia</i>	Silver	50	5
5	<i>Bacillus licheniformis</i>	Silver	50	5
6	<i>Corynebacterium sp.</i>	Silver	10-15	5
7	<i>Bacillus subtilis</i>	Silver	5-60	5
8	<i>Geobacter sulfurreducens</i>	Silver	200	5
9	<i>Morganella sp.</i>	Silver	20±5	5
10	<i>Escherichia coli</i>	Silver	1-100	5
11	<i>Proteus mirabilis</i>	Silver	10-20	5
12	<i>Bacillus sp.</i>	Silver	5-15	5
13	<i>Bacillus cereus</i>	Silver	4-5	5
14	<i>Staphylococcus aureus</i>	Silver	1-100	5
15	<i>Lactic acid bacteria</i>	Silver	11.2	5
16	<i>Brevibacterium casei</i>	Silver	50	5
17	<i>Enterobacter cloacae</i>	Silver	50-100	6
18	<i>Proteus mirabilis</i>	Silver	10-20	6
19	<i>Pseudomonas stutzeri</i>	Copper	50-150	7
20	<i>Bacillus subtilis</i>	Gold	5-25	8
21	<i>Pseudomonas aeruginosa</i>	Gold	15-30	8
22	<i>Rhodococcus sp.</i> (Actinomycete)	Gold	5-15	8
23	<i>Thermoanaerobacter ethanolicus</i>	Magnetite	36-65	8
24	<i>Magnetospirillum magnetotacticum</i>	Magnetite	50-100	8
25	<i>Rhodopseudomonas capsulate</i>	Gold	10-20	9
26	<i>Corynebacterium glutamicum</i>	Silver	5-50	9
27	<i>Ureibacillus thermosphaericus</i>	Gold	50-70	9
28	<i>Pyrobaculum islandicum</i>	Cobalt	-	9
29	<i>Desulfovibrio desulfuricans</i>	Lead	50	9
30	<i>Nocardia farcinica</i>	Gold	15-20	10
Fungi				
Sr. No.	Microorganism	Nanoparticle	Size in nm	Reference
1	<i>Pseudomonas stutzeri</i> AG259	Silver	200	5

2	<i>Lactobacillus Strains</i>	Silver	500	5
3	<i>Bacillus megaterium</i>	Silver	46.9	5
4	<i>Klebsiella pneumonia</i>	Silver	50	5
5	<i>Bacillus licheniformis</i>	Silver	50	5
6	<i>Cladosporium cladosporioides</i>	Silver	10-100	5
7	<i>Fusarium semitectum</i>	Silver	10-60	5
8	<i>Trichoderma asperellum</i>	Silver	13-18	5
9	<i>Trichoderma viride</i>	Silver	5-40	5
10	<i>Penicillium fellutanum</i>	Silver	1-100	5
11	<i>Penicillium brevicompactum</i> WA2315	Silver	23-105	5
12	<i>Fusarium solani</i>	Silver	5-35	5
13	<i>Fusarium acuminatum</i>	Silver	5-40	5
14	<i>Aspergillus clavatus</i>	Silver	10-25	5
15	<i>Verticillium sp.</i>	Silver	25	6
16	<i>Phoma sp.</i> 32883	Silver	75	6
17	<i>Coriolus versicolor</i>	Silver	350-600	6
18	<i>Fusarium oxysporum and Verticillium sp.</i>	Magnetite	20-50	8
19	<i>Fusarium oxysporum</i>	Gold	20-40	8
20	<i>Fusarium oxysporum</i>	Cadmium Sulfide	5-20	8
21	<i>Fusarium oxysporum</i>	Zirconia	3-11	8
22	<i>Colletotrichum sp.</i>	Gold	20-40	8
23	<i>Yarrowialia polytica</i>	Gold	15	9
24	<i>Phaenerochaete chrysosporium</i>	Silver	50-200	9
25	<i>Neurospora crassa</i>	Gold	32	9
Yeast				
Sr. No.	Microorganism	Nanoparticle	Size in nm	Reference
1	<i>Candida glabrata</i>	Cadmium sulfide	2	8
2	<i>Schizosaccharomyces pombe</i>	Cadmium sulfide	1-1.5	8
3	<i>MKY-3</i>	Silver	2-5	8
4	<i>Candida albicans</i>	Silver	50-100	9
Algae				
Sr. No.	Microorganism	Nanoparticle	Size in nm	Reference
1	<i>Shewanella algae</i>	Gold	10-20	9
2	<i>Sargassum wightii</i>	Gold	8-12	9
3	<i>Shewanella oneidensis</i>	Gold	12±5	9
4	<i>Plectonema boryanum</i>	Gold	25-100	9
5	<i>Plectonema boryanum</i>	Silver	1-200	9

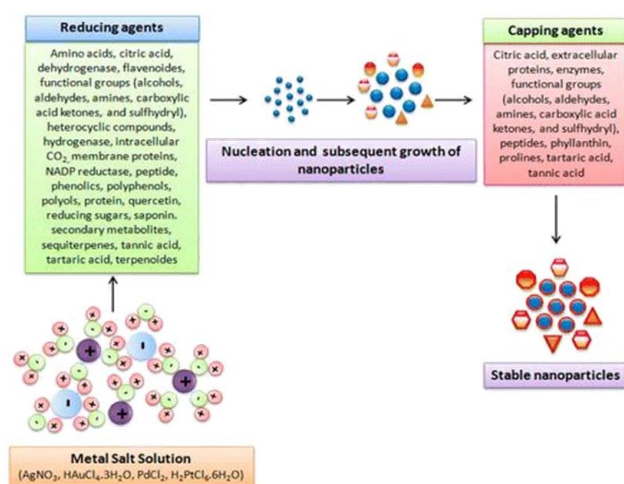


## Plant Mediated Synthesis of Metal Nanoparticles

Plant mediated synthesis of metal nanoparticles is gaining importance owing to its simplicity, rapid rate of synthesis of nanoparticles of diverse morphologies and elimination of elaborate maintenance of cell cultures and eco-friendliness.<sup>11</sup>

The mechanism for synthesis of nanoparticles in principle remains same for microorganisms and plants both. Metal salts comprising of metal ion is first reduced to atoms by means of a reducing agent. The obtained atoms then nucleate in small clusters that grow into particles.

Shanker have reported the presence of proteins and secondary metabolites in the water-soluble fractions of geranium leaves and postulated that terpenoids contributes to the reduction of silver ions and oxidized to carbonyl groups. Fourier transfer infrared spectroscopy (FTIR) analysis of the study suggested ester C=O group of chlorophyll acting as a reducing agent and a protein involved in the surface capping of gold nanoparticles synthesized using geranium leaf extract.<sup>12, 13</sup>



**Figure 1:** Flow chart for mechanism of nanoparticle synthesis.<sup>14</sup>

There is growing interest in the synthesis of metal nanoparticles by 'green' methods, biomass or extracts of different plants have been tried with success as reducing agents.<sup>15</sup>

Certain medicinal plants have also been reported for synthesis of silver nanoparticles.<sup>16</sup> A list of some plants reported for synthesis of nanoparticles is compiled in Table - 2.

### Proposed Mechanisms for Biosynthesis of Nanoparticles

Although lot of reports for synthesis of nanoparticles using the biological route are available very few have data for understanding the exact mechanism for the same.

A generalized interpretation is, involvement of proteins like enzymes and cofactors that have redox potential as well as act as electron shuttles play key role in metal reduction.

**Table 2:** List of plants reported for synthesis of nanoparticles.

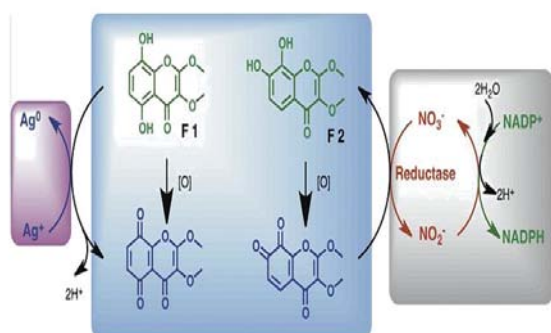
Leaves				
Sr. No.	Plant	Nanoparticle	Size in nm	Reference
1.	<i>Argemone maxicana</i>	Silver	30	15
2.	<i>Acalypha indica</i>	Silver	20-30	18
3.	<i>Mangifera indica</i>	Silver	20	19
4.	<i>Cassia fistula</i>	Silver	50-60	20
5.	<i>Catharanthus roseus</i>	Silver	48-67	21
6.	<i>Doiropyros kaki</i>	Platinum	2-12	22
7.	<i>Clerodendrum inerme</i>	Silver	5-60	23
8.	<i>Gardenia jasminoides</i>	Palladium	3-5	24
9.	<i>Murraya koenigii</i>	Silver & Gold	10-25	25
10.	<i>Aloe vera</i>	Silver & Gold	15.2	26
11.	<i>Piper betle</i>	Silver	3-37	27
12.	<i>Ocimum tenuiflorum</i>	Silver	25-40	28
13.	<i>Coleus aromaticus</i>	Silver	44	29
14.	<i>Apiin(henna leaves)</i>	Silver & Gold	21&39	30
15.	<i>Camellia sinensis</i>	Gold	40	31
16.	<i>Eucalyptus camaldulensis</i>	Gold	5.5 – 7.5	32
17.	<i>Pelargonium</i>	Gold	5.5 – 7.5	32
18.	<i>Azadirachta indica</i>	Gold	5.5 – 7.5	32
19.	<i>Glycine max</i>	Palladium	15	33
Fruit				
Sr. No.	Microorganism	Nanoparticle	Size in nm	Reference
1.	<i>Emblica officinalis</i>	Silver & Gold	10-20&15-25	34
2.	<i>Carica papaya</i>	Silver	15	35
3.	<i>Tanacetum vulgare</i>	Silver & Gold	16&11	36
4.	<i>Musa paradisiaca</i>	Palladium	50	37
Flower				
Sr. No.	Microorganism	Nanoparticle	Size in nm	Reference
1.	<i>Nyctanthes arbortristis</i>	Gold	19.8	38
Bark				
1.	<i>Cinnamon zeylanicum</i>	Silver	31 – 40	39
2.	<i>Pinus resinosa</i>	Palladium	16 – 20	40
3.	<i>Pinus resinosa</i>	Platinum	6 – 8	40
4.	<i>Cinnamom zeylanicum</i>	Palladium	15 – 20	41
Seed				
1.	<i>Jatropha curcas</i>	Silver	15 – 50	42
2.	<i>Syzygium cumini</i>	Silver	73 – 92	43
3.	<i>Cuminum cyminum</i>	Gold	1 – 10	44

In a recent study, biosynthesis of silver nanoparticles by *Bacillus stratophericus* spores nitrate reductase, laccase and catalase were assayed to elucidate the mechanism.

The enzymatic activity of nitrate reductase was nil and that of laccase and catalase were found to be negligible. Further experimentation revealed production of dipicolinic acid (DPA) during the process of sporulation and varying concentrations of DPA affected the synthesis of silver nanoparticles. The hypothesis was confirmed by using standard DPA for synthesis of nanoparticles. The reduction of metal by DPA having two carboxylic groups was effective as carboxylic acid groups in acetic acid are known for formation of silver nanoparticles. Thus, the study proposed dipicolonic acid moiety as main mechanism for production of silver nanoparticles by *Bacillus stratophericus* spores.<sup>45</sup>

Jain take a step ahead in interpreting the mechanism by studying the protein profile of the cell free filtrate. The SDS – PAGE profile revealed presence of two extracellular proteins of 32 and 35 kDa found to be responsible for synthesis and stability of silver nanoparticles. These results were affirmed by the UV – Vis spectroscopy and FTIR analysis of the cell free filtrate. Although the proteins have not been identified and characterized which is reportedly underway, results of same will definitely clarify and significantly help in understanding the mechanism of synthesis of silver nanoparticles by the fungal strain used in the study.<sup>46</sup>

Recently Mittal have employed following strategies in order to investigate the mechanism behind the formation of AgNPs using *Syzygium cumini* fruit extract. They did solvent partition of *S. cumini* fruit extract using water, hexane, ethyl acetate (EtOAc), chloroform and dichloromethane (DCM). Upon solvent partition studies, the order of the yield of AgNPs was found to be as follows: fraction EtOAc > fraction chloroform > fraction DCM > fraction water > fraction hexane. Hence they have used Ethyl acetate fraction for column chromatography followed by purification using TLC preparative plate. Among several isolated fractions, two relatively non polar pure fractions (eluted using hexanes/ethyl acetate 9:1) produced good yield of AgNPs. Upon FTIR, NMR, GC–MS and HR-MS analysis, the fractions revealed presence of the members of flavonoids family (molecules F 1 and F 2) as major components which were mainly responsible for the synthesis of nanoparticles.



**Figure 2:** Prospective mechanism of silver nanoparticle biosynthesis (structures of isolated flavonoid molecules are shown) by the fruit extract of *Syzygium cumini*.<sup>47</sup>

They have hypothesized the mechanism showing the reduction of cationic silver to metallic silver by the flavonoid compounds through redox reaction. Additionally, biomolecules from fruit extracts were responsible for the aggregation and stabilization of the AgNPs as FTIR spectra of both fruit extract and AgNPs showed the presence of similar functionalities.<sup>47</sup>

## Common Methodologies for Synthesis of Metal Nanoparticles using Microbes

### Extracellular Mechanism

The test strain (culture) is grown in suitable media and incubated on orbital shaker at 150 rpm at 37°C. After incubation the broth is centrifuged and the supernatant is used for synthesis of nanoparticles. The supernatant is added to separate reaction vessels containing the metal ions in suitable concentrations and incubated for a period of 72 h.

The colour change of the reaction mixture suggests the presence of nanoparticles in the solution, and bio reduction of silver ions in the solution is monitored by sampling the aqueous solution and measuring the absorption spectrum using a UV-Visible spectrophotometer.

The morphology and uniformity of silver nanoparticles are investigated by X-ray diffraction (XRD) and Scanning electron microscopy (SEM). While the interaction between protein and silver nanoparticles (AgNPs) are analyzed using Fourier transform infrared spectroscopy (FTIR).<sup>48</sup>

### Intracellular Mechanism

The culture is grown in suitable liquid media incubated on shaker at optimal temperature. After incubation the flask is kept at static condition to allow the biomass to settle following which the supernatant is discarded and sterile distilled water is added for washing the cells. The flask is kept steady for 30 minutes to settle the biomass post which the supernatant is again discarded. This step is repeated for three times. The biomass is then separated from the sterile distilled water by centrifugation for 10 minutes.

The wet biomass is exposed to 50 ml of sterilized aqueous solution of metals at various dilutions and incubated on shaker at suitable temperature till visual colour change is observed. The change in colour from pale yellow to brownish colour indicates the formation of silver nanoparticles, pale yellow to pinkish colour indicates the formation of gold nanoparticles and the formation of whitish yellow to yellow colour indicates the formation of manganese and zinc nanoparticles.<sup>49</sup>

## Methodology for Synthesis of Metal Nanoparticles using Plant Extracts

Fresh leaves of plant are collected from the pesticide and pollution free area. The leaves are washed thoroughly with the tap water twice and atleast once with the



distilled water. Either fresh or dried leaves are taken for extract preparation. Different solvents can be used for the preparation of extracts in which our phytochemical of interest can be extracted well. These extracts are used for the synthesis of metal nanoparticles. Different concentrations of the metal ion solutions are challenged with the plant extracts at 37°C under agitation at 150rpm for 24-48hrs. The colour change indicate the synthesis of nanoparticles. The reduction of metal ions is monitored by measuring the UV-Vis spectrum of the reaction medium.

Plant extracts are known to have various reducing agents and stabilizing agents that play key role in the nanoparticle synthesis. The nature of plant extract affects the type of nanoparticles synthesized to a great extent. With particularly the source of plant extract being the most vital factor affecting the morphology of synthesized nanoparticles. Also, this is so because different plant extracts contain different concentrations of biochemical reducing agents. The change in concentration of biochemical reducing agents differs regionally as well as seasonally in most plant extracts. This variation will lead to difference in nanoparticle synthesized in every batch.<sup>50</sup>

To overcome this difficulty researchers have now progressed to different level in plant mediated synthesis of nanoparticles, with involvement of plant tissue culture. Successful development of silver nanoparticles, from *in vitro* plant and callus culture has been reported from *Costus speciosus*.<sup>51</sup>

The reason for plant derived nanoparticle synthesis remains less popular for bulk production is that plant cell culture is relatively difficult as compared to microbial cultures and imparts to complicity of the process.<sup>50</sup>

Thus scaling up of nanoparticle synthesis for bulk production definitely requires employment of such methods wherein, stock culture of reducing agent plant or microbe is available persistently. And, to avoid batch to batch variation in nanoparticle morphology development of such *in vitro* methods are prerequisite.

### Factors Affecting Synthesis of Nanoparticles

Shape and size of nanoparticles depends on the physical and chemical factors. The optimum metal ion concentration, pH and temperature of reaction mixture play key role in nanoparticles synthesis.

#### Concentration of Metal Ion

Increasing the concentration of silver ions from 1mM to 5 mM in reaction mixture. Revealed, that in 1 mM concentration the nanoparticles synthesis and size reduction started quickly due to more availability of functional groups in the extract as per *Vanaja*. While increasing the substrate concentration the large size and aggregation of nanoparticles occurred due to the occurrence of compete between silver ions and functional groups.<sup>52</sup>

#### pH

pH plays an important role in the nanoparticles synthesis, this factor induces the reactivity of extract with silver ions. In a study reported, alkaline pH 8.2 showed a sharp peak at 460 nm with maximum production of AgNPs. The sharp peak indicated formation of spherical shape of silver nanoparticles. Thus indicating alkaline pH is more suitable for synthesis of silver nanoparticles.<sup>10</sup> Several reports propose pH plays role in shape and size control in nanoparticle synthesis. Another report suggests increase in absorption while a decrease in pH and indicated the production of bigger particles with decrease in pH.<sup>53</sup>

#### Temperature

Temperature is one of the important physical parameter for synthesis of nanoparticles. Synthesis of nanoparticles increases while increasing the reaction temperature. The higher rate of reduction occurs at higher temperature due to the consumption of metal ions in the formation of nuclei whereas, the secondary reduction stops on the surface preformed nuclei. The broadening peak obtained at low temperature shows formation of large sized nanoparticles and the narrow peak obtained at high temperature, indicates the nanoparticles synthesized are smaller in size. Thus, it can be established that higher temperature is optimum for nanoparticles synthesis.<sup>52</sup>

#### Time

In a study, synthesis of nanoparticles at various time intervals was studied after reaction for 1 hour, the Ag-NPs obtained showed a UV-vis absorption peak, the intensity of the peak increased as the reaction time increased, which indicated the continued reduction of the silver ions. The increase of the absorbance with the reaction time indicates that the concentration of AgNPs increases. When the reaction time reached 3 hours the absorbance increased, and the  $\lambda_{max}$  value was slightly shifted. This phenomenon continued for reaction times of 6 and 24 hours, indicating that the size of particles was decreased. At the end of the reaction i.e. 48 hours the absorbance was considerably increased and there was no significant change in  $\lambda_{max}$  (430 nm), compared with the 24 hour reaction time. The Transmission electron microscopy (TEM) results indicate that the samples obtained over a longer time period retained a narrower particle size distribution; the average size of all prepared AgNPs was 20nm.<sup>54</sup>

### Characterization of Nanoparticles

#### To determine the size, shape and conformity of nanoparticle synthesized

##### SEM

The scanning electron microscope images the sample surface by scanning it with a high energy beam of electrons. When the beam of electrons strikes the surface of the specimen and interacts with atoms of sample, signals in form of secondary electrons, back scattered



electrons and characteristic X-rays are generated that contain information about sample's surface topography, composition etc.<sup>55</sup>

Advantages of SEM are its two dimensional imaging, ease of sample preparation and provision of digital data forms. Its limitations are improper sample preparation can lead to confusion between artifacts and actual data. And obvious limitations are size, cost and maintenance.

### **TEM**

In TEM the crystalline sample interacts with electron beam mostly by diffraction rather than by absorption. The intensity of diffraction depends on orientation of planes of atoms in a crystal. This produces a variation in the electron intensity that reveals information of the crystal structure. Along with distribution & dispersion, exfoliation, intercalation & orientation of nanoparticles can also be visualized using a TEM micrograph.<sup>55</sup>

Advantages of TEM include high quality, detailed and powerful magnification of element and compound structures. Its limitations are laborious sample preparation, artifacts from sample preparation and definitely large and expensive.

### **HRTEM**

High resolution transmission electron microscope (HRTEM) is an imaging mode of TEM that allows imaging of crystallographic structure of samples at an atomic scale. In HRTEM electron wave after interacting with sample undergoes phase change and interacts with image wave in the imaging plane. Thus, individual atoms and crystalline defects can be imaged clearly using HRTEM.<sup>55</sup>

### **AFM**

Atomic force microscopy is ideal for qualitatively measuring surface roughness and visualizing the surface of nanoparticles. It has a very high three dimensional spatial resolution. The surface of the sample is scanned using a probe and the oscillation amplitude is used to measure the surface characteristics of the sample.<sup>55</sup>

Advantages are AFM provides higher resolution than SEM. It gives true atomic resolution comparable scanning tunneling microscopy and transmission electron microscopy.

Limitations include single scan image size, AFM cannot scan images as fast as a SEM and image artifacts.

### **XRD**

X-ray diffraction data provides information about crystallinity, crystallite size, orientation of the crystallites and phase composition and aid in molecular modeling to determine the structure of the material.<sup>55</sup>

Advantages of XRD are simplicity of sample preparation, rapidity of measurement, analyze mixed phases and determine sample purity. Its limitations are requirement

of homogenous and powdered material, peak overlays lead to unclear data.

### **DLS**

Dynamic light scattering is a well established technique for measuring the size of molecules and particles. The fluctuation in the intensity of the scattered light from laser illuminated particles is size dependent and hence, size of particles can be analyzed. Thus, size and size distribution of particles can be studied by DLS.<sup>55</sup>

Advantages include measurement of particle sizes of 1 nm, precision of  $\pm 1\%$ , repeatable analysis, no sample preparation & liquid sample. Its limitations are low resolution of polydisperse samples and multiple light scattering.

### **To determine the functional group of nanoparticle synthesized**

#### **EDX**

This technique is used in conjugation with SEM. The characteristic X-rays used to identify composition of sample by a technique known as Energy Dispersive X-ray (EDX) thus, giving an overall mapping of sample.<sup>55</sup>

Advantages of EDX are it improves quality control and helps in process optimization, identification of contaminant, gives higher production yield. Its limitations are quantitative analysis requires standards of known composition and that fluorescence of emitted x-rays limit the precision.

#### **UV-Vis Spectroscopy**

Metal nanoparticles scatter optical light because of collective resonance of the conduction electrons in the metal known as surface plasmon resonance (SPR). This SPR peak is shown in UV absorption spectra by these nanoparticles. The magnitude of peak, wavelength and spectral bandwidth associated with nanoparticles are dependent on size, shape and material composition.<sup>55</sup>

Advantages of UV-Vis spectroscopy are rapid means of analysis, it provides very high precision and accuracy, useful for a wide variety of chemicals and can be used both quantitatively and qualitatively. Its limitation is non selective for compounds if they absorb at the same wavelength.

#### **FTIR**

Fourier transform infrared spectroscopy gives data of proteins and other compounds present in the mixture that interact with metal ions. The identification of functional groups leads to determine the reducing agent and the capping agent responsible for synthesis and stability of nanoparticles.<sup>48</sup>

Advantages of FTIR are identifying and detecting changes in protein secondary structures, can resolve between similar components. Its limitations are overlapping peaks



makes it difficult to distinguish, difficult to quantify, better results with solid components.

## CONCLUSION

A large number of biological entities are being reported for synthesis of nanoparticles, it can be projected that in future a biosynthetic mode of nanoparticle synthesis will be largely accepted. And, can become potential bio-factories for attaining the enormous demand of nanoparticles for its various applications. Scaling up the protocols for biosynthesis of nanoparticles and achieving the requisite yields for bringing it at par with physical and chemical methods is the eventual objective to accomplish. Great contributions of researchers all over the world towards developing a simpler and environmentally benign method for nanoparticle synthesis, provides an aid in task at hand. With greater understanding of probable mechanisms involved in the biosynthetic mode and various instruments to comprehensively characterize the material the future of nanotech industry can be the biosynthetic nanoparticles.

## REFERENCES

- Geethalakshmi R, Sarada DVL, Synthesis of plant-mediated silver nanoparticles using *Trianthema decandra* extract and evaluation of their antimicrobial activities, International Journal of Engineering Science and Technology, 2(5), 2010, 970-975.
- Varshney R, Bhadauria S, Gaur MS, A Review: Biological synthesis of silver and copper nanoparticles, Nano Biomed. Eng., 4(2), 2012, 99-106.
- Sadowski Z, Maliszewska I, Grochowalska B, Polowczyk I, Koźłowski T, Synthesis of silver nanoparticles using microorganisms, Materials Science-Poland, 26(2), 2008, 419-424.
- Sadowski Z, Silver Nanoparticles, David PP, In Tech, 2010, 257-276., Available from: <http://www.intechopen.com/books/silver-nanoparticles/biosynthesis-and-application-of-silver-and-goldnanoparticles>.
- Venkataraman D, Kalimuthu K, Sureshbabu RKP, Sangiliyandi G, Metal nanoparticles in Microbiology Rai M, Duran N, Vol.-XI, Springer, 2011, 17-35.
- Sukumaran P, Eldho KP, Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects, International Nano Letters, 2(32), 2012, 2-10.
- Ratnika V, Bhadauria S, Gaur MS, Renu P, Copper Nanoparticles Synthesis from electroplating industry effluent, Nano Biomed. Eng., 3(2), 2011, 115-119.
- Abhilash, Revati K, Pandey BD, Microbial synthesis of iron-based nanomaterials—A review, Bull. Mater. Sci., 34(2), 2011, 191–198.
- Xiangjian L, Huizhong X, Chen Z, Chen G, Biosynthesis of nanoparticles by Microorganisms and their applications, Journal of Nanomaterials, 2011, 2011, 1-16.
- Oza G, Pandey S, Gupta A, Kesarkar R, Sharon M, Biosynthetic reduction of gold ions to gold nanoparticles by *Nocardia farcinica*, Journal of Microbiology and Biotechnology Research, 2(4), 2012, 511–515.
- Ghosh, *Gnidia glauca* flower extract mediated synthesis of gold nanoparticles and evaluation of its chemo-catalytic potential, Journal of Nanobiotechnology, 2012, 10-17.
- Shankar SS, Ahmad A, Pasricha R, Sastry M, Bioreduction of chloroaurate ions by *Geranium* leaves and its endophytic fungus yields gold nanoparticles of different shapes, J. Mater. Chem., 13(7), 2003, 1822–1826.
- Shankar SS, Ahmad A, Sastry M, *Geranium* leaf assisted biosynthesis of silver nanoparticles, Biotechnol. Prog., 19(6), 2003, 1627–1631.
- Akhtar MS, Panwar J, Sang Yun Y, Biogenic Synthesis of metallic nanoparticles by plant extracts, ACS Sustainable Chem. Eng. 1, 2013, 591–602.
- Singh, A, Jain D, Upadhyay MK, Khandelwal N, Verma HN, Green synthesis of silver nanoparticles using *Argemone Mexicana* leaf extract and evaluation of their antimicrobial activity, Dig. J. Nanomater. Biostruct., 5(2), 2010, 483–489.
- Karnai R, Chowdhary A, Biosynthesis of silver nanoparticle by eco-friendly method, Indian Journal of Nanoscience, 1(2), 2013, 25–31.
- Baker S. Plants: Emerging as Nanofactories towards facile route in synthesis of nanoparticles, Bio-Impacts, 3(3), 2013, 111-117.
- Krishnaraj C, Jagan EG, Rajasekar S, Selvakumar P, Kalaichelvan PT, Mohan N. Synthesis of silver nanoparticles using *Acalypha indica* leaf extracts and its antibacterial activity against water borne pathogens, Colloids Surf B, 76, 2010, 50-56.
- Phillip D, *Mangifera indica* leaf-assisted biosynthesis of well-dispersed silver nanoparticles, Spectrochim. Acta, Part A, 78(1), 2011, 327–331.
- Lin L, Wang W, Huang J, Li Q, Sun D, Yang X, Nature factory of silver nanowires: Plant mediated synthesis using broth of *Cassia fistula* leaf, Chem Eng J, 2010, 162, 852-858.
- Mukunthan KS, Elumalai EK, Trupti NP, Ramachandra VM, *Catharanthus roseus*: A natural source for the synthesis of silver nanoparticles, Asian Pac J Trop Biomed, 11, 2011, 270-274.
- Song JY, Kwon E Y, Kim BS, Biological synthesis of platinum nanoparticles using *Diopyros kaki* leaf extract. Bioprocess Biosyst. Eng. 33(1), 2010, 159–164.
- Farooqui MDA, Chauhan PS, Krishnamoorthy P, Shaik J, Extraction of silver nanoparticle from the leaf extracts of *Clerodendrum inerme*. Dig J Nano-mater Biostruct. 5, 2010, 543-549.
- Jia L, Zhang Q, Li Q, Song H, The biosynthesis of palladium nanoparticles by antioxidants in *Gardenia jasminoides Ellis*: Long life time nanocatalysts for p-nitrotoluene hydrogenation, Nanotechnology, 20, 2009, 385601.
- Christensen L, Biosynthesis of silver nanoparticles using *Murraya koenigii* (curry leaf): An investigation on the effect of broth concentration in reduction mechanism and particle size, Adv. Mat. Lett., 2(6), 2011, 429-434.
- Chandran SP, Chaudhary M, Pasricha R, Ahmad A, Sastry M., Synthesis of gold nanotriangles and silver nanoparticles using Aloe vera plant extract, Biotechnol Prog. 22, 2006; 577-583.
- Mallikarjuna, K, Dillip GR, Narasimha G, Sushma NJ, Raju BDP, Phytofabrication and characterization of silver nanoparticles from Piper betle broth. Res, Journal of Nanoscience and Nanotechnology, 2(1), 2012, 17–23.
- Patil RS, Kokate MR, Kolekar SS, Bioinspired synthesis of highly stabilized silver nanoparticles using *Ocimum tenuiflorum* leaf extract and their antibacterial activity. Spectrochim. Acta A, 91, 2012234–238.
- Vanaja M, Annadurai G, *Coleus aromati cus* leaf extract mediated synthesis of silver nanoparticles and its bactericidal activity, Appl. Nanosci. 2012, DOI: 10.1007/s13204-012-0121-9.
- Kasthuri J, Veerapandian S, Rajendiran N, Biological synthesis of silver and gold nanoparticles using *Apiin* as reducing agent, Colloids Surf A, 68, 2009, 55-60.
- Sharma NC, Sahi SV, Nath S, Parsons JG, Gardea T, Pal T, Synthesis



- of plant-mediated gold nanoparticles and catalytic role of biomatrix-embedded nanomaterials. *Environ. Sci. Technol.* 41(14), 2007, 5137–5142.
32. Ramezani N, Ehsanfar Z, Shamsa F, Amin G, Shahverdi HR, Esfahani HRM, Shamsaie, A, Bazaz RD, Shahverdi AR, Screening of medicinal plant methanol extracts for the synthesis of gold nanoparticles by their reducing potential. *Z. Naturforsch.* 63, 2008, 903–908.
  33. Petla RK, Vivekanandhan S, Misra M, Mohanty AK, Satyanarayana N, Soybean (*Glycine max*) leaf extract based green synthesis of palladium nanoparticles, *J. Biomater. Nanobiotechnol.*, 3(1), 2012, 14–19.
  34. Ankamwar B, Damle C, Ahmad, A, Satry M, Biosynthesis of gold and silver nanoparticles using *Emblica officinalis* fruit extract, their phase transfer and transmetallation in an organic solution. *J. Nanosci. Nanotechnol.* b, 5(10), 2005, 1665–1671.
  35. Jain D, Daima HK, Kachhwala S, Kothari SL, Synthesis of plant-mediated silver nanoparticles using Papaya fruit extract and evaluation of their antimicrobial activities, *Dig. J. Nanomater. Biostruct.* 4(3), 2009, 557–563.
  36. Dubey SP, Lahtinen M, Sillanpaa M, Tansy fruit mediated greener synthesis of silver and gold nanoparticles, *Process Biochem.* 45(7), 2010, 1065–1071.
  37. Bankar A, Joshi B, Kumar AR, Zinjarde S, Banana peeled extract mediated novel route for the synthesis of palladium nanoparticles, *Mater. Lett.*, 64(18), 2010, 1951–1953.
  38. Das RK, Gogoi N, Bora U, Green synthesis of gold nanoparticles using *Nyctanthes arbortristis* flower extract. *Bioprocess Biosyst. Eng.* 34(5), 2011, 615–619.
  39. Sathishkumar M, Sneha K, Won SW, Cho CW, Kim S, Yun YS, *Cinnamon zeylanicum* bark extract and powder mediated green synthesis of nano-crystalline silver particles and its bactericidal activity. *Colloids Surf.* 73(2), 2009, 332–338.
  40. Shenton W, Douglas T, Young M, Stubbs G, Mann S, Inorganic – organic nanotube composites from template mineralization of tobacco mosaic virus. *Adv. Mater.* 11(3), 1999, 253–256.
  41. Sathish kumar M, Sneha K, Kwak IS, Mao J, Tripathy SJ, Yun YS, Phyto-crystallization of palladium through reduction process using *Cinnamon zeylanicum* bark extract. *J. Hazard. Mater.* 171(1–3), 2009, 404–404.
  42. Bar, H.; Bhui, D. K.; Sahoo, G. P.; Sarkar, P.; Pyne, S.; Misra, A., Green synthesis of silver nanoparticles using seed extract of *Jatropha curcas*, *Colloids Surf.*, A 348(1), 2009, 212–216.
  43. Kumar V, Yadav SC, Yadav SK, *Syzygium cumuni* leaf and seed extract mediated biosynthesis of silver nanoparticles and their characterization, *J. Chem. Technol. Biotechnol.*, 85(10), 2010, 1301–1309.
  44. Krishnamurthy S, Sathishkumar M, Lee SY, Bae MA, Yun YS, Biosynthesis of Au nanoparticles using cumin seed powder extract, *J. Nanosci. Nanotechnol.* 11(2), 2011, 1811–1814.
  45. Hosseini-Abari A, Emtiazi G, Lee SH, Kim BG, Kim JH, Biosynthesis of silver nanoparticles by *Bacillus stratosphericus* spores and dipicolinic acid in this process, *Applied Biochem Biotechnol.* 174, 2014, 270–282.
  46. Jain N, Bhargava A, Majumdar S, Tarafdar JC, Panwar J, Extracellular biosynthesis and characterization of silver nanoparticles using *Aspergillus flavus* NJP08: A mechanism perspective, *Nanoscale*, 3, 2011, 635–641.
  47. Mittal AK, Bhaumik J, Kumar S, Banerjee UC, Biosynthesis of silver nanoparticles: Elucidation of perspective mechanism and therapeutic potential, *Journal of Colloid and Interface Science*, 415, 2014, 39–47.
  48. Jeevan P, Ramya K, Edith R, Extracellular biosynthesis of silver nanoparticles by culture supernatant of *Pseudomonas aeruginosa*, *Indian journal of Biotechnology*, 11, 2012, 72-76.
  49. Waghmare SS, Deshmukh AM, Kulkarni SW, Oswaldo LA, Biosynthesis and characterization of manganese and zinc nanoparticles, *Universal journal of environmental research and technology*, 1, 2011, 64-69.
  50. Malik P, Shankar R, Malik V, Sharma V, Mukherjee TK, Green Chemistry based benign routes for nanoparticle synthesis, *Journal of Nanoparticles*, 2014, 2014, 1-15.
  51. Malabadi RB, Meti NT, Mulgund GS, Nataraja K, Vijaya Kumar S, Synthesis of silver nanoparticles from *in vitro* derived plants and callus cultures of *Costus speciosus* (Koen.); Assessment of antibacterial activity, *Research in Plant Biology*, 2(4), 2012, 32-42.
  52. Vanaja M, Rajeshkumar S, Paulkumar K, Gnanajobitha G, Malarkodi C, Annadurai G, Kinetic study on green synthesis of silver nanoparticles using *Coleus aromaticus* leaf extract, *Advances in applied science research*, 4(3), 2013, 50-55.
  53. Namita S, Prakash S, Factors affecting the geometry of silver nanoparticles synthesis in *Chryso sporiumtropicum* and *Fusariumoxysporum*, *American journal of nanotechnology* 2(1), 2011, 112-121.
  54. Darroudi M, Ahmad MB, Zamiri R, Zak AK, Abdullah AH, Ibrahim NA, Time-dependent effect in green synthesis of silver nanoparticles, *International journal of nanomedicine*, 6, 2011, 677–681.
  55. Joshi M, Bhattacharyya A, Ali SW, Characterization techniques for nanotechnology applications in textiles, *Indian journal of fibre & textile research*, 33, 2008, 304–317.

**Source of Support: Nil, Conflict of Interest: None.**

