PREPARATION OF SILVER NANOPARTICLE BY MICROORGANISM AND ITS APPLICATION IN PHARMACY

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Summary

Materials by changing their size from normal to nanometer range shows a great change in their physicochemical properties primarily due to their small size and large specific surface area. A number of techniques have been reported for the preparation of metallic nanoparticles but there is a great need to develop environmentally benign nanoparticle synthesis processes without use of toxic chemicals. Therefore use of biological systems for preparation of nanoparticle is considered as a best solution where use of toxic chemicals and harsh preparation methods can be avoided. Silver nanoparticles can be prepared by bioreduction process using bacteria, actinomycetes and fungi etc very efficiently. The silver nanoparticles formed are having efficient antimicrobial activity.

Keywords: Silver nanoparticle, Microorganism, Bioreduction, Extracellular reductase enzymes.

Introduction

Nanotechnology provides the ability to engineer the properties of materials by controlling their size, and this has driven research toward a multitude of potential uses for nanomaterials. Metal nanoparticles are having number of uses in pharmaceutical field mostly in cancer therapy, used as antimicrobial agents and also used in preparation of biosensors. Among noble metal nanomaterials, silver nanoparticles have received considerable attention due to their attractive physicochemical properties. The surface Plasmon resonance and large effective scattering cross section of individual silver nanoparticles make them ideal candidates for molecular labeling, where phenomena such as surface enhance Raman scattering (SERS) can be exploited. Silver in various chemical forms exhibit strong toxicity to a wide range of microorganisms and silver nanoparticles have recently been shown to have promising antimicrobial activity.[1] Several techniques to prepare silver nanoparticles are in use. However, atomistic, molecular and particulate processing in a vacuum or in a liquid medium is usually employed. Most of the techniques are tedious, costly, use harsh preparation conditions as well as inefficient in materials and energy use.[2]

Metal nanoparticles prepared by chemical methods uses reducing agents for reduction of metal ions and a protective agents or phase transfer agents to stabilize the nanoparticles.[3] Several types of toxic reducing agents containing boron commonly have been employed to produce metal

nanoparticles from inorganic salts therefore the resulting metal nanoparticles are contaminated with borides and use of such nanoparticles are restricted for use in biological and medical purposes. Quaternary ammonium compounds are used for stabilizing metal nanoparticles and the particles are not dispersible in water because their anionic particle's surface is surrounded by the hydrophobic tetraoctyl quaternary ammonium ions.[4]

To make the nanoparticles hydrophilic water-based synthesis of nanoparticles are tried but the preparation process is having problems such as ionic interactions, low reactant concentration and stabilizers are difficult to remove from the prepared nanoparticles.[5] It is well known that biological systems can provide a number of metal or metal-containing particles in the nanometer size range. For example, the synthesis of magnetite nanoparticles by magneto tactic bacteria[6], siliceous materials by diatoms[7] and gypsum and calcium carbonate layers by S-layer bacteria.[8] Therefore more and more attention has been paid to bioreduction as an efficient and environment friendly approach. For silver bioreduction, examples include the fungus *Verticillium* sp. and *Fusarium oxysporu*m, which were able to reduce the silver metal ions into silver nanoparticles intracellularly and extracellularly, respectively.[9]

It was believed that the enzymes of the microorganisms played an important role in the reduction process. Another study showed that fungi of *Penicillium* genus are having extreme potential for the synthesis of silver nanoparticles. The preparation of silver nanoparticle in this process is done probably by an extracellular mechanism. Nanoparticles obtained possess negative zeta potential and are fairly stable at pH value above 8 due to the electrostatic repulsion.[10] However; some studies proved that dried cells of some microorganisms could also reduce silver ions, where the processes of reduction were probably nonenzymatic. For example, Fu et al., showed that dried cells of *Bacillus megaterium* D01, *Lactobacillus* sp. A09, were capable of reducing silver ions through the interaction between silver ions and functional groups present on microbial cell wall.[11]

Silver nanoparticles in the size range of 10-15nm were produced by treating dried cells of *Cornebacterium* sp. SH09 with diammine silver complex. The ionized carboxyl group of amino acid residues and the amide of peptide chains were the main groups trapping $[Ag(NH_3)_2]^+$ onto the cell wall and some reducing groups, such as aldehyde and ketone, were involved in subsequent bioreduction for formation of nanoparticle.[12] In the present study preparation of silver nanoparticle by bioreduction process using microbial enzymatic system will be discussed in detail.

Principle

The principle of preparation of silver nanoparticles by using microorganism is a bioreduction process; the silver ions are reduced by the extracellular reductase enzymes produced by the microorganisms to silver metal in nanometer range.[13]

The reaction involved in this process is shown in **Fig-I**. It is concluded from protein assay of microorganisms that the reductase involved in bioreduction for preparation of silver nanoparticles is an NADH-dependent reductase. The enzyme reductase gains electrons from NADH and oxidizes it to NAD+. The enzyme is then oxidized by the simultaneous reduction of

Silver ions forming silver metal in nanoform. In some cases a nitrate-dependent reductase is responsible for the bioreduction process whereas in the case of rapid extracellular synthesis of nanoparticles the reduction happens within few minutes, therefore a complex electron shuttle materials may be involved in the biosynthesis process.[14]

Method

The specific microorganism is cultured in specific culture medium and incubated at the specific culture conditions for overnight. The sample culture medium is then centrifuged to separate the cell mass. The supernatant is separated and preserved for future experiment. 50ml of supernatant solution is taken in a 250ml Erlenmeyer flask and the pH is adjusted to 8.5. To that 50ml aqueous solution of 1mM Silver nitrate ($AgNO_3$) is added. The samples are prepared in duplicate. A control is prepared by mixing 50ml aqueous solution of 1mM Silver nitrate and 50 ml distilled water. All the prepared samples are kept in a shaker at 40°C (200 rpm) for 5 days and maintained in the dark condition. The sample mixture that is added with bacterial enzymes changes its colour to black or dark brown indicating formation of silver nanoparticles as shown in **Fig-II** compared with control where there is no colour change.[10] At different time intervals sampling is done and colorimetric observations are carried out to detect the time of maximum production of nanoparticles. The reaction mixture is then centrifuged at 15000 rpm to separate the prepared silver nanoparticles. The prepared nanoparticles are to be characterized by Transmission electron spectroscopy (TEM), Scanning electron spectroscopy (SEM), and Photon correlation spectroscopy (PCS) etc. In chemical nanoparticle synthesis methods, a stabilizer is necessary to prevent the aggregation of fine particles to make them stable for a long period of time but with use of biological systems, it is clear from the Transmission electron spectroscopy study that even aggregated nanoparticles don‘t have direct contact with one another. This is due to the fact that nanoparticles are stabilized in solution by capping proteins, which are secreted from microorganisms. One important enzyme that may be responsible for this is Cytochrome C. The silver nanoparticles formed by this process are quite stable due to capping by bacterial proteins for a period of 5 months at 25°C.[13]

Application

Silver may have an important advantage over conventional antibiotics in that it kills all pathogenic microorganisms, and no organism has ever been reported to readily develop resistance to it. Researchers believe that the potential of colloid silver is just beginning to be discovered.[15] Silver ions interact with various components of bacterial, protozoal and fungal cells at very low concentrations with bactericidal activity.[16] Studies have also demonstrated that silver ions interact with sulfydryl (-SH) groups of proteins as well as the bases of DNA leading either to the inhibition of respiratory processes[17] or DNA unwinding[18] leading to death of microorganism. Inhibition of cell division and damage to bacterial cell envelopes is also recorded[19] and interaction with hydrogen bonding processes has been demonstrated to occur.[20] Antiviral activity of silver ions has been recorded and interaction with –SH groups has been implicated in the mode of action.[21]

Silver metal in nanoscale range add antibacterial properties on to a given product. Generally, this involves the

application or incorporation of nanosized silver particles into or on the surface of products like cleaning sprays, skin creams, ATM buttons, and sports clothing etc. Nanosilver technologies appear in a variety of manufacturing processes and also in end products. It can appear imbedded in a coating which is applied to the product by the manufacturer. Some products come in a liquid form and are meant to be applied to form a coating. Nanosilver can be presented in a liquid form such as a homeopathy colloid or contained within a shampoo. It can also be embedded in a solid such as a polymer master batch or be suspended in a bar of soap. Nanosilver can also be utilized in the textile industry by incorporating it into the fiber or produced as a powder.[22]

Silver nanoparticles also used for its antiviral properties. Silver nanoparticles undergo a size dependent interaction with HIV-1, with nanoparticles exclusively in the range of 1-10 nm attached to the virus. The regular spatial arrangement of the attached nanoparticles, the center-to-center distance between nanoparticles, and the fact that the exposed sulfur-bearing residues of the glycoprotein knobs would be attractive sites for nanoparticle interaction suggest that silver nanoparticles interact with the HIV-1 virus via preferential binding to the gp120 glycoprotein knobs. Due to this interaction, silver nanoparticles inhibit the virus from binding to host cells.[1]

Conclusions

The use of microorganisms such as bacteria, yeasts, algae, fungi and actinomycetes in the biosynthesis of metal nanoparticles has been described, with particular emphasis on the externally produced reductase enzymes. Extracellular secretion of enzymes offers the advantage of obtaining large quantities in a relatively pure state, free from other cellular proteins associated with the organism and can be easily processed by filtering of the cells and isolating the enzyme for nanoparticles synthesis from cell-free filtrate. Therefore more research is needed to isolate microorganisms producing smaller size nanoparticles efficiently.

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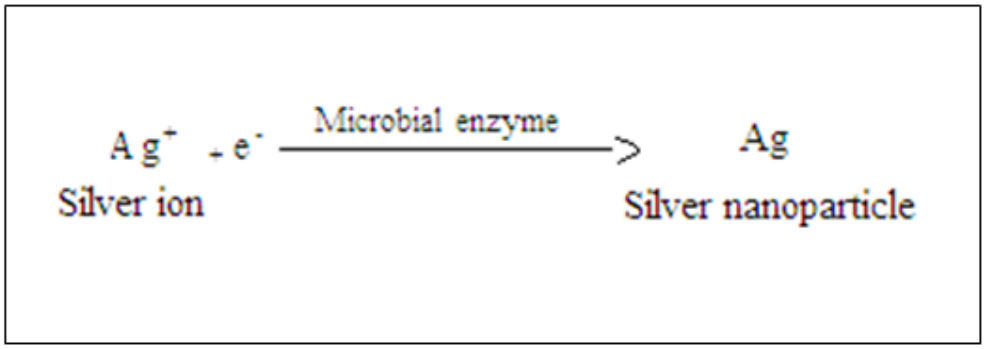


Fig-I: **Reaction showing preparation of silver nanoparticles.**

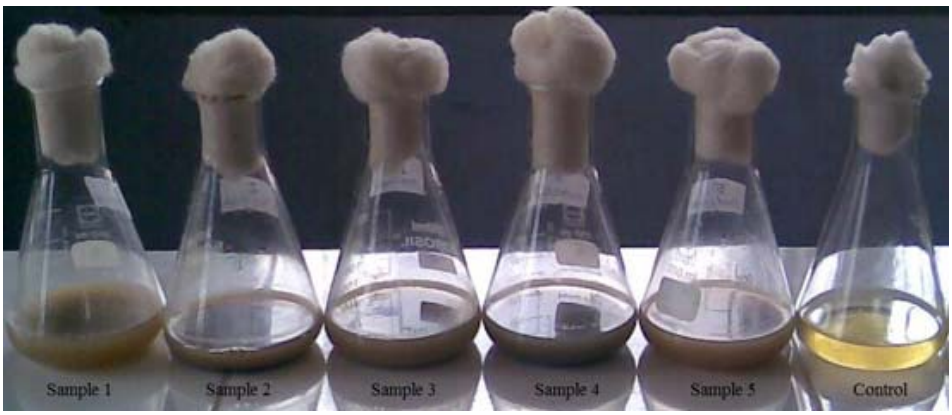


Fig-II: **Sample 3 and 4 shows a colour change indicating formation of silver nanoparticles.**