Synthesis Methods of Silver Nanoparticles by Chemical, Biological Synthesis-Bacteria, Fungi and Plants

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Abstract: *Silver nanoparticles are increasingly used in various fields of biotechnology, bio sensor materials, antimicrobial applications and applications in the medicine. We revise some of the most relevant and widely used synthetic methods available for the preparation of metallic silver nanoparticles namely chemical method, synthesis of Ag nanoparticles by bacteria, fungi, and plant extracts etc. The biological approach is the most emerging approach of preparation, as this method is easier than the other methods, eco-friendly and less time consuming.*

Key Words: *Silver Nanoparticles, Chemical method, bacteria, fungi, plant extracts.*

1. INTRODUCTION:

Silver nanoparticles are one of the promising products in the nanotechnology industry. The development of consistent processes for the synthesis of silver nanoparticles is an important aspect of current nanotechnology research. Silver nanoparticles are of interest because of the unique properties (e.g., size and shape depending optical, electrical, and magnetic properties) which can be incorporated into antimicrobial applications, biosensor materials, composite fibers, cryogenic superconducting materials, cosmetic products, and electronic components. Several physical and chemical methods have been used for synthesizing and stabilizing silver nanoparticles [1]. Silver is a nontoxic, safe inorganic antibacterial agent used for centuries and it has the capability of killing different type of diseases causing microorganisms. Silver has been known to be a potent antibacterial, antifungal and antiviral agent, but in recent years, the use of silver as a biocide in solution, suspension, and especially in Nano particulate form has experienced a dramatic revival. Due to the properties of silver at the nano level, Nano silver is currently used in an increasing number of consumer and medical products. The remarkably strong antimicrobial activity is a major reason for the recent increase in the development of products that contain nanosilver[2].Green synthesis approaches include mixed-valence polyoxometalates, polysaccharides, Tollens, biological, and irradiation method which have advantages over conventional methods involving chemical agents associated with environmental toxicity. Ag nanoparticles can be synthesized using various approaches including chemical, physical, and biological approach. Although chemical method of synthesis requires short period of time for synthesis of large quantity of nanoparticles, but requires capping agents for size stabilization of the nanoparticles. Chemicals used for nanoparticles synthesis and stabilization are toxic and lead to non-ecofriendly by products. The need for environmental non-toxic synthetic protocols for nanoparticles synthesis leads to the developing interest in biological approaches which are free from the use of toxic chemicals as by products. Thus, there is an increasing demand for “green nanotechnology” [3]. Many biological approaches for both extracellular and intracellular nanoparticles synthesis have been reported till date using microorganisms including bacteria, fungi and plants [4,5].The production of nanoparticles by living organisms or material of biological origin for example, nanoparticles may be synthesized using living bacteria or fungi, or using plant extracts. These techniques have advantages over more traditional methods of synthesizing nanoparticles as these are environmental friendly, can take place around room temperature or lower, and require little intervention or input of energy. In these methods, organisms involved are generally easily cultured in simple organic media, are a renewable resource, and can usually simply be left to do their work. Research has focused heavily on prokaryotes as a means of synthesizing metallic nanoparticles. Due to their abundance in the environment and their ability to adapt to extreme conditions, bacteria are a good choice for study. They are also fast growing, inexpensive to cultivate and easy to manipulate. Growth conditions such as temperature, oxygenation and incubation time can be easily controlled [6]. The Ag-NPs are in the range of 30 nm in dimensions, and are stabilized in the solution by proteins secreted by the fungus. It is believed that the reduction of the metal ions occurs by an enzymatic process, thus creating the possibility of developing a fungus-based method for the synthesis of nanoparticles. It has been well established that microorganisms are a virtually unlimited source and have potential therapeutic applications [7]. The major advantage of using plant extracts for silver nanoparticle synthesis is that they are easily available, safe, and nontoxic in most cases, and have a broad variety of metabolites that can aid in the reduction of silver ions, and are quicker than microbes in the synthesis.Plants provide a better platform for nanoparticles synthesis as they are free from toxic chemicals as well as provide natural capping agents. Moreover, use of plant extracts also reduces the cost of micro-organisms isolation and culture media enhancing the cost competitive feasibility over nanoparticles synthesis by microorganisms [3]

2. SYNTHESIS METHODS OF SILVER NANO PARTICLES:

2.1. CHEMICAL SYNTHESIS:

The chemical synthesis of silver nanoparticles is a well-established research field. The most common approach for synthesis of silver nanoparticles is chemical reduction by organic and inorganic reducing agents. In general, different reducing agents such as sodium citrate, ascorbate, sodium borohydride (NaBH4), elemental hydrogen, polyol process, Tollens reagent, N, N-dimethylformamide (DMF), and poly (ethylene glycol)-block copolymers are used for reduction of Silver ions (Ag+) in aqueous or non-aqueous solutions. Generally, the chemical synthesis process of Ag nanoparticles in solution usually employs the following three main components: (1) metal precursors, (2) reducing agents and (3) stabilizing /capping agents. Silver nanoparticles were prepared through a very simple chemical reduction route by using $N_2H_4.H_2O$ as reducing agent, aqueous solution of $AgNO_3$ and sodium acetate is taken precursor as show in Fig.2.1.[8]

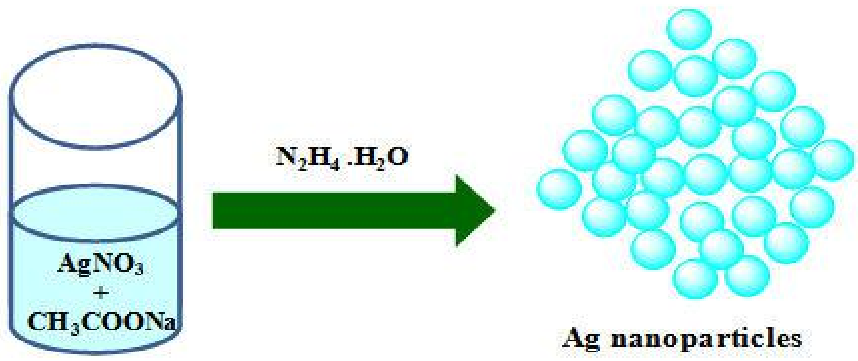

Fig.2.1.Schematic Illustration of synthesis of Ag nanoparticles.

2.2. BIOLOGICAL SYNTHESIS METHODS:

The Biological method can be used to synthesize silver nanoparticles without the use of any harsh, toxic and expensive chemical substances. The bioreduction of metal ions by combinations of biomolecules found in the extracts of certain organisms (*e.g.,* enzymes/proteins, amino acids, polysaccharides, and vitamins) is environmentally benign, yet chemically complex. Many studies have reported successful synthesis of silver nanoparticle using organisms (microorganisms and biological systems).

Silver nanoparticles were biologically synthesized by the culture supernatant Escherichia coli. The bacterial strain Escherichia coli were cultured, to produce the biomass for biosynthesis in nutrient broth. The collected 100 ml supernatant was added into the reaction vessel containing 1 mm of silver nitrate, control was also run containing only silver nitrate in water and blank was also run containing only biomass.All the reactions were carried out in bright condition for 24 hours. Color change from yellow to brown in the silver nitrate containing flask indicated the formation of silver nanoparticles . [9].

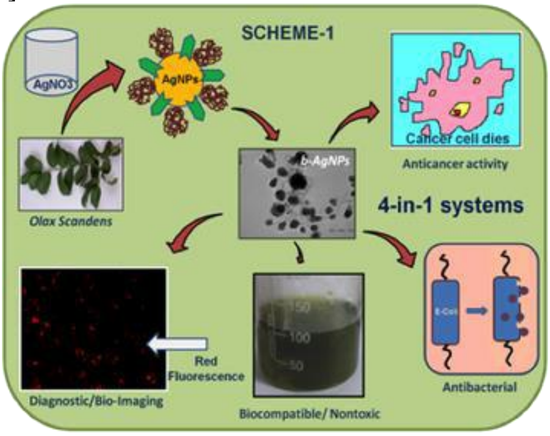

Fig.2.2.A general representation of the synthesis and applications of biogenically synthesized silver nanoparticles

2.3. SYNTHESIS OF SILVER NANOPARTICLES BY BACTERIA:

Microbial synthesis of nanomaterials utilizes of biological components, primarily prokaryotes and eukaryotes. Microbes play direct or indirect roles in several biological activities. Metals and non-metals present on earth are in constant association with biological components. The most abundant organisms in our biosphere are bacteria. Slight climate changes can potentially be disastrous to the life processes of bacteria; this can result in the prolific advantage for the production of nanoparticles.[10].The first evidence of bacteria synthesizing silver nanoparticles was established using the Pseudomonas stutzeri AG259 strain that was isolated from silver mine [11]. 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 [12]. 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. Some known examples of bacteria synthesizing inorganic materials include magnetotactic bacteria and S layer bacteria which produce gypsum and calcium carbonate layers. Several microorganisms are known to produce nanostructured mineral crystals and metallic nanoparticles with properties similar to chemically synthesized materials, while exercising strict control over size, shape and composition of the particles. Some examples are the formation of magnetic nanoparticles by magnetotactic bacteria, the production of silver nanoparticles within the periplasmic space of *Pseudomonas stutzeri* and the formation of palladium nanoparticles using sulphate reducing bacteria in the presence of an exogenous electron donor. In the most cases of bacteria, most metal ions are toxic and therefore the reduction of ions or the formation of water insoluble complexes is a defense mechanism developed by the bacteria to overcome such toxicity. Bacteria take up silver nanoparticles in its cell wall as shown in fig.2.3 and SEM visualization allows the measurement of the size and shape of the synthesized nanoparticles, SEM image of biologically synthesized AgNPs using bacterial extract confirm that the obtained AgNPs are cubical in shape with diameter ranging from 5 nm to 15 nm is shown in fig.2.4.[13]

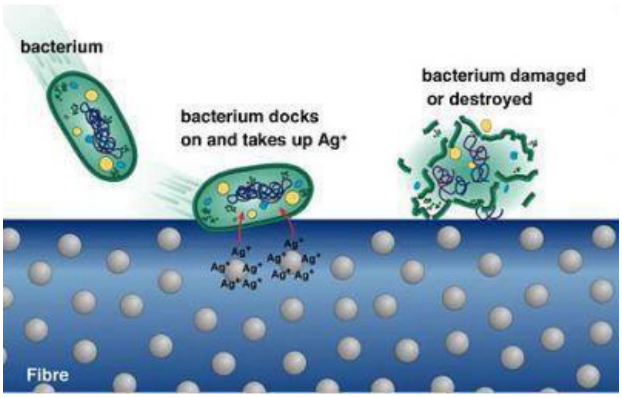

Fig.2.3

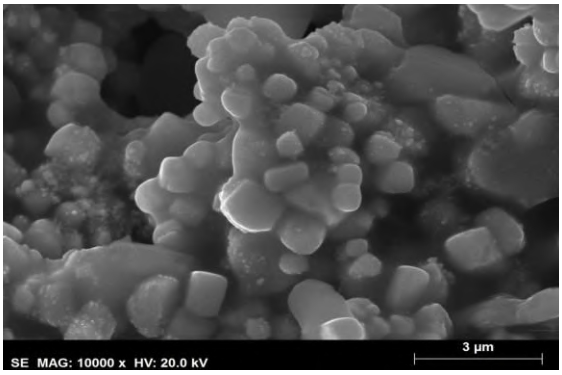

Fig.2.4

Fig.2.3. Bacteria take up silver nanoparticles in its cell wall.
Fig.2.4. SEM micrograph of AgNPs.

2.4. SYNTHESIS OF SILVER NANOPARTICLES BY FUNGI:

In addition to monodispersity nanoparticles can be obtained using fungi as compared to bacteria, fungi could be used as a source for the production of large amount of nanoparticles because they can secrete larger amounts of proteins, which directly translate to higher productivity of nanoparticles. The mechanism of Silver nanoparticle production by fungi is said to follow the steps such as trapping of Ag+ ions at the surface of the fungal cells and the subsequent reduction of the Silver ions by the enzymes present in the fungal system. The extracellular enzymes like naphthoquinones and anthraquinones are said to facilitate the reduction. Considering the example of F.oxysporum, it is believed that the NADPH-dependent nitrate reductase and a shuttle quinine extracellular process are responsible for nanoparticle formation. Though the exact mechanism involved in Silver nanoparticle production by fungi is not fully deciphered, it is believed that the above-mentioned phenomenon is responsible for the process. Compare to plant and bacteria, fungi are the best candidates for the production of Silver nanoparticles because of their more enzyme secreting activity, easy availability and culture and maintenance is very simple. Promising synthesis of nanoparticles appears by the use of specific enzymes secreted by fungi. This would lead to the possibility of genetically engineering microorganisms to over express specific reducing molecules and capping agents and thereby control the size and shape of the biogenic nanoparticles. [14, 15]. It was observed that aqueous silver ions, when exposed to the fungus

Pleurotus Ostreatus, are reduced in solution, thereby leading to the formation of an extremely stable silver particle. The Ag-NPs are in the range of 30 nm in dimensions, and are stabilized in the solution by proteins secreted by the fungus [16].AgNPs was synthesized using a reduction of aqueous Ag+ with the culture supernatants of Aspergillus terreus at room temperature [17]. The fungal inoculates were prepared in potato dextrose agar (PDA) media (a common microbiological media for culturing fungus) at 28°C in Petri plates. [18].the extracellular synthesis of stable silver nanoparticles can be done using the white rot fungus, Schizophyllum radiatum with Gene Bank Accession no HE 863742.1. and corresponding SEM images are shown in fig.2.5.[19].

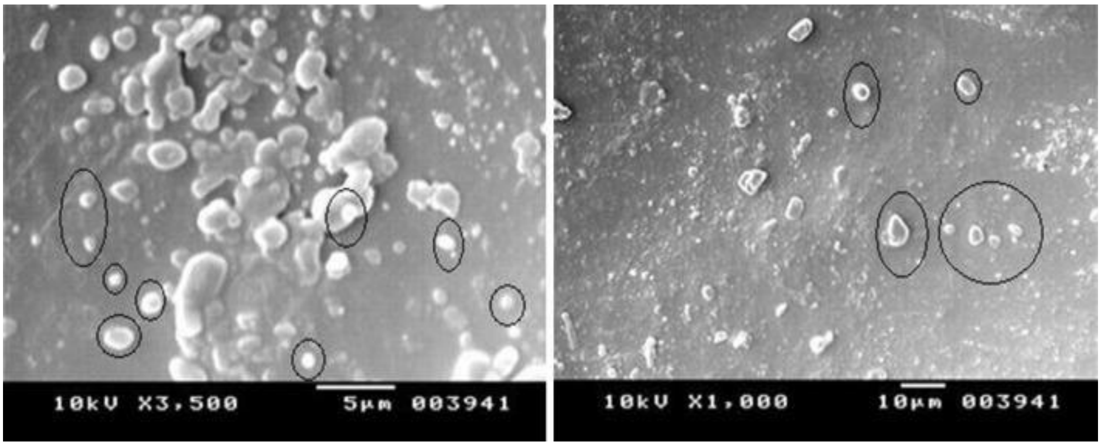

Fig.2.5.SEM micrographs of silver nanoparticles synthesized from fungal extracts.

2.5. SYNTHESIS OF SILVER NANOPARTICLES BY PLANTS:

Nanoparticles of silver, nickel, cobalt, zinc and copper are synthesized inside the live plants of Brassica juncea (Indian mustard), Medicago sativa (Alfa alfa) and Heliantusannus (Sunflower). Silver nanoparticles have also been synthesized by Azadirachta indica (Neem) leaves, Acorus calamus (Sweet flag) rhizome. Certain plants are known to accumulate higher concentrations of metals compared to others and such plants are termed as hyper-accumulators. Of the plants investigated, Brassica juncea had better metal accumulating ability and later assimilating it as nanoparticles. The major advantage of using plant extracts for silver nanoparticle synthesis is that they are easily available, safe, and nontoxic in most cases, have a broad variety of metabolites that can aid in the reduction of silver ions, and are quicker than microbes in the synthesis. The main mechanism considered for the process is plant-assisted reduction due to phytochemicals. The main phytochemicals involved are terpenoids, flavones, ketones, aldehydes, amides, and carboxylic acids. Flavones, organic acids, and quinones are water-soluble phytochemicals that are responsible for the immediate reduction of the ions. Studies have revealed that xerophytes contain emodin, an anthraquinone that undergoes tautomerization, leading to the formation of the silver nanoparticles. In the case of mesophytes, it was found that they contain three types of benzoquinones: cyperoquinone, dietchequinone, and remirin. It was suggested that the phytochemicals are involved directly in the reduction of the ions and formation of silver nanoparticles.[20]. Then plant extract was prepared by mixing of plant extract with deionized water in a conical flask. Then the solution was incubated for some time and then subjected to centrifuge for few minutes at room temperature. The supernatant was separated and filterd with (mm filter paper pore size) filter paper with the help of vaccume filter. Then the solution was used for the reduction of silver ions Ag^+) to silver nanoparticles (Ag^o).The leaf exerts of three Indian medicinal plants, M. balbisiana (banana),A. indica (neem) and O. tenuiflorum (black tulsi) are separately used to produce silver nanoparticles by adding Aqueous solution (1 mM) of silver nitrate (AgNO3).[21]. TEM images of silver nanoparticles formed by the leaf extract of banana, neem and tulsi leaves [21] are shown in fig.2.6. Silver nanoparticles were synthesized from AgNO3 through a simple green and natural route using *Calotropis gigantea* leaf[22]. Plant extract is used synthesis of silver nanoparticles contain alkaloids, flavonoids, saponins, steroid compounds, acts as reducing and stabilizing agents[23]. The silver nanoparticle, synthesized from natural plant extracts, as it is cost effective, eco-friendly, stable and safe in cancer treatment [24]. The different types of antioxidants presented in the pineapple juice synergistically reduce the Ag metal ions, as each antioxidant is unique in terms of its structure and antioxidant function. The reaction process was simple for formation of silver nanoparticles and AgNPs presented in the aqueous medium were quite stable, even up to 4 months of incubation.[25]. The use of plants as the production assembly of silver nanoparticles has drawn attention, because of its rapid, ecofriendly, non-pathogenic, economical protocol and providing a single step technique for the biosynthetic processes. [26].

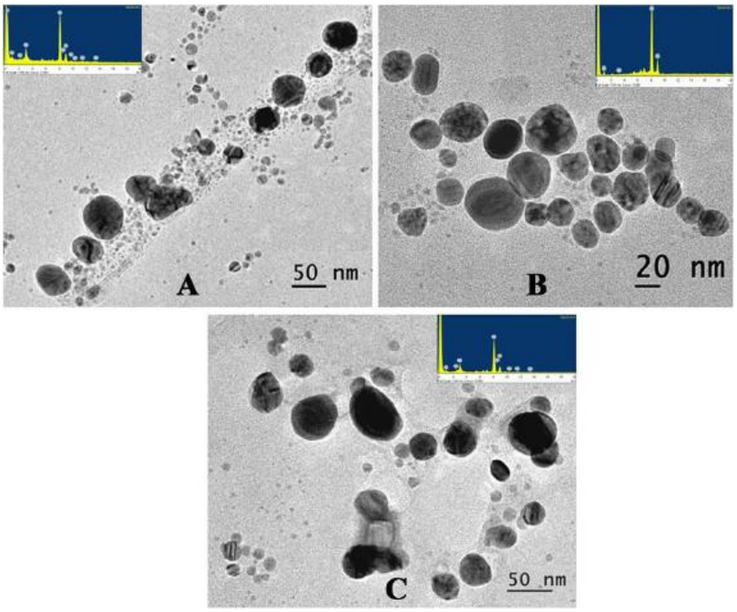

Fig.2.6.TEM images of silver nanoparticles Formed by the leaf extract of (A) banana. (B) neem and (C) tulsi leaves respectively.

3. CONCLUSIONS:

Synthesis of nanoparticles to have a better control over particles size, distribution, morphology, purity, quantity and quality, by employing as environment friendly economical processes has always been a challenge for the researchers. Biologically synthesized silver nanoparticles have many applications like coatings for solar energy absorption and intercalation material for electrical batteries, as optical receptors, as catalysts in chemical reactions, for biolabelling, and as antimicrobials due to the antibacterial activity. Synthesis of Silver nanoparticles by using of bacteria has emerged as a novel approach, Several microorganisms are known to produce nanostructured mineral crystals and metallic nanoparticles with properties similar to chemically synthesized materials, while exercising strict control over size, shape and composition of the particles. Several Fungi have been widely used for the biosynthesis of nanoparticles and the mechanistic aspects governing the nanoparticle formation have also been explained for few of them. In addition to monodispersity nanoparticles can be obtained using fungi as compared to bacteria, fungi could be used as a source for the production of large amount of nanoparticles. synthesis of nanoparticles using plant extracts is the most adopted method of green, eco-friendly production of nanoparticles and also has a special advantage that the plants are widely distributed, easily available, much safer to handle and act as a source of several metabolites.

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