A Study of Synthesis and Characterization of Photochemical Synthesis and Biological Properties of Silver Nanoparticles

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Abstract

Nobel metal nanoparticles have drawn significant attention for a wide range of new applications in various fields including biology and medicine. Research work during the last two decades have clearly demonstrated that the properties of nanoparticles, in particular silver nanoparticles are strongly influenced by shape, size and size distribution, which is dictated by the synthetic method adopted. In this review, we enumerate various top-down and bottom up approaches to synthesize nanoparticles. Chemical reduction method is one of the simple and facile approaches for bottom-up synthesis of silver nanoparticles and the stability of the synthesized nanoparticles has been found to be influenced by the type and amount of reducing agent and type of stabilizer used. Some of the capping reagents discussed including citrate salts, oleic acid, amino silanes, and polyelectrolytes so as to stabilize the nanoparticles. Instead of using polyelectrolytes to conjugate nanoparticles, bio macromolecules have been used to stabilize nanoparticles so that it renders the nanoparticles bioactive and biocompatible as well as provides additional functionalities for further biological interactions. Surface modification of nanoparticles with proteins such as Bovine Serum Albumin (BSA) is an effective approach to providing electrostatic stability to silver nanoparticles. We highlight the various pathways by which stabilized nanoparticles promote antibacterial activity and describe the impact of stabilized nanoparticles on mammalian cells. More importantly, in this review we describe the possibility of a concentration window at which nanoparticles are toxic to bacteria and not to mammalian cells, so that the nanoparticles loaded matrix could be designed with the intent that nanoparticles when released in the physiological medium can maintain a sterile environment against microorganisms while not inhibiting the growth of mammalian cells in the site specific region of intended application. Additionally, methodologies used to characterize the composition, morphology and biological properties of synthesized nanoparticles by multiple techniques have been presented.

Keywords: *Synthesis, Photochemical Synthesis, Biological Properties, Silver Nanoparticles, Chemical reduction.*

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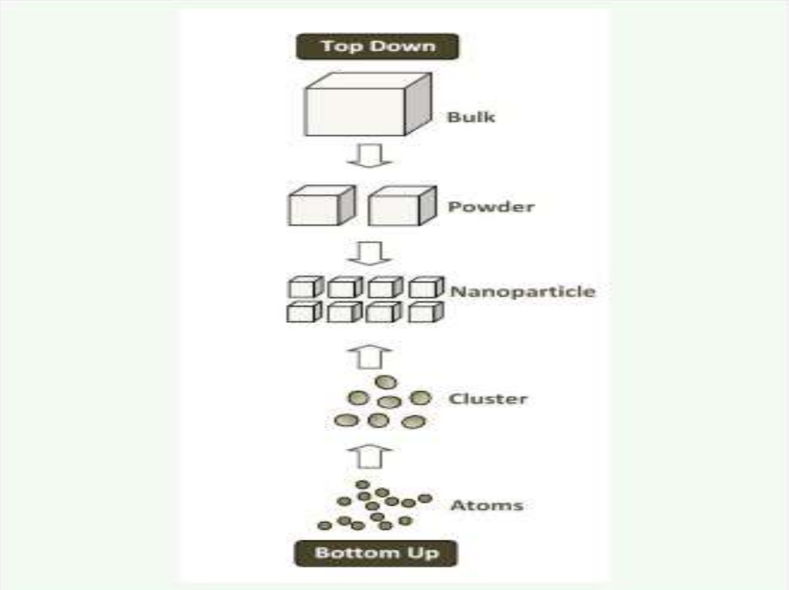
1. INTRODUCTION

The effective prevention and treatment of an ever-increasing range of infections caused by bacteria, viruses, fungi, and parasites are a priority to public health officials and a big challenge to pharmaceutical industry [1]. Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa are the etiological agents of several infectious diseases [2]. Infections caused by these and other pathogenic bacteria decreased during the twentieth-century due in part to the discovery and therapeutic use of antibacterial antibiotics [3]. Antibacterial antibiotics are classified by their specific mechanism of action. Examples of antibacterial antibiotics include bacterial cell wall inhibitors, e.g., (beta lactams - penicillins and cephalosporins), vancomycin, cycloserine, and bacitracin; bacterial DNA gyrase/topoisomerase inhibitors, e.g., quinolones; bacterial RNA polymerase inhibitor, e.g., rifampin; RNA elongation inhibitor, e.g., actinomycin; bacterial protein synthesis inhibitors, e.g., 50 S ribosome inhibitors - macrolides and chloramphenicol and 30 S ribosome inhibitors - tetracyclines and aminoglycosides; folic acid metabolism inhibitors , e.g., trimethoprim and sulfonamides; and cell membrane inhibitors, e.g., polmyxins. Moreover, antibiotic resistance has emerged as a prevalent problem due in part to the misuse of existing antibiotics and the lack of novel antibiotics [4,5]. Conventional antibiotics no longer inhibit bacterial growth because bacteria have developed antibiotic resistance via mutational and/or several adaptive mechanisms that include decreasing the antibiotic concentration via efflux pumps (tetracycline efflux pumps), antibiotic inactivation via enzymatic modification, (beta lactamase cleavage of the beta lactam rings present in penicillin and cephalosporins or acetylation of chloramphenicol via chloramphenicol acetyl transferase), and or alteration of the bacterial drug targets, e.g., altered penicillin binding proteins or bacterial ribosome subunits. Additionally, antibacterial resistance genes reside on either the bacterial genome or on extra chromosomal plasmids and these resistance genes may be transferred between bacteria. Increasing antibiotic resistance has emerged as a consequence [4]. Bacterial resistance to conventional antibiotics has prompted the development of alternative strategies to prevent and treat bacterial infections. Among them, nanoscale materials have emerged as an alternative approach to treat bacterial infections caused by the antibiotic resistant bacterial strains. Stabilized nanoparticles act on bacteria via multiple mechanisms [6,7]. Bioconjugated nanoparticles are able to attach to the membrane of bacteria by electrostatic interaction and damage the integrity of the bacterial peptidoglycan and/or cell membrane [8]. Among the several metal nanoparticles, silver nanoparticles have received considerable attention due to their broad inhibitory behavior towards nearly 650 species of microbes, and more importantly against antibiotic resistant bacterial strains. In one of the findings, it was shown that silver nanoparticles showed superior antibacterial activity against E. coli and S. aureus when compared to gold nanoparticles Furthermore, the chemistry and morphology of silver nanoparticles can be easily tuned to improve their antibacterial efficacy. The worldwide production of silver nanoparticles is estimated to be 320 tons per year.

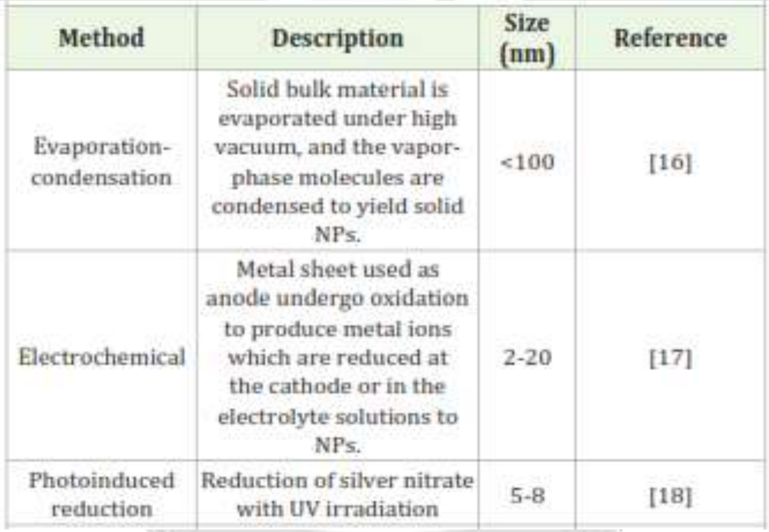
2. SYNTHESIS OF NANOPARTICLES

A top-down approach to nanofabrication is based on the synthesis of the nanomaterials from the bulk system, while bottom-up synthesis of nanomaterials is based on packing of several atoms, or molecules with molecules, or clusters with clusters. A representation of the top down and bottom up approach is shown in Scheme (1). Table (1) summarizes the advantages and disadvantages of various methods used in the syntheses of nanoparticles. Procedures used in top-down synthesis of nanoparticles include etching, grinding, ball milling, laser ablation, photo-lithography, and electron beam lithography. Unlike top-down approach, bottom up approach is based on organization of small constituents (atoms or molecules). This method is guided by physicochemical interaction of neighboring constituents, the surface chemistry and self-assembly principles of the constituents that makeup the nanoscopic material. Bottomup approach offers a better chance to obtain nanostructures with less defects, more homogeneous chemical composition, potentially better short and long range order. Some examples of bottom-up approach include biological, photochemical, and chemical synthetic routes. Here, we describe the bottom up method which is the primary focus of the study.

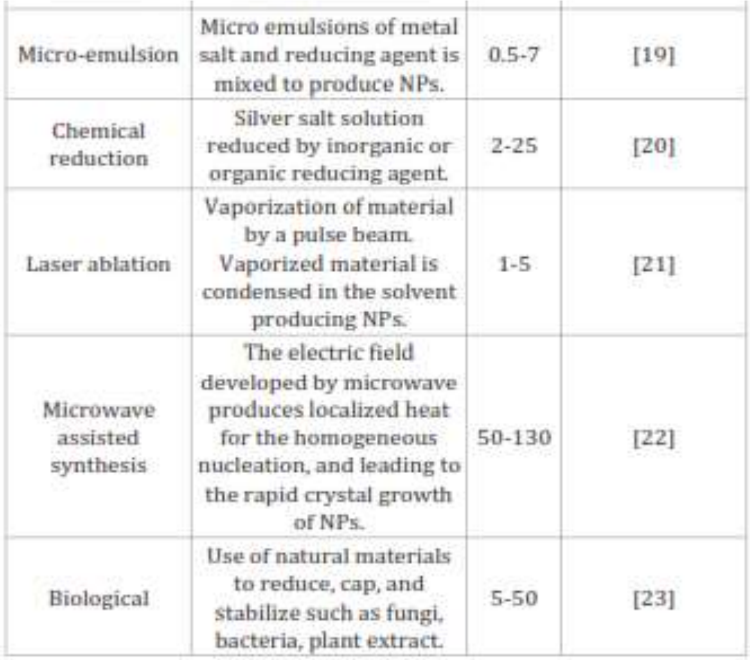


Scheme 1 Schematic representation of formation of nanostructure via Top-down Vs. Bottom-up.

Table 1: Summary of methods used for the Ag NPs synthesis.



Method	Description	Size (nm)	Reference
Evaporation-condensation	Solid bulk material is evaporated under high vacuum, and the vapor-phase molecules are condensed to yield solid NPs.	<100	[16]
Electrochemical	Metal sheet used as anode undergo oxidation to produce metal ions which are reduced at the cathode or in the electrolyte solutions to NPs.	2-20	[17]
Photoinduced reduction	Reduction of silver nitrate with UV irradiation	5-8	[18]



Micro-emulsion	Micro emulsions of metal salt and reducing agent is mixed to produce NPs.	0.5-7	[19]
Chemical reduction	Silver salt solution reduced by inorganic or organic reducing agent.	2-25	[20]
Laser ablation	Vaporization of material by a pulse beam. Vaporized material is condensed in the solvent producing NPs.	1-5	[21]
Microwave assisted synthesis	The electric field developed by microwave produces localized heat for the homogeneous nucleation, and leading to the rapid crystal growth of NPs.	50-130	[22]
Biological	Use of natural materials to reduce, cap, and stabilize such as fungi, bacteria, plant extract.	5-50	[23]

3. PHOTOCHEMICAL SYNTHESIS OF SILVER NANOPARTICLES

In the photochemical approach, the nanoparticles are synthesized from ionic precursors. For example, when an aqueous solution of silver salt, acetone, propanol and polymer stabilizer is UV irradiated, polymer capped silver nanoparticles are formed. UV illumination is believed to generate ketyl radicals via initial excitation of acetone and subsequent hydrogen atom abstraction from 2-propanol according to Equation 1:

$$CH_3COCH_3^* + (CH_3)_2CHOH \text{ -----> } 2(CH_3)_2C^{\cdot}OH \qquad (1)$$

The short lived radicals serve as strong reductants. It releases electron and a proton in the process of regeneration of acetone. The electrons could subsequently reduce silver salt to form silver atom, according to Equation 2 and 3. Polymers effectively stabilize the clusters of silver atoms to form polymer capped nanoparticles.

$$(CH_3)_2C^{\cdot}OH \text{ -----> } CH_3COCH_3 + H^+ + e^- \qquad (2)$$

$$(CH_3)_2C^{\cdot}OH + Ag^+ \text{ -----> } (CH_3)_2CO + H^+ + Ag^0 \qquad (3)$$

Alternatively, the synthesis of silver nanoparticles may involve direct photo-reduction of AgNO3 in the presence of sodium citrate with different light sources at room temperature. It was demonstrated that depending upon the wavelength of light source used in photochemical reduction i.e. UV or white or green light, nanoparticle suspension with distinctive optical properties could be formulated. These nanoparticles differed in size and shape. Occasionally, in a UV photo-activation method, a reagent is used in the preparation of stable silver nanoparticles which serves as reducing agent as well as stabilizing agent. In fact, when silver nanoparticles were prepared along with aqueous Triton X-100, Triton X-100 served the dual purpose of reducing agent and stabilizing agent. Likewise, when silver nanoparticles were synthesized in an alkaline solution of AgNO3 /carboxymethylated chitosan (CMCTS) with UV light, CMCTS served the dual role as a reducing agent for silver cation reduction and a stabilizing agent/ surfactant for silver nanoparticles. Studies have shown that surfactants play an important role in the photochemical reduction of silver salt solution to form uniform sized nanoparticles. The surfactant solution acts as stabilizer in the preparation of welldefined nanoparticles (by increasing the surface tension at the solvent– nanoparticles interface). The major merits of the photochemical synthesis route are: (i) clean, (ii) controlled formation of nanoparticles triggered by the photo irradiation and (iii) significant versatility in the photochemical synthesis of nanoparticles in various mediums including emulsion, surfactant micelles, etc.. Some of the factors that can influence the overall composition of synthesized nanoparticles include the wavelength and intensity of irradiation beam, and exposure time of the reagent solution to irradiation. In the absence of proper control, there is a possibility of localized heating of the reagent solution leading to inhomogeneity in synthesized nanoparticles composition.

4. BIOLOGICAL SYNTHESIS OF SILVER NANOPARTICLES

When silver nanoparticles are produced by biological route, the living organisms or proteins act as reducing agent and/or stabilizing agent. Bacteria such as Shewanella oneidensis has been noted to interact with silver nitrate solution, resulting in the formation of nearly monodispersed nanoparticles in the size range of 2 to 10 nm with an average size of 4 nm. The bacteria assisted synthesis of nanoparticles is economical, simple, reproducible, and requires less energy when compared to other synthetic routes. Silver nanoparticles have also been synthesized using the Lactobacillus species where Lactobacillus species serves as reducing and capping agent. Sintubin et al showed Lactobacillus species accumulated and subsequently reduced Ag+ to produce Ag0 species inside the cell. [31]. The mean diameter of the biogenic silver nanoparticles produced by this method varied depending upon the Lactobacillus spp. used. The recovery of silver nanoparticles and the reduction rate of silver ions were found to be pH dependent. Other researchers have used plant based compounds to synthesize silver nanoparticles. For example, Kumar et al., developed an eco-friendly and sustainable green route for the synthesis of stable silver nanoparticles (AgNPs) using aqueous leaf extract of plants as both reducing as well as a stabilizing agent [32-35]. Most of the AgNPs were spherical and in the range of 8 nm to 24 nm having an average size distribution of 15.5 nm. The biological method of synthesizing silver nanoparticles is a low cost approach and less energy intensive process. Generally, it is not easy to produce a large quantity of silver nanoparticles by using biological approach.

5. CHEMICAL SYNTHESIS OF SILVER NANOPARTICLES

Among the various known methods, the chemical method has been the most widely studied because of the general versatility of the technique. For example, silver nanocubes in large amounts have been synthesized by reducing silver nitrate with ethylene glycol in the presence of stabilizing agent, the so-called polyol process. Ethylene glycol serves as both reductant and solvent. Based on the molar ratio of stabilizer relative to silver nitrate and the experimental conditions used in the synthesis, the geometric shape and size of the nanoparticles could be varied significantly. The polyol process has also been used to synthesize spherical silver nanoparticles with a controllable size and high monodispersity. Alternatively, spherical silver nanoparticles can be synthesized using oleyl amine - liquid paraffin mixture. The use of a high boiling point liquid e.g. paraffin, offers the flexibility to effectively use reaction temperature to generate silver nanoparticles of varying size without changing the solvent. The size of nanoparticles in the solution is strongly dependent on the duration of the individual stages of synthesis i.e., synthesis of silver nuclei and subsequent growth accompanying nuclei formation. For the synthesis of monodispered silver nanoparticles with uniform size distribution, it is preferable to form the nuclei at similar time. The initial nucleation and the subsequent growth step of initial nuclei can be controlled by adjusting the reaction parameters such as reaction temperature, pH, type of metal precursors, reducing agents (e.g. NaBH4 , ethylene glycol, glucose) and stabilizing agents (e.g. sodium citrate). Reduction of silver salts to form nanoparticles has been achieved using sodium citrate and/or borohydride. The use of sodium borohydride (a strong reductant) usually results in the formation of somewhat monodispersed smaller sized silver nanoparticles while the use of only citrate (a weaker reductant) usually results in the formation of somewhat polydispersed larger sized silver nanoparticles because of slower reduction rate. Reduction of silver ion by sodium citrate is shown below

$$4Ag^{+} + C_6H_5O_7Na_3 + 2H_2O \text{ -----> }$$

$$4Ag^{0} + C_6H_5O_7H_3 + 3Na^{+} + H^{+} + O_2\uparrow \qquad (4)$$

Reactions 5, 6, and 7 provide the individual steps and overall reaction step in the formation of silver nanoparticles upon reduction with sodium borohydride.

$$2Ag^{+} + 2e^{-} \text{ -----> } 2Ag \qquad (5)$$

$$2BH_4^{-} \text{ -----> } B_2H_6 + H_2 + 2e^{-} \qquad (6)$$

$$2AgNO_3 + 2NaBH_4 \text{ -----> } 2Ag + 2H_2 + B_2H_6 + NaNO_3 \qquad (7)$$

Solomon et al. proposed a mechanism of nanoparticle formation based on sodium borohydride reduction and stabilization (without stabilizing agent). The nanoparticle formation is based on the temporary stabilization of smaller sized silver nanoparticles by excess BH4 - species. Figure 1 shows structure of stabilized silver nanoparticles with a shell of excess borohydride anion. However, with time, there is the collapse of the stabilized shell around the nanoparticles that causes the nanoparticles to aggregate which is largely attributed to the degradation of BH4 - accompanied by hydrogen gas evolution as mentioned in equation 8.

$$BH_4^- + 4H_2O \text{ -----> } B(OH)_4 + 4H_2 \qquad (8)$$

Given the borohydrydate anion degradation in sodium borohydride capped nanoparticles, a number of alternative capping agents have been studied to stabilize nanoparticles with or without dispersants. A nice review on the common capping agents commonly used in nanoparticle synthesis and their impact is presented by Niu and Li et al.. Recently aminosilanes have been used as capping agent to stabilize the nanoparticles as well as serve as coupling agent to couple with other moieties. Li et al., took a different approach to stabilize nanoparticle by dispersing oleic acid capped silver nanoparticles with different dispersion agents. Interactions between dispersant and capping agent determine the extent of dispersion of silver nanoparticles. H-bonding between dispersant and capping agent effectively results in enhanced agglomerations of Ag nanoparticles. Other studies have evaluated the stability of stabilized silver nanoparticles at various pH conditions. It was established that citrate anion as capping molecule may not be enough to maintain the stability of citrate stabilized nanoparticles over a wide pH range There is a strong likelihood for the nanoparticles to aggregate depending upon the pH despite the nanoparticles stabilized by small molecules such as citrate anion. Therefore alternative routes to stabilize nanoparticles have been studied.

6. CONCLUSION

Silver nanoparticles have received considerable attention due to the strong toxicity to a wide range of microorganisms, including gram-positive and gram-negative bacteria. The properties (size, shape, morphology, composition, aggregation level) of silver nanoparticles play an important role in the nanoparticles antibacterial activity. Properties of nanoparticles can be influenced by a number of factors such as the method of selection for synthesis of nanoparticles and type of stabilizer used. Generally, there are two basic methods of synthesis of nanoparticles. They are classified as top-down and bottom-up approaches. Several methods of synthesis of silver nanoparticles have been reported in the literature, ranging from photochemical reduction, biosynthesis, γ irradiation to chemical reduction. Chemical reduction method is one of the common approach for bottom-up synthesis of silver nanoparticles, and is simple and facile. The stability of the nanoparticles is important so that the nanoparticles can be used for their intended application. The stability of the synthesized nanoparticles can also be affected by the type and amount of reducing agent and type of stabilizer used. For example, the size of nanoparticle core can be tuned from few nanometers to greater than 10nm based on the composition of reagent used in the synthesis. Nanoparticles corona can be modified either through adsorption or in-situ method with ligands/bio macromolecules so as to create surface specific receptors for further conjugation with other biomolecules or other ligands. There are three modes of stabilizing the nanoparticles: electrostatic charge stabilization, steric stabilization and their combination electrosteric stabilization. Electrosteric stabilization is the most preferred method of stabilization of nanoparticles especially when dealing with high ionic strength biological medium. However, electrostatic stabilization may not be enough to maintain the stability of the nanoparticles over a variety of conditions such as variation in pH value and electrolyte concentration that is especially found in biological medium.

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