2.1 History of Nanoparticles

During the 1970-80's, when the first thorough fundamental studies with "nanoparticles" were underway in the USA [Granqvist *et al.* (1976)] and Japan, (within an ERATO Project) [Hayashi *et al.* (1997)] they were called "ultrafine particles" (UFP). However, during the 1990s before the National Nanotechnology Initiative was launched in the USA, the new name, "nanoparticle" had become fashionable (see, for example the same senior author's paper 20 years later addressing the same issue, lognormal distribution of sizes) [Kiss *et al.* (1999)]. A so-called **Elixir of Life**, a potion made from gold, was discussed, if not actually manufactured, in ancient times. Colloidal gold has been used since Ancient Roman times (8th century BC) to color glass intense shades of red or mauve, depending on the concentration of gold, and in Hindu Chemistry, for various potions.

The preparations of NPs are usually carried out by various physical and chemical methods like laser ablation, pyrolysis, lithography, chemical vapor deposition, sol-gel technique, and electrodeposition which are very expensive and hazardous. Therefore, scientists are looking forward for greener methods [Roopan *et al.* (2013); Roy *et al.* (2010)]. Phytosynthesis of transition metal nanoparticles is gaining importance due to their biocompatibility, low toxicity, green approach, and environment-friendly nature [Narayanan *et al.* (2008). The increasing environmental concern necessitates the development of new and eco-friendly techniques for the synthesis of bimetallic nanoparticles.

Biological substrates have shown promising abilities for interaction with metal ions [Koh *et al.* (2005), Mukhopadhyay *et al.* (2007), Nayak *et al.* (2006)]. Proteins and carbohydrates of cell wall provide good sites for metal binding [Brady *et al.* (1994)]. They appear to be the safer alternatives towards designing greener technology for the future [Hutchison (2008)]. Synthesis of nanoparticles using biological entities has great interest due to their

unusual optical [Krolikowska *et al.* (2003)], chemical [Kumar et al. (2003)], photo electrochemical [Chandrasekharan and Kamat (2000)] and electronic [Peto *et al.* (2002)] properties. Advantages of biological methods include tightly controlled, highly reproducible syntheses: biocompatible particles: and the avoidance of toxic surfactants or organic solvents. Consequently, researchers in the field of nanoparticle preparation have been looking at biological systems [Merroun *et al.* (2007)] such as those that allow a commercially viable and environmentally clean synthesis of highly stabilized gold particles [Mukherjee *et al.* (2008)]. Beveridge and Murray (1980) first demonstrated that the exposure of *Bacillus subtilis* treated with gold chloride ($AuCl_4$) resulted in the synthesis of AuNPs. Biosynthesis of nanoparticles can be categorized into intracellular and extracellular synthesis according to the place where nanoparticles are formed [Simkiss *et al.* (1989), Mann (1996)]. The **intracellular** method consists of transporting ions into the microbial cell to form the nanoparticles in the presence of enzymes. In the **extracellular** method, microbes have been employed for generating nanostructured mineral crystals and metallic nanoparticles, and the control of the size, shape, composition and monodispersity of particles were also studied. Nair and Pradeep (2002) reported that common *Lactobacillus strains* found in buttermilk assisted the growth of microscopic gold, silver, and gold-silver alloy crystal of well-defined morphology.

The synthesis of metallic NPs are currently being explored through bacteria [Joerger *et al.* (2000)], yeast [Kowshik *et al.* (2003)], fungi [Mandal *et al.* (2006)], plant biomass [Armendariz *et al.* (2004); Sharma *et al.* (2007)], live plants [Bali *et al.* (2006)], and plant extracts [Vilchis *et al.* (2008)]. However, the synthesis of **plant**-based nanoparticles can be advantageous over other microbial methods since the reaction rate for the synthesis of nanoparticles is very high and there is no need to grow microbes [Kumar *et al.* (2012)]. The use of these biological systems for the preparation of nanoparticles offers several

advantages since the methods are easier to carry out and more economical than traditional ones.

Metallic Nanoparticles like Gold (Au), Silver (Ag), Palladium (Pd), Platinum (Pt), Cerium (Ce), Selenium (Se) etc. can be biosynthesized respectively from *Rhizopus oryzae, Aspergillus clavatus, Shewanella oneidensis, E. coli, Leptothrix discophora* & *Bacillus subtilis*. **Multimetallic** Nanoparticles like Gold-Silver (Au-Ag), Gold-Silver-Copper (Au-Ag-Cu), Copper alumina ($CuAlO_2$), Iron oxide (Fe_3O_4), Lead Sulphide (PbS), Zinc Sulphide (ZnS), Cadmium Sulphide (CdS), Cadmium-Tellurium (CdTe) etc. can also be biosynthesized respectively from *Saccharomyces cerevisiae, Brassica juncea* seed, *Humicola sp., Magnetospirillum Gryphiswaldense, Cocci, Bacillus, Schizosaccharomyces pombe* & *Escherichia coli* [Schrofel *et al.* (2011)].

2.2 Silver Nanoparticles

2.2.1 Silver Nanoparticles from Bacteria

The first evidence of bacteria synthesizing silver nanoparticles was established using the *Pseudomonas stutzeri* strain that was isolated from silver mine. There are some microorganisms that can survive metal ion concentrations and can also grow under those conditions, and this phenomenon is due to their resistance to that metal. The mechanisms involved in the resistance are efflux systems, alteration of solubility and toxicity via reduction or oxidation, biosorption, bioaccumulation, extracellular complex formation or precipitation of metals, and lack of specific metal transport systems. There is also another aspect that though these organisms can grow at lower concentrations, their exposure to higher concentrations of metal ions can induce toxicity.

The most widely accepted mechanism of silver biosynthesis is the presence of the nitrate reductase enzyme. The enzyme converts nitrate into nitrite. In in vitro synthesis of silver using bacteria, the presence of alpha-nicotinamide adenine dinucleotide phosphate reduced

form (NADPH)-dependent nitrate reductase would remove the downstream processing step that is required in other cases. During the reduction, nitrate is converted into nitrite and the electron is transferred to the silver ion; hence, the silver ion is reduced to silver (Ag^+ to Ag^0). This has been said to be observed in *Bacillus licheniformis* which is known to secrete NADPH and NADPH-dependent enzymes like nitrate reductase that effectively converts Ag^+ to Ag^0. The mechanism was further confirmed by using purified nitrate reductase from *Fusarium oxysporum* and silver nitrate along with NADPH in a test tube, and the change in the color of the reaction mixture to brown and further analysis confirmed that silver nanoparticles were obtained. There are also cases which indicate that there are other ways to biosynthesize silver nanoparticles without the presence of enzymes. It was found that dried cells of *Lactobacillus sp.* A09 can reduce silver ions by the interaction of the silver ions with the groups on the microbial cell wall (Table 1). Silver nanoparticles synthesized from *Bacillus megaterium* (46.9)**,** *Enterobacter cloacae* (50-100 nm)**,** *Escherichia coli* (5-25 nm)**,** *B. licheniformis* (50 nm)**,** *Lactobacillus fermentum* (11.2)**,** *Klebsiella pneumonia* (50 nm)**,** *Proteus mirabilis* (10-20 nm)**,** *Brevibacterium casei* (50 nm) etc. [Prabhu *et al.* (2012)].

2.2.2 Silver Nanoparticles from Molds

Silver nanoparticles synthesized from *Verticillium sp.* (25nm) [Mukherjee *et al.* (2001)], *Phoma sp.*(70 nm) [Chen *et al.* (2003)], *F. oxysporum* (20-50 nm) [Duran *et al.* (2005), Selvi *et al.* (2012), Birla *et al.* (2013), Korbekandi *et al.* (2013)], Mohammadhassan *et al.* (2014), Husseiny *et al.* (2015)], *Penicillium fellutanum* (5-25 nm) [Kathiresan *et al.* (2009)], *Aspergillus fumigatus* (5-25 nm) [Navazi *et al.* (2010)], *Aspergillus flavus* (7-10 nm) [Jain *et al.* (2010), Saeed Moharrer *et al.* (2012)], *Fusarium semitectum* (10-60 nm) [Basavaraja *et al.* (2008)], *Fusarium solani* (5-35 nm) [Ingle *et al.* (2009)], *Aspergillus clavatus* (10-25 nm) [Verma *et al.* (2010)], *Aspergillus terreus* [Li *et al.* (2012)],

Aspergillus niger (20-70 nm) [Sagar *et al.* (2012), Soni *et al.* (2013)], *Trichoderma* (8-60 nm) [Vahabi *et al.* (2011), Devi *et al.* (2013)], *Aspergillus tubingensis* [Rodrigues *et al.* (2013)], *Ganoderma neo-japonicum Imazeki* (10-70 nm) [Gurunathan et al. (2013)], *Epicoccum nigrum* (1-22 nm) [Qian et al. (2013)], *Aspergillus foetidus* (20-40 nm) [Roy *et al.* (2013)], *Penicillium citrinum* (90-120 nm) [Honary *et al.* (2013)], *Humicola* sp. [Sayed et al. (2013)], *Penicillium nalgiovense* (15-25 nm) [Maliszewska *et al.* (2014)], *Penicillium chrysogenum* (19-60 nm) [Pereira *et al.* (2014)], *Aspergillus oryzae* [Phanjom *et al.* (2015)], *Penicillium atramentosum* (5-25 nm) [Sarsar *et al.* (2015)] and *Penicillium italicum* (14.5-23.3 nm) [Majeed *et al.* (2018)].

2.2.3 Silver Nanoparticles from Yeast

A green approach for synthesizing the nanoparticles using the yeast have been suggested as promising ecofriendly alternative to chemical methods. Ecofriendly biosynthesis of extracellular silver nanoparticles was carried out using *Candida utilis*. Characterization of synthesized AgNPs was done by UV–visible spectroscopy, Scanning electron microscopy and antibacterial activity. AgNPs are found spherical in shape with size in the range of 20–80 nm. AgNPs showed antibacterial activity against pathogenic organisms such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*. The SEM analysis confirms the antibacterial activity of Ag nanoparticles due to damage of cytoplasmic membrane. AgNPs synthesized by *C. utilis* could be applicable in the development of antibacterial water filters for treatment of water [Sonawane *et al.* (2015)].

Extracellular biosynthesis of silver nanoparticles (Ag-NPs) using *Kluyveromyces marxianus*, *Candida utilis* and evaluating the antibacterial and antifungal efficacy against *Staphylococcuc aureus* ATCC 6538, *Escherichia coli* ATCC 10536, *Pseudomonas flourescence* ATCC 50090, *Candida albicans*, *Candida glabrata*, *Candida krusei* as multi-drug resistant human pathogens. Morphological observation and characterization of

biosynthesized silver nanoparticles were performed by UV-visible spectroscopy, Transmission electron microscopy and Fourier transform infrared spectroscopy. The biosynthesized silver nanoparticles from each yeast strains showed a maximum absorption in the visible region at 430-450 nm and at 400-430 nm respectively and the size was ranged from 3-12 nm and 6-20 nm respectively. The interaction between protein and Ag-NPs was analyzed and the stabilization of Ag-NPs by protein is a clear possibility. Further more, Ag-NPs have the highest antibacterial and antifungal efficacy against all the tested microorganisms. Silver nanoparticles from each strains have great potential to be an effective to antibacterial and/or antifungal agents for future therapies in multi-drug resistant human pathogens of bacteria and *Candida* infections [Ashour (2014)].

2.3 Gold Nanoparticles

2.3.1 Gold nanoparticles from Bacteria

Recently, bacterial cell supernatant of *Psudomonas aeruginosa* was used for the reduction of gold ions resulting in extracellular biosynthesis of GNPs [Husseiny *et al*. (2007)]. Morphological control over the shape of GNPs has been achieved by using *Plectonema boryanum* UTEX 485, a filamentous cyanobacterium. When it was reacted with aqueous $Au(S_2O_3)^{3-}{}_2$ and $AuCl_4^-$ solutions at 25-100 ºC for up to 1 month and at 200 ºC for 1 day resulted in the precipitation of cubic GNPs and octahedral gold platelets, respectively [Lengke et al. (2006a)]. The mechanisms of gold bioaccumulation by cyanobacteria (*Plectonema boryanum* UTEX 485) from gold (III)-chloride solutions have documented that interaction of cyanobacteria with aqueous gold (III)-chloride initially promoted the precipitation of NPs of amorphous gold (I)-sulfide at the cell walls, and finally deposited metallic gold in the form of octahedral (III) platelets near cell surfaces and in solutions [Lengke et al. (2006b)]. Adding further to the mechanism, a sulfate-reducing bacterial enrichment was used to destabilize gold(I)-thiosulphate complex to elemental gold and

proposed that this could occur by three possible mechanisms involving iron sulfide, localized reducing conditions, and metabolism [Lingke *et al*. (2006)].

Usually the chemical methods followed are carried out under very high temperatures to form gold nanoparticles and also result in environmental pollution due to toxicity of the reagents (reducing agents like borohydrates and acetylene) used [Wang *et al.* (2007)]. Consequently, researchers in the field of nanoparticle preparation have been looking at biological systems [Merroun *et al.* (2007)] such as those that allow a commercially viable and environmentally clean synthesis of highly stabilized gold particles [Mukherjee *et al.* (2008). Beveridge and Murray (1980) first demonstrated that the exposure of *Bacillus subtilis* treated with gold chloride ($AuCl_4$) resulted in the synthesis of gold nanoparticles, which revealed the presence of 5-25 octahedral nanoparticles inside the cell wall. Biosynthesis of gold nanoparticles has been carried out using *Rhodopseudomonas capsulata* [He *et al.* (2007)], *Fusarium oxysporum* [Mukherjee *et al.* (2002), *Sargassum wightii* [Singaravelu *et al.* (2007)], *Lactobacillus sp.* [Binoj *et al*. (2002)] and *Helminthosporum solani* [Kumar *et al.* (2008)]. Advantages of biological methods include tightly controlled, highly reproducible syntheses: biocompatible particles: and the avoidance of toxic surfactants or organic solvents. Moreover, bacteria are easy to handle and can be manipulated genetically. Considering these advantages, a bacterial system could prove to be an excellent alternative for the synthesis of gold nanoparticles [Sweeney *et al.* (2006)].

2.3.2 Gold Nanoparticles from Molds

Conversion of gold salt into AuNPs by following molds have already been done: *Verticillium* [Ahmad *et al.* (2001)], *Fusarium oxysporium* [Mukherjee *et al.* (2002), Ahmad *et al.* (2003)], *Trichothecium* [Ahmad *et al.* (2005)], *V. luteoalbum* [Gericke *et al.* (2006)], *Helminthosporium solani* [Kumar *et al.* (2008)], *Rhizopus oryzae* [Das *et al*. (2009)],

Aspergillus niger [Bhambure *et al.* (2009)], *Penicillium sp.* [Liangwei *et al.* (2011)], *Trichoderma viridae* [Fayaz *et al.* (2011)], *Penicillium citrinum* [Alapatt *et al.* (2012)], *Aspergillus fumigatus* [Vasanthi *et al.* (2012)], *Alternaria alternata* [Sarkar *et. al.* (2012)], *Chrysosporium* tropicum [Soni *et al.* (2012)], *Trichoderma sp.* [Mishra *et al.* (2014)], *Aspergillus sydowii* [Vala (2014)], *Alternaria sp.* [Dhanasekar *et al.* (2015)], *Penicillium acculiatum* [Barabadi *et al.* (2017)], *Streptomyces sp.* [Skaladanowski *et al.* (2017)] & *Aspergillus sp.* WL Au [Shen *et. al.* (2017)].

2.3.3 Gold nanoparticles from Yeast

Yeast strains (*P. jadinii*) have been identified for their ability to produce gold nano particles, whereby controlling growth and other cellular activities controlled size and shape of the nanoparticles was achieved [Gericke *et al.* (2006a, 2006b)].

Among various eukaryotic organisms yeasts play an important role as a model of eukaryotic cells for biochemical and physiochemical experiments. For example, the effects of some heavy metals on the growth of some soil-yeasts were studied [Falih (1998)]. As a useful means of bioremediation of environmental chromium contamination, yeasts were used to treat Cr containing effluents in order to remove toxic compounds from waters and soils [Pepi *et al.* (1992)]. Earlier, we have also reported extraction of 152 Eu, a long-lived fission product, by yeast cells, *Saccharomyces* [Roy *et al.* (2008)]. The dry waste biomass of powder of *Saccharomyces cerevisiae* obtained from beer fermentation was studied for Au^{3+} biosorption [Lin *et al.* (2005)].

The size of nanoparticles can be manipulated by controlling parameters such as pH, temperature, gold concentration and exposure time to gold solution. On the other hand, the effects of ionic gold on *S. cerevisiae*, was determined by long-term and short-term interactions [Karamushka (1999)]. The addition of low concentrations of gold as tetra chloroaurate salt to growth medium resulted in the formation of a dispersed phase over 10–

12 h incubation. Transmission electron microscopy revealed no differences in ultra structure. No gold deposits were observed in transmission electron micrographs of cells grown in presence of gold, in contrast to numerous gold particles located outside the cell.

Yarrowia lipolytica has emerged as important non-conventional yeast with significant biological relevance and biotechnological applications [Barth *et al.* (1997), Fickers *et al.* (2005)]. Literature survey shows that considerable biomass of *Y. lipolytica* is generated during waste treatment procedures [Oswal *et al.* (2002)] and this fungus displays biological features required for interactions with metals [Garcia *et al.* (2002), Strouhal (2003)]. We hypothesized that *Y. lipolytica* may be a promising candidate for nanoparticle synthesis and this work is a report on gold crystal and nanoparticle production by this fungus.

2.4 Bimetallic Nanoparticles

Biological synthesis of Bimetallic Nanoparticles

The use of chemical and physical methods in the synthesis of nanoparticles was very expensive and cumbersome which leads to the presence of some toxic chemicals absorbed on the surface that may have adverse effects in applications. Hence, there was a growing need to develop environmentally benign nanoparticles. Researchers have used biological extracts for the synthesis of nanoparticles, by adopting simple protocols, involved in the process of the reduction of metal ions by using biological extracts as a source of reductant either extracellularly or intracellularly. The synthesis of nanoparticles may be triggered by several compounds such as carbonyl groups, terpenoids, phenolics, flavonones, amines, amides, proteins, pigments, alkaloids, and other reducing agents present in the plant extracts and microbial cells [(Xiao *et al.* (2011), Du *et al.* (2009), Chandran *et al.* (2006), Kun *et al.* 2012)].

The green synthesis/biosynthesis of bimetallic nanoparticles is a good, low-cost, and nontoxic method compared to physical and chemical methods which showed excellent

biological activities. At this juncture, biosynthetic methods are gaining more interest for the synthesis of bimetallic nanoparticles.

The biosynthesis of Au-Ag bimetallic nanoparticles was carried out with 1.5 mL of plant extracts mixed with 30 mL of $AgNO_3$ and $HAuCl_4$ (1 mM/mL) and incubated at 28 °C for 24 h (Ali *et al.* 2011). The reaction mixtures were centrifuged at 6,000 rpm for 10 min and resuspended. The pellet in small amount of sterilized double-distilled water. The color reaction can be observed in which a clear $AgNO_3$ solution changed into brown color, whereas pale yellowish $HAuCl_4$ solution turned to a ruby red-colored solution which indicates the formation of the corresponding nanoparticles (Tripathy *et al.* 2010). The synthesized bimetallic nanoparticles were caracterized by UV, XRD, SEM, and TEM analyses.

Au-Pd bimetallic nanoparticles

Fe-Pd bimetallic nanoparticles

Ti-Ni bimetallic nanoparticles

Sol-gel method

The Co-cerium (Ce) oxides were prepared by the sol-gel route (samples $CeCu_2O_4$) using the reaction of aqueous solutions containing stoichiometric amounts of Ce(III) and Cu(II) nitrates in the presence of urea (Mahia *et al.* (1997). The solutions were kept on a hot plate at 80 °C with the continuous stirring until gel formation and kept aside for further cooling. The gel was then decomposed at 250°C to yield precursor $CeCu_2O_4$. And then it was calcinated at 600 and 900°C, respectively.

Microwave irradiation method

Fe-Ru bimetallic nanoparticles

The Fe-Ru aqueous solutions were prepared by using $RuCl_3 \cdot H_2O$ and $FeCl_3 \cdot 6H_2O$ in 45 mL of 1,2-propanediol under stirring to form a dark red solution; it was then added to 5 mL

of 1,2-propanediol solution. Ammonia (0.1 M) was added to it dropwise under vigorous stirring. The resultant solution was irradiated under microwave condition. A transparent dark brown homogenous colloidal solution of Fe-Ru bimetallic nanoparticles was obtained without any precipitate after microwave heating at 300W for 3 min. The prepared polyol dispersions of Fe-Ru bimetallic nanoparticles were precipitated by excess of acetone. The precipitates were washed with anhydrous acetone in order to remove chloride ions and then dried under vacuum and redispersed into ethanol-water mixture prior to characterization [Du *et al.* (2009)].

Micelle synthesis method

It includes Au-Ni, Fe-Co & Ni-Pt bimetallic nanoparticles.

Gold-Nickel bimetallic nanoparticles

Gold-Nickel (Au-Ni) bimetallic nanoparticles had been prepared by the micelle method. The prepared nanoparticles were used as an electrocatalyst (Au-Ni) for the electrochemical oxidation of borohydride. The authors have used water-in-oil microemulsion of water/AOT/n-heptane. The effects of the molar ratios of water to water-in-oil on the particle size and catalytic performance were systemically investigated. It has been found that particle size increased with the increase of Ru value and the particle size decreased when >10 due to twophase separation of the microemulsion. The morphology and structure of prepared Au-Ni electrocatalysts are examined by transmission electron microscopy (TEM) and XRD. It was confirmed that all Au-Ni nanoparticles were spherical in shape with average diameters in the range of 3–9 nm and uniformly distributed on the surfaces of carbon [He *et al.* (2012b)].

Iron-cobalt bimetallic nanoparticles

Iron-cobalt (Fe-Co) bimetallic nanoparticles were prepared by the reverse micelle method [Mhlanga *et al.* (2008)], in which $CaCo_3$ was supported by Fe-Co. Three different methods

such as wet impregnation, deposition-precipitation, and reverse micelle impregnation were adopted. The authors have tailored the nanofabrication of **carbon nanotubes (CNTs)** using 5 wt% Fe-Co/$CaCO_3$ by chemical vapor deposition. High yields of multiwalled nanotubes with 100 % selectivity and excellent reproducibility were obtained. The reverse micelle technique enabled a more precise control over the size of the CNTs.

Electrochemical method

It includes Cu-Ag, Au-Pt, Cu-Ni, Co-Pd & Pt-ruthenium bimetallic nanoparticles.

Au-Pt bimetallic nanoparticles

Monometallic Pt/SiO_2 catalyst (1.96 wt% Pt) can prepared by incipient wetness impregnation (IWI) of SiO_2 with an aqueous solution of 0.04 mol L^{-1} $Pt(NH_3)_4(NO_3)_2$ using a volume of solution of 2.5 mL g^{-1} of SiO_2 [Delannoy *et al.* (2006)]. After aging at room temperature for 6 h, the catalyst was dried overnight at 120 °C. Au/SiO_2 (0.70 wt% Au) was also prepared by IWI of SiO_2 with an aqueous solution of 0.02 mol L^{-1} $HAuCl_4$. After aging at room temperature for 6 h, the sample was washed with a 1-mol L^{-1} aqueous NH_3 solution which was adjusted to pH 8.5 with HCl to remove the chloride ions and avoid the formation of large gold particles after thermal treatment. This was followed by washing with water several times, until a test by $AgNO_3$ no longer showed the presence of residual Cl^-.

Cu-Ag bimetallic nanoparticles

The Cu-Ag bimetallic nanoparticles were synthesized by using a facile process of electrical method. The advantage of this synthetic method includes its production of water dispersible copper and copper/silver nanoparticles at room temperature under inert atmosphere. The authors have investigated the resulting nanoparticles (Cu-Ag) by XRD, UV-Vis spectroscopy, and TEM. The nanometallic dispersions were characterized by surface plasmon resin absorbance measurement at 420 and 572 nm for Ag and Cu nanoparticles,

respectively. TEM results showed that the formed Cu-Ag nanoparticles are in the range of 10–30 nm with a spherical shape [Danhui *et al.* (2013)].

Laser irradiation method

It includes Pt-Au & Ag-Ni bimetallic nanoparticles.

Pt-Au bimetallic nanoparticles

The Pt-Au bimetallic nanoparticles have been prepared by using the laser irradiation method [Chau *et al.* (2013)]. Pt-Au precursor was prepared by dissolving chloroauric (III) acid tetrahydrate and chloroplatinic (IV) acid hexahydrate in deionized water with a concentration of 1.0×10^{-2} wt%. All the preparations were done in a dark room because of the photosensitive property of the gold and platinum precursor molecules.

Ag-Ni bimetallic nanoparticles

The Ag-Ni bimetallic nanoparticles were synthesized by laser ablation of solid targets, which were prepared by compressing a mixture of powder silver and nickel. A variety of techniques, including SEM, EDAX, and XRD, have been used to characterize the morphology, composition, and construction of synthesized bimetallic nanoparticles [Xiao *et al.* (2011)].

Reduction method

It includes Fe-Pd & Ru-Pt bimetallic nanoparticles.

Fe-Pd bimetallic nanoparticles

The Fe-Pd nanoparticles were prepared by the reduction of borohydride with carboxymethyl cellulose (CMC) as a stabilizer [Dong *et al.* (2011)]. For the preparation of Fe-Pd bimetallic nanoparticles, $FeSO_4\cdot7H_2O$ (0.02 M) aqueous solution was mixed with 70 mL of CMC (sodium carboxymethyl cellulose). The mixture was plugged with N_2 for 15 min to remove the dissolved oxygen and to maintain an environment for the formation of

Fe^{2+}-CMC complex. The following mechanism has been adopted by ferrous ion with KBH_4.

When the evolution of visible gas ceased, the solution was left aside for 10 min. The bimetallic particles can be synthesized by loading a trace amount of K_2PdCl_6 (0.15 mM, 0.1 % (*w/w*) Fe) aqueous solution onto the wet iron particles under stirring and nitrogen atmosphere according to the equation below [Xiuli et al. (2008)]. The synthesized nanoparticles were characterized by UV, IR, XRD, SEM, and TEM analyses.

Co-reduction method

Bimetallic nanoparticles can also be prepared with the co-reduction method, in which 100 mL of corresponding metal was dissolved in 10 mL of 0.1 M hexane to give microemulsion A. Microemulsion B was prepared by mixing 200 mL hydrazine hydrate (0.4082 M) with 10 mL of 0.1 M hexane. To both microemulsions A and B, excess water (100 mL) was added separately in order to maintain the same value as above. Both solutions were stirred until they became clear. Then, microemulsion B was added dropwise to microemulsion A under cold condition (5°C) for the preparation of bimetallic nanoparticles. The particles now can be extracted from the microemulsion solution by centrifugation at higher rpm. The synthesized particles were then washed with ethanol for five times and finally dispersed in water. The aqueous dispersion of these individual metals and bimetallic nanoparticles can be used as a catalyst for the reduction of microemulsion B in aqueous medium [Singh et al. (2013)]. It only includes Platinum-copper bimetallic nanoparticles.

Platinum-copper bimetallic nanoparticles

The platinum-copper (Pt-Cu) bimetallic alloy nanoparticles were synthesized by using the reverse micelle method. Bimetallic alloy nanoparticles were prepared by water-in-oil (w/o) microemulsions of water/cetyltrimethylammonium bromide (CTAB)/isooctane/n-butanol by the co-reduction of H_2PtCl_6 and $CuCl_2$ with hydrazine at room temperature. These

samples were characterized by high-resolution transmission electron microscopy (HRTEM), X-ray diffraction (XRD), and X-ray photoelectron spectroscopy (XPS). The XRD results showed that there is a peak in the pattern of bimetallic nanoparticles, corresponding to the (111) plane of the $PtCu_3$ bulk alloy. The authors have confirmed the size of the bimetallic nanoparticles to be 1.6 nm in diameter by HRTEM analysis [Weihua *et al.* (2005)].

Radiolytic co-reduction method

It includes Au-Pt, Pt-Au & Ag-Pd bimetallic nanoparticles.

Au-Pt bimetallic nanoparticles

The bimetallic Au-Pt nanoparticles were synthesized by using the radiolytic co-reduction of aqueous solution of $HAuCl_4$ and H_2PtCl_6 in the presence of stabilizing polymers, polyacrylic acid, and polyvinyl alcohol at a ratio of 1:1 to which 2-propanol was added which acts as a radical scavenger [Remita *et al.* (2005)]. Fresh solutions of Au-Pt in the ratio of 1:1 were reacted under N_2 in the absence of light to prevent further photochemical reduction of Au and to remove oxygen. The radiolyses of the solutions were carried out by using ^{60}Co panoramic gamma source [Ksar *et al.* (2009)] with a radiation dose rate of 2.2 kGy h^{-1}. The deposition of the radiolytical nanoparticles can be achieve by stirring the SiO_2 solution for 24 h. Synthesized nanoparticles were confirmed by TEM, XRD, and UV analyses.

Pt-Au bimetallic nanoparticles

Pt-Pd and Pt-Au bimetallic nanoparticles were prepared by utilizing octa (diabetic amino phenyl) silsesquioxanes (OAAPS) by reducing ethanol. The synthesized nanoparticles were subjected to characterization studies like UV, XRD, and TEM analyses which showed that the size of nanoparticles was in 2.6±0.5 nm in diameter. Synthesized nanoparticles were

used as catalysts for the hydrogenation of phenyl aldehydes to phenyl alcohols with dihydrogen under mild conditions [Xiuli *et al.* (2008)].

Ag-Pd bimetallic nanoparticles

The synthesis of Ag-Pd bimetallic nanoparticles was carried out by the co-reduction of $AgNO_3$ and $(NH_4)_2PdCl_6$ in aqueous solution with Triton X-100. The authors have synthesized Ag-Pd nanoparticles and have characterized them by TEM, SAED, and XRD. The electrochemical results showed that the Ag-Pd bimetallic nano alloys possess much better electrocatalytic activity and better long-term performance than Ag nanoparticles [Chunling *et al.* (2011)].

Sonochemical co-reduction method

Au-Ru bimetallic nanoparticles

The gold-ruthenium bimetallic nanoparticles can be synthesized by using the sonochemical co-reduction of Au (III) and Ru (III) ions in aqueous solutions. It contained varying mole fractions of the metal ions using polyethylene glycol as a stabilizer. The synthesized bimetallic nanoparticles were characterized by UV, IR, XRD, SEM, and TEM analyses [Kumar *et al.* (2010)].

2.5 Bimetallic (Gold-Silver) Nanoparticles

Synthesis of Au-Ag Bimetallic nanoparticles

Several studies show that the stability and size of nanoscale colloidal particles effectively depend on the technique and the experimental conditions followed. The novel Ag/Ag–Au BMNPs were produced by the replacement reaction between Ag NPs and $HAuCl_4$. Au–Ag NPs were synthesized by method of reduction of changeable mole fractions of $HAuCl_4$ and $AgNO_3$ using sodium borohydride in the presence of sodium citrate, in water .The exchange of Ag-NPs into Ag/Ag–Au BNPs involved numerous sequential processes [Lu *et al.* (2007), Chen *et al.* (2006), Park *et al.* (2009)]:

i. oxidative dissolution of Ag atoms,

ii. reduction of $AuCl^-_4$

iii. deposition of Au atoms.

Metal NPs can be produced in two different ways, that is, by subdivision of bulk metals (a physical method) and by the growth of particles obtained from metal atoms, which are from molecular or ionic precursors (a chemical method).

Core–shell

Core–shell and multishell Au–Ag BNPs have been produced by successive reduction of metal salts with ascorbic acid on pre-made seeds in the presence of a cationic surfactant, cetyltrimethylammonium bromide. In thickness-proscribed synthesis of core–shell structured Au/Ag or Ag/Au NPs, the external metal surface can be tuned as a performance of the internal metal surface, provided that the outer shell is thin enough [Gonzalez *et al.* (2005), Cao *et al.* (2001), Rivas *et al.* (2000), Daniel *et al.* (2004), Ferrer *et al.* (2007), Kim *et al.* (2005)].

Co-reduction

The co-reduction of Au and Ag precursors is the simplest method of preparing Au–Ag alloy NPs. Since two metal precursors are involved in the reduction reactions, the influence of synthesis conditions on the rates of precursor reduction, and the nucleation and growth of the alloy NPs are more complex than in the case of monometallic NPs [Wang *et al.* (2009), Wilson *et al.* (2005), Kim *et al.* (2003), Mallin *et al.* (2002), Chen *et al.* (2002), Link *et al.* (1999), Hostetler *et al.* (1998), Han *et al.* (1998)].

Biogenic Synthesis

The synthesis of Au-Ag bimetallic synthesized by *Neurospora crassa* (Longoria *et al.* 2011) & *Volvariella volvacea* [Philip (2009)]. This extract plays an important role in the reduction of Ag^{+2} and Ag ions, which leads to the synthesis of Au-Ag bimetallic

nanoparticles. They confirmed the structure, shape, and size by UV, XRD, FTIR, and TEM analyses.

The synthesis of Au-Ag alloy bimetallic nanoparticles from the aqueous extract of dried leaves of mahogany (*Swietenia mahogani* JACQ) by using a clean, nontoxic, and environment-friendly method. The aqueous leaf extract of *S. mahogani* acts as a reducing agent and also the stabilizing agent for the rapid formation of stable metal nanoparticles with various compositions, shapes, sizes, and high monodispersity. (Mondal *et al.* 2011). The synthesis of Ag-Au bimetallic nanoparticles was done using *Piper pedicellatum* leaf extracts (Tamuly *et al.* 2013). The prepared nanoparticles are spherical in shape along with few triangular, hexagonal, and pentagonal nanoparticles which in the range of 2–30 nm. The biosynthesis of Au-Ag bimetallic nanoparticles was done by using the aqueous extract and dried powder of *Anacardium occidentale* leaf (Sheny *et al.* 2011). In this work, the authors have reported the effects of quantity of extract/ powder, temperature, and pH on the formation of bimetallic nanoparticles.

Laser-assisted synthesis of Au–Ag alloy NP

One of the simple and convenient methods for synthesizing Au–Ag BNPs is "laser irradiation" method, which is a bottom-up approach comparable with the approach of laser ablation of bulk materials in solution for producing NPs. Spherical shapes and crystallized NPs can effortlessly be obtained in one-step procedures with no succeeding heat treatments, due to high energetic state of irradiated species without production of by-products [Takami *et al.* (1999)].

Replacement reactions

Replacement reaction is a simple method to prepare Ag–Au BNPs, which occurs between Ag-NPs and $HAuCl_4$ at elevated temperatures [Sun *et al.* (2002), Liang *et al.* (2005), Sun *et al.* (2004)].

Bimetallic (Au-Ag) Nanoparticles is also synthesized from *olive* [Longoria *et al.* (2011)], *Camellia sinensis* [Mostafa *et al.* (2012)], *Lansium domesticum* [Shankar *et al.* (2014)], *Trichoderma harzianum* [Tripathi *et al.* (2015)], *Bacillus Safensis* [Ojo *et al.* (2016)], *Antigonon leptopus* [Ganaie *et al.* (2016)], *Lyngbya majuscule* [Roychaudhary *et al.* (2016)] and *Annona squamosa* [Syed *et al.* (2018)].

Since there is very less study done on the synthesis of Gold, Silver and Bimetallic (Gold-Silver) Nanoparticles from molds. Also no one had done the synergistic effect of that nanoparticles on the Antibiotics. This thing I have done in my research work.

Objectives of Current Study:

The first phase of work involves screening of different biological methods for conversion of Gold, Silver & Bimetallic salts into nanoparticles.

The second phase of the work deals with the optimization of the different parameters of the selected process viz: pH, Temperature, gold salt, silver salt concentration etc. Growth pattern of the selected Microorganism with and without Gold/Silver/Bimetallic salt solutions.

The third phase of the work deals with the synthesis of Gold, Silver & Bimetallic nanoparticles using above mentioned processes. Application of selected Microorganism for conversion of Gold, Silver salts and Bimetallic salts into Metallic Nanoparticles.

The fourth phase of the work involves analysis of Gold, Silver & Bimetallic nanoparticles using different instruments (UV Spectrophotometric, SEM, XRD etc.). Characterization of synthesized Nanoparticles.

The final phase of the work involves therapeutic application of the produced Gold, Silver & Bimetallic nanoparticles.