Int. J. Nano Dimens., 7 (2): 97-108, Spring 2016

REVIEW PAPER

The Green route of Silver nanotechnology: Phytosynthesis and applications

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Received 07 July 2015; revised 30 September 2015; accepted 05 October 2015; available online 02 May 2016

ABSTRACT: Silver nanoparticles (AgNPs) are amongst the most investigated materials in nanotechnology in view of their unique physio-chemical features and and applications in restorative science. Their progressive usage in different fields of science and some predominant restrictions with conventional methods for their synthesis have demanded analysts to discover green courses for their creation. Biological methods are the preferred to combat with the issues concerned with the nanoparticle synthesis. Within this decade, thousands of plants have been screened to analyze the final impact on characterization and morphology of AgNPs compared to general modes of their synthesis. Phytosynthetic method is an eco-friendly route that can lead to an advanced production of silver nanoparticles with controlled morphology. We herein reviewed the present aspects of phytosynthesis of AgNPs and their importance in modern science. Moreover, overviews of proposed mechanisms in this technology have also been included, which ultimately provide some insights of their safe use and demand for further research.

Keywords: *Ecofriendly; Green synthesis; Nanotechnology; Nanomaterials; Phytosynthsesis; Plant extract; Silver nanoparticles*

How to cite this article:
Ahmad H, Rajagopal K, Hussain Shah A. The green route of Silver nanotechnology; phytosynthesis and applications. Int. J. Nano Dimens. 2016; 7(2): 97-108. DOI: 10.7508/ijnd.2016.02.001

INTRODUCTION

Nanotechnology is the branch of technology that deals with dimensions and tolerances of less than 100 nanometres, especially the manipulation of individual atoms and molecules. Nanomaterials often have a significant degree of difference in physico-chemical and biological properties when compared to their macroscale counterpart, in spite of the similar chemical compositions they possess [1].

Among the various inorganic metal nanoparticles, silver nanoparticles [AgNPs] have emerged as one of the most intensively studied areas in the field of nanotechnology due to their well-known effectiveness in various fields [2]. AgNPs applications in biomedical [3-5], drug delivery [6], food industries [7], agriculture [8], textile industries [9], water treatment [10], catalysis and surface-enhanced Raman scattering [11] have been reviewed more. Thus, their applications in various fields have demanded researchers to create an ecofriendly route for their mass production [12, 13]. By way of green synthesis methodology, it is expected to develop and implement the nano products and processes to reduce or eliminate the use and generation of substances that are hazardous to human health and the environment.

Nevertheless, the use of plants for the synthesis of AgNPs is in focus of intensive research because of its eco-friendly nature. The use of plants boasts of several advantages such as elimination of elaborate processes of maintaining cell cultures, easy scale up for large-scale synthesis and cost-effectiveness. Moreover, plant extracts may act as both reducing agents and stabilizing agents in the synthesis of nanoparticles [14]. In this article, we aim to

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elaborate the different ways of how plants have been used to generate the silver nanoparticles.

Further, the article covers some important mechanisms proposed by different researchers that have been briefly explained here.

Phytosynthesis of silver nanoparticles

Compared to other green sources, plants are emerging as advantageous because the presence of broad and variable bio-molecules in plants can act as capping and reducing agents and thus increases the rate of reduction and stabilization of nanoparticles. Since plants differentiate in their concentrations and combinations of organic reducing agents, the source of the plant extract is known to influence the characteristics and morphology of the nanoparticles [14, 15].

Parameters of phytosynthesis of silver nanoparticles and their role

The reduction reaction, which brings about AgNPs development, is basically controlled by specific parameters, which impact the rate of synthesis and characterization of AgNPs. Researchers call them as altering factors in the nanoparticle formation and morphology, have revealed these parameters, which include reaction temperature, pH, substrate concentration, and exposure time to the substrate [16, 17]. The controlled production of some essential morphology like plates, discs, rods, wires and/or size, and size distribution of AgNPs heavily alters their application like electromagnetic field enhancement property. Thus, it became necessary to review the parameters which mainly influence the properties of nanoparticles.

I. Reaction temperature

As indicated by studies the reaction temperature can significantly impact response rate, and thus the particle qualities [18, 19 and 20]. The size of silver nanoparticles increases with temperature [17, 19 and 21]. The profile and growth trend of measurements of silver particles are apparently different at different temperatures. The higher the reaction temperature the faster the silver nanoparticles grow and with the temperatures increasing, the triangular silver nanoplates and spheres grow larger. This is because of the phenomenon of particle fusion mechanism [19, 22, and 23]. Moreover, a high temperature leads to reaction too faster because some of the reducing agents of reaction mixture become more active at higher temperatures [17, 24 and 25]. Thus, in general, the variation in temperature of the reaction system heavily affects the reaction capability of components in reduction, surfactant adsorption/desorption, and complexion stability, the formation and growth rate, and hence the morphological distributions [19].

II. pH

Like temperature, pH is one of the most important factors for nanoparticle formation. There is a parallel relation of nanoparticle formation with increasing pH [17, 26 and 20]. The alkaline pH is seen to be favorable for the nanoparticle formation than the acidic pH. The possible reason may be because more precipitation or agglomeration occurs due to lack of stabilizing agents and may be due to the ionization of the phenol group present in the extract at neutral or alkaline pH. Thus, the rate of formation of AgNPs is high in basic pH than in acidic pH [27]. According to some studies, the formation of AgNPs occurs rapidly in neutral and basic pH, which is evident from observation, and this may be due to the ionization of the active groups present in the PE [27]. However, at acidic pH, the electrostatic repulsion of anions present in the solution may slow down the rate of formation and aggregation of AgNPs at this pH [28].

III. Substrate concentration

The effect of increasing molarities of $AgNO_3$ and plant extract broth concentration on the phytosynthesis and thus characterization of AgNPs has been revealed [17, 20 and 29]. The increase in concentration of $AgNO_3$ in the reaction mixture marks a gradual up of Surface Plasmon Resonance (SPR) bands [20, 30]. It was seen that the increase in metal concentration enlarges the AgNPs size while as with increase in PE quantity decreases the particle size of the AgNPs [20, 31].

IV. Role of Phytogenic source

Since the phyto-chemistry of plant kingdom is quiet large. This variation is seen among various species of same family to different parts of the same plant source [32]. Different authors have used separate plant parts for phytosynthesis showing a perfect result of AgNPs formation and it was seen that the specific plant part acts differently to reduce Ag to Ag^0 [33- 35]. With this phenomena it can be empathized that each plant part and /or plant source has a steady impact upon the amalgamation of AgNPs [Table 1.].

Table 1. Use of different plants for phytosynthesis of AgNPs and their characterization

Plant used	Reaction Temperature (°C)*	Size (nm)	Shape**	References
Moringa oleifera	RT	57	NA	[36]
Trachyspermum ammi	RT	87-99	Triangular	[37]
Papaver somniferum	25°C	3-8	Spherical	
Acalypha indica	25°C	20-30	Spherical	[38]
Terminalia chebula	RT	25	Crystalline	[39]
Sorbus aucuparia	25°C	16-18	Spherical, Triangular and Hexagonal	[40]
Boswellia serrata	RT	7.5	Spherical	[41]
Allium sativum	RT	4-22	Spherical	[42]
Datura metel	RT	16-40	NA	[43]
Coleus aromaticus	RT	44	Spherical	[17]
Aloe vera	RT	50-350	Spherical, Triangular	[44]
Pinus desiflora	RT	15-500	Cubic	[25]
Annona squamosa	RT	20-100	Spherical	[84]
Cissus quadrangularis	70°C	37-44	Spherical, rod and Triangular	[17]
Euphorbia hirta	25°C	40-50	Spherical	[45]
Desmodium tri?orum	25°C	5-20	NA	[46]
Anacardium occidentale	27°C	6	NA	[47]
	100°C	17	NA	
Musa paradisiaca	25°C	20	NA	[48]
Desmodium tri?oru	25°C	16-40	multistructural	[43]
Ceratonia siliqua	RT	5-40	Spherical	[49]
Chenopodium album	25°C	10-30	Quasi-Spherical	[50]
Trachyspermum copticum	RT	6-50	NA	[51]
Artemisia nilagirica	RT	70-90	Spherical, Triangular, Hexagonal	[51]
Jatropha curcas	RT	10-20	Crystalline	[33]
Jatropha curcas	RT	15-50	Spherical	[33]
Argemone maxicana	RT	30	Cubic, Hexagonal	[52]
Syzygium cumini	RT	29-92	Spherical	[53] [129]
Acalypha indica	RT	20-30	Crystalline	[38]
Swietenia mahogani	RT	20-100	NA	[54]
Coscinium fenestratum	RT	28.5-68.0	Rod shaped	[55]
Rhododedendron dauricam	RT	25-30	Spherical	[56]
Nelumbo nucifera	RT	25-80	Spherical, Triangular	[57]
Sesuvium portulacastrum	RT	5-20	Spherical	[32]
Rumex hymenosepalus	RT	2-40	FCC, Hexagonal	[58]
Mentha piperita	RT	5-150	Spherical	[59]
Ocimum tenuiflorum	RT	25-40	Spherical	[60]
Platanus orientalis	RT	15-500	Cubic	[25]
Ocimum sanctum	RT	157.2	NA	[61]
Ginko biloba	RT	15-500	Cubic	[25]
Mentha piperita	28°C	90	Spherical	[62]
Murraya keenigii	RT	10	Crystalline, Spherical	[63]
Pelargonium graveolens	RT	16-40	Crystalline	[64]
Citrus sinensis	25°C	35	Spherical	[65]
	60°C	10		
Memecylon edule	25°C	20-50	Triangular, Circular,l	[66]

Continued Table 1.

Plant used	Reaction Temperature (°C)*	Size (nm)	Shape**	References
Rosa rugosa	25°C	30-60	NA	[40]
Piper pedicellatum	RT	2.0-30	Spherical	[44]
Catharanthus roseus	25°C	48-67	NA	[67]
Camelia sinensis	25°C	30-40	NA	[68]
Lawsonia inermis)	25°C	7.5-65	Spherical, Triangular, quasiSpherical	[69]
Gelidiella acerosa	25°C	22	NA	[27]
Solanum torvum	RT	1-100	Spherical	[70]
Eclipta prostrata	RT	35-60	Triangular, Pentagonal, Hexagonal	[71]
Cinnamomum camphora	RT	55-80	NA	[72]
Euphorbiaceae latex	RT	18	NA	[73]
Garcinia mangostana	RT	35	NA	[74]
Solanum trilobatum	RT	15-20	Cubic and Hexagonal	[75]
Boswellia ovalifoliolata	RT	30-40	NA	[76]
Catharanthus roseus	RT	48-67	Spherical	[15]
Coleus aromaticus	RT	44	NA	[77]
Cassia auriculata	RT	20-40	Spherical	[18]
Trianthema decandra	RT	17.9-59.6	Spherical	[79]
Artocarpus heterophyllus	121℃	10.78	Irregular	[41]
Mangifera indica	RT	20	Spherical, Triangular, Hexagonal	[80]
Coleus amboinicus Lour	RT	25	NA	[81]
Azadirachta indica	>100℃	20-50	Cubic	[82]
Citrullus colocynthis	RT	31	Spherical	[83]
Zingiber offcinale	RT	6-20	Spherical	[34]
Piper betle	RT	3-37	Spherical	[84]
Svensonia hyderabadensis	RT	45	Spherical	[85]
Moringa oleifera	25°C	58	NA	[87]
Pelargonium graveolens	25°C	16-40	NA	[149]
Carica papaya	25°C	25-50	NA	[86]
Nicotiana tobaccum	RT	8	Crystalline	[87]

*Where RT is meant for room temperature and has been decleared where some authors did not mention reaction temperature.
** NA is placed where authors have not mentioned the shape of AgNPs.

Proposed mechanism for Silver Nanoparticles phytosynthesis

I. Metal reduction by phytochemicals

It has been analyzed that phytochemicals are the key sources of nanoparticle synthesis. The reduction of Ag ions to Ag^0 is likely dependent upon type of active compounds present in a plant extract.

Mostly the reduction occurred due to the presence of flavonoids [88], phenols [89, 39, 54], proteins, flavones and terpenoids [32], saponins [66], polyhydroxy limonoids [90], quinones [91] or other active ions like amine group [-NH2], carbonyl group, -OH groups [92], polyol components [72], chlorophyll [63], in the respective plant extract solution. Thus, according to phytochemical reduction concept the synthesis of silver nanoparticles can be possibly achieved by any plant extract owing to the presence of active components in the plant source under consideration [93, 64].

II. Concept of photosynthesis of AgNPs

According to some researchers, synthesis of silver nanoparticles is a photochemical reaction where green plant parts are used as photo centers. As in photosynthesis, here many chlorophyll pigments serve as an antenna, collecting light and transferring its energy to the reaction center. In the reaction center the silver ions, which entered in to the plant cell via the H+ ATPase protein embedded in the thylakoid membrane, is reduced to form silver nanoparticles by proteins embedded inside the plasma membrane. According to some authors [43], the following four

observations were recorded in the reaction system; a) Silver [Ag+] ions act as an electron donor molecule. b) Plastoquionone [PQ] acts as an electron acceptor molecule.c) Chlorophyll pigments act as a stabilizing agent by stabilizing reaction between electron donor and electron acceptor molecules. d) Plasto-hydroquinone or quinol [alcoholic agent] acts as a main reducing agent for reduction of silver ion to silver metal, both oxidation and reduction reactions carried out by different are reducing and oxidizing agent.

III. Metal reduction by reductases

In plant extracts, the NADH-dependent reductase can act as an electron carrier to transport electrons from NADH to metallic silver for their reduction. One such probable mechanism for AgNPs biosynthesis has been reported by Kalimuthu K et al. in 2008 and Ahmad K et al in 2010 [94, 95]. The biosynthesis of nanoparticles by reductases as reported earlier suggest that the nitrate reductase catalyzes the reduction of $AgNO_3$ to silver nanoparticles utilizing NADPH as reducing agent [38, 96 and 97].

Biological applications of Silver Nanoparticles

(a) Application in cancer therapy

The production of Silver nanoparticles through phytosynthesis has shown advanced cytotoxic effect against various cancer cell lines. This property of AgNPs has thus attracted oncologists for their use since it targets the mitochondrial system thereby limiting ATP and altering other biological mechanistic pathways for cell survival [98].

Different cell types investigated for their cytotoxicity using AgNPs including NIH 3T3 fibroblast cells [99], HeLa cells [100] and human glioblastoma cells [101]. The cytotoxic effect of AgNPs synthesized using *Piper longum* also had signiûcant cytotoxic effect [94%-500ug/ml] against invasive cells of Hep-2 cell line [102].

The AgNPs synthesized using *Annona squamosa* [27], *Coscinium fenestratum* [55], *Melia azedarach* [103], *Piper longum* [104], *Eucalyptus chapmaniana* [105] and *Albizia adianthifolia* [106] had some advanced results against HEp-2 , MCF-7[human breast cancer], HeLa [cervical cancer], HEp-2, HL-60 [human acute promyelocytic leukemia] and A549 [lung cancer] cancer cell lines, respectively. These results clarify that AgNPs generally show cytotoxicity to carcinoma cells when compared with normal cell lines. Mechanism of action: MTT and LDH assays are the mostly used techniques to determine cytotoxicity of AgNPs. The mechanisms for AgNP-induced toxicity may be related to mitochondrial damage, oxidative stress, DNA damage and induction of apoptosis [102]. It is also proposed that the cytotoxic effect of AgNPs is due to the physicochemical interaction of silver atoms with the functional groups of intracellular proteins, as well as with the nitrogen bases and phosphate groups in DNA [83, 107].

(b) Application in anti-microbial activity

Antibacterial activity: Bio-synthesized silver nanoparticles exhibited better antibacterial activity against most human pathogens [108]. Antimicrobial activity of silver nanoparticles synthesized by specific commercial plant extracts has been investigated against various pathogenic organisms. Extracts of *C. sinensis* and *C. asiatica* were used to study the effect of AgNPs against *Streptococcus aureus, P. aeruginosa, E. coli* and *K. pneumoniae* [109]. AgNPs synthesized from *Trianthema decandra* were more pronounced in Gram-negative bacteria than Gram-positive ones [110]. The AgNPs synthesized using extract of *Ocimum sanctum* were effective against a resistant *of Streptococcus aureus* and *B. megaterium* being resistant against Ampicillin, Penicillin, Cloxacillin, Ceftazidime, Methicillin and Ceftazidime and Cloxacillin, respectively [61]. The AgNPs synthesized using *Ocimum sanctum* leaf extracts had significant antimicrobial activity against both Gram-negative [*E. coli*] and Gram-positive [*Streptococcus aureus*] microorganisms [111] which corresponds with that of *Ocimum tenuiûorum* being active against Gram-negative and Gram-positive bacteria [60]. Moreover the antibacterial activity against ampicillin-resistant *Escherichia coli* and multi-drug resistant *E. coli, S. aureus,* and *Salmonella typhi* strains was also investigated [112]. About 10-35 nm size AgNPs synthesized using peel extract of *Citrus sinensis* reported a broad spectrum antibacterial activity [65].

Anti-fungal activity of AgNPs: The anti-fungal activity of AgNPs has been reported against both human and some phytopathogens. Padma S. et al evaluated AgNPs against some crop pathogens like *Fusarium oxysporum* and *Alternaria brassicicola* using agar diffusion method [113]. The disease incidence of powdery mildew in cucumbers and pumpkins was efficiently controlled by AgNPs both *in vitro* and in field conditions when compared to commercial

fungicides [114]. Some other findings also support the effectivity of AgNPs against phytopathogens [115-119]. However, some studies support cytotoxicity and genotoxicity effects of AgNPS on the plant anatomy [120-128]. On the other hand, substantial inhibition of fungal species was obtained in terms of zone of inhibition in the plate against human pathogens namely *Candida albicans* [79,129*], Candida lipolytica* [48], *Candida parapsilosis* [79, 130 and 75]. In addition it was also demonstrated that these nanoparticles [from *Solanum trilobatum*] when mixed with shampoo enhance the anti-dandruff effect against dandruff causing fungal pathogens namely *Pityrosporum ovale* and *Pityrosporum folliculitis* [131].

Anti-plasmodial activity of AgNPs: According to some studies, AgNPs synthesized using *Ammannia baccifera* show significant toxic effects against fourth instar larvae of two mosquito species, *Anopheles subpictus* and *Culex quinquefasciatus.* The larvae were exposed to varying concentrations of AgNPs for 24 h to analyze the larvicidal effect. The AgNPs were toxic against the larvae of *A. subpictus* with an LC50 = 29.54 ppm and against the larvae of *C. quinquefasciatus* with LC50 = 22.32 ppm [132]. The larvicidal activity of AgNPs against ulariasis and malaria vectors was also reported using *Eclipta prostrate* as biogenic agent [133].

Mechanism of antimicrobial action of AgNPs

The exact mechanism for the growth inhibition by Ag nanoparticles has not yet been elucidated, but many possible mechanisms have been proposed. The effect of silver ions on microbe can be observed by the structural and morphological changes. Some of the researchers have tried to generate the mechanism of AgNPs upon targeting microbes, which can be explained under two broad concepts (Fig. 1).

Extra-cytol aberration concept

According to this concept, AgNPs get attached to the surface of bacterial membrane thus disturbing its permeability (Fig. 2). This also interferes with the respiration ability of a cell during their interaction [134]. This concept is widely accepted while researchers propose that AgNPs has a higher tendency to react with phosphorus and sulfur compounds present in the membrane of bacterial cells, which readily causes the bacteria to lose its ability to replicate [135]. The electrostatic attraction in the cell wall membrane with negatively charged and silver nanoparticles with positive charge makes them to interact and get attached [136-38]. After attachment, the silver nanoparticles associated with thiol groups of cell wall result in generation of reactive oxygen species and disrupt the cell [139- 143] or AgNPs may form 'pits' in the cell wall after attachment and finally affects the permeability, and cause cell death [144]. Moreover, the attachment ruptures the cell wall, which leads to denaturation of proteins and finally leading to cell death [145]. It may also cause accumulation of envelope protein precursors or affect the function of membrane-bound enzymes on the cell wall, which results in dissipation of the proton motive force.

This leads to destabilization of the outer membrane after which it ruptures and finally causes depletion of intracellular ATP [36, 144, 146 and 147].

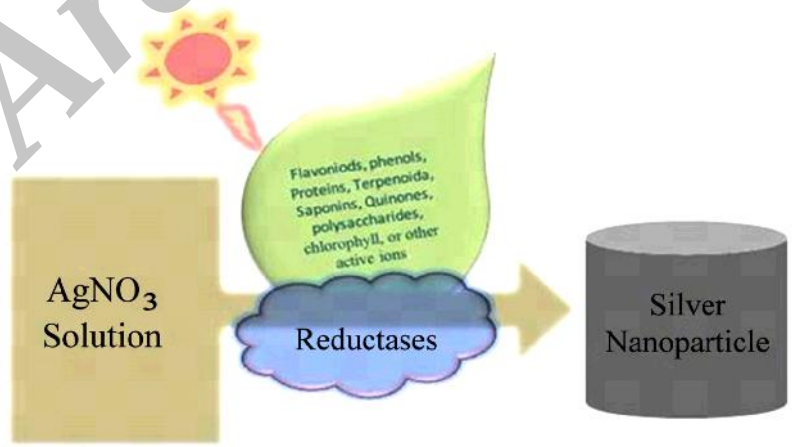

Fig. 1: Mechanism of AgNP Phytosynthesis by using concepts of Metal reduction, AgNP photosynthesis and Reduction by Reductases.

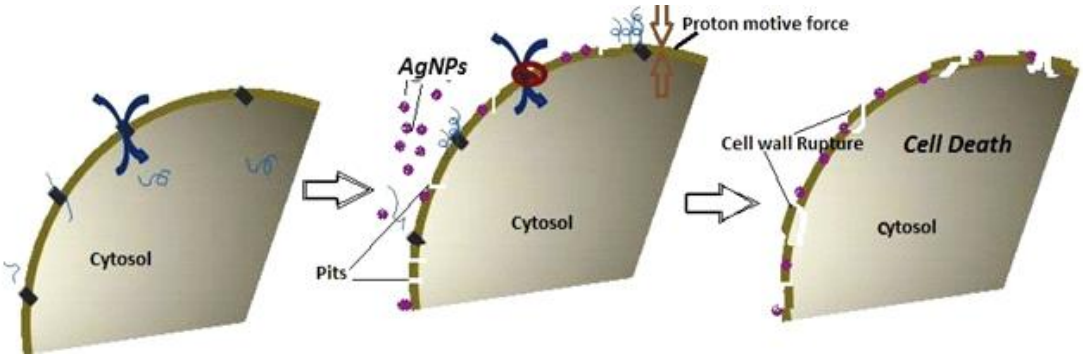

Fig. 2: Extra-cytol aberration of microbial cell by AgNPs.

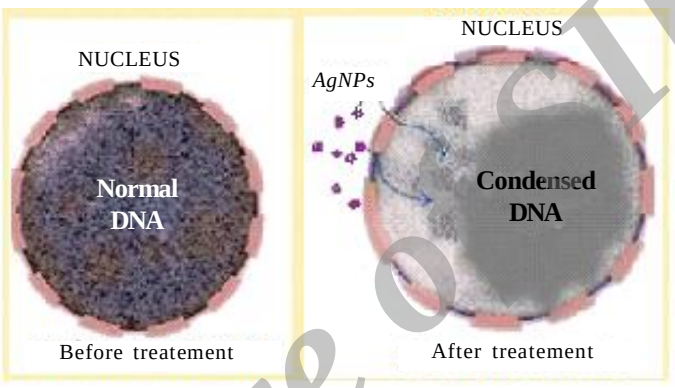

Fig. 3: Intra-cytol aberration of microbial cell by AgNPs.

Intra-cytol aberration concept

This concept takes into account the interference of AgNPs with the macro molecules like proteins, DNA and RNA. It is assumed that the DNA loses its replication ability in condensed form, which can be achieved by it when the silver ions penetrate inside the microbial cell (Fig. 3). This condensation inhibits the replication ability of DNA, which ultimately leads to cell death [148]. The malfunction of the bacterial replication system can also happen when Ag ions released from AgNP normally penetrate into bacterial cell and cause damage on its main components such as the peptidoglycan, DNA, and proteins [3 and 112].

CONCLUSION

Silver products have a long history for their solid inhibitory and bactericidal impacts, and in addition an expansive range of antimicrobial exercises. They have been used from centuries to prevent and treat various diseases, particularly infections [149]. The performance of AgNPs in biomedical science has pulled researchers to adapt the phytogenic way for their synthesis. According to reports, the phytogenic resource and reaction parameters do impact upon the characterization and thus the affectivity of AgNPs. The phytosynthetic approach provides an eco-friendly platform to generate AgNPs with controlled growth and development. Since the reaction proceeds easily within minutes and at room temperature, there is no much energy loss by the reaction system. Consequently, with a specific end goal to accomplish better results in a controlled way, a considerable measure of examination is in need to figure out the real component of their synthesis and method of activity.

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