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# A Mini Review on the Synthesis of Ag-Nanoparticles by Chemical Reduction Method and their Biomedical Applications

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## Abstract

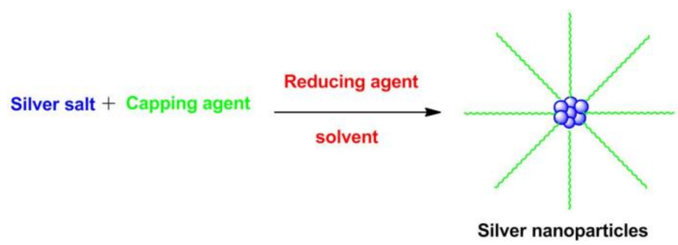
This mini-review provides an adequate amount of information about the synthesis of silver nanoparticles through various routes. Among the competitors, Sodium-, borohydride and citrate, water, polyvinyl pyrrolidone and sodium citrate and silver nitrate are the most commonly employed reducing agents, solvent, capping agents and silver salt precursor. The particles thus formed when incorporated to form blended materials can be used in advanced applications, especially in medical devices, preventing the adhesion of microorganisms over them. Herein, the similar function of silver, either distributed over the polymeric material surfaces as a polished layer or equally dispersed therein, will be presented. The marked examples include incorporation of silver nanoparticles into fluff pulp through sonochemical impregnation and blending the silver nanoparticles synthesized through solvent-free, greener routes into poly(dicyclopentadiene).

**Keywords:** Antimicrobial properties, Blended materials, Green synthesis, Silver nanoparticles

## Introduction

The Silver is one of the important coinage metals in group 11 of the periodic table and is already known since ancient times due to its remarkable applications in making tools, utensils, aesthetically designed ornaments, and for medicinal applications [1]. Recently, the demanded application of silver has significantly increased when gigantic metal composites are broken down into small ultra-refined nanoparticles. The scope of this metal in the form of nanomaterials has been expanded in both academia and in industry, especially for chemiluminescent materials, microchips and biocidal applications [2]. In the latter case, this application is important, when even tiny amounts of silver in materials make them practically useful against adhesion of microorganisms like a virus, bacteria and fungi over them [3]. Besides, antimicrobial activity, high stability of silver nanoparticles at ambient temperatures and low volatility make them practically more applicable than any other material. The coating on polymers can be accomplished in both forms either only at polymer surfaces or real impregnation of silver into whole material [4]. The impregnation method is preferable due to more reliability and durability of material for long lasting protection of inner and outer core of the materials while Ag-polished materials get chances of being deactivated by protein anions [5].

The amount of silver is also important in the manufacturing process of such materials as toxic applications due to an excess amount of silver and less cleaning action due to insufficient quantity of silver lie at two different ends of the scale. For Example, the concentrations more than 5 ug/mL of silver in nano- form has been reported effective against necrosis or apoptosis of mouse spermatogonial stem cells [6]. Similarly, while studying antimicrobial characteristics of 1w% silver nanoparticles incorporated in nylon 6.6, insufficient antimicrobial activities were examined [7]. Thereby, the amount of silver in either form is important for incorporation in the materials. Besides, the addition of transition metals to the environment has also been counted for a major cause of contaminations for the systems accommodating living creatures and the contribution of silver is lesser as compared to other hazardous metals [8]. Accounting all these endeavours, the use of silver in all forms has significantly increased and it is becoming more demanding material with the passage of time. Keeping the same in view, a mini review is presented covering the major areas of different routes for silver nanoparticles, their impregnation in different polymer composites and fibers and finally, the biomedical application including antimicrobial activity and toxic applications is presented.




**Scheme 1: The general reaction scheme for the synthesis of silver nanoparticles**

Nanoparticles due to their narrow size and sharp approach have emerged widely into modern applications in photonics, catalysis, construction materials, biomedical and in energy saving devices. The role of silver is equally important in the manufacturing of diverse ranged nanoparticles [9]. Various routes including chemical, physical, photochemical and biological are being employed for the synthesis of these particles [10]. The syntheses routes have preferences over one another due to manufacturing costs, particle size, size distribution and scalability. Besides chemical method, all others need a critical condition like high temperature and/or vacuum environments that make the syntheses complicated and expensive [11]. Therefore, the chemical method is the most commonly employed syntheses mechanism for the development of silver nanoparticles. The ultra-refined pure silver nanoparticles with sharp size and distribution and control over three-dimensional orientations of nanoparticles make the chemical synthesis method preferable over other routes. The inexpensive equipment and chemicals and ease of formation are also among the additionally counted factors.

## Chemical syntheses of silver nanoparticles

The nanoparticles of silver in the range of 1-100 nm are generally synthesized by treating silver salt with capping agents and reducing agents after solubilizing in an optimal solvent. In a synthesis step, these factors combined with respective concentrations and followed experimental procedures define the size distribution and 3D-orientation of nanoparticles [12]. Due to the low price, ease of availability and high chemical stability, $AgNO_3$ is the most commonly applied metal salt. Both, sodium borohydride and sodium citrate separately applied in different experiments contribute one-third among all the reducing agents reported in the literature [13]. Besides, common laboratory chemicals like carbohydrates, acids, amines, bio-based substrates like organisms and special treatment methods like irradiation of reaction mixture have also been applied [14]. As solubilizers, many organic and inorganic solvents have been reported for nanoparticles formation but according to different data available, more than 80% of the syntheses have been accomplished in water as a solvent [15]. Organic solvents like ethanol, dimethylformamide, ethylene glycol and amines are among other commonly applied solvents [16]. The capping agents, also named as stabilizing agents are generally added to prevent aggregation of the nanoparticles after the formation. Polymer-based composites are useful precursor employed for the same purpose. Poly (vinylpyrrolidone) (PVP) is second most abundantly applied polymer after sodium citrate (27%) for such purposes [17]. Similarly, poly (*n*-isopropyl acrylamide) (PNIPAM) is used as reducing agent when nanoparticles of demanded low critical solution temperature applications are required [18]. The arbitrary literature available for demanded application provides a wide range of data for nanoparticles syntheses for both general and specific applications but a greener route for synthesis where least number of chemicals applied in ideally no solvent and within shorter durations of time, were still demanded. Counting the same factors, the solvent-free synthesis of oleylamine capped silver nanoparticles was established in our laboratory [19]. Oleylamine was used as both solubilizing and stabilizing agent and heating ten folds excess of it with $AgNO_3$ at 165$^o$C for thirty minutes, black precipitates of silver nanoparticles were obtained. After precipitation and washing three times in ethanol, the nanoparticles were characterized to study the UV-absorbance, size measurements and stability of volatile ligands attached after dissolving in chloroform. The particles synthesized through this method were also readily soluble in many organic solvents like toluene, dichloromethane and diethyl ether. The various route selected for syntheses of silver nanoparticle are reported in **Table 1**.

**Table 1: Selected Model for the Syntheses of Silver- Nanoparticles from Silver Nitrate.**

| Reducing-Agent | Solvent | Capping Agent | Size (nm) | Reference |
|---|---|---|---|---|
| Sodium borohydride | Water | Polyvinyl alcohol (PVA) | Variable | [20] |
| | | Trisodium Citrate | 4.4 -5 | [21] |
| | | Sodium borohydride | 12 | [22] |
| | | Oleic acid | 8 | [23] |
| | | Polyvinyl pyrolidone (PVP), Trisodium Citrate | < 3 | [24] |
| | Water/ Chloroform | Hexadecyl amine | Variable | [25] |
| | Ethanol/Toluene | Trisodium Citrate + Dodecylamine | 7 | [26] |
| | Ethanol | Poly-Methylmethacrylate(PMMA) | 5-8 | [27] |
| Bacterium Bacillus licheniformis | Water | Bacterium Bacillus licheniformis | 50 | [28] |
| Capsicum annuum L. extract | | Capsicum annuum L. extract | 10 | [29] |
| Spent mushroom substrate | | Spent mushroom substrate | 30.5 | [30] |
| Fructose, Glucose, Sucrose | | Fructose, Glucose, Sucrose | Variable | [31] |
| Vitamin E | | N.A | 3-14 | [32] |

| | | | | |
|---|---|---|---|---|
| Vitamin $B_2$ (Riboflavin) | | Vitamin $B_2$ (Riboflavin) | 6.1 | [33] |
| Sodium carboxymethyl cellulose | | Sodium carboxymethyl cellulose | 15 | [34] |
| Dodecylamin& Formaldehyd | Water/ Cyclohexane | Dodecylamine & Formaldehyde | 4 | [35] |
| Octadecylamine | Octadecylamine | Cetyltrimethylammonium bromide | 4.7 | [36] |
| Ethylene glycol | Ethylene glycol | Polyvinylpyrrolidone | Variable | [37] |
| Oleylamine | Oleylamine | Oleylamine | 63 | [19] |
| Ascorbic acid | Water | Cetyltrimethylammonium bromide | 35 | [38] |
| $H_2$ Gas | Water/Toluene | PVP/PVA/ Starch | Variable | [39] |
| ã- irradiation | Water | PVP | Variable | [40] |
| Microwave irradiation | Ethylene Glycol | PVP | Variable | [41] |
| Sonochemical irradiation | Water | Fluff pulp | N.A | [42] |

With sodium borohydride, as a reducing agent and water as a solvent, the most commonly employed capping agents are polyvinyl alcohol, trisodium citrate, oleic acid, polyvinylpyrrolidone and borohydride itself [20-23]. The substantial examples are also available where the combination of two or more capping agents was utilized for the demanded applications and ultra-refined nanoparticles of less than 3 nm sized-range were synthesized [24]. Both Immiscible and immiscible organic solvents in neat and/or in combination with water have been used as the solubilizing media for nanoparticles syntheses [25-27]. However, for the natural extracts like *Bacterium Bacillus licheniformis*, *Capsicum annuum L.* extract, *Spent mushroom substrate*, various sugars, vitamins and carboxymethylcellulose, the aqueous medium is the best-suited environment [28-34]. In most of the examples, the extract from natural resources act as both capping- and reducing agents, however, additional reducing could also be introduced as per special needs for the properties of nanoparticles [35-37]. Besides solid and liquid reducing agents, $H_2$-gas may also be utilized for a source for reduction [38]. The nanoparticles with variable sizes were obtained through this method. The irradiation methods like microwave, UV-irradiation, gamma radiations and sonochemical impregnation were also beneficial in some cases for the production of nanoparticles [39-42]. The special measures were taken for irradiation that it is not a source for complete reduction rather it provides free electrons to initiate the formation of nanoparticles [40]. One of the very basic tools for evaluation of silver nanoparticle synthesis is UV-visible spectroscopy where a typical curve of 380-420nm represents the formation of silver nanoparticles. The surface plasmon absorption resonance frequency of silver is responsible for such graph through this technique (*cf.* Figure 1b). Other optical instrumental methods for the confirmation of nanoparticle synthesis are scanning electron microscope (SEM) and transmission electron microscope (TEM) images captured for the materials at different resolutions. The orientation of nanoparticles in any of the one-, two- or three-dimensional configurations clearly predicts the nature of nanoparticles. X-ray diffraction pattern and Atomic force microscopy are also among the tools in hand for the confirmation and identification of surface morphology of nanoparticles. One very decent example of the size determination and distribution of nanoparticles through dynamic light scattering is oleylamine capped nanoparticles prepared in neat conditions [19].

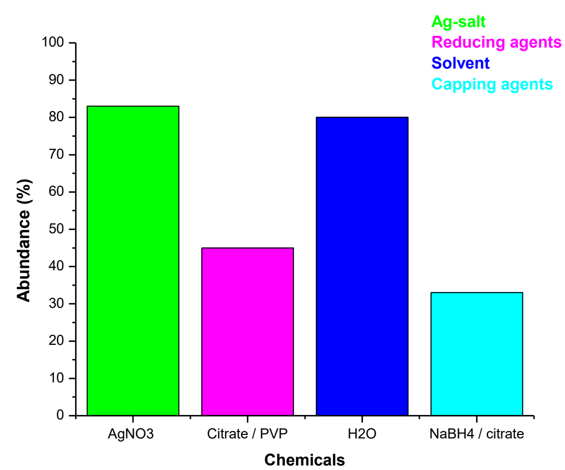



**Fig. 1a: Most frequently employed chemicals for the synthesis of Ag nanoparticles [19].**

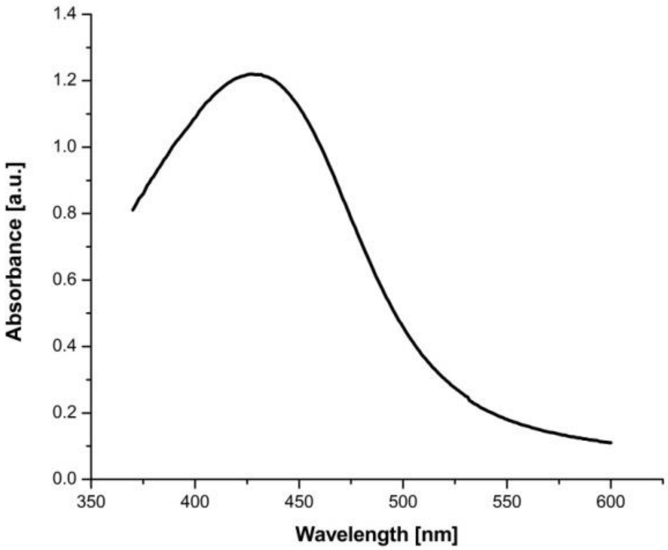



**Fig. 1b: An exemplary UV-visible spectrograph for silver nanoparticles [42]**

## Materials equipped with silver for antimicrobial applications

The strong antimicrobial activity of materials containing silver is followed either with the release of silver ion or silver nanoparticles in the solution to behave strongly against microorganisms [43]. The detailed mechanism is still unknown but different assumptions are available for silver ions and nanoparticles for their activity against microorganisms. For example, according to many authors silver ion binds strongly to the nitrogen, sulphur and oxygen in different proteins and causes the killing of the cell wall of bacteria and hence reducing the cell count [5]. Similarly, in an activity, the cell functions like permeability and respiration are also disturbed when silver ion makes complexes with proteins and DNA [44]. Such activity has been observed in Ag-zeolites complexes which behave as strong antimicrobial agent. The slow dissociation of silver ion in the saliva of mouth is helpful in reducing the plaque in a dental application with least harm to the human health [45]. Different counts of 2.5-7.5 wt% of zinc- and silver-zeolite have been reported for strongly antimicrobial and favorable for denture based resins but all concentrations above 2.5wt% showed a significant change in mechanical properties of the material [46].

In past, three different techniques have been applied for nanocomposite formation: the first one includes the spreading of silver nanoparticles over the polymer matrix, in second process in-situ preparation of silver nanoparticles by electronically active polymers used as both reducing agents and entrapping agents are established, and in third nanoparticles are mixed with the monomer before polymerization process. In the last described technique, nanoparticles are ideally equally distributed among the whole core of the polymer matrix and show more efficiency than the other methods [5]. In some cases, materials releasing silver nanoparticles produce stronger effects against bacterial activity than silver ion releases. For example, in comparison of silver nano-based poly(methylmethacrylate) (PMMA) and sulphadiazine / $AgNO_3$, the former showed stronger kill rates for E. coli and S. aureus bacteria by releasing the silver nanoparticles sizing 7 nm range on average [47]. The matrix was also developed using poly(vinyl alcohol)/ poly(methyl methacrylate) and similar polymers for advanced antibacterial applications. Additionally, no black precipitates were formed in the later case whereas, this phenomenon is commonly observed in experiments releasing Ag-ions.

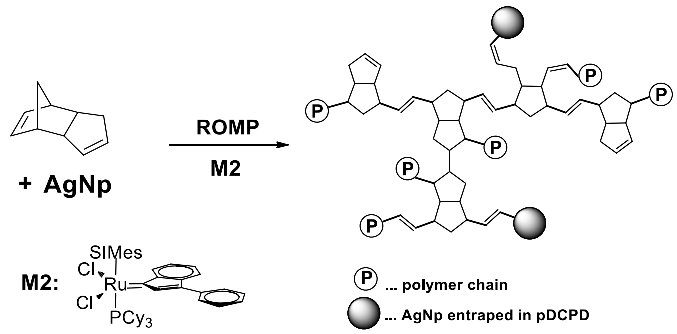



**Fig. 2: Specimen example for ROMP of DCPD in presence of 1w% Oleylamine stabilized silver nanoparticles [19]**

The studies have also been evaluated for block copolymers, e.g. poly(urethane)/poly(ethylene glycol) and $TiO_2$ based polymers. These polymers when attached with silver, yielded materials of high activity against *E.coli* and *S.auserus* bacteria [48]. Recently, poly(dicyclopentadiene) with high mechanical strength, low cost and ease of formation have been emerged as an advanced duroplastic material [49]. A way to create a poly(dicyclopentadiene) bearing silver nanoparticles and hence antimicrobial properties were recently established [19]. Polymerization of dicyclopentadiene via ring opening metathesis polymerization in presence of 1w% of silver nanoparticles yielded an antimicrobial equipped thermosetting material, as exemplified by the complete killing of E.coli colonies (as determined according to the Japanese international standard Z2801:2000) [50].

In an example when silver films and solution of physiological saline were immersed together where the dissolution of $Ag_2O$ occurs and formation of Silver causes the inhibition of growth of *E. coli*, *S. aurses* and *P. aruginosa* [51]. When PVC is modified with NaOH and $AgNO_3$ it inhibits the production of *P.aeruginosa* colonies in endotracheal tubes [52]. Similarly, the in-situ process for the production of fine colloidal silver particles is accomplished where poly(ethylene glycildimethylacrylate) was used with control surface area and surface functionality. This material showed high antimicrobial activity for different bacterial strains [53]. In an another example, Silver nanoparticles fabricated Chitosan/polyvinylalcohol blended webs were prepared by the electrospun method. The fibers manufactured by refluxing method were more refined and showed better antimicrobial properties than by annealing method [54]. Poly (3,4-ethylene di oxithiophene) / poly (styrene sulphonate) (PEDOT/PSS) aqueous dispersions were mixed with the aqueous silver nanoparticle colloids to obtain silver nanoparticle films.

**Table 2: Highlighted Blends of Silver with Antimicrobial Properties**

| Sr. No | Ag-particles Production Method | Size (nm) | Dopped with | Antimicrobial Properties | Reference |
|---|---|---|---|---|---|
| 1 | Electrostatic | NA[a] | Zeolite/ Acrylic resin | *P. gingivalis, P. intermedia and A. actinomycetemcomitans* | [45-46] |
| 2 | radical-mediated dispersion polymerization | 7 | poly(methylmethacrylate) (PMMA)/ poly(vinyl alcohol) and sulphadiazine | *E. coli* and *S. aureus* | 47 |
| 3 | Solution casting method | 400 | poly(urethane)/poly (ethylene glycol) and $TiO_2$ | *E. coli* and *B. subtilis* | 48 |
| 4 | Solvent-free synthesis in presence of oleyl amine | 63 | Poly(dicyclopentadiene) | *E. coli* and *S. aureus* | 19 |
| 5 | $Ag_2O$ and NaOH (reduction method) | N.A | Saline | *E. coli, S. aurses* and *P. aruginosa* | 51 |
| 6 | Reduction in NaOH | N.A | Poly(vinylchloride) | *P.aeruginosa* (in endotracheal tubes) | 52 |
| 7 | Colloidal method | 2-10 | poly(ethylene-glycil-dimethylacrylate) | *E. coli, S. aurses* and *P. aruginosa* | 53 |
| 8 | Electrospun reflux method | Variable | Chitosan/polyvinyl alcohol | *E. coli* | 54 |
| 9 | Microemulsion method | N.A | Poly(3,4-ethylenedioxithiophene)/poly(styrene sulphonate) (PEDOT/PSS) | N.A | 55 |
| 10 | Thermal reduction | <50nm | Polyethylene/super absorbant (PE/SAP) | Limited applications | 56 |
| 11 | Electrospinning reflux | 15nm | carboxymethylcellulose CMC and polyvinyl alcohol | N.A. | 57 |
| 12 | Reduction in trisodium citrate | variable | Wool | *S. aureus* | 58 |

The results showed the typical optical properties of the metal nanoparticles and the excitation of the bipolaron band of the conducting polymer [55]. Polyethylene/super absorbent (PE/SAP) materials could also be functionalized with silver nanoparticles via thermal reduction of a silver ion from its acetate salt. The major portion of silver nanoparticles was located at the interface of PE and SAP particles and a very controlled release of silver ion were observed in this case. Such controlled release of the silver nanoparticle was the reason of low antimicrobial characteristics of the matrix [56]. Silver nanoparticles blended with carboxymethylcellulose and polyvinyl alcohol by electrospinning method develops the nanofibers. The advanced research can open new horizons for the antimicrobial material development in tissue engineering and medical and dental care applications [57]. Among the natural polymers and fibers, textile materials and accessories incorporated with silver nanoparticles and the cherished antimicrobial properties for such materials have also been reported [58].

## Conclusion

The basic information about the synthesis of silver nanoparticles, incorporated materials and activity of such materials against microorganisms is presented. The multiple routes for the chemical syntheses of silver -nanoparticle include silver nitrate and water as the major components for silver salt-resource and as a solvent respectively. For reducing agents; sodium borohydride, natural extracts, reducing sugars and electromagnetic radiations have been utilized, whereas for the capping agents; polymers, carbohydrates, long chain fatty acids and natural extracts can be used. The analysis of different products developed through incorporation of silver nanoparticles clearly depicts that material contains property of disinfecting microbes.

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