

## Review Article

## Biological Routes for the Synthesis of Silver Nanoparticles

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## Abstract

Silver nanoparticles (AgNPs) play a vital role in nano-science and technology field owing to their unique physical and chemical properties such as optical, electrical, thermal, high electrical conductivity, and biological properties. The AgNPs are usually synthesized through chemical and biological processes. The major drawback of using chemical techniques is the waste generated during preparation is very high which is toxic, hazardous and causes environmental pollution. To overcome these drawbacks green synthesis/biological routes are most preferred. The ultimate aim of green synthesis is to prepare AgNPs with minimal waste in a sustainable process to ensure safety through the eco-friendly and relatively non-toxic process. There are two major practices to prepare AgNPs via green synthesis such as micro-organism based synthesis and plant extract mediated synthesis where plant extract mediated synthesis are widely used owing to its low toxicity, facile technique, time effective, tunable preparation techniques, etc. which are discussed elaborately in this review.

Keywords: Silver nanoparticles, green synthesis, micro-organism mediated synthesis, plant extract mediated synthesis.

## Overview

Silver nanoparticle (Ag Nps) has a wide range of applications in the field of nano-science due to their high catalytic activity, high chemical stability, good conductivity; and stable physio-chemical properties.<sup>1</sup> Silver is well known for its anti-microbial activity for the past few generations. Likewise, AgNPs also poses enhanced anti-microbial activity owing to their capability to attach on to the microbial membrane and generate oxidative stress which leads to cellular disruption.<sup>2</sup> This characteristic of AgNPs is widely used in the food industry, textile industry and many other processes to eradicate the influence of microbes for spreading infectious diseases. For these applications, AgNPs are prepared through various methods and technologies such as physical vapor deposition, ball milling, wet chemical synthesis, sol gel synthesis, sono-chemical synthesis, etc. of which bottom-up approaches are widely preferred. For bottom-up approach, there are some common requirements which are i) solvent, ii) reducing agent, and iii) stabilizing agents. The solvent is chosen in such a

way that the precursor salt, reducing agent and stabilizing should be readily dissolved. The reducing agent is a compound that has excess electrons (e-) to be shared or termed as e-donor that donate its excess e- to Ag+ ions. The reducing power of reducing agent determines the degree of reduction of Ag+ to Ag<sup>0</sup>.<sup>3</sup> The stabilizing agent is also called as capping agent/surfactant which controls the growth rate of Ag Nps and prevents aggregation by promoting Brownian motion.<sup>4</sup> Usually, chemical compounds such as hydrazine hydrate, polyvinyl pyrrolidone, sodium citrate, polyvinyl alcohol, etc. are used as reducing and stabilizing agents for wet chemical synthesis. Even though, these chemicals don't pose acute toxicity, when continuously discarded into the environment can lead to ecotoxicity. To overcome such drawbacks, green synthesis technique is introduced where Ag Nps are prepared using eco-friendly methods. Green synthesis follows the principles behind the green chemistry i.e., the principles to reduce and control the hazardous waste entering our environment. Green synthesis concentrates on all the three important parameters such as solvent medium,

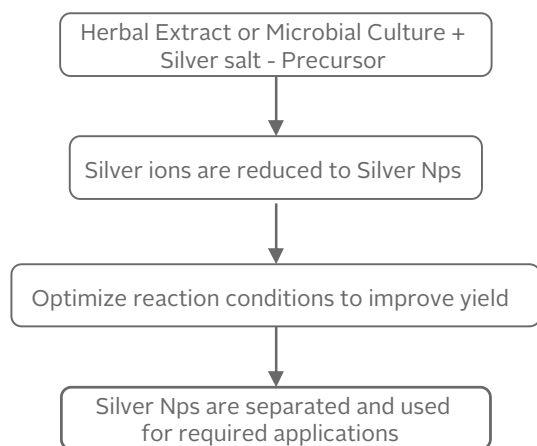


Figure 1: Flow chart explaining the synthesis of Ag Nps through biological route

reducing agent, and stabilizing agents to be non-toxic materials. Green synthesis of Ag Nps is carried out majorly by two processes i.e. microorganism-based synthesis and plant extract-based synthesis.

### Microorganism based synthesis

Microorganisms both unicellular and multi-cellular have a tendency to nucleate and grow inorganic materials inside their cellular structure. The primary process that takes place is that metallic ions like Ag<sup>+</sup> ions are captured on the surface of the microbe's cell membrane, get slowly penetrated into the cellular structure by establishing electrostatic interactions between Ag<sup>+</sup> ions and negatively charged cell wall.<sup>5</sup> Thus, once these metallic ions penetrate to the cell, reduction of ions occurs owing to the presence of biological moieties like sugar, proteins, etc.<sup>6</sup> that are

natural reducing agents where nucleation and crystal growth happens. Most of the biological metabolism reaction inside the cell induces bio-reduction to form Ag Nps.<sup>7</sup> However, the full process of Ag Nps growth inside the microbe's cell is still under study. For example, Guangquan et al. synthesized the Ag Nps by reducing Ag<sup>+</sup> ions from the culture supernatant of *Aspergillus terreus*. The nicotinamide adenine dinucleotide present in the cell acts as the reducing agent and causes enzymatic reaction process for this biosynthesis.<sup>8</sup> Here, the culture is maintained in water and therefore, acts as a solvent. In another study, Sable et al. reported that during microorganism-based synthesis, proteins and peptides from the culture media act as the capping agent.<sup>9</sup> Some examples of microorganism-based synthesis of Ag Nps are listed in Table 1.

The microorganism-based synthesis of Ag Nps poses major advantage of low toxicity compared to chemically synthesized counterpart and they have wide applications such as antimicrobial agents, biomedical device coatings, drug-delivery carriers, imaging probes, and diagnostic and optoelectronic platforms.<sup>20</sup> However, some major drawbacks set back the commercial potentiality of the microorganism-based synthesis technique. The bacterial culture preparation is a tedious process that requires a hygienic environment.<sup>21</sup> Maintaining aseptic condition involves sophisticated instruments. Also, after the preparation process, the culture has to be treated before discarding.<sup>22</sup> Further, the preparation of microbial culture is time-consuming where it takes 1 to several days depending on the microbe strain, growth, and media.<sup>23</sup>

Sl. No.	Size	Microorganism used	Application
1	10 nm <sup>10</sup>	<i>Gluconobacter roseus</i>	Antiplatelet activity
2	34-90 nm <sup>11</sup>	<i>Phanerochaete chrysosporium</i>	Antibacterial activity
3	20-100 nm <sup>12</sup>	<i>Candida utilis</i>	Antimicrobial activity
4	27.5 nm <sup>13</sup>	<i>Pseudomonas aeruginosa</i>	Antibacterial property
5	8-12 nm <sup>14</sup>	<i>Acinetobacter calcoaceticus</i>	Antibacterial property
6	20-30 nm <sup>15</sup>	<i>Streptomyces hygroscopicus</i>	Antibacterial property
7	10 nm <sup>16</sup>	<i>Sclerotinia sclerotiorum</i>	Antibacterial property
8	6-13 nm <sup>17</sup>	psychrophilic bacteria	Antibacterial property
9	41-68 nm <sup>18</sup>	<i>Bacillus brevis</i>	Antibacterial property
10	10-50 nm <sup>19</sup>	<i>Stenotrophomonas</i>	Antimicrobial activity

Table 1: Microorganism based AgNps synthesis

## Plantextract-based synthesis

In the plant extract-based synthesis method, the bio-molecules that are present in plants are extracted and used as reducing and capping agent. The reduction of Ag<sup>+</sup> to Ag<sup>0</sup> can be achieved more rapidly without any sophisticated instruments.<sup>24</sup> It overcomes the drawbacks of microorganism-based synthesis such as difficulty in growth, maintaining culture, slow growth process, etc.<sup>25</sup> Here, the extract is obtained from the plant leaf, stem, or roots and mixed with silver precursor solution to obtain Ag Nps. The plant extracts withhold phytochemicals that tend to reduce the Ag<sup>+</sup> ion much faster than the microbes. The phytochemicals include flavones, sugar, ketones, aldehydes, carbohydrates, proteins, etc. all acts as reducing agent.<sup>26</sup> The formation of Ag Nps through plant extract-based synthesis undergoes three phases

### Activation phase

In the activation phase, the bio-reduction of Ag<sup>+</sup> ions to Ag<sup>0</sup> occurs which leads to the nucleation process of the reduced metal atoms. The degree of bio-reduction depends on the reducing power of phytochemicals.<sup>27</sup> Some plant extract readily reduces the ions but, in some reactions, some activation energy is required to start the reaction that solely depends on the concentration of plant extract and type of phytochemicals present.<sup>28</sup>

### Growth phase

The growth phase of Nps is mainly due to a phenomenon known as Ostwald ripening. In the aqueous solution containing nucleated particles, the smaller particles tend to re-deposited over larger particles for reducing the overall surface energy influenced by thermodynamics [Fig. 2]. Thus, the ripening of smaller particles occurs.<sup>29</sup>

### Termination phase

In this phase, the plant extract stabilizes the metal Nps growth by the favorable conformation of size, shape and morphological character and minimizes the Gibbs free energy.<sup>30</sup> The plant extract also acts as a capping agent which declines the aggregation of Nps and maintains the morphological characters of Nps. The bio-capping agents are negatively charged thus they get trapped on the positively charged surface of AgNps.<sup>31</sup>

Most preferably two types of solvents are used to extract phytochemicals from plant parts. The first one is the use of ethanol termed as ethanolic extract and the latter is aqueous extract where water is used as a solvent. The type of solvent determines the type of phytochemicals in the extract. In a study reported by Azwanida et. al. stated that ethanolic extract of *Psidium guajava* L. leaves is rich in alkaloids, saponins, carbohydrates, tannins and flavonoids whereas the water display similar constituents with the exception of alkaloids.<sup>32</sup>

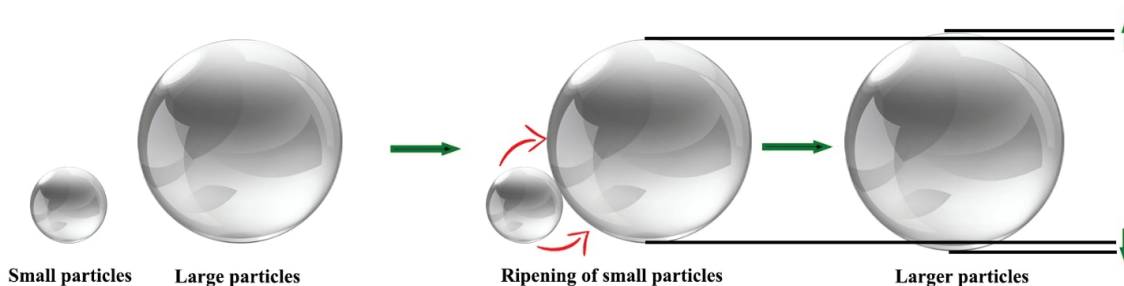


Figure 2: Schematic representing the Ostwald ripening process

Sl. No.	Size and shape	Plant used	Aqueous/ ethanolic extract	Application
1.	Spherical shape; 20-30 nm <sup>33</sup>	<i>Cissusquadrangularis</i> (whole plant is used)	Aqueous extract	Antibacterial activity
2.	Spherical shape; 20-44 nm <sup>34</sup>	<i>Prosopis cineraria</i> (leaf extract)	Ethanolic extract	Antibacterial activity
3.	15 nm <sup>35</sup>	<i>Skimmialaureola</i> (leaf extract)	Ethanolic extract	Biomedical application
4.	11-16 nm <sup>36</sup>	<i>Tanacetum vulgare</i> (leaf extract)	Ethanolic extract	Antibacterial activity and sensor

to be followed...

Sl. No.	Size and shape	Plant used	Aqueous/ ethanolic extract	Application
5.	55-80 nm <sup>37</sup>	<i>Tinospora Cordifolia</i> Miers (leaf extract)	Ethanolic extract	Pediculocidal and larvicidal
6.	28-68 nm <sup>38</sup>	<i>Citrus sinensis</i> (Orange peel extract)	Aqueous extract	Antimicrobial
7.	30-50 nm <sup>39</sup>	<i>Chenopodium murale</i> (leaf extract)	Ethanolic extract	Antibacterial activity
8.	10-60 nm <sup>40</sup>	<i>Morindacitrifolia</i> (leaf extract)	Ethanolic extract	Inhibiting human pathogens
9.	Spherical shape; 5-30 nm <sup>41</sup>	<i>Melia azedarach</i> (bark extract)	Aqueous extract	Antimicrobial
11.	40-60 nm <sup>43</sup>	<i>Anacardium occidentale</i> (leaf extract)	Aqueous extract	Detection of Cr
12.	Polygon shape; 100-150 nm & hexagon shape; 10-20 nm <sup>44</sup>	<i>Dendropanaxmorbifera</i> (leaf extract)	Ethanolic extract	Anticancer
13.	Spherical shape; 6-8 nm <sup>45</sup>	<i>Tamarindus indica</i> (fruit extract)	Ethanolic extract and few Aqueous groups	Antibacterial activity
14.	2-115 nm <sup>46</sup>	<i>Erythrina suberosa</i> (leaf extract)	Aqueous extract	Antimicrobial, antioxidant and cytotoxicity
15.	4-35 nm <sup>47</sup>	<i>Albiziaadanthifolia</i> (leaf extract)	Aqueous extract	Anticancer
16.	Irregular shape; 10.78 nm <sup>48</sup>	<i>Artocarpusheterophyllum</i> (seed extract)	Aqueous extract	Antibacterial activity
17.	22 nm <sup>49</sup>	<i>Rhinacanthusnasutus</i> (leaf extract)	Aqueous extract	Antimicrobial
18.	20-30 nm <sup>50</sup>	<i>Ziziphus jujuba</i> (leaf extract)	Ethanolic extract	Antimicrobial
19.	6.45 nm <sup>51</sup>	<i>Coriandrum sativum</i> (leaf extract)	Ethanolic extract	Biomedicine
20.	15.5 nm <sup>52</sup>	<i>Momordica cymbalaria</i> (fruit extract)	Ethanolic extract	Antimicrobial

Table 2:

Therefore, depending upon the type of phytochemical required, the solvent is chosen. There is much literature that dealt with the synthesis of Ag Nps using both ethanolic and aqueous extract of different plant parts. Some of the data are summarized in Table 2.

Thus, Table 1 depicts that different part of the plant extract is used to synthesis AgNps, which is of aqueous or ethanolic extract. Each plant has its own activation phase and growth phase which keenly depends on the phytochemicals that are present in the extract<sup>28,29</sup> and therefore, the size of the resultant Ag Nps varies upon the particle interaction during nucleation phase.<sup>53</sup> It is also clear that we can optimize the size of Ag Nps by controlling the

precursor concentration, temperature, pH, the amount of reducing and stabilizing factors.<sup>54</sup>

### Advantages of using plant extract-based synthesis

The major advantages of using plant extract-based green synthesis are facile, non-toxicity, time consumption is low, high stability, and cost-efficient. The Nps produced via this process enhances the biocompatible characteristics and can be used for in-vivo studies also because they all are produced from natural reducing agents and they are non-toxic when compared to chemically synthesized Nps.<sup>55-57</sup> The better biocompatible nature of Ag Nps synthesized using green synthesis is due to the slow ionic

release facilitated by the presence of natural capping agent.

## Summary

Overall, this review explains that the biological process of preparation AgNps is more efficient and produces low pollutants than conventional chemical techniques. The plant extract-based synthesis provides more practical feasibility than microbe assisted synthesis of Ag Nps due to various factors such as low cost, eco-friendly, no sophisticated environment required, time-efficient, etc. Further, the biocompatibility of Ag Nps synthesized through plant extract mediated synthesis promotes selective toxicity towards microbes facilitating in vivo study.

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