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Review Article

Synthesis of Silver Nanoparticles in Photosynthetic Plants

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Nanobiotechnology is emerging as a field of applied biological science and nanotechnology. Synthesis of nanoparticles is done by various physical and chemical methods but the biological methods are relatively simple, cost-effective, nontoxic, and environmentally friendly methods. The present review focuses on the synthesis of nanoparticles with special emphasis on the use of plants parts for the synthesis process, its applications, and future prospectus.

1. Introduction

Nanotechnology focuses mainly on the design, synthesis and manipulation of structure and size of the particles with dimensions smaller than 100 nm [1]. Nanotechnology combines the principles with physical and chemical procedures to generate nanosized particles with the specific function. Nanotechnology is now creating a growing sense of excitement in the life sciences especially biomedical devices and medicine. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, shape, and orientation [2].

The biological synthesis of nanoparticles is a cost-effective and ecofriendly methods and has ability to replace the physical and chemical methods because these methods are toxic and costly. Consequently, nanomaterials have been synthesized using microorganisms and plant extracts. The use of plant extracts for synthesis of nanoparticles is potentially advantageous over microorganisms due to the ease of scaling up the biohazards and elaborate process of maintaining cell cultures [3, 4]. For the last two decades extensive work has been done to develop new drugs from natural products because of the resistance of microorganisms to the existing drugs [1]. Integration of nanoparticles with biological molecules has led to the development of diagnostic devices and important tools in cancer therapy. Biological methods can employ either microorganism cells or plant extracts for nanoparticles production. Biosynthesis of nanoparticles is an exciting recent addition to the large repertoire of nanoparticles synthesis methods and, now, nanoparticles have entered a commercial exploration period. Au, Ag, Zn, and Cu have been used mostly for the synthesis of stable dispersions of nanoparticles, which are useful in areas such as photocatalysis, diodes, piezoelectric devices, fluorescent tubes, laser, sensor, optoelectronics, photography, biological labeling, photonics, and surface-enhanced Raman scattering detection [5–7]. Moreover, the biocompatible and inert nanomaterials have potential applications in cancer diagnosis and therapy. Nowdays, nanotechnology methods are used to enhance the properties of material coatings which are resistant to corrosion, high mechanical, and electrocatalytic properties. These characteristics make nanostructured coatings strong contenders in oil and gas for applications requiring high corrosion protection coupled with high mechanical properties [8, 9].

2. Plants Are Better Synthesizers for Nanoparticles

Biological methods of synthesis have covered the greener synthesis of nanoparticles. These have proven to be better methods due to slower kinetics and they offer better manipulation on control over crystal growth and their stabilization. This has motivated an increase in research on the synthesis routes that allow better control of size and shape for wide variety of nanotechnological applications. The use of environmentally benign materials, namely, plant extract, microorganisms, and enzymes for the synthesis of AgNPs [10–12] which offer plentiful benefits such as ecofriendliness, biocompatibility, non-toxic, and cost effective method [13].

In the present scenario of the nanotechnology, plants are better synthesizers as compared to the other biological methods due to the abundance of the availability of the plant resources when compared to the other forms of biological resources. Also, plants provide a better platform for nanoparticles synthesis as they are nontoxic chemicals and provide natural capping agents. Additionally, use of plant extracts also reduces the cost of microorganism isolation and culture media enhancing the cost competitive feasibility over nanoparticles synthesis by microorganisms [14, 15]. The advantage of using plants for the synthesis of nanoparticles is that they are easily available and safe to handle and possess a broad variability of secondary metabolites. A number of plants are being currently investigated for their role in the synthesis of nanoparticles. Gold nanoparticles with a size range of 2–20 nm have been synthesized using the live Alfalfa plants. Nanoparticles of Ag, Ni, Co, Zn, and Cu have also been synthesized inside the living plants of *Brassica juncea* (Indian mustard), *Medicago sativa* (Alfalfa), and *Helianthus annuus* (Sunflower). The research group of Gardea-Torresdey from the University of Texas at El Paso first reports on the formation of Ag and Au nanoparticles by living plants *Medicago sativa* and it opened up new and exciting ways to fabricate nanoparticles. It showed connecting link to materials science and biotechnology in the new emerging field of nanobiotechnology [16]. Certain plants are known to accumulate higher concentrations of metals compared to others and such plants are termed as hyperaccumulators. *Brassica juncea* had better metal accumulating ability and later assimilating it as nanoparticles, *Brassica juncea* when hydroponically grown in solution of $AgNO_3$, $Na_3Ag(S_2O_3)_2$, and $Ag(NH_3)_2NO_3$ synthesized AgNPs of 2–35 nm [17]. The concentrations of plant extracts which are aqueous or alcoholic extracts play an important role in maintaining the shape and size of the nanoparticles; for example, an aqueous seed extract of *Jatropha curcas* produced spherical AgNPs of between 15 and 50 nm. The latex of *J. curcas* served as reducing and capping agent in the synthesis of AgNPs of size in between 10 and 20 nm [18]. Ag and AuNPs were synthesized by reducing aqueous solution of $AgNO_3$ and $AuCl_4$ with clove extract, respectively. Sun-dried biomass of *Cinnamomum camphora* leaf extract, when treated with aqueous silver or gold precursors at ambient temperature, produced silver and gold nanoparticles in the range of 55–80 nm. The polyol component and the water-soluble heterocyclic components present in the *C. camphora* were mainly responsible for the reduction and stabilization of silver or chloroaurate in nanoparticles [19]. The reduction of silver ions and stabilization of the AgNPs were thought to occur through the involvement of proteins.

3. Phytofabrication of Nanoparticles

The term phytofabrication indicates the synthesis of the nanoparticles with the help of the plant constituents. Plant constituents include the enzymes and protein contents such as reductases which are involved in the biological reduction of the substrates, such as silver nitrate, aurum chloride, and titanium chloride, into their corresponding nanoparticles such as silver, gold, and titanium. These nanoparticles have a wide range of the applications in the fields of physical, chemical, material, and biological sciences.

Plant extract has been used as reducing and capping agent for the synthesis of nanoparticles. It could be advantageous over microbial synthesis because there is easy culturing and maintenance of the cell. It has been shown that many plants can actively uptake and bioreduce metal ions from soils and solutions during the detoxification process, thereby forming insoluble complexes with the metal ion in the form of nanoparticles. Plant leaf extract had been used for synthesis of silver and gold nanoparticles, which lead to formation of pure metallic nanoparticles of silver and gold [20, 21]. Some of the plant extracts are given in Table 1.

4. Mechanism of Nanoparticles Formation

Still up to date there is no proper mechanism for the synthesis of silver nanoparticles. The proposed hypothetical mechanism behind the synthesis of nanoparticles is an enzymatic reaction in which the plant extract contains the complex of reducing enzymes which reduce the chemicals such as silver nitrate into silver ions and nitrate ions [22].

Plants contain a complex network of antioxidant metabolites and enzymes that work together to prevent oxidative damage to cellular components. It was reported that plants extracts contain biomolecules including polyphenols, ascorbic acid, flavonoids, sterols, triterpenes, alkaloids, alcoholic compounds, polysaccharides, saponins, β-phenylethylamines, glucose and fructose, and proteins/enzymes which could be used as reductant to react with silver ions and therefore used as scaffolds to direct the formation of AgNPs in the solution. Hypothetically, biosynthetic products or reduced cofactors play an important role in the reduction of respective salts to nanoparticles. However, it seems probable that some glucose and ascorbate reduce $AgNO_3$ and $HAuCl_4$ to form nanoparticles [13, 22–24]. In neem leaf broth, terpenoids are the surface active molecules stabilizing the nanoparticles and reaction of the metal ions is possibly facilitated by reducing sugars [25]. *Capsicum annuum* extract also indicated that the proteins which have amine groups played a reducing and controlling role during the formation of AgNPs in the solutions and that the secondary structure of the proteins changed after reaction with silver ions [20]. *Ficus benghalensis* leaf contains antioxidants and polyphenols (flavonoids) and it can also directly scavenge molecular species of active oxygen. Antioxidant action of flavonoids resides mainly in their ability to donate electrons or hydrogen atoms, that is, change keto group to enol form. Proteins, enzymes, phenolics, and other chemicals within plant leaf extract reduce silver salts and also provide excellent tenacity against agglomeration, which can be further studied to understand the mechanism of evolution by biological systems [24, 26].

Table 1: Synthesis of metal nanoparticles by photosynthetic plants.

Plant species	Plant parts	Nanoparticles	Size (nm)	Reference
Sorghum bicolor	Bran	Ag and Fe	—	[3]
Vitex negundo	Leaf	Ag	—	[4]
Ocimum sanctum	Leaf	Ag	—	[15]
Medicago sativa	Leaf	Au and Ag	20–40	[16]
Jatropha curcas	Latex	Ag	10–20	[18]
Cinnamomum camphora	Leaf	Au and Ag	55–80	[19]
Capsicum annuum	Leaf	Ag	15–20	[20]
Allium cepa	Extract	Ag	—	[21]
Azadirachta indica	Leaf	Ag, Au, and Ag/Au bimetallic	50–100	[25]
Avena sativa	Leaf	Au	25–85	[46]
Sesbania drummondii		Au	6–20	[22]
Pelargonium graveolens	Leaf	Ag	16–40	[47]
Aloe vera	Leaf	Ag	15–15.6	[23]
Emblica officinalis	Leaf	Ag and Au	10–20 and 15–25	[48]
Tamarindus indica	Leaf	Au	20–40	[49]
Chilopsis linearis	Leaf	Au	—	[50]
Humulus lupulus	Leaf	Au	—	[51]
Trapa bispinosa	Peel	Ag	—	[52]
Brassica juncea	Leaf	Au	—	[53]
Gliricidia sepium	Leaf	Ag	10–50	[54]
Euphorbia hirta	Leaf and bark	Ag	—	[55]
Argemone mexicana	Leaf	Ag	—	[56]
Boswelliaovali foliolata	Bark and leaf	Ag	—	[57]
Cycas circinalis	Leaf	Ag	2–6	[58]
Nerium indicum	Leaf	Ag	—	[59]
Bacopa monniera	Leaf	Ag	15–120	[60]
Rhizophora apiculata	Leaf	Ag	13–19	[61]
Nicotiana tobaccum	Leaf	Ag	8	[62]
Memecylonedule	Leaf	Ag and Au	50–90 and 10–45	[63]
Saraca indica	Leaf	Ag	13–50	[26]
Bauhinia variegata L.	Leaf	Au	43–145	[64]
Ficus benghalensis	Leaf	Ag	16	[24]
Murraya koenigii	Leaf	Ag	10–25	[65]
Dalbergia sissoo	Leaf	Au and Ag	50–80 and 5–55	[66]
Ipomea carnea	Leaf	Ag	30–130	[67]
Phyllanthus maderaspatensis	Leaf	Ag	59–76	[68]
Santalum album	Leaf	Ag	80–200	[69]
Syzgium cumini	Leaf	Ag	100–160	[70]
Syzgium cumini	Bark	Ag	20–60	[71]

5. Factors Affecting Phytofabrication of Nanoparticles

There is enormous interest in metal nanoparticles because of their unpredicted physical and chemical properties revealed at the nanoscale level. The formation of nanoparticles is depending upon certain physiochemical properties such as temperature, time, pH, optical, concentration of the substrate, and enzyme sources. These factors play an important role in the phytofabrication of nanoparticles such as the shape, size, and distribution. The effect of these factors is explained as follows.

5.1. Effect of Temperature. Temperature is the basic physical factor that affects the formation of the nanoparticles. Vigneshwaran et al. (2006) reported that the silver nanoparticles synthesize by autoclaving the solution of silver nitrate and starch at 15 psi and 121°C temperature [27]. Njagi et al. (2011) reported the synthesis of silver and iron nanoparticles at room temperature using green extracts such as aqueous sorghum bran [3]. Table 2 provides some of the plant extracts which synthesized AgNPs at different temperatures.

The particle size in the solution is performed by several calculations with the computer simulation program "Mie plot v. 3.4" [28]. From the absorbance spectra we can determine

Table 2: Plant extracts which produce AgNPs depending on the temperature.

S. number	Name of the plant	Temperature	Time (hrs)	References
1	*Ocimum sanctum*	Room temperature	48	[27]
2	*Vitex negundo*	Room temperature	48	[4]
3	*Boswellia ovalifoliolata*	50–95°C	1	[57]
4	*Argemone mexicana*	Room temperature	4	[56]
5	*Euphorbia hirta*	Room temperature	7	[59]
6	*Nerium indicum*	Room temperature	7	[59]

size of the particles compared with the one calculated by using Mie theory. One can find the useful information by analyzing the optical spectra; the change in the absorbance shows change of absorbing species in a solution and variations of the quantity of the AgNPs. The position of the peak directly depends upon the size of the nanoparticles [28, 29].

Mie theory:

$$I = I_0 \left(\frac{1 + \cos^2\theta}{2R^2} \right) \left(\frac{2\pi}{\lambda} \right)^4 \left(\frac{n^2 - 1}{n^2 + 2} \right)^2 \left(\frac{d}{2} \right)^6, \quad (1)$$

where R is the distance between the particle and the observer, θ is the scattering angle, n is the refractive index of the particle, and d is the diameter of the particle.

The stability of the nanoparticles also depends on the temperature, the AgNPs which are formed are maintained at the temperature between 18 and 25°C for two to three months, and these solutions are stable at these temperature ranges [28].

5.2. Effect of Concentration of the Substrate and the Reducing Agents. The effect of pH can be better understood by the preparation of nanoparticles from the chemical methods such as preparation by Tollens' reagent, that is, ammoniacal silver nitrate, which reduces the carboxyl group of the sugar substrate, such as glucose and ribose; the size of the nanoparticles depends on the concentration of ammonium; in this reaction $Ag(NH_3)_2{}^+$ is a stable complex ion resulting from ammonia's strong affinity for Ag^+; therefore the ammonia concentration and nature of the reductant must play a major role in controlling the AgNPs [30].

5.3. Effect of pH. pH is physical factor that affects the size and the distribution of the nanoparticles [30, 31]. The difference in structure of monosaccharide and disaccharides influences the particle size with disaccharides giving on average smaller particles than monosaccharide at pH 11.5. Furthermore, particles obtained at pH 11.5 were smaller than those at pH 12.5. Polydispersity also decreased by lowering the pH [31].

5.4. Effect of Time. The synthesis of nanoparticles depends upon the rate of the reaction and the stability of the nanoparticles depends upon the reaction time. Synthesis of AgNPs revealed that glucose was able to reduce Ag^+ ions to Ag^0 and through this reaction, glucose can be oxidized to gluconic acid. The gradual formation of AgNPs was investigated by UV visible spectroscopy, which has proven to be a useful spectroscopic method for the detection of prepared NPs over time. In UV visible spectra, the AgNPs can be shown by a SPR peak at around 400 nm, but a small shift (blue shift or red shift) in the wavelength of the peak could be related to obtaining Ag-NPs in different shapes, sizes, or solvent dependences of prepared AgNPs [32]. Concerning the colloid stability of the prepared silver hydrosols, it was found to be strongly dependent on particle size. The colloids with particle sizes below 100 nm, prepared at lower ammonia concentrations, are stable for several months, while larger particles reveal a high level of instability as a result of the relatively quick sedimentation process. The colloid stability of silver particles prepared at lower ammonia concentrations is well documented by the time dependencies of absorption spectra, exhibiting a sharp plasmon absorption maximum even three months after preparation [33].

6. Applications

Nanoparticles application in catalysis, sensors, and medicine depends critically on the size, shape, and composition of the particles. Thus, different routes leading to the synthesis of nanoparticles of various shapes and sizes have extended the choice of properties that can be obtained. Various applications of nanoparticles are listed below.

6.1. Environmental Sciences. Due to the large surface area and relatively high surface energy, once released into the environment, AgNPs transformation takes place such as oxidation, aggregation, sulfurization, and chlorination. Also, environmental transformation related AgNP toxicity and stability should be investigated. Because environmental systems are always variable, AgNPs have limited stability and tendency of being easily oxidized and releasing silver ion; it is hard to predict the fate and transport of AgNPs. Furthermore, in the presence of dissolved organic matter, dissolved silver ion can also be reduced to AgNPs. Previous study revealed that silver ion release is mediated by dissolved oxygen and protons, but present study revealed that the dissolved oxygen would generate superoxide anion in natural waters under sunlight and significantly promote the reformation of AgNPs. Given their complicated behaviour in the environment, we must make great effort to broaden our knowledge of the transformation of AgNPs so as to correctly forecast their environmental and human health risks [34]. Photodynamic cancer therapy is based on the destruction of the cancer cells by laser generated atomic oxygen, which is cytotoxic [35].

6.2. Antibacterial Activity. The AgNPs are well known for their excellent antibacterial ability and superior physical properties and are widely used in a growing number of applications ranging from home disinfectants and medical devices to water purificants. The AgNPs have been an effective biocide against broad-spectrum bacteria including both Gram-negative and Gram-positive bacteria. Silver is known for antimicrobial properties and has been used in the medical field and also has been shown to prevent HIV from binding to host cells. AgNPs can be exploited in medicine and pharmacy for dental materials, burn treatments, coating stainless steel materials, and sunscreen lotions [36]. In general, therapeutic effects of AgNPs depend on important aspects, including particle size (surface area and energy), particle shape (catalytic activity), particle concentration (therapeutic index), and particle charge (oligodynamic quality) [27]. Mechanisms of antimicrobial effects of AgNPs are still not fully understood, but several studies have revealed that AgNPs may attach to the negatively charged bacterial cell wall and rupture it, which leads to denaturation of protein and finally cell death. The cell death due is also related to uncoupling of oxidative phosphorylation, induction of free radical formation, interference with respiratory chain at cytochrome C level, interaction with protein thiol groups and membrane bound enzymes. Additionally, interaction with phosphorus and sulphur containing compounds such as DNA and protein. AgNPs act as an antibacterial, antiviral, and antifungal agent when incorporated in coatings, nanofiber, first aid bandages, plastics soap, and textiles, in self-cleaning fabrics, and as conductive filler [27]. In hospitals, infection is the most common complication and cause of death in patients. Therefore, antibacterial effects of AgNPs have been incorporated into various medical applications. Plastic catheters coated with AgNPs prevent biofilm formation from *Escherichia coli, Enterococcus faecalis, Staphylococcus aureus, Candida albicans, Staphylococci,* and *Pseudomonas aeruginosa* and also show significant *in vitro* antimicrobial activity [37]. Microorganisms that are exposed to pollutants in the environment such as metal ions have remarkable ability to fight with metal stress. These metal-microbe interactions have already found to be beneficial role in nanobiotechnological applications.

6.3. Nanobiosensors. Nanoparticles are excellent labels for the biosensors because they can be detected by numerous techniques, such as optic absorption fluorescence and electric conductivity. Using the surface plasmon resonance effect the AgNPs gain a very high sensitivity and the measurements can be conducted in real time [38]. The unique physicochemical properties of metals at the nanoscale have led to the development of a wide variety of biosensors, such as nanobiosensors for point of care disease diagnosis, nanoprobes for *in vivo* sensing/imaging, cell tracking, and monitoring disease pathogenesis or therapy monitoring, and other nanotechnology-based tools that benefit scientific research on basic biology [35].

6.4. Agricultural Engineering. Agriculture is the backbone of most developing countries, with more than 60% of the population dependent on it for their occupation. Nanosized lignocellulosic materials have been obtained from crops and trees which had opened up a new market for innovative and value added nanosized materials and products. These can be applied in food and other packaging, construction, and transportation vehicle body structures. Nanofertilizer, nanopesticides including nanoherbicides, nanocoating, and smart delivery system for plant nutrients are being used extensively in agriculture with several industries making formulations which contain 100–250 nm nanoparticles that are more soluble in water thus increasing their activity [39]. Nanofertilizers are able to synchronize the release of nutrients with their plant uptake, thus avoiding nutrient losses and reducing the risks of groundwater pollution. Additionally, the nanofertilizers should release the nutrients on demand while preventing them from prematurely converting into chemical/gaseous forms that cannot be absorbed by plants. This can be achieved by preventing nutrients from interacting with soil, water, and microorganisms and releasing nutrients only when they can be directly internalized by the plant [40, 41].

6.5. Nanoparticles Impregnated Fabrics for Clinical Clothing. Because nanoparticles possess a large surface area to volume ratio as well as a high surface energy the use of nanoparticles could provide high durability for treated fabrics that lead to an increase in durability of the textile functions. The film thickness is in the range of 50–80 nm and acts as a catalyst to self-cleaning the fabric. Titanium nanoparticles with a smooth surface may be used as an antiadhesive coating for windows or spectacles lenses and application in textiles. AgNPs have been used to produce self-cleaning or antiodour clothes, furniture textiles, kitchen cloths, towels, antibacterial wound dressings, patient dresses, bed lines or reusable surgical gloves and masks, protective face masks, suits against biohazards, cosmetic products, ultrahydrophobic fabrics with potential applications in the production of highly water repellent materials, and sportswear. Freeman et al. (2012) investigated the effect of silver impregnation of surgical scrub suits on surface bacterial contamination during use in a veterinary hospital. It was observed that silver-impregnated scrubs had significantly lowered bacterial colony counts compared with polyester/cotton scrubs. The results showed that silver impregnation appeared to be effective in reducing bacterial contamination of scrubs during use in a veterinary hospital [42–45].

7. Conclusions and Future Prospects

Plants as a biological system for the fabrication of nanoparticles have emerged as simple, cost-effective, ecofriendly, and rapid technique. They could be a more efficient biological system than microorganisms along with physiochemical methods employed for the fabrication process. Nanoparticles of Ag, Au, and Pt have been successfully prepared using a simple and efficient green chemistry methodology.

Nanoparticles had a wide application in the field molecular research to study the nucleic acid structures and their functions in the intact of the cell. Nanoparticles also act as biological markers included in the proteins, nucleic acids, hormones, and all other biological active molecules. The application of nanoparticles in the agriculture opens a new scope for the preparation of biologically degradable synthetic pesticides and having the target of the pesticide action for the agricultural pests. Nanoparticles suspensions with different ranges of nanoparticles had different mode of application in the agricultural biotechnology for the preparation of disease resistant and drought resistance plants in the future which can replace the classical experiments.

The antimicrobial activity of the nanoparticles had a wide scope in the preparation of target based drug delivery and clinical diagnostics system. The identification of the specific mechanism of the AgNPs inhibition of the microbial growth or the lethal effect provides major application in the medicine, environment, soil fertility, and water quality. Moreover, the synthesis of nanoparticles with different sizes and shapes is the basic challenging task in the green synthesis of nanoparticles; this requires a basic understanding of the nuclei formation and the influence of reaction species in nuclei morphology, but still there is dormancy about the actual mechanism of the nanoparticles synthesis from the biological mode of synthesis.

Nanobiotechnology has emerged as the present and the future technology of the era, with profound variety of applications that include fields such as quantum dots, opto-electronics, medicine, therapeutics, biosensors, and many more.

Conflict of Interests

The author declares that he has no conflict of interests.

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