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GREEN SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES: A REVIEW

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ABSTRACT
Recent advances in nanoscience and nanotechnology radically changed the way we diagnose, treat, and prevent various diseases in all aspects of human life. Silver nanoparticles (AgNPs) are one of the most vital and fascinating nanomaterials among several metallic nanoparticles that are involved in biomedical applications. AgNPs play an essential role in nanoscience and nanotechnology, particularly in Nanomedicine. To avoid the chemical toxicity, biosynthesis (green synthesis) of metal nanoparticles is proposed as a cost-effective and environmentally friendly alternative. Reaction parameters under which the AgNPs were being synthesized hold a noticeable impact on their size, shape, and application. Available published information on AgNPs synthesis, effects of various parameters, characterization techniques, properties and their use are summarized in this review.

KEYWORDS: Nanoscience and AgNPs.

INTRODUCTION

Nanobiotechnology is one of the natural scientific developments in which plants and different plant products are found to be used in the synthesis of nanoparticles (NPs). These processes have been developed as an essential research field in all therapeutic areas. Nanoparticles are synthesized by using nanotechnology because of its ecofriendliness, involvement of nontoxic molecules, solvents, and a suitable process for large-scale production. Nanoparticles are referred to be particles with a size less than 100 nm with dcharacteristics such as size, distribution, and morphology in comparison to the larger particles of the mass material.[1] Nanotechnology can be applied to medicine, therapeutics, drug delivery and also in treatment for many diseases and disorders. Although nanotechnology is a novel field of research, nanoparticles are known to be used for centuries. For example, Chinese used Gold nanoparticles to introduce red color into ceramic porcelains. The colours in Roman glass artifacts contained metal nanoparticles, used for decoration of cathedral windows. Nanoparticles of noble metals have applications in electronics, magnetic, optoelectronics and information storage.[2,3] Among these, silver nanoparticles (Ag- NPs) are found to possess unique characteristics of high antimicrobial activity to develop nanosilver-based disinfectant products.[4] Silver nanoparticles (AgNPs) are increasingly used in many fields, including medical, food, healthcare, and industrial purposes, due to their novel physical and chemical characteristics.[5-7] they are used as antibacterial agents, in industrial, household, and healthcare products, in consumer products, medicinal device coatings, optical sensors, and cosmetics, in the pharmaceutical manufacturing, the food enterprise, in diagnostics, orthopedics, drug delivery, as anticancer agents, wound dressings, and biomedical devices.[6,9,10] and have ultimately improved the tumor-killing effects of anticancer drugs.[8] Nanosized metallic particles can modify physical, chemical, and biological characteristics due to their surface-to-volume ratio.[11,12] Conventional physical and chemical processes seem to be very expensive and hazardous to human health and environment.[5,13] Biologically-prepared AgNPs show high yield, solubility, and high stability.[5] Among synthetic methods for AgNPs,. biological processes seem to be simple, rapid, non-toxic, dependable, and green synthesis approaches that can produce well-defined size and morphology under optimized conditions for translational research..

CHEMICAL REDUCTION

Silver NPs are synthesized by using organic and inorganic reducing agents such as sodium citrate, ascorbate, sodium borohydride (NaBH4), polyol process, N, N-dimethylformamide (DMF), and poly (ethylene glycol)-block copolymers in aqueous or non-aqueous solutions. These agents reduce Ag+ and lead to the formation of metallic silver (Ag0), followed by agglomeration into oligomeric clusters which eventually lead to the formation of metallic colloidal silver particles. These methods produce pure silver NPs, it is important to and protects the NPs from binding onto nanoparticle surfaces, avoiding their agglomeration.

Oliveira[14] prepared dodecanethiol-capped silver NPs, according to Brust procedure[15] reported that small changes in synthetic factors lead to modifications in nanoparticle structure, average size, size distribution width, stability and self-assembly patterns. Kim and colleagues[16] synthesized spherical silver NPs with a controllable size and high monodispersity using the polyol process and a modified precursor injection method. Zhang and coworkers[17]used a polymer to produce colloids of silver. Chen and colleagues[18] have shown the formation of monodispersed silver NPs in three stages: growth, incubation, and Oatwald ripening stages.

GREEN SYNTHESIS

Green synthesis is similar to chemical reduction chemical reducing agent is replaced by the extract of a natural product such as leaves of trees/crops or fruits for the synthesis of metal or metal oxide Nanoparticles. Biogenic reduction of metal precursors to corresponding Nanoparticles is eco-friendly, free of chemical contamination, less expensive and can be used for mass production. Biosynthesis of Nanoparticles allows recycling of expensive metal salts like gold and silver contained in waste streams. The biological molecules, mostly proteins, enzymes, sugars and even whole cells stabilize Nanoparticles easily allow to interact with other biomolecule and thus increase the antimicrobial activity by improving the interactions with microorganisms..

The green synthesis of AgNPs requires a silver metal ion solution and a reducing biological agent. The reducing agents or other constituents present in the cells acts as the stabilizer and capping agents. The Ag+ ions concentration range between 0.1 - 10 mm obtained from many water-soluble salts of silver along with aqueous $AgNO_3$ solution is used. The AgNPs have been synthesized using prokaryotic organisms unicellular organisms with a true nucleus, fungi, Plantae and Animalia. Due to this restriction, green synthesis of AgNPs has been reviewed under headings microorganisms, plants, and biopolymers. Green syntheses of AgNPs have been made using plant extracts, microbial cell biomass or cell-free growth medium and biopolymers. The plants used for AgNps synthesis range from algae to angiosperms; however, insufficient reports are available for lower plants, and the most suitable choice is the angiosperm plants. Parts like leaf, bark, root, and stem have been used for the AgNP synthesis. The medicinally valuable plants like *Boerhaavia diffusa, Tinospora cordifolia, Aloe vera, Terminalia chebula, Catharanthus roseus, Ocimum tenuiflorum, Azadirachta indica, Emblica* Officinalis, *Cocos nucifera*, common spices like Piper nigrum and *Cinnamon zeylanicum*. Some exotic weeds like Partheniumhysterophorus have also been used for AgNP's synthesis. The other group includes alkaloids (*Papaver somniferum*) and essential oils (*Menthapiperita*) producing plants. Metabolites, proteins and chlorophyll present in the plant extracts were found to be acting as capping agents for synthesized AgNPs. The most chosen solvent for extracting reducing agents from the plant is water. Other organic solvents like methanol, ethanol and ethyl acetate are used. Some researchers pre-treated the plant materials in saline or acetone atmospheres before extraction. Nanoparticles of well-defined structure shape, and morphology are obtained from bark, tissue and whole plant. The problem lies in growth, culture maintenance, and inoculums size standardization. The AgNPs synthesis mainly uses extracellular substances secreted in the growth medium utilizing microbial cell biomass directly.. The biological synthesis of AgNP is due to the presence of organic chemical like fat, carbohydrate, proteins, enzymes& coenzymes, phenols flavonoids, terpenoids, gum, etc. capable for the reduction of Ag+ to Ag0. The active component for the reduction of Ag+ ions depends upon organism/extract used. For nano- transformation of AgNPs, electrons are assumed to be derived from dehydrogenation of alcohols and acids (ascorbic acid) in hydrophytes, keto to enol conversion in mesophytes or both mechanisms in xerophytes plants.[19-21]

CHARACTERIZATION

The physicochemical characteristics of nanoparticles are characterized to evaluate the behavior, bio-distribution, safety, and efficacy of the synthesized particles.

UV-Visible Spectroscopy

UV-visible spectroscopy is used to monitor the synthesis and stability of AgNPs. This is a fast, sensitive, simple, selective method which calibrates the particles in a short period. The absorption band is due to the oscillation of electrons of silver nanoparticles in resonance with the light. The absorption depends on the dielectric medium, particle size, and chemical surroundings. The observation in the peaks ranges from 2 to 100 nm.[23]

X-ray Diffraction (XRD)

X-ray diffraction (XRD) is used for the analysis of both molecular and crystal structures, qualitative analysis, quantitative analysis, measuring the degree of crystallinity, isomorphous substitutions, particle sizes, etc. When X-ray light reflects on any crystal, it leads to the production of many diffraction patterns, and the models reflect the physicochemical characteristics of the crystal structures. XRD has been used to define and identify both bulk and XRD is applied for the identification of the crystalline nature. X-ray powder diffraction is a nondestructive technique with great potential for the characterization of both organic and inorganic crystalline materials. XRD is based on the wide-angle elastic scattering of X-rays. Although XRD has several merits, it has some demerits like becoming the crystals and the ability to get results pertaining only to single conformation/binding state, low intensity of diffracted X- rays compared to electron diffractions.[24]

Dynamic Light Scattering

DLS is used to determine the particle size and size distributions in aqueous or physiological solutions. This process depends on the interaction of light with particles. A light scattered from laser passes through a colloid and measured by Rayleigh scattering. Next, the modulation of the scattered light intensity as a function of time is analyzed, the hydrodynamic size of particles can be defined. DLS is used to get the average diameter of nanoparticles dispersed in liquids. The advantage is probing a lot of particles simultaneously.[25]

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectroscopy is used to find out whether biomolecules are included in the synthesis of nanoparticles. FTIR has also been carried out for the confirmation of functional molecules covalently grafted onto silver, carbon nanotubes, and gold nanoparticles, or interactions happening between substrate and enzyme during the catalytic process. The advantages of FTIR are rapid data collection, strong signal, large signal-to-noise ratio, and less sample heat-up. Therefore, FTIR is a suitable, valuable, non-invasive, cost-effective, and simple method to know the role of biological molecules in the reduction of silver nitrate to silver.[26]

X-ray Photoelectron Spectroscopy (XPS)

XPS is a quantitative analysis used to estimate empirical formulae. XPS is also known as electron spectroscopy for chemical analysis (ESCA). XPS gives qualitative, quantitative/semi- quantitative information concerning the sensor surface. XPS is performed under high vacuum conditions. X-ray irradiation leads to the emission of electrons, and the measurement of the kinetic energy and the number of electrons escaping from the surface gives XPS spectra. Specific groups of macromolecules such as P=S, aromatic rings, C–O, and C=O can be identified and characterized by XPS.[27]

Scanning Electron Microscopy

Among various electron microscopy techniques, SEM is a surface imaging method of resolving different particle sizes, size distributions, nanomaterial shapes, and the surface morphology of the particles at the micro and nanoscales. Using SEM, we derive a histogram either by measuring and counting the particles manually or using specific software. The limitation of SEM is the internal structure cannot be resolved, but the purity and degree of particle aggregation can be valued.[24]

Transmission Electron Microscopy

TEM explains size distribution, and morphology. The ratio of the distance between the objective lens and the specimen and the distance between the objective lens and its image plane gives the resolution and the capability for analytical measurements. The disadvantages are it required large vacuum, thin sample section, here sample preparation is time-consuming. Therefore, sample preparation is essential to obtain the highest-quality images possible.[22]

Atomic Force Microscopy

AFM is used to study the dispersion of nanomaterials, their shape, size, sorption, structure and to describe the interaction of nanomaterials with supported lipid bilayer. AFM does not require oxide-free, electrically conductive surfaces for measurement, does not cause damage to native surfaces, and it can measure up to the sub-nanometer scale in aqueous fluids. However, a drawback is the overestimation of the lateral dimensions of the samples due to the size of the cantilever.[23]

Localized Surface Plasmon Resonance (LSPR)

LSPR is a coherent collective spatial oscillation of the conduction electrons in a metallic nanoparticle. This method is used to investigate the processes of nanoparticles, molecular detection or (bio)-imaging tools with developed single-molecule sensitivity. LSPR analyze faster with higher sensitivity, wavelength tunability, smaller sensing volumes, and lower instrumentation cost.[24]

APPLICATIONS OF SILVER NANOPARTICLES

Antibacterial The AgNPs shows bactericidal action against both Gram-positive and Gram-negative bacteria.. The activity of AgNPs is may be due to either (i) formation of pores in the cell wall or (ii) the silver ion penetrate does not damage the cell membranes; rather denatures the ribosome and the production of ATP and DNA thus resulting in cell death.[25]

Antifungal

Fungal infections are reasons of particular disease and mortality of immune-compromised patients. One of the most common is Candida species that causes nosocomial infection.[26] The literature revealed that AgNPs are effective against *C. glabrata, C. albicans, C. krusei, C. parapsilosis* and *T. mentagrophytes*.[27] Recently studies showed that the (Ocimum sanctum L.) mediated AgNPs exhibited antifungal activity against an opportunistic human fungal pathogen. Hence AgNPsis considered as potent and a fast-acting fungicide against a broad spectrum of standard fungi including *Aspergillus, Candida*, and *Saccharomyces*.[28]

Antiviral

The cytoprotective characteristics of silver are well known and have been employed for the prevention of HIVinteraction to the host cells. Recent studies reported that AgNPs interact with HIV-1 by binding preferentially to gp120 glycoprotein knobs. This sort of interaction of AgNPs specifically inhibits the binding of the virus to host cells.[28]

MEDICAL DEVICES

Wound dressing

AgNPs in topical ointments as well as creams used to prevent wounds, burns, and infections. AgNPs are used in medical devices, implants, consumer products such as colloidal silver gel and silver-embedded fabrics used in sporting equipment. Silver coated biomedical devices, textile fibers are glass windows and other surfaces to maintain sanitization and hygienically conditions. Metallic AgNPs are effective microbicidal, so they have received significant consideration in multiple products ranging from paints to textiles.[29]

Catheters

The plastic catheters are coated with bioactive AgNPs. The researchers have developed a coating method for a thin layer of nanoparticles of silver on the surface of the catheters. The nanoparticles coated catheters are biocompatible and have a sustained release of silver at the implantation site. The infection risk is highly reduced in these catheters due to significant in vitro antimicrobial activity by the inhibition of biofilm formation using *Escherichia coli, Enterococcus,* Staphylococcus aureus, coagulase-negative *staphylococci, Pseudomonas aeruginosa* and *Candida albicans.*[30]

Bone cement

AgNPs with additive poly (methyl methacrylate) (PMMA) has been used as a bone cement. Such bone cement is antibacterial due to the presence of AgNPs. Bone cement is the material for annexing prostheses like hip and knee replacement surgery that has high infection rates, i.e., approximately 1.0–4.0%. So another type of bone cement which greatly reduce the rate of infection to 0.4% and 1.8% is required. It has been demonstrated that nanosilver -PMMA bone cement decreases the incidence of resistance by the versatile mode of action, and has also known for its antibacterial activity and low cytotoxicity. These bone cements are known to have effective antibacterial agents against methicillin-resistant *S.epidermidis* and *S.aureus* and showed retarded biofilm growth. The biocompatibility of these bone cement was demonstrated against mouse fibroblasts or human osteoblasts having very low cytotoxicity, suggesting good biocompatibility.[31]

Tumor

Reactive oxygen species (ROS) can induce loss of cellular components such as proteins lipids and DNA and eventually lead to the death of the cell. It has been found that AgNPs can induce cell death and oxidative stress in skin carcinoma cells and human fibrosarcoma. AgNPs is also known to induce a p53-mediated apoptotic pathway through which majority of the chemotherapeutic drugs triggers apoptosis. Antiproliferative characteristic of piperidine from Piper nigram against HEp2 cancer cell line has also been studied. A small dose of AgNPs from Piper longum extracts can effectively show the cytotoxic effect on HEp-2 cell line, thus indicating that AgNPs can also be used for anti-cancerous drug preparations.[32]

Water purification

The AgNP's can be employed for purification of water filtering apparatus which may be due to its enhanced antimicrobial nature. Preventing the growth of harmful microorganisms by modifying or coating the surfaces with antimicrobial agents is applied in biomedical devices and health, food and hygiene industries. Antimicrobial coatings should possess antibacterial efficacy, ease of fabrication along with low toxicity. But the continuous exposure of these agents might leads to the increased occurrence of resistance to treatment.[33]

Antimicrobial nano paints

The production of nanofibre with AgNPs are highly stable and can withstand high temperature, i.e., up to 200°C by resisting oxidation and aggregation, which improves their application in the production of silver nanoparticle embedded homogeneous paints.[28]

Biosensors

Based on the plasmonic characteristics of AgNPs like its size, shape and dielectric medium, they are applied in biosensing. When these biomolecules adhere on the surface of AgNPs, it increases the refractive index and shifting the absorption spectrum. These are advantageous over commercial thin, plasmonic, continuous films. Haes and Van produced triangular AgNPs and coated on a glass substrate which was used to detect interactions between biomolecules, such as biotin-streptavidin and two biomolecules related to Alzheimer's disease. Protein interactions are sensed by the cubical or rhombohedral silver nanoparticles. AgNPs based biosensors are applied in cancer detection. The silica-coated nanosilver is used as biosensors for the detection of bovine serum albumin (BSA).[34]

Bioimaging

The AgNPs due to its plasmonic characteristics is photostable thus they can be used as biological probes to monitor events continuously. Lee et al. demonstrated AgNPs are incubated with cells to determine physical interactions and uptake, or to functionalization of the biomolecule on the surface of AgNPs thus increasing specificity for the cell membrane. AgNPs are examined in neuroblastoma cells under dark-field illumination. AgNPs attached on iron oxide nanoparticles were detected easily by incubating with macrophages by two-photon imaging after their cell uptake.[35]

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