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Silver Nanoparticles: potential and promising means to combat pathogenic microorganisms

Rini Roy [a1] and Aditi Nag Chaudhuri [b2]

[a] *Post Graduate Department of Microbiology, Bidhannagar College, Kolkata, W.B., India*

[b] *Post Graduate Department of Microbiology, Lady Brabourne College, Kolkata, W.B., India*

Abstract: Nanotechnology is a rapidly expanding field with its applications in biomedical sciences and is associated with the engineering and production of materials at the atomic and molecular level. Study of silver nanoparticles has a wide application in various fields. Metal nanoparticles are the most promising due to their anti-bacterial properties which, occurs because of the high surface to volume ratio. Silver nanoparticles (AgNPs) are particularly excellent because of their potential applications in health care, textile fibers, food packaging, and antibacterial fields. When used in relatively low concentrations at the same size and shape Ag-NPs are non-toxic or less harmful to mammalian tissues and environmentally friendly. In this study an overview on the synthesis of AgNPs from different sources and their antibacterial activity has been given. The physical and chemical methods and green synthesis as well as microbial synthesis of AgNPs are mentioned here. It has been shown that synergistic action of AgNPs and antibiotics have antibacterial effect on pathogenic bacteria [1].

Keywords: silver nanoparticles; pathogenic bacteria; reducing agents; antibiotics

1 Introduction

Nanotechnology is a significant research field which deals with particle structures ranging from approximately 1-100 nm as also their design, synthesis, and manipulation. Nanobiotechnology is a multidisciplinary aspect where nanoparticles used in biological systems encompasses the disciplines of biology, biochemistry, chemistry, engineering, physics and medicine. Moreover, the nanobiotechnology also involves clean, nontoxic, and eco-friendly procedures for the synthesis of metal NPs having the intrinsic ability to reduce metals by specific metabolic pathways [2-7]. Green synthesis approaches include mixed valence polyoxometalates, polysaccharides, Tollens, biological, and irradiation method which renders less environmental toxicity. Solvent medium selection and selection of eco-friendly nontoxic reducing and stabilizing agents are the main criteria in green synthesis of NPs [8].

The importance of silver NPs results from the unique properties which can be incorporated into antimicrobial applications, biosensor materials, composite fibers, cryogenic super-conducting materials, cosmetic products, and electronic components. Some important applications of silver NPs are in pharmaceutics, medicine, and dentistry. There are many physical and chemical methods used for synthesizing and stabilizing silver NPs.

Silver nanoparticle based antibacterial agents are the need of the hour in the present scenario of multidrug resistance in microorganisms. The antibacterial mechanisms of silver nanoparticles are dependent on different structural factors including surface chemistry, size, and shape [9]. Silver is least toxic among other elements with respect to oligodynamic effect against microorganisms. So, silver-based materials like silver nanoparticles are useful exhibiting antimicrobial properties by damaging not only the key enzymes in the pathogenic bacterial cell membranes but also by penetrating the bacteria [10].

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[1] Email: for_rini_roy@yahoo.co.in
[2] Email: odditybiom@gmail.com

Silver nanoparticles (AgNps) have been found to be effective in case of hospital isolated *Pseudomonas aeruginosa* which is generally considered as a multidrug resistant strain [11].

2 Preparation of silver nanoparticles

2.1 Physical methods

Evaporation-condensation and laser ablation are the most important physical methods for the preparation of AgNPs. The advantages of physical synthesis methods in comparison with chemical processes are the absence of solvent contamination in the prepared thin films and the uniformity of NPs distribution.

A small ceramic heater with a local heating area has been used to synthesize AgNPs [12]. To evaporate source materials the small ceramic heater was used. The evaporated vapor can cool at a suitable rapid rate since the temperature gradient in the vicinity of the heater surface is very steep compared to that of a tube furnace.

The formation of small NPs in high concentration results from this method. As the fluctuation of temperature of the heater surface does not take place with time the particle generation is very stable This physical method can be used for two purposes - as a nanoparticle generator for long-term experiments for inhalation toxicity studies, and as a calibration device for nanoparticle measurement equipment [12].

Laser ablation of metallic bulk materials in solution is a technique by which silver NPs could be synthesized [13]. The wavelength of the laser impinging the metallic target, the duration of the laser pulses (in the femto-, pico- and nanosecond order), the laser fluence, the ablation time duration and the effective liquid medium, with or without the presence of surfactants are the parameters on which the ablation efficiency and the characteristics of produced nano-silver particles depend [14].

One important advantage of laser ablation technique in comparison with other methods for production of metal colloids is the absence of chemical reagents in solutions.

Tien and co-workers [15] used the arc discharge method to synthesize silver NPs suspension in deionized water with no added surfactants. In this method, silver wires (Gredmann, 99.99%, 1 mm in diameter) were submerged in deionized water and used as electrodes. With a silver rod consumption rate of 100 mg/min, yielding metallic silver NPs of 10 nm in size, and ionic silver obtained at concentrations of approximately 11 ppm and 19 ppm, respectively.

The synthesis of silver NPs by direct metal sputtering into the liquidmedium was shown by Siegel and colleagues [16]. An interesting alternative to time-consuming, wet-based chemical synthesis techniques is the method combining physical deposition of metal into propane-1,2,3-triol (glycerol).

2.2 Chemical methods: Chemical reduction

The chemical reduction by organic and inorganic reducing agents is the most common approach for synthesis of AgNPs. Different reducing agents have been used in aqueous or non-aqueous solutions, leading to formation of metallic silver (Ag), which is followed by assembly into nanoparticles. These different reducing agents include sodium citrate, ascorbate, sodium borohydride (NaBH4), elemental hydrogen, polyol process, Tollens reagent, N, N-dimethylformamide (DMF), and poly (ethylene glycol)-block copolymers. Reduction of Ag^{+} to metallic silver (Ag^{0}) by these reducing agents is followed by agglomeration into oligomeric clusters which eventually lead to the formation

of metallic colloidal silver particles [17]. Some protective agents are used to stabilize dispersive NPs during metal nanoparticle preparation and protect the NPs that can be absorbed on or bind onto nanoparticle surfaces, avoiding their agglomeration [18]. The presence of surfactants consisting functional groups like thiols, amines, acids, and alcohols are responsible for interactions with particle surfaces which can stabilize particle growth. Thus, these surfactants protect particles from sedimentation, agglomeration, or losing their surface properties.

Polymeric compounds such as poly (vinyl alcohol), poly (vinylpyrrolidone), poly (ethylene glycol), poly (methacrylic acid), and polymethylmethacrylate have been found to be the effective protective agents to stabilize NPs. Oliveira and co-workers[18] in one study prepared dodecanethiol-capped silver NPs based on Brust procedure [19]where a phase transfer of an Au^{3+} complex from aqueous to organic phase in a two-phase liquid-liquid system occurred, which was followed by a reduction with sodium borohydride in the presence of dodecanethiol as stabilizing agent. Dodecanethiol binding onto the NPs surfaces, avoided their aggregation and made them soluble in certain solvents. Dramatic modifications in nanoparticle structure, average size, size distribution width, stability and self-assembly patterns were observed due to small changes in synthetic factors. Kim and colleagues [20] showed production of spherical silver NPs with a controllable size and high monodispersity using the polyol process and a modified precursor injection technique. The injection rate and reaction temperature were important factors for producing uniform-sized silver NPs with a reduced size in the precursor injection method.

In the process of preparation of AgNPs at room temperature, the corresponding metal ions are mixed with reduced polyoxometalates which serves as reducing and stabilizing agents. Polyoxometalates are soluble in water. They have the capability of undergoing stepwise, multielectron redox reactions without disturbing their structure. It was shown that AgNPs were produced by illuminating a deaerated solution of polyoxometalate/S/Ag^{+} [21]. It has been reported that green chemistry-type one-step synthesis and stabilization of silver nanostructures with MoV–MoVI mixed-valence polyoxometalates occur in water at room temperature [22].

2.3 Microemulsion techniques

Microemulsion techniques are used to synthesize uniform and size controllable AgNPs.The initial spatial separation of reactants (metal precursor and reducing agent) in two immiscible phases is the basis for the NPs preparation in two-phase aqueous organic systems. A quaternary alkyl-ammonium salt mediates the interface between the two liquids and the intensity of inter-phase transport between two phases. This affects the rate of interactions between metal precursors and reducing agents. Due to surface coating with stabilizer molecules occurring in the non-polar aqueous medium metal clusters formed at the interface are stabilized and transferred to the organic medium by the inter-phase transporter [23]. The use of highly deleterious organic solvents is one of the major disadvantages.

Thus, separation and removal of large amounts of surfactant and organic solvent from the final product is necessary. Use of dodecane by Zhang and co-workers [24] as oily phase (a low deleterious and even nontoxic solvent), reduced the necessity to separate the prepared silver solution from the reaction mixture. Colloidal NPs prepared in nonaqueous media for conductive inks are well-dispersed in a low vapor pressure organic solvent which readily wet the surface of polymeric substrate without any aggregation. Metal nanoparticles are applied as catalysts to catalyse most organic reactions being conducted in non-polar solvents. Transferring metal NPs to different physicochemical environments are important in practical applications [25].

2.4 UV-initiated photoreduction

UV-initiated photoreduction is a simple and effective method for synthesis of silver NPs in the presence of citrate, polyvinylpyrrolidone, poly (acrylic acid), and collagen. Silver NPs produced by Huang and Yang via photoreduction of silver nitrate in layered inorganic laponite clay suspensions served as stabilizing agent for prevention of NPs aggregation. The properties of produced NPs are expressed as a function of UV irradiation time. When irradiated under UV for 3 h, bimodal size distribution and relatively large silver NPs were obtained. The silver NPs disintegrated into smaller sizes with a single distribution mode on further irradiation. This disintegration continued until a relatively stable size and size distribution was obtained [26]. Poly (vinyl alcohol) served as protecting and stabilizing agent for preparation of the Silver NPs (nanosphere, nanowire, and dendrite) by UV irradiation photo reduction technique at room temperature. Significant contribution in the growth of the nanorods and dendrites depended on concentration of both poly (vinyl alcohol) and silver nitrate [27].

2.5 Photoinduced reduction

Photoinduced or photocatalytic reduction methods can be used to synthesize silver NPs because this process has high spatial resolution, convenience of use, and great versatility. Photochemical synthesis can design the NPs in various mediums including cells, emulsion, polymer films, surfactant micelles, glasses, etc. Poly (styrene sulfonate)/poly (allylamine hydrochloride) polyelectrolyte capsules are used as microreactors for preparing Nano-sized silver particles with an average size of 8 nm by photoinduced reduction [28]. Photoinduced method converts silver nanospheres into triangular silver nanocrystals (nanoprisms) with desired edge lengths in 30-120 nm range [29]. Particle growth process was regulated using dual-beam illumination of NPs. Citrate and poly (styrene sulfonate) act as stabilizing agents in this process.

In the presence of sodium citrate (NaCit), the direct photo-reduction process of $AgNO_3$ was conducted with different light sources (UV, white, blue, cyan, green and orange) at room temperature. This light-modification process results in a colloid with distinctive optical properties relating to the size and shape of the particles [30]. UV photo-activation method has been used for the preparing stable silver NPs in aqueous Triton X-100 (TX-100)[31]. TX-100 molecules serve dual role of reducing agent and of NPs stabilizer through template/capping action.

Furthermore, surfactant solution by decreasing the diffusion or mass transfer co-efficient of the system carry out the process of NPs growth in the diffusion-controlled way. It governs NPs size distributions by increasing the surface tension at the solvent-NPs interface.

2.6 Irradiation methods

Silver NPs with a well-defined shape and size distribution are obtained by laser irradiation of an aqueous solution of silver salt and surfactant [32]. Use of laser in a photo-sensitization synthetic method of making silver NPs required benzophenone. Low laser powers produced silver NPs of about 20 nm, but an increased irradiation power synthesized NPs of about 5 nm. Light sources used for production of silver NPs were laser and mercury lamp [33]. Photo-sensitized growth of silver NPs using thiophene (sensitizing dye) was observed in visible light irradiation studies and by illumination of $Ag(NH3)^+$ in ethanol silver nanoparticle formation has been done [34, 35].

2.7 Microwave-assisted synthesis

Microwave-assisted synthesis is a method which is worthy to mention for synthesis of AgNPs. For consistently yielding nanostructures with smaller sizes, narrower size distributions, and a higher degree of crystallization microwave heating is better than a conventional oil bath [36]. The

advantages of microwave heating are shorter reaction times, reduced energy consumption, and better product yields preventing the agglomeration of the particles formed [36].

Using carboxymethyl cellulose sodium as reducing and stabilizing agent AgNPs could be synthesized by microwave-assisted synthesis method. Concentration of sodium carboxymethyl cellulose and silver nitrate control the size of AgNPs. The produced AgNPs remained uniform and stable at room temperature for 2 months without any visible changes [37]. Pt seeds, polyvinyl pyrrolidine and ethylene glycol help in the synthesis of AgNPs [38].

2.8 Green synthesis

Metal nanoparticles produced by Green synthesis of using various plants like extracts of *Cacumen platycladi*, *Eucalyptus citriodora* and *Ficus bengalensis, Ocimum sanctum* leaf and plant products has recently been successfully accomplished. The AgNPs synthesized from these plants or plant parts are shown to show the antimicrobial activities against pathogenic microorganisms [39-41].

2.9 Microbial synthesis

Waste material from the corn industry has been utilised for the synthesis of silver nanoparticles to demonstrate the antibacterial activities against food borne pathogens like *Bacillus cereus* ATCC 13061, *Staphylococcus aureus* ATCC 49444, *Listeria monocytogenes* ATCC 19115, *Escherichia coli* ATCC 43890, and *Salmonella Typhimurium* ATCC 43174. Microorganisms are also able to synthesize AgNPs. The characterization of biosynthesized AgNPs were done by UV-Vis spectrophotometry along with surface plasmon resonance at 450 nm as also by using scanning electron microscope, X-ray diffraction, Fourier-transform infrared spectroscopy and thermogravimetric analysis [42].

The shape and size of the resulting AgNPs largely depended on experimental parameters like temperature, concentration of the Ag(I) compound, pH solution. In the case of biological synthesis, the shape and size are depended on the direct object used to produce AgNPs [43].

3 Mode of action of AgNPs on cells

There are different mechanisms by which AgNPs exert its antibacterial effect/ adhesion on the surface of the bacterial cell wall and membrane. The mode of penetration into the cell and disruption of intracellular organelles and biomolecules, induction of oxidative stress, and modulation of signal transduction pathways have been studied for AgNps[44]. On the cell surface for Gram-negative bacteria the adhesion and accumulation of AgNPs takes place. There are water-filled channels called porins in the outer membrane of Gram-negative bacteria through which AgNPs can penetrate bacterial cells. Passive transport of hydrophilic molecules of various sizes and charges across the membrane occurs through porins. The thicker cell wall of Gram-positive bacteria is responsible for the penetration of silver ions into the cytoplasm, therefore the effect of AgNPs is more pronounced in Gram-negative bacteria than in Gram-positive bacteria. The presence of lipopolysaccharides attributes to the structural integrity of the Gram-negative bacteria cell wall, making such bacteria more sensitive to silver nanoparticles because the negative charge of the lipopolysaccharides promotes AgNP adhesion. It has been predicted that the ability of silver nanoparticles to attach to the bacterial cell wall is due to the electrostatic interaction between positively charged silver ions and the negatively charged surface of the cell membrane due to the carboxyl, phosphate, and amino groups, give an

opportunity to subsequently penetrate it, thereby causing structural changes in the cell membrane and, as a result, its permeability [45].

Thus, proton motive force (PMF) is dissipated and then membrane is destroyed. AgNPs may also act as a carrier to transport Ag^+ more efficiently to bacterial cells whose proton motive force would consequently reduce the local pH and increase Ag^+ release. Silver nanoparticles damage the cell membrane by forming free radicals upon contact with bacteria thus making it porous. However, in the view of others AgNPs adhere to the surface of bacteria and change the membrane properties, while inside the bacterial cell, they can lead to DNA damage.

Inhibition of transcription occurs due to the penetration of AgNPs into the cell where they could associate with intracellular elements such as lipids, proteins and DNA [45].

4 Applications of AgNPs

Due to antibacterial, antifungal, antiviral, larvicidal, antiplasmodial, anthelmintic and leishmanicidal activity AgNPs have versatile applications [45].

AgNPs have their application in dental medicine, cardiology and dermatology by virtue of their antimicrobial activity [46].

The smaller the particle size of Ag-NPs, the smaller the value of the minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC), indicating the greater the antibacterial activity [47].

The aqueous extract of *Murraya koenigii* leaves was used for synthesis of silver nanoparticlesand their antibacterial potential was evaluated on multiple Extended-spectrum β-lactamase (ESβL) producing genteric bacteria and Methicillin Resistant *Staphylococcus aureus* (MRSA). The AgNps prepared from plant extract inhibited the growth of the test pathogens on nutrient agar plates with varying zones of inhibition [48].

Rapid synthesis of silver nanoparticles can be done by the combination of culture supernatant of bacteria. The AgNP synthesized from *S.aureus* was tested for antimicrobial activity by well diffusion method, against pathogenic organisms such as MRSA, MRSE, *Streptococcus pyogenes, Salmonella typhi*, *Klebsiella pneumoniae* and *Vibrio cholorae*. The zone of inhibition was observed [49].

There are different studies which revealed that the plant and fungus can also produce silver nanoparticles. Using *Penicillium purpurogennum* silver nanoparticles have been successfully produced [50]. They found that increase in concentration of silver nitrate solution increases the formation of silver nanoparticles. They have also reported that, the change in pH of the reaction mixture led to the change in the shape and size of the silver nanoparticles [49].

In our study [1] the purpose was to prepare different silver nanoparticles and to observe the antimicrobial effect of only the silver nanoparticles on pathogenic microorganisms like *E. coli*, *Salmonella* sp., *Vibrio cholera* isolated from the water samples collected from the East Kolkata Wetland. In our studies experiments were designed to observe the antimicrobial effect of silver nanoparticles along with different types of chemicals (SDS, lysozyme) on pathogenic microorganisms and to observe the antimicrobial effect of silver nanoparticles along with different types of antibiotics like Ampicillin, Streptomycin, Tetracycline, Chloramphenicol, Gentamycin, Erythromycin and Kannamycin on pathogenic microorganisms.

5 Conclusion

The silver nanoparticles prepared with various reducing agents in our study were found to be effective antimicrobial agent, effective against pathogenic bacteria. The silver nanoparticle prepared along with sodium citrate was found to be highly effective in retarding the growth of pathogenic bacteria in comparison to the AgNPs prepared with sodium borohydride and that prepared with pyrogallol [1]. When AgNPs prepared with sodium citrate was used along with SDS, it effectively reduced the size of the pathogenic bacteria and retarded their growth.

When AgNPs prepared with sodium citrate was used along with antibiotic ampicillin, it was seen that *E. coli* cells which were ampicillin resistant turned out to be ampicillin sensitive strains [1]. A possible cause for this phenomenon could be that the silver nanoparticle helped in the entry of ampicillin inside the cells, or it can also be that the silver nanoparticle helped to destroy the thiol (–SH) of beta lactamase thus turning the ampicillin resistant strains to ampicillin sensitive strains.

The prepared silver nanoparticles can thus be effectively used as antimicrobial agents along with antibiotics and the different structures may attribute to their different mode of action. As AgNPs are cheap and have low cytotoxicity, so they can be used as an alternative antimicrobial agent.

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