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Biosynthesis and application of silver and gold nanoparticles

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1. Introduction

Nanotechnology has dynamically developed as an important field of modern research with potential effects in electronic and medicine (Glomm 2005, Chan 2006, Boisselier and Astruc 2009). Nanotechnology can be defined as a research for the design, synthesis, and manipulation of structure of particles with dimension smaller than 100nm. A new branch of nanotechnology is nanobiotechnology. Nanobiotechnology combines biological principles with physical and chemical procedures to generate nano-sized particles with specific functions. Nanobiotechnology represents an economic alternative for chemical and physical methods of nanoparticles formation. These methods of synthesis can be divided on intra cellular and extracellular (Ahmad et al. 2005).

This integration of nanoparticles with biological molecules has lead to the development of diagnostic devices, contrast agents, and important tools in cancer therapy. Nanobiotechnology describes an application of biological systems for the production of new functional material such as nanoparticles. Biosynthetic methods can be employed either microorganism cells or plant extract for nanoparticles production. Biosynthesis of nanoparticles is an exciting recent addition to the large repertoire of nanoparticles synthesis methods and now, nanoparticles have entered a commercial exploration period. Gold and silver nanoparticles are presently under intensive study for applications in optoelectronic devices, ultrasensitive chemical and biological sensors and as catalysts. This chapter is devoted to biosynthesis and application of gold and silver nanoparticles

2. Biosynthesis of silver and gold nanoparticles by microorganisms

2.1 Synthesis of nanoparticles by bacteria

An important part of work in nanobiotechnology concerns the synthesis of nanoparticles of different chemical compositions, sizes, shapes, and polydispersity. Many microorganisms produce inorganic materials either intra- or extracellularly. Well-known example is magnetotactic bacteria which are able to synthesize magnetic nanoparticles (Bazylinski and Frankel 2004). Magnetotactic bacteria are motile, prokaryotes that move along geometric field lines. They produce magnetosomes, unique intracellular structure contains a magnetic

particle, in narrow range of very low oxygen concentration. Magnetotactic bacteria usually mineralize either oxide magnetite Fe_3O_4 or iron sulfide Fe_3S_4 – greigite.

An extracellularly preparation of metal nanoparticles generally involves the reduction of metal ions in solution.

The formation of extracellular and intracellular silver nanoparticles by bacteria (*Pseudomonas stutzeri*, *Escherichia coli*, *Vibrio cholerae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, and *Staphylococcus aureus*) has been investigated (Langke et al. 2007).

Various microbes are known to reduce metal ions to the metals. The formation of extracellular silver nanoparticles by photoautotrophic cyanobacterium *Plectonema boryanum* had been described (Langke et al. 2007). The procedure of synthesis was as follows: 5 mL of silver solution (approximately 560 mg/L) was added to 5 mL of washed cyanobacteria culture (approximately 10 mg dry weight). The synthesis was conducted at 25, 60 and 100°C for up to 28 days in the dark. Only, at 100°C, the soluble silver was completely precipitated from solutions within 28 days. A greyish-black silver particles adhered to bacterial cells were observed macroscopically. The reaction products were analyzed using transmission electron microscopy (TEM) and X-ray photoelectron spectroscopy (XPS). The addition of AgNO_3 caused the precipitation both inside and outside the microbial cells. At 60°C, silver nanoparticles were deposited at the cell surface. At 100°C, the cyanobacterial cells were incrustated by silver nanoparticles. The size of nanoparticles inside the cell was ranging from 1 to 40 nm. The size of nanoparticles of silver which were precipitated outside the bacteria cells was in the range of 1 -200nm.

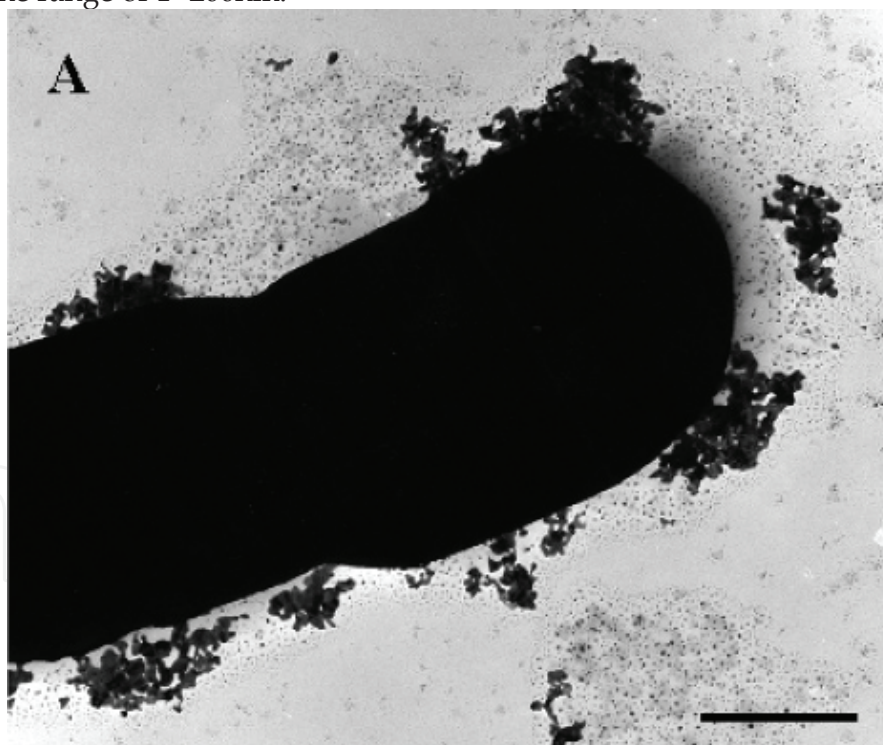
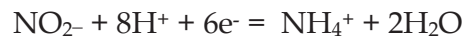


Fig. 1. Precipitated silver nanoparticles on the cyanobacteria cell surface (from Langke et al. 2007)

The bioreduction of the Ag^+ ions could be associated with metabolic processes utilizing nitrate by reducing nitrate to nitrile and ammonium (Langke et al. 2007).

Cyanobacteria commonly use nitrate as the major source of nitrogen. Nitrate was reduced by cyanobacteria metabolic process.



It suggests that Ag^+ ions could be reduced by an intracellular electron donor (Lengke et al., 2007).

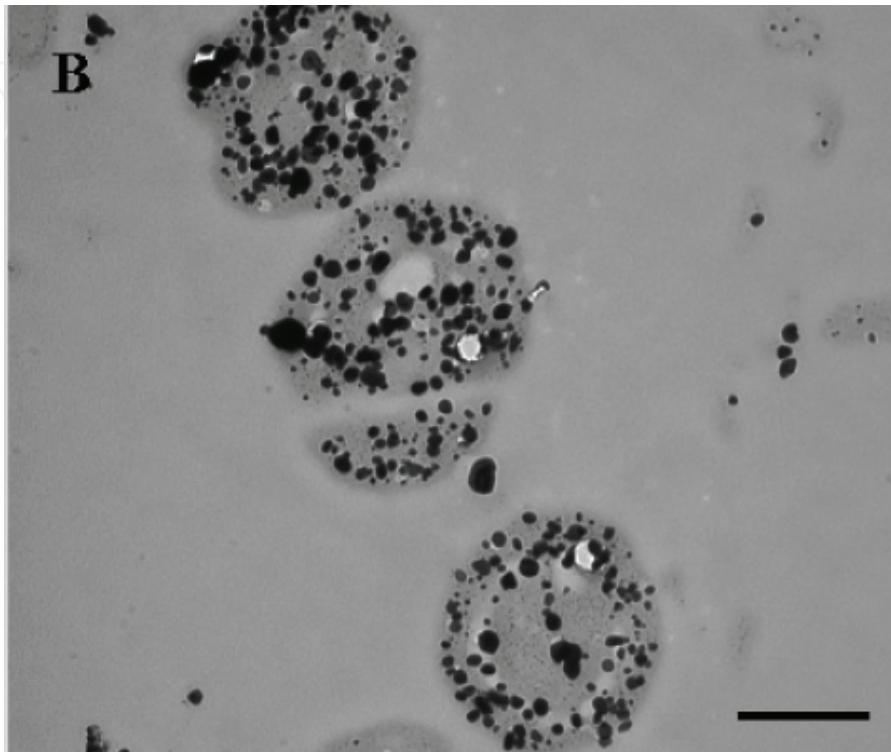


Fig. 2. Cyanobacteria cells with nanoparticles of silver inside the cell (from Lengke et al. 2007)

The intracellular recovery of gold by microbial reduction of AuCl_4^- ions using the anaerobic bacterium *Shewanella algae* has been investigated (Konishi et al. 2006). The solution turned light yellow after 1h, indicating the initial formation of gold nanoparticles.

Silver nanoparticles in the range of 50 nm were synthesized by supernatant of *Bacillus licheniformis* (Kalishwaralal et al. 2008). *Bacillus licheniformis* is a gram positive, thermophilic bacterium, commonly found in the soil. As was showed previously, during the visual observation culture supernatant incubated with silver nitrate showed a color change from yellow to brown. The appearance of brown color suggested the formation of silver nanoparticles. The XRD pattern shows four characteristic peaks in the whole spectrum. The peaks at 2Θ values of 38.48° , 44.48° , 64.69° and 77.62° corresponding to 111, 200, 220 and 311 planes for silver crystal, respectively (Fig. 3).

Recently, a rapid method for synthesizing silver nanoparticles by treating the aqueous silver nitrate solution with culture supernatants of different strains of *Enterobacteria* such as *Klebsiella pneumonia* has been described (Shahverdi et al. 2007 and Mokhtari et al. 2009). The process of synthesis was quite fast. As it has been presented, the silver nanoparticles were formed within 5 min of the silver ions coming in contact with the culture supernatant. The particle size histogram of silver nanoparticles showed the particle range in size from 28.2 nm to 122nm with the average size value of 52.5 nm.

Enterobacteria is a Gram-negative bacteria, usually associated with intestinal infections. The investigations which have been realized by Mokhtari and coworkers showed that piperitone (3 methyl-6-1 methylethyl)-2 cyclohexan-1-one) can be responsible for the silver ions reduction to metal (Mokhtari 2009). This conclusion supports the hypothesis that nitroreductase enzymes may be involved in silver ions reduction process.

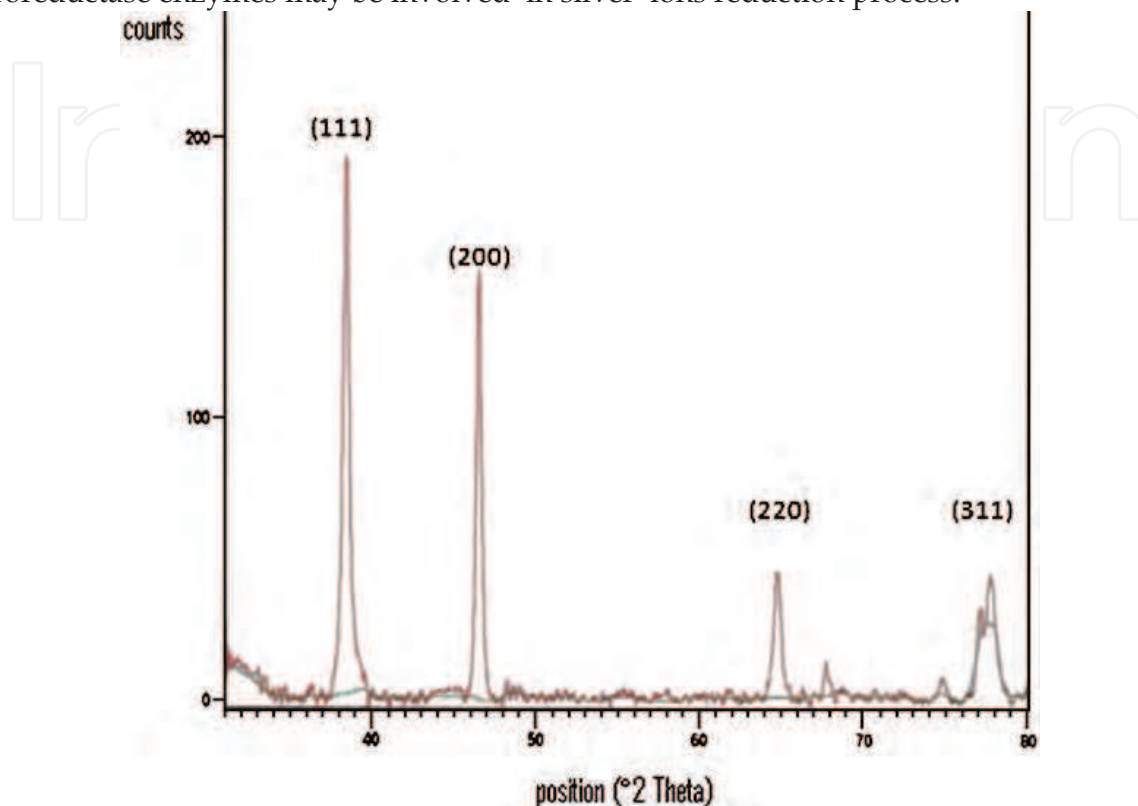


Fig. 3. XRD pattern of silver nanoparticles formed after reaction of *B. licheniformis* culture supernatant with AgNO_3 ($1 \cdot 10^{-3}$ M) (from Kalishwaralal et al. 2008)

The effect of visible-light irradiation on the synthesis of silver nanoparticles has been recently investigated (Mokhtari et al., 2009). The following procedure was used for the silver nanoparticles formation using a supernatant of *B. pneumonia* in the presence of light. The first step was silver chloride suspension preparation. The sodium chloride solution (50mL, 140 mg/L) was added to 50 mL of silver nitrate solution (340 mg/L) in a dark pale. The sediment fraction of AgCl was separated, cleaned and redispersed in distilled water. Then 1 mL of culture supernatant of *K. pneumonia* was added to the suspension. The silver nanoparticles fabrication was realized in the presence of various visible light intensities, generated by a 75 W halogen lamp. The experimental results confirmed a proposed mechanism involving the conversion of AgCl into Ag nanoparticles. This conversion of AgCl to silver nanoparticles by culture supernatant of *K. pneumonia* in a bright condition is presented at Fig. 4. Generally, AgCl is treated as the main intermediate compound during the bioreduction of the silver ions.

Kalimuthu and coworkers (Kalimuthu et al. 2008) have investigated the process of synthesis silver nanoparticles using bacteria *Bacillus licheniformis* and sonification of reacting mixture. *Bacillus licheniformis* were isolated from sewage collected from municipal wastes. Ultrasonic destruction of bacteria cell was carried out with ultrasonic processor over three 15 s periods.

The interval between periods was 45 s. Fabricated silver nanoparticles had a size ranging from 2 nm to 100 nm. The average particle size was found to be around 50 nm.

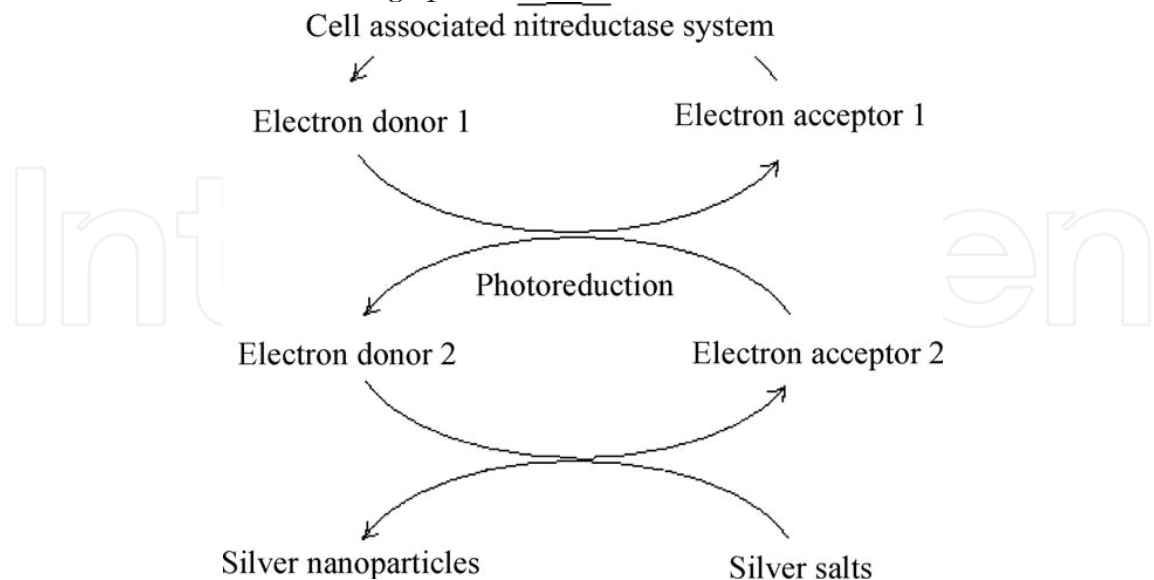


Fig. 4. Hypothetical mechanism of silver nanoparticles synthesis using the culture of *B.licheniformis* (from Mokhtari et al. 2009)

The enzyme involved in the fabrication of nanoparticles can belong to nitrate reductase, presented in *B.licheniformis*. This enzyme reduces the silver ions to metallic silver. It is known that NADH-dependent nitrate reductase enzymes are an important factor in the biosynthesis of metal nanoparticles. The possible mechanisms of reduction of silver ions using nitrate reductase, as it was presented in Fig.5.

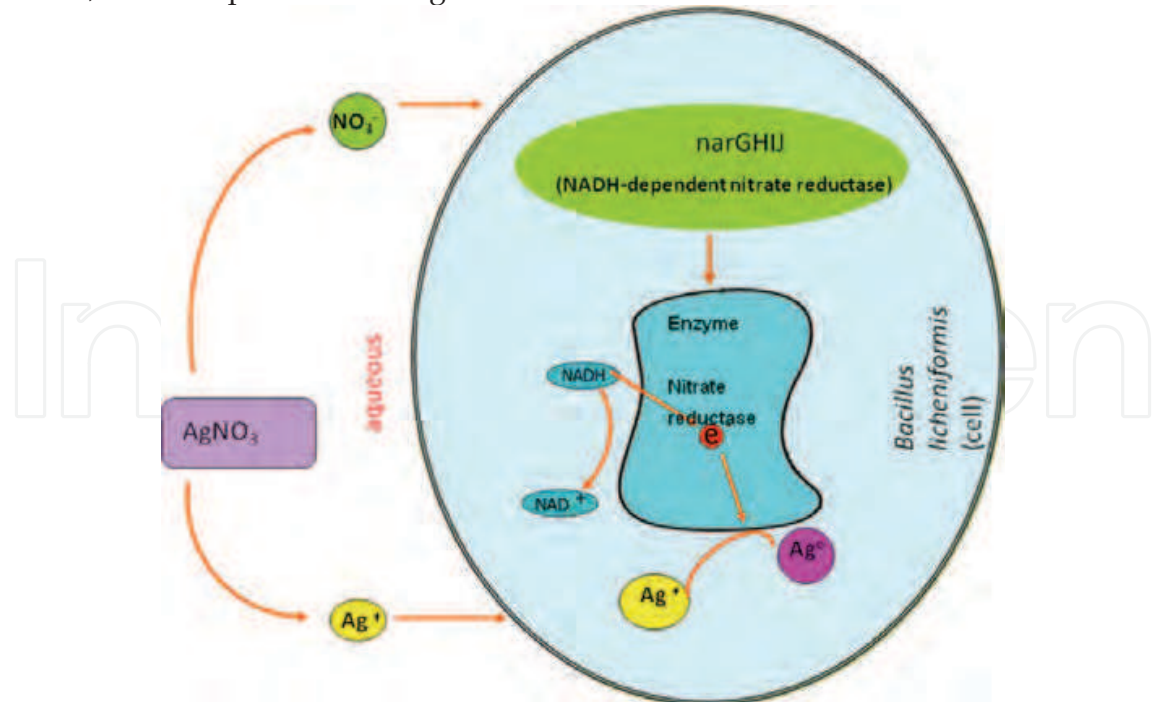


Fig. 5. Possible mechanism for silver nanoparticles synthesis using *Bacillus licheniformis* (from Kalimuthu et al. 2008)

Minaeian and coworkers (Minaeian et al. 2008) used different cultures which were sterilized and inoculated with fresh culture of the strains (*Bacillus subtilis*, *Lactobacillus acidophilus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter doacae*, *Staphylococcus aureus*, *Candida albicans*). The biosynthesized silver nanoparticles have the size range 50-100 nm.

Novel method of biosynthesis of silver nanoparticles using a combination of culture supernatant of *Bacillus subtilis* and microwave irradiation was proposed by Saifuddin and coworkers (Saifuddin et al. 2009). The formation of nanoparticles by this method was extremely rapid. The samples (supernatant and AgNO_3 solution) were subjected to several short burst of microwave irradiation at the frequency of 2.45 GHz, at power output of about 100 W in a following cyclic mode on 15 s off 15 s to prevent of overheating. The synthesized nanoparticles had the size range of 5 -50 nm.

The gold nanoparticles were synthesized using similar procedure. The two isolated strains of *Pseudomonas aeruginosa* were adopted to synthesis of gold nanoparticles (Husseiny et al. 2007). The synthesis of stable gold nanocubes by the reduction of aqueous AgCl_4^- by *Bacillus licheniformis* has been described (Kalishwaralal et al. 2009).

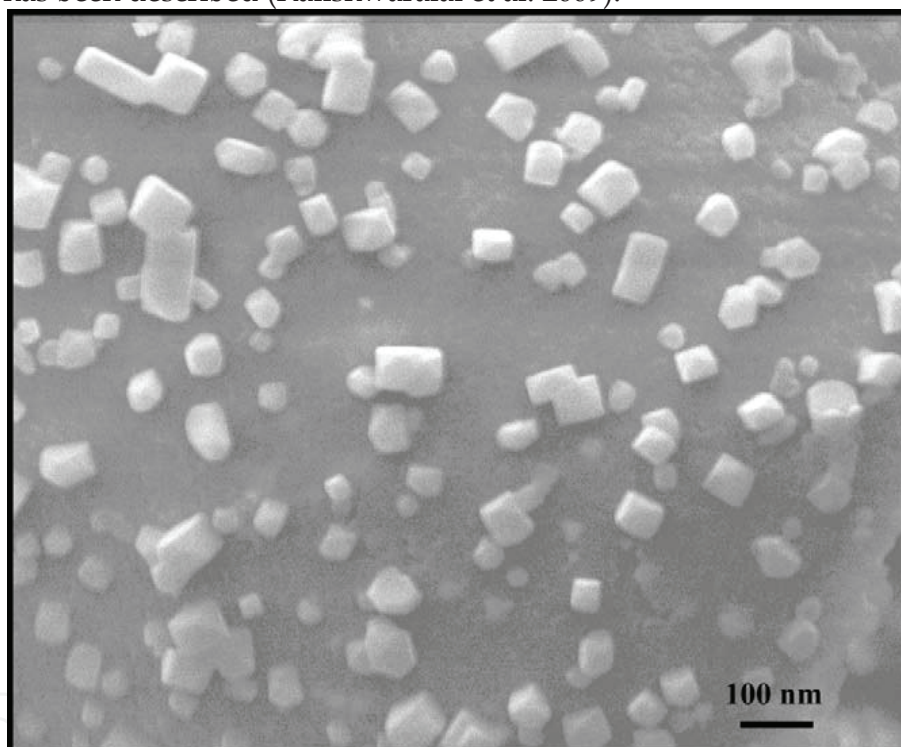


Fig. 6. SEM image of gold nanocubes fabricated by *Bacillus licheniformis* (from Kalishwaralal 2009)

The gold nanoparticles were prepared on the surface of bacteria cells as a result of incubation of bacteria with AuCl_4^- ions. Procariotic bacteria *Rhodopseudomonas capsulate* were adapted to bioreduction of gold ions (He et al. 2007). The aqueous chloroaurate ions were reduced during exposure to the bacteria *R. capsulate* biomass. The reaction was completed after 48 h of incubation. It was showed that the shape of gold nanoparticles was controlled by pH of solution. For the explanation of this behavior, the following mechanism was discussed. The aqueous chloroaurate ions were reduced during took a contact with bacterial cell groups. These groups such as amino, sulfhydryl and carboxylic had a positive charge. These positive charges depend on the solution pH. The adsorption of AuCl_4^- ions onto the

cell surface was supposed. Bioreduction of gold ions seems to be initiated by electron transfer from NADH by NADH-dependent reductase as electron carrier. Then, the gold ions obtained electrons reduced to gold.

2.2 Synthesis of nanoparticles by the fungal systems

The fungi are extremely good candidates in the synthesis of metal nanoparticles. The synthesis of silver particles using two *Aspergillus niger* strains was investigated (Sadowski et al. 2008 A and B). These strains were isolated from a soil. Inoculated fungi were prepared in Petri dishes at the room temperature using 2% malt extract with 0.5% yeast extract. Fungal biomass preparation was grown aerobically in the liquid medium containing (g/L): KH_2PO_4 7.0; K_2HPO_4 2.0; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.1; $(\text{NH}_4)_2\text{SO}_4$ 1.0; yeast extract 0.6 and glucose 10.0. After the incubation, the biomass was separated and extensively washed with distilled water. Fresh and clean biomass was collected with 100 mL of Milli-Q deionized water and new incubation was carry out. After the incubation, the supernatant was obtained by passing suspension through Whatman filter paper No. 1. For synthesis of silver nanoparticles, AgNO_3 1mM solution of the final concentration was mixed with 50mL of cell filtrate in an Erlenmeyer flask and agitated at 25° C in the dark. Sample 1 mL was with drown at different time intervals and absorbance was measured using UV-visible spectrophotometer. The spectra recorded at different times of biosynthesis is presented in Figure 7.

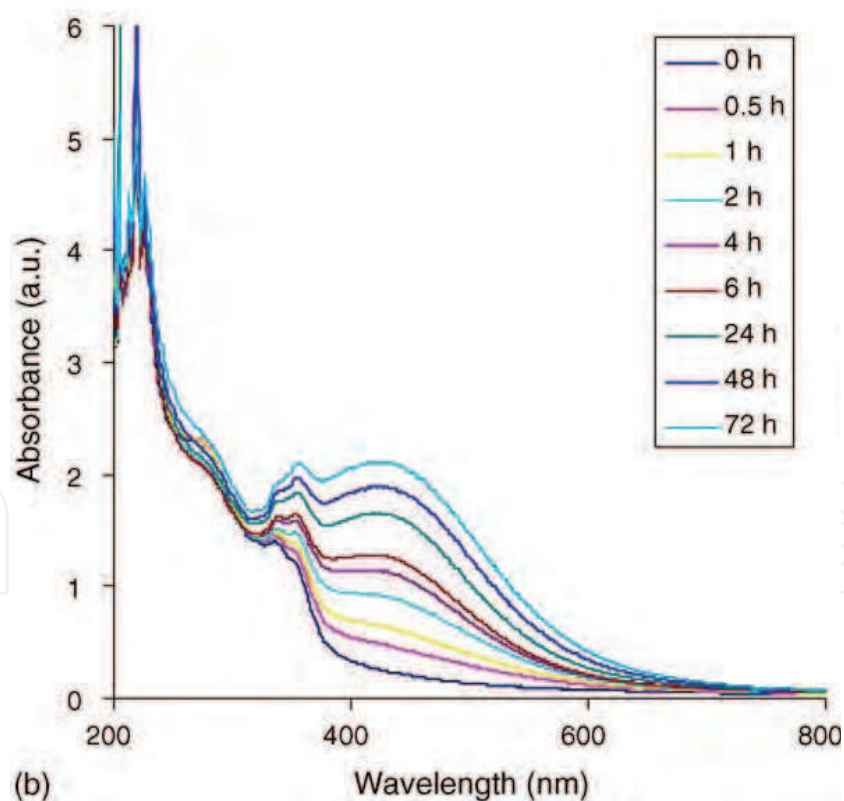


Fig. 7. UV-vis spectrum of aqueous medium during the synthesis of silver nanoparticles (from Sadowski et al. 2008A)

The electrokinetic measurements indicated that zeta potential of silver nanoparticles was negative value (Sadowski et al. 2008B).

The extracellular synthesis of silver and gold-silver nanoparticles by fungus *Fusarium oxysporum* biomass had a contribution on the formation of nanoparticles (Ahmad et al. 2003). The reduction of silver ions by *Fusarium oxysporum* strains has been attributed to a nitrate-dependent reductase and a shuttle quinone extracellular process. In a typical biosynthesis, 10g of fungal biomass was taken in Erlenmeyer flask containing 10 ml of distilled water. A corresponding quantity of AgNO_3 was added to Erlenmeyer flask to yield the concentration of Ag^+ ions equal 10^{-3} M. The reaction was carried out in the dark. Periodically, 5 mL of the reaction solution was removed and subjected to UV-vis spectroscopic measurements. Independently, it was observed that the biomass suspension has a yellow color before reaction with the silver ions and brown color on completion of the reaction.

The extracellular biosynthesis of silver nanoparticles using the filamentous fungus *Aspergillus fumigatus* has been investigated (Bhainsa and D'Souza 2006). This study included kinetics of synthesis, spectroscopic and microscopic characterization of the silver nanoparticles. The fungus *Aspergillus fumigatus* (NCIM 902) was grown aerobically in a liquid media containing (g/L) KH_2PO_4 , 7.0; K_2HPO_4 , 2.0; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1; $(\text{NH}_4)_2\text{SO}_4$, 1.0; yeast extract, 0.6; and glucose, 10.0. The biomass was harvested after 72 h of growth, then it was extensive washing with distilled water. 20g of fresh biomass was contacted with 200 mL of deionized water for 72 h and agitated in the same condition as first sample. After incubation, the suspension was filtered using Whatman filter paper No. 1.

The mechanism of leading to formation of silver nanoparticles is not definitely understood at the moment. One hypothesis supports that a first step involve trapping of the Ag^+ ions onto the surface of the fungal cells. It can be realized by electrostatic interaction between Ag^+ and a negative charged carboxylate groups on the cell surface. The reduction of metal ions occurs on the surface by the enzymes presented in the cell wall (Mukherjee et al. 2001). The extracellular enzymes such as naphthoquinones and anthraquinones showed an excellent redox properties, they can act as electron shuttle in silver ions reduction.

It was presented (Duran et al. 2005) that enzyme hydrogenase is present in a filtrate broth obtained from *Fusarium oxysporum* growth. The silver nanoparticles production capacity has been depended on the reductase/electron shuttle relationships.

Next paper presents the extracellular synthesis of stable silver nanoparticles using the fungus *Penicillium brevicompactum* WA 2315 (Shaligram et al. 2009). The analysis of data obtained from transmission electron microscope showed the average size of nanoparticles to be 58.35 ± 17.88 nm. Figure 8 shows the FTIR spectrum of the freeze-dried powder of silver nanoparticles formed after 72 h of incubation with the fungus supernatant. The band seen at 3356 cm^{-1} and 2922 cm^{-1} were assigned to the stretching vibration of primary and secondary amines, respectively. The bands at 1622 cm^{-1} and 1527 cm^{-1} correspond to the stretch molecule vibration. The two bands existing at 1412 cm^{-1} and 1029 cm^{-1} can be assigned to the C-N stretching vibrations of aromatic and aliphatic amines. This FTIR spectrum supports the presence of proteins in the synthesis of silver nanoparticles.

The use of fungus *Fusarium semitectum* for the extracellular synthesis of silver nanoparticles has been reported by Basavaraja and co-workers (Basavaraja et al. 2008). The formation and stability of the reduced silver nanoparticles in colloidal solution was monitored by using UV-vis spectral analysis. It was observed from spectra that the silver surface plasmon resonance band occurred at 420nm and this absorption steadily increased in intensity as a

function of time of reaction. IR spectroscopic study has confirmed that amino acid and peptides have formed a coat covering the silver nanoparticles to prevent agglomeration.

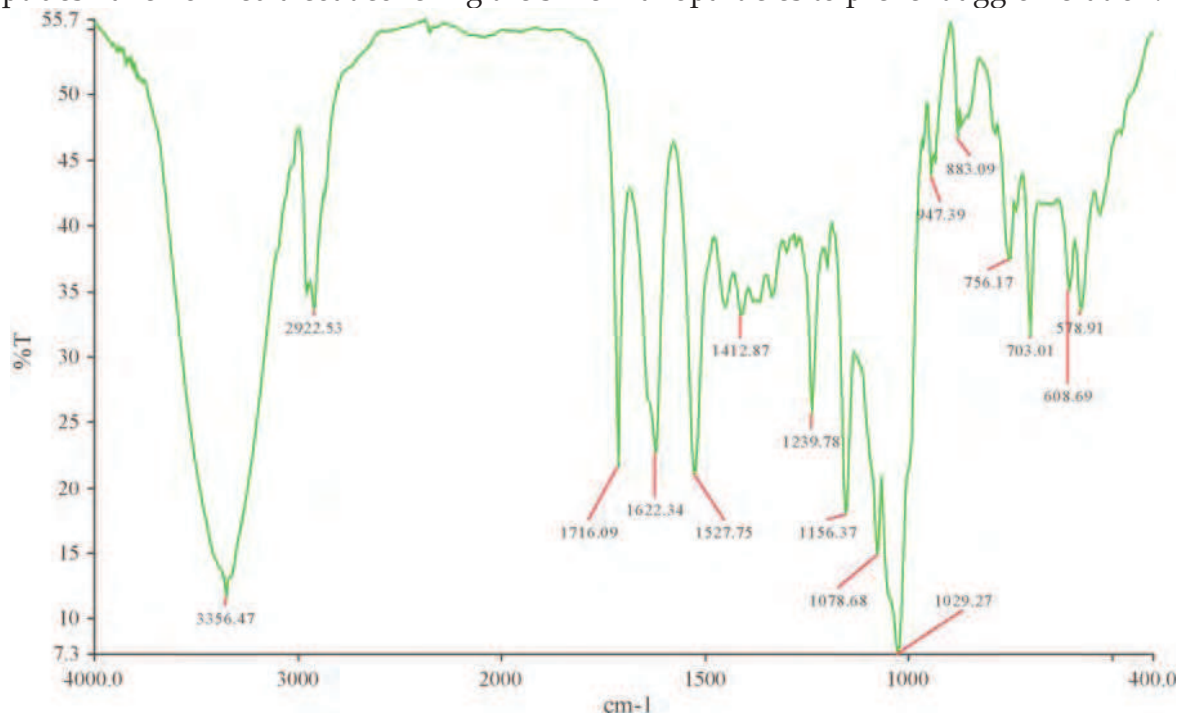


Fig. 8. FTIR spectrum of silver nanoparticles formed after 72 h of incubation of the supernatant of *P. brevicompactum* WA 2315, (from Shaligram et al. 2009)

The mean particles diameter of silver nanoparticles was calculated from the XRD pattern using Scherrer equation. The calculated average particles size of silver nanoparticles was found to be 35 nm.

The extracellular synthesis of silver nanoparticles by a marine fungus *Penicillium fellutanum* has been described by Kathiresan and coworkers (Kathiresan et al. 2009). The fungus *P. fellutanum* was isolated from a costal mangrove sediments. The procedure of biosynthesis of silver nanoparticles was analogous with the procedure has early described. For synthesis of silver nanoparticles AgNO₃ 1mM solution was mixed with 50 mL of cell filtrate and agitated in dark. The present of silver nanoparticles in reacting mixture was confirmed by absorption peak at 430 nm. The obtained silver nanoparticles were spherical in shape with size ranging from 5 to 25 nm. The TEM micrograph of silver nanoparticles synthesized by *P. fellutanum* is presented in Fig.9.

Cladosporium cladosporioides is a commonly available fungus found in marshland regions, This fungus was employed to biosynthesis of silver nanoparticles (Balaji et al. 2009). To prepare biomass for the synthesis, the fungus was grown aerobically in liquid medium containing (g/L): K₂HPO₄, 2.5; KNO₃, 5.0; MgSO₄ 7 H₂O, 1.00; MnSO₄ H₂O 0.001; CuSO₄ 5H₂O, 0.003; ZnSO₄ 7H₂O, 0.01; Na₂MoO₃ 2H₂O 0.0015; FeCl₃ 0.02 and glucose, 40.0. The fungus was grown for 1 week then the broth was filtered and washed with distilled water. 10 mL of pure solution were brought in contact with 100 mL of double distilled water containing 0.01 mL Ag⁺ metal ion solution. The mixture was agitated and kept on a shaker at 27°C for 78 h. Size and morphology of obtained nanoparticles were analyzed by employing TEM and Fourier transform infrared spectroscopy (FT-IR).

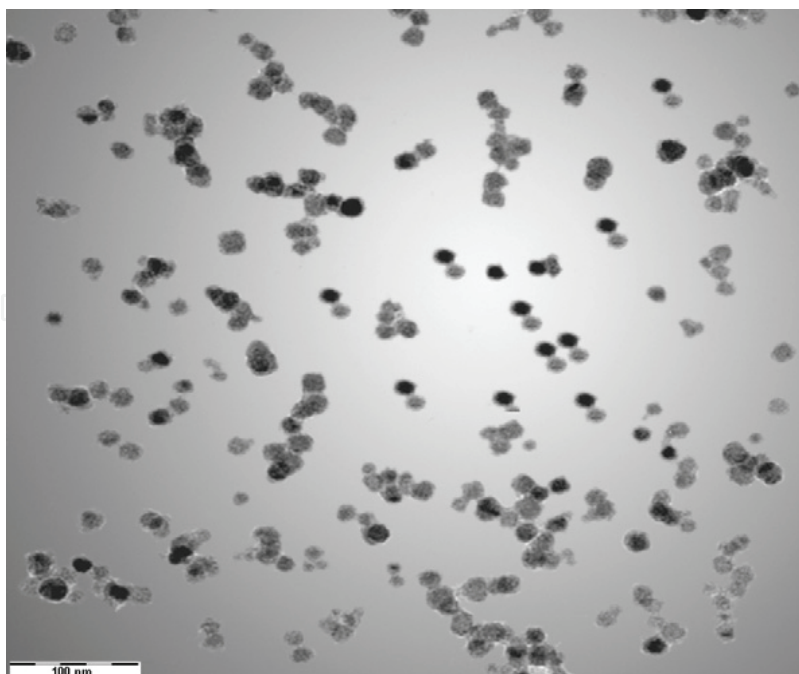


Fig. 9. TEM micrograph of silver nanoparticles synthesized by *Penicillium fellutanum* (from Kathiresan et al. 2009)

The extra- and intracellular biosynthesis of gold nanoparticles by fungus *Trichothecium* sp. was reported by Absar and coworkers (Absar et al. 2005). It was observed that when the gold ions reacted with the *Trichothecium* sp. fungal biomass under stationary conditions results in the rapid extracellular formation of gold nanoparticles of spherical rod-like and triangular morphology whereas reaction of the biomass under shaking conditions resulted in intracellular growth of the gold nanoparticles.

The biosynthesis of gold nanoparticles using marine alga *Sargassum wightii* has been investigated (Singaravelu et al. 2007). The stable gold nanoparticles were obtained by reduction of aqueous AuCl_4^- ions by extract of marine alga. Seaweed were collected from Mandapad Camp south east coast of Tamil Nadu, India. Collected seaweed were cleaned and dried for 3-5 days. Dried material was ground to powder in glass mortar. Synthesis of gold nanoparticles was carried out by taking 1g of seaweed powder in 500mL Erlenmeyer flask with 100 mL of 10^{-3} M aqueous HAuCl_4 solution. Aliquots of the reaction solution were investigated, the absorption of solution was measured.

The 95 % of the gold recovery occurred after 12 h of reaction. UV-vis spectra were recorded from the aqueous chloroauric acid and algae reaction medium. The bends at 527 nm corresponds to the surface plasmon resonance and showed the formation of nanoparticles. These particles were illustrated by TEM micrographs. The diameter of gold nanoparticles was ranging from 8 to 12 nm.

The synthesis of gold nanoparticles by the reduction of gold nanoparticles by the reduction of gold ions using a kind of Chinese herbal extract – *Barbated Skullcup* has been reported (Wang, et al. 2009)

The study on edible mushroom as reducing and protective agent for both silver and gold nanoparticles has been carried out by Philip (Philip 2009). Edible mushroom *Volvariella volvacea* was used for the metal nanoparticles synthesis. 68g of finely cut mushroom was boiled for 2 min in 300 mL water. Then the solution was filtered. This filtrate was cooled to

room temperature and used as reducing agent. 30 mL aqueous solution of $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ and 6 mL of mushroom extract were mixed together. Slow reduction taken place and was total in 2.5 h period of time. Gold nanoparticles colloid had a stable purple color. It was showed that the size and shape of Au nanoparticles can be controlled by varying the temperature and relative concentration of the extract with respect to the metal ion. In the case of silver nanoparticles biosynthesis, 35mg of AgNO_3 dissolved in 250 mL of water was contacted with a various volume (from 6 to 25 mL) of mushroom extract. The bioreduction was complete in 6 h. Ag-Au bimetallic nanoparticles were prepared by the simultaneous reduction of Au^{3+} and Ag^+ ions using excess of mushroom extract.

The extremophilic actinomycete, *Thermomonos* when exposed to gold ions reduced the metal ions extracellularly, yielding gold nanoparticles (Sastry et al. 2003). For the synthesis gold nanoparticles, the actinomycete was grown in 250 mL Erlenmeyer flasks containing 50 mL of agar slants. Sodium carbonate was used for pH adjusted. *Thermomonos* sp. had an optimum growth at pH=9 and temperature 50°C

3. Metal nanoparticles synthesis using plant extracts

An important branch of biosynthesis of nanoparticles is the application of plant extract to the biosynthesis reaction. Fig. 10 shows some popular plants using to the extract preparation.

A rapid reduction of the silver ions was observed when the silver nitrate solution was contacted with geranium (*Pelargonium graveolens*) leaf extract (Shiv Shankar et al. 2003). The extract used for reduction of Ag^+ ions to Ag^0 was prepared by taking 20g of thoroughly washed and finely cut geranium leaves in a 500 mL Erlenmeyer flask with 100 mL of



Fig. 10. Plants used for biosynthesis of metal nanoparticles

distilled water. The suspension was boiling for 1 min. 5 mL of pure broth was added to 100 mL of 10^{-3} M aqueous solution of AgNO_3 . The bioreduction of the Ag^+ ions was monitored by measuring the UV-vis spectra of the solution.

Fig. 11 shows the UV-vis spectra recorded from the aqueous silver nitrate-geranium leaf extract reaction medium as a function of the reaction time.

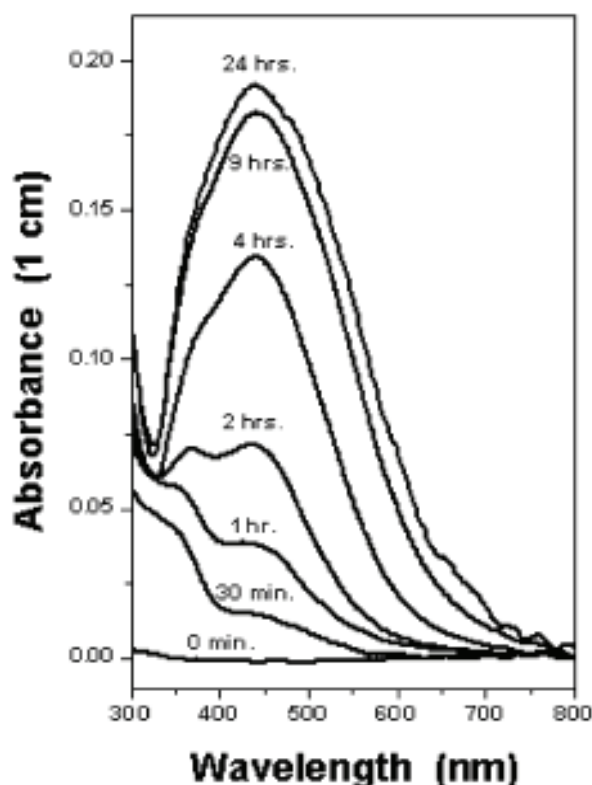


Fig. 11. UV-vis spectra as a function of reaction time of silver ions with *P. graveolens* leaf broth (from Shiv Shankar et al., 2003)

In the case of Neen leaf extract a competition reduction of Au^{3+} and Ag^+ ions presented simultaneously in solution was observed. It has lead to the synthesis of bimetallic Au core-Ag shell nanoparticles in solution (Shiv Shankar et al. 2004).

Silver nanoparticles ranging from 55 to 80 nm in side and triangular or spherical gold nanoparticles were fabricated using the novel sundried biomass of *Cinnammum canphora* leaf (Huang et al. 2007). It was found that formation of gold nanotriangles by *C. camphore* leaf at ambient temperature strongly depended on the amount of dried biomass. This biomass offered sufficient protective biomolecules.

A simple procedure applying *Aloe vera* leaf extract has been used for gold nanotriangle and spherical silver nanoparticles synthesis (Chandran et al., 2006). The kinetics of gold nanoparticles formation was monitored by UV-vis absorption spectroscopy and transmission electron microscopy (TEM). The effect of the amount of leaf extract on the synthesis of gold nanotriangles was investigated by observation of product formed. Addition of *Aloe vera* extract to 10^{-3} M aqueous solution of HAuCl_4 led to the appearance of a red color in solution after bout 5 h of reaction. An analysis of the percentage of triangles formed in the reaction medium as a function of varying amounts of the *Aloe vera* extract showed that more spherical particles were formed with increasing amount of added extract. *Eclipta* (known as Bhingraj) belongs to the family Asteraceae. It is a common weed growing mostly in a shade area (Jha et al., 2008). Extract from *Eclipta* leaf has used as medically important herb. The plant is rich in flavonoids, belonging to the group of phenolic compounds. The sample of 5 g of freshly collected leaves of *Eclipta* was washed for 10 min and ringed briefly in distilled water. Prepared biomaterial was taken in 250 mL capacity beaker having 200mL of 50% Et-OH and was placed on boiling steam bath for 15 to 20 min.

till color of the solvent changes to dark green. The cold extract was treated with 20 ml of 0.025 (M) AgNO_3 solution and warmed again on the steam bath for 10 min until the color of solution changes. The formation of silver nanoparticles was monitored by UV-vis spectrophotometry and X-ray diffractometer.

Biosynthesis of silver nanoparticles was also conducted using *Cycas* leaf extract. *Cycas* belongs to the *Cycadaceae* family. It is a common gymnospermic plant and is a commercial source of sago (Jha and Prasad 2009). This plant is rich in flavonoids broadly belonging to the class of phenolic compounds. The procedure of *Cycas* leaf broth preparation was like to made *Eclipta* extract. The *Cycas* extract solution was treated with 20 mL of 0.25 M AgNO_3 solution and warmed on the steam bath for 20 min until the color of solution changes to brown.

The particle size histogram obtained silver nanoparticles shows broad distribution of particle size. The size particle ranged from 2-6 nm and the average particle size comes out to be 3.29 ± 0.22 nm. The X-ray diffraction pattern obtained for silver nanoparticles synthesized by *Cycas* leaf broth shows that the silver nanoparticles are crystalline in nature.

Silver nanoparticles were successfully synthesized using the latex of *Jatropha curcas* (Bar et al. 2009). The plant, *Jatropha curcas* is commercially important one as bio diesel is extracted from its seeds on industrial scale. Crude latex was obtained by cutting the green stems of *J. curcas* plants. Milky white latex was stored in the refrigerator. For biosynthesis, 20 mL of 3% of latex solution was mixed with 20 mL of 5×10^{-3} M aqueous silver nitrate solution. The reacting mixture was heated at 85°C with constant stirring for 4h.

The bark powder and water extract from *Cinnamomum zeylanicum* tree were used for silver synthesis (Sathishkumar et al. 2009). The bark was cut into small pieces powdered in a mixer and then sieved using a 20-mesh sieve. The final sieved fraction of powder was used for further experiments. For the production of extract, 2.5 g of powder was added to a 500 mL Erlenmeyer flask with 100 mL distilled water and then boiled for 5 min. For the silver nanoparticles synthesis 100, 500 and 1000 mg of powder were added to 50 mL of 1mM aqueous AgNO_3 solution in a 250 mL Erlenmeyer flask. The flasks were then incubated in the dark at 25°C . In the second way 1, 2.5 and 5 mL of extract were used for the biosynthesis of Ag nanoparticles from 50 mL of 1 mM aqueous AgNO_3 solution.

The first report on the formation of gold nanoparticles by living plants was presented by Gardea-Torresdey and coworkers (Gardea-Torresdey et al. 2002). The alfalfa seeds were soaked to avoid fungal contamination in 3 % formaldehyde for 15 min. and washed three times with deionised water. Approximately 100 seeds were transferred to a mason jar and autoclaved for a sterile conditions. The nutrient solution had a composition: $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (3.57×10^{-4} M); H_3BO_3 (2.31×10^{-5} M); $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (2.14×10^{-3} M); KH_2PO_4 (9.68×10^{-4} M); KNO_3 (2.55×10^{-4} M); MgClO_4 (1.04×10^{-3} M); FeCl_3 (6.83×10^{-5} M); $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ (7.69×10^{-6} M); MoO_3 (1.0×10^{-5} M); $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$ (7.69×10^{-5} M); $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (1.6×10^{-6} M), and agar-agar 1g per 200 mL. Gold(III) ions from potassium tetrachloroaurate was used at concentrations of 0, 5, 10, 20, 40, 80, 160, and 320 ppm. Also, the alfalfa plants were harvested after two weeks of growth.

Leaf extracts of two plants *Magnolia kobus* and *Diopyros kaki* were used for extracellular synthesis of gold nanoparticles (Song et al. 2009). The gold nanoparticles were formed by treating an aqueous HAuCl_4 solution by the plant extract. Only a few minutes were required for > 90% recovery of gold nanoparticles at a reaction temperature of 90°C .

It has also been published that living alfalfa plants have the capability to take up silver from liquid media (Gardea-Torresday et al. 2003). The alfalfa plant samples grown in silver ions

rich media were embedded in a synthetic resins and dried in an oven at 65^o C for 24 h. TEM analysis suggested that silver atoms accumulated inside the alfalfa plant tissue under nucleation and nanoparticles formation as a correlated processes.

The extract from Black Tea has been employed as a reducing agent for the synthesis of Au and Ag nanoparticles (Begum et al. 2009). Three different extracts were prepared from Black Tea: (i) tea leaf broth, (ii) ethyl acetate extract and (iii) CH₂Cl₂ extract. Metal nanoparticles were synthesized by adding aqueous solution of AgNO₃ or HAuCl₄ to any of the three extracts. The formation and growth of the nanoparticles was monitored with the help of absorption spectroscopy and transmission electron microscopy.

4. Application of silver and gold nanoparticles

Gold and silver nanoparticles synthesized by various technique have received special attention because they have found potential application in many fields such as catalysis, sensors, drugs delivery system. Additionally, silver nanoparticles possess an excellent biocompatibility and low toxicity.

Nanocatalysis has recently been a rapidly growing field which involves the use of nanoparticles as catalysts. The catalysis properties of gold and silver nanoparticles varied from their sizes and synthesis method. It is well-known that metals such as Au, Ag and Pt and metal ions can catalyze the decomposition of H₂O₂ to oxygen. In addition, these metal ions can catalyze luminal-H₂O₂ systems. It was observed, when the Ag colloid was injected, chemiluminescence emission from the luminal-H₂O₂ system was greatly enhanced (Guo, et al. 2008). Silver is also the most popular catalyst for the oxidation of ethylene to ethylene oxide and methanol to formaldehyde.

When Au nanoparticles less than 5 nm in size are supported on base metal oxide or carbon, very active catalysts are produced. Understanding the interaction between Au nanoparticles and their support material is a key issue (Hvolbeek et al., 2007). Au nanoparticle catalysts are highly active for the oxidation many compounds, particularly CO and trimethylamine. Gas sensors based on Au nanoparticles have been developed for detecting a number of gases, including CO and NO_x (Thompson 2007).

The most catalytically active material has a Au core (submonolayer Pd shell) nanostructure. Pd-coated silver nanoparticles are very effective catalyst for remediation of trichloroethene (TCE) and common organic pollutant in ground water (Nutt et al., 2005).

One of the potential advantages that Au catalysts offer compared with other precious metal catalysts is lower cost and greater price stability, Au being substantially cheaper and considerably more plentiful than Pt.

The extraordinary optical properties of noble metal nanoparticles have been taken advantage of optical biosensors and chemosensors. One of the research subjects focused on the measurement of biological binding signals between antigen and antibody using the triangular Ag-nanoparticles (Zhu et al., 2009).

It is well-known that the polymer-gold nanoparticles composites possess interesting electrical properties (Gou and Wang, 2007). The nanocomposites composed of Au and biopolymer are employed as a novel biosensor. This biosensor exhibited a fast amperometric response and wide linear range of concentrations from 5.0 × 10⁻⁶ M to 4.01 × 10⁻⁷ M.

For biological applications, nanoparticles and quantum dots are conjugated with biospecific molecules such as antibodies, DNA, or enzymes. The binding event is detected by

monitoring nanoparticles property change. Most of these applications are based on the specific optical properties of gold or silver (Huo 2007).

Silver nanoparticles have important applications in the field of biology such as antibacterial agents and DNA sequencing. Silver has been known to exhibit strong toxicity to wide range of microorganisms (antibacterial applications). Antibacterial property of silver nanoparticles against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* has been investigated (Rai et al. 2009). Silver nanoparticles were found to be cytotoxic to *E. coli*. It was showed that the antibacterial activity of silver nanoparticles was size dependent. Silver nanoparticles mainly in the range of 1 -10 nm attach to the surface of cell membrane and drastically disturb its proper function like respiration and permeability (Morones et al., 2005).

The fluorescent bacteria were used to investigate the antibacterial properties of silver nanoparticles (Gogoi et al. 2006). The green fluorescent proteins (GFPs) were adapted to these studies. The general understanding is that silver nanoparticles get attached to sulfur-containing proteins of bacteria cell causes the death of the bacteria. The fluorescent measurements of the cell-free supernatant reflected the effect of silver on recombination of bacteria.

The high synergistic activity of silver nanoparticles and antibiotics was observed with erythromycin against *Staphylococcus aureus* (Shahverdi et al., 2007b). The antibacterial properties of the biosynthesized silver nanoparticles when incorporated on textile fabric were investigated (Kong and Jang 2008). The silver nanoparticles were also used for impregnation of polymeric medical devices to increase their antibacterial activity. Silver impregnated medical devices like surgical masks and implantable devices showed significant antimicrobial efficiency (Furno et al. 2004).

The current investigation that use of silver ion or metallic silver as well as silver nanoparticles can be exploited in medicine for burn treatment, dental materials, coating stainless steel materials, textile fabrics, water treatment, sunscreen lotions, etc. (Duran et al, 2007).

Gold nanoparticles are excellent labels for biosensors because they can be detected by numerous technique, such as optic absorption fluorescence and electric conductivity. Au nanoparticles have been primarily used for labeling application. Gold nanoparticles are a very attractive contrast agent. The interaction of gold nanoparticles with light can be used for the visualization of particles (Sperling et. 2008). In this way, the therapeutic application of metallic nanoparticles is also possible. The metallic structures can be used for hyperthermia therapy. Absorbed light by gold nanoparticles leads to heating of these particles and upon transport subsequently to heating of the particle environments. The resulting localized heating causes irreversible thermal cellular destruction (Pissuwan et al. 2006). The plasmonic photothermal therapy is a minimally-invasive oncological treatment strategy (Dickerson et al. 2008).

Generally, gold nanoparticles provide non-toxic routes to drug and gene delivery application. Gold nanoparticles are capable of delivering large biomolecules (peptides, proteins, or nucleic acids like DNA or RNA) (Ghosh et al. 2008) . The gold nanoparticle protected by a thin layer of fluorinated amphiphilic thiols is presented in Fig. 12. It is an example of water soluble gold nanoparticles protected by polymer layer.

Gold nanoparticles can be applied to amplify the biorecognition of the anticancer drug (Shen et al. 2008). Dacarbazine [5-(3, 3-dimethyl-1-triazenyl) imidazole-4-carboxamide; DTIC] is a commonly used anticancer drug. Gold nanoparticles were stabilized by PPh_3 with negative charge. The oxidized DTIC is positive charged. Thus, DTIC could be easily

assembled onto the surface of gold nanoparticles. The specific interactions between anticancer drug DTIC and DNA or DNA bases were facilitated by gold nanoparticles.

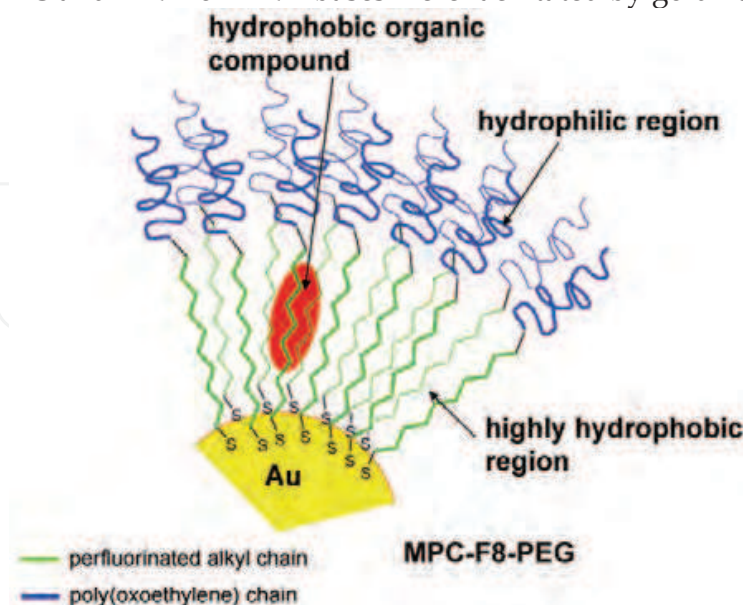


Fig. 12. Schematic of water soluble gold particles (from Agbenyega, 2008)

5. Conclusion

A green chemistry synthetic route has been used for both silver and gold nanoparticles synthesis. The reaction occurred at ambient temperature. Among the nanoparticles biological organism, some microorganisms such as bacteria, fungi, and yeast have been exploited for nanoparticles synthesis. Several plant biomass or plant extracts have been successfully used for extracellular biosynthesis of silver and gold nanoparticles. Analytical techniques, such as ultraviolet-visible spectroscopy (UV-vis), X-ray powder diffraction (XPD), transmission electron microscopy (TEM) and zeta potential measurements were applied to characterize the nanoparticles morphology.

Silver and gold nanoparticles have a number of applications from electronics and catalysis to biology, pharmaceutical and medical diagnosis and therapy. The antibacterial activity of silver ions is well known, however, the antibacterial activity of elementary silver, in the form of nanoparticles has been developed. The antimicrobial activity of Ag nanoparticles was investigated against yeast, *Escherichia coli*, and *Staphylococcus aureus*.

Gold nanoparticles may be supported by a matrix to act as a perfect catalyst. Nanoscale drug delivery systems have the ability to improve a distribution of medicines. The gold nanoparticles were utilized to facilitate the specific interactions between anticancer drugs and DNA. This may create a valuable application of metal nanoparticles in the relative biomedical area.

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Nanotechnology will be soon required in most engineering and science curricula. It cannot be questioned that cutting-edge applications based on nanoscience are having a considerable impact in nearly all fields of research, from basic to more problem-solving scientific enterprises. In this sense, books like “Silver Nanoparticles” aim at filling the gaps for comprehensive information to help both newcomers and experts, in a particular fast-growing area of research. Besides, one of the key features of this book is that it could serve both academia and industry. “Silver nanoparticles” is a collection of eighteen chapters written by experts in their respective fields. These reviews are representative of the current research areas within silver nanoparticle nanoscience and nanotechnology.

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