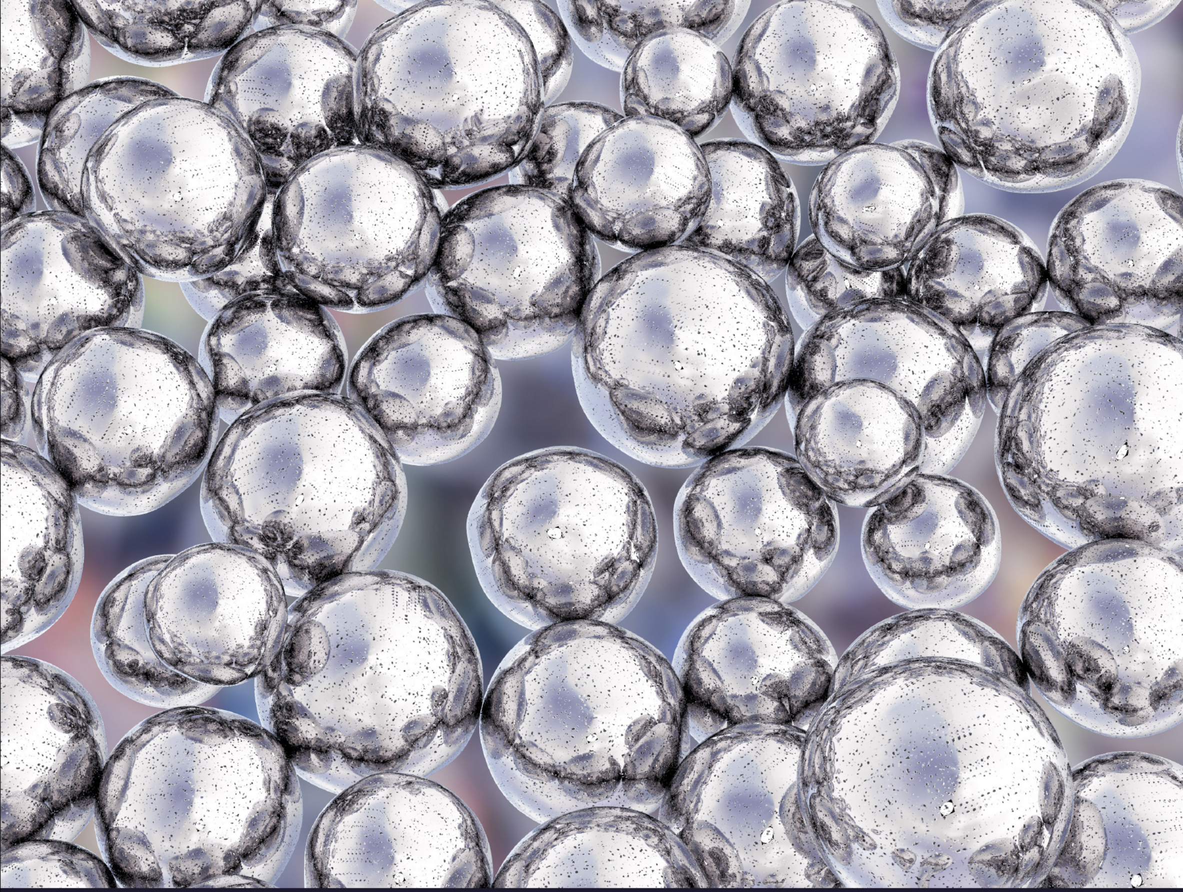

CHEMISTRY RESEARCH AND APPLICATIONS

Applications of SILVER NANOPARTICLES

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Contents

Preface

Nanotechnology has become very present in the field of science and scientific research. As a result, this book focuses on the green synthesis approach and latest applications of silver nanoparticles. The book also covers their potential uses in the environment, biosensing, food biotechnology, in water treatment as well as in daily life.

Chapter 1 - Nanoparticles, exhibit significantly changed physical, chemical, and biological properties due to their high surface-to-volume ratio, which makes them valuable for a wide range of applications. Among the various metal nanoparticles, silver nanoparticles (AgNPs) have attained a pinnacle position due to their enhanced electrical, optical, and thermal properties. AgNPs have proven application in various fields like chemistry, medicine, agriculture, space, electronics, and environment. These NPs have been synthesized by various physical, chemical, and biological routes. The chemical methods, although very common, versatile, and inexpensive to produce AgNPs in definite sizes and shapes but are toxic and non-eco-friendly. The physical methods, although superior in terms of greenness, require very high temperatures, consume enormous amounts of energy, and are expensive, which makes them unsuitable for synthesis. On the other hand, the biosynthesis of AgNPs using plant extracts, microbes, algae, fungi, etc. has opened an eco-friendly, cost-effective, and safer way to synthesize AgNPs. Biosynthesis methods have the least impact on human health and environment and can produce uniform-sized AgNPs of definite shape without any contamination. In this chapter, the authors have compiled and discussed the applications of biosynthesized AgNPs for various environmental purposes such as the removal of organic pollutants from water, removal of toxic metal ions, for food packaging bioplastic, an alternative to chemical pesticides and fertilizers, biosensors, and solar cells, etc. The chapter also provides a comprehensive description of the latest research on the biosynthesis of AgNPs, and includes mechanistic insight into biosynthesis methods, properties of biosynthesized AgNPs and scopes for further improvements.

Chapter 2 - In recent decades, nanotechnology has emerged as a rapidly growing scientific field because of its excellent properties and tremendous applications in every possible field of material science and biology. Typically, nanotechnology deals with the nanoparticles (NPs) having dimension of 1-100 nm. The large surface to volume ratio of NPs from its bulk counterpart leads to some unique properties in the nano dimension and therefore have a wide range of applications in some challenging areas such as sensing, imaging, pharmaceuticals, catalysis, soler cell, environmental remedies, water treatments, etc. Furthermore, tuning of size, shape, composition, structure, etc. of NPs, helps in enhancing their properties and possibilities of applications. Over the years, different approaches are applied in the nanomaterial synthesis, however, the green synthesis approach has drawn special attention because of the use of less toxic and hazardous materials and for allowing sustainable development. Among different types of nanoparticles, the authors' focus is on silver due to its less toxicity towards human health, antibacterial activity, greater stability, catalytic activity, good conductivity, etc. In this chapter, the synthesis of silver nanoparticles using green synthesis approach will be discussed along with the possible applications of silver nanoparticles in sensing, imaging, and biomedical field of research.

Chapter 3 - The applications of silver nanoparticles (AgNPs) derived from their physical and chemical properties are widely studied. Biosensing is one of the areas that has attracted the most attention, where the emphasis of studies is placed on detecting molecules of biological interest, such as glucose, peroxides, drugs, and enzymes, among others. The importance of AgNPs in biosensing relies on the fact that the molecule of interest (target molecule) can be present in low concentration within a complex matrix of various analytes, where analytical techniques may not detect it. The surface plasmon resonance (SPR) of AgNPs plays an essential role in detecting analytes since when the target molecule interacts with the surface of the AgNPs, the detection signal can be increased. The SPR is a physical phenomenon that has been extensively studied since the optical properties of such nanoparticles derive from it, and it depends on the size and morphology of the AgNPs. This review presents the use of AgNPs as biosensors of molecules such as glucose, peroxides, and drugs, among others. Likewise, the synthesis processes for the obtention of AgNPs of various morphologies (i.e., prisms, nanoflowers, nanowires); and a discussion on how the different shapes modify the electronic properties of AgNPs and, therefore, their ability for sensing is described. On the other hand, this review includes sensing technologies like fluorescence spectroscopy, Surface-Enhance Raman Scattering (SERS), and electrochemical techniques, where AgNPs have been implemented to improve or generate a signal. Additionally, the use of composite nanomaterials (AgNPs mixed with other materials) for biosensing is discussed.

Chapter 4 - Nanotechnology has drawn scientists from a wide range of disciplines in recent decades. When a substance is shrunk down to the nanoscale range, its properties undergo a significant change that forms the basis of nanotechnology. Due to their excellent physical, biological, and chemical properties—aside from their extremely small size—metal nanoparticles are the subject of active research. Due to their wide range of benefits above its bulk material, they have become the economical material of choice in many applications to date. An outstanding plasmonic metal with built-in anti-bacterial and anti-fungal properties is silver. Since more than a century ago, nanoscale silver particles have been used in a variety of commercial products, including pigments, photo catalysts, UV absorption, photo graphics, conductive or antistatic composites, and biocides for the treatment of medical conditions. However, the term "nano" has only recently been coined. This extensive, multifaceted research history supports silver's distinctiveness and potential over numerous other noble metals used in nanotechnology today.

The preparation of nano silver material can be done in a number of physico-chemical and biological ways. The change in the form, size, and size distribution of the silver nanoparticles, which was primarily influenced by the synthesis technique, has resulted in noticeable differentiation in their functional properties such as electromagnetic, catalytic, anti-bacterial, optical, etc. Currently, a green-synthesis biogenic concept is developing as the best protocol for converting silver ions into silver nanoparticles at room temperature in comparison to conventional chemical or physical procedures. This protocol is simple, quick, one step, ecological, and nontoxic. Today, silver nano is a trademark for antibacterial assurance and is included into products like refrigerators, air conditioners, air purifiers, water filters, environmental hygiene devices, and diagnostic equipment. In addition to this, the increased functionality brought about by these nanoscale structures has enabled a major expansion in their range of applications. Conductive (paste, ink), optical (Raman scattering, fluorescence), household (waste water treatment, pesticides), and antimicrobial textiles are among the important industries on this list (garments, towels, socks, home furnishing).

Chapter 5 - In recent times, the biological synthesis of silver nanoparticles (AgNPs) has become a viable alternative to conventional physical or chemical methods, since it is easy, fast and environmentally friendly. In addition, this synthesis carried out from plants or microorganisms, have been found to give nanoparticles colloidal stability, as well as to control their size and shape. Microbial-based synthesis may be advantageous due to the large number of metabolites produced, thus increasing productivity. These organisms are widely used as reducing and stabilizing agents, due to their tolerance to heavy metals and their ability to internalize and bioaccumulate metals. It also has advantages from the industrial point of view, such as the production of large amounts of proteins and extracellular enzymes, ease in handling biomass, good dispersion of AgNPs and easy handling in large-scale production. Microorganisms are the source of the stabilizing agent (capping) that constitutes the biogenic AgNPs. This capping agent provides stability and can also present biological activity, acting in synergy with the effect of the core of the AgNPs. For example, it has recently been shown that in addition to providing greater antimicrobial activity, this stabilizing agent would be responsible for less cytotoxicity and genotoxicity, a fundamental factor to consider for its use as a therapeutic agent in human and animal health.

Several fungal AgNPs have shown broad antimicrobial activity against Gram-negative and Gram-positive bacteria and plant pathogenic fungi. Furthermore, the effectiveness of these microbial nanoparticles in the eradication of microbial biofilms was reported. Moreover, the antimicrobial potential of biogenic silver nanoparticles is very promising in the agri-food area, in food preservation or in the control of phytopathogens with a high impact in agriculture. The antimicrobial action mechanisms of biogenic nanoparticles are not completely elucidated and depend on their characteristics (shape, size, composition, surface charge). That is why there have been advances in recent years in studies of interactions of biogenic nanoparticles with microbial cells as well as their possible antimicrobial mechanisms of action. All these advances in the knowledge of the characteristics and biotechnological properties of microbial silver nanoparticles will encourage new challenges in the development of nanomaterials, in their production on a larger scale, as well as in their expanded use in the area of human and animal health, agriculture, food and materials.

Chapter 6 - Silver is a soft, white and lustrous transition metal used since 4000 BC, due to its recognized antimicrobial properties. Considering the benefits of silver, together with the advance of nanotechnology, silver has been thoroughly formulated in nanosystems. During the past decades, silver nanoparticles (AgNP), have been considered the most commercialized nanosystems worldwide, being applied in diverse sectors, from medicine to the food industry. AgNP assume a special relevance in the food industry, due to the perception of its safe nutritional attributes and improvement of food quality. As a result, the risks associated with their potential migration into foods are a major concern. This chapter comprehensively reviews the use of silver nanoparticles in daily consumer products, with a special emphasis on their incorporation during food processing and packaging.

Chapter 7 - Silver nanoparticles have recently become the most frequently encountered nanomaterials in many areas of biotechnology. They are known as eco-friendly nanomaterials and have some important impacts on plant growth. Their effects on seed germinations and biomass accumulations have rarely been investigated and remain unclear yet. In this study, clove-derived biosynthetic silver nanoparticles, at 1, 4, 7 and 10 mg/L concentrations were applied to determine the effects on seed germination and the growth of seedlings of *Linum usitatissimum* in in vitro conditions. The seed germination percentages were irresponsive to the rising concentration of biosynthetic silver nanoparticles. The addition of nanoparticles into the culture media increased the biomass accumulation in *L. usitatissimum* seedlings. Root and shoot fresh weights were enhanced 3.8 times and 1.6 times higher in 10 mg/L silver nanoparticle-added medium, respectively. Root and shoot dry weights were also enhanced in coordination with the ascending silver nanoparticle concentrations. The highest shot fresh weight was approximately 1.2 times higher than the control group. The results exhibit the positive effects of biosynthetic silver nanoparticles on *L. usitatissimum* growth and suggest that eco-friendly biosynthetic silver nanoparticles can be used as plant biomass enhancers.

Chapter 8 - Colloidal silver is known as one of the most widely used nanomaterials for the control of microorganisms. The authors' minimum inhibitory concentration (MIC) assays conducted for *E. coli*, *S. aureus*, *B. subtilis* and *P. phoeniceum* have shown that the antimicrobial activity of silver ions was superior to that of silver nanoparticles. As silver nanoparticles can be more suitable in some bactericidal applications than silver ions, the efficacy of nanosilver as an antimicrobial agent against a range of microbes on the surface of water paints and cotton fabrics has been studied. The efficacy of nanosilver as an antimicrobial agent has been also estimated against a range of microbes on the surface of fiber ion exchange sorbents used for water treatment applications.

Chapter 9 - The main objective of present work was to synthesize Canagliflozin nanosuspension and to develop and validate simple, sensitive, precise, rapid and cost effective method for Canagliflozin nanosuspension with the help of simple double beam UV Spectrophotometer. Canagliflozin nanosuspension was synthesized by solvent evaporation method using poly vinyl alcohol surfactant and methanol as a solvent. Canagliflozin showed maximum absorption at 290 nm by using methanol as diluents. The particle size of nanosuspension was analyzed by Particle size analyzer. The Canagliflozin nanosuspension exhibits excellent uniformity. Maximum absorbance for Canagliflozin in methanol occurs at 290 nm using UV Spectrophotometer. Particle size analysis showed the Z-Average of the nanosuspension at 920.2 and the Polydispersity index at 0.364, indicating extremely high homogeneity in the synthesized Canagliflozin. Percentage purity of Canagliflozin nanoparticles were 84.90%. The correlation coefficient of synthesized Canagliflozin nanosuspension was found to be 0.993 from calibration curve that shows a positive, linear relationship between concentration and absorbance. The LOD and LOQ for Canagliflozin nanoparticles were found to be 10.17 µg/ml and 10.00 µg/ml respectively. The method for Canagliflozin nanoparticles was quantitatively determined with respect to linearity, precision, accuracy, LOQ and LOD. The developed method proved simple, accurate and economical, and it may be used for standard laboratory analysis.

Chapter 10 - Nanotechnology is currently an emerging field of science to possess unique properties. Due to its different potential growth, nanotechnology is gaining more importance and technological significance in various industries. Microbial contamination is one of the major problems faced mainly by food industries. Synthesis of silver nanoparticles (AgNPs) is widely used against microbial agents in food industries as AgNPs has antimicrobial, antifungal, anti-viral and antiyeast properties. Recent research on synthesis of silver nanoparticles from microbes and plants is carried out due to the non-usage of hazardous materials and ecofriendly nature to the environment. Application of silver nanoparticles in the field of food packaging technologies involves wide range of activities against food borne pathogens and biofilm formation, to develop much safer food products, to increase the shelf-life and quality of the products. United States Food and Drug Administration and the European Food Safety Authority have regulated the application of silver nanoparticles in the food packaging technologies. However, the application of silver nanoparticles in food packaging has to be studied extensively and should be implemented widely in the area of effective levels. Moreover, continuous research in the current aspect is more challenging and inspiring as silver nanoparticles draw special attention towards the potential biocidal activity against the broad range of microorganisms.

Chapter 1

Environmental Applications of Biosynthesized Silver Nanoparticles

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Abstract

Nanoparticles, exhibit significantly changed physical, chemical, and biological properties due to their high surface-to-volume ratio, which makes them valuable for a wide range of applications. Among the various metal nanoparticles, silver nanoparticles (AgNPs) have attained a pinnacle position due to their enhanced electrical, optical, and thermal properties. AgNPs have proven application in various fields like chemistry, medicine, agriculture, space, electronics, and environment. These NPs have been synthesized by various physical, chemical, and biological routes. The chemical methods, although very common, versatile, and inexpensive to produce AgNPs in definite sizes and shapes but are toxic and non-eco-friendly. The physical methods, although

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superior in terms of greenness, but require very high temperatures, consume enormous amounts of energy, and are expensive, which makes them unsuitable for synthesis. On the other hand, the biosynthesis of AgNPs using plant extracts, microbes, algae, fungi, etc. has opened an eco-friendly, cost-effective, and safer way to synthesize AgNPs. Biosynthesis methods have the least impact on human health and environment and can produce uniform-sized AgNPs of definite shape without any contamination. In this chapter, we have compiled and discussed the applications of biosynthesized AgNPs for various environmental purposes such as the removal of organic pollutants from water, removal of toxic metal ions, for food packaging bioplastic, an alternative to chemical pesticides and fertilizers, biosensors, and solar cells, etc. The chapter also provides a comprehensive description of the latest research on the biosynthesis of AgNPs, and includes mechanistic insight into biosynthesis methods, properties of biosynthesized AgNPs and scopes for further improvements.

Keywords: silver nanoparticles, biosynthesis, environment

Introduction

The applications of metal nanoparticles (NPs) have emerged in various fields of day-to-day life due to their exceptional biological and physicochemical properties. In the present era of nanotechnology silver nanoparticles (AgNPs) have a significant contribution (Sharifi-Rad et al., 2021). AgNPs have shown their potential and importance in various fields, majorly the food packaging, instrumentation, medicine, textile, engineering, energy, cosmetics, surgery, electronics, agriculture, and environment (Kasi et al., 2014; Akter et al., 2018). The physicochemical properties of AgNPs like the large surface area to volume ratio, capability to bind with different extents with a ligand, easy customizations, and catalytic behaviors are among some properties that make them highly applicable in diverse fields (Jain et al., 2008; Akter et al., 2018).

There are several approaches to synthesize AgNPs - physical, chemical, photochemical, and biological. Each technique has its advantages and disadvantages. The physical methods use radiations which do not involve the use of any chemical but on the other hand, these are expensive, and the yield is less along with high energy requirements (Sharma et al., 2022). The most frequently used technique is the chemical approach which uses silver metal precursor ($AgNO_3$), reducing agents like ethylene glycol, and lastly stabilizing or capping agents like polyvinyl pyrrolidone. The use of costly, toxic, and non-eco-friendly chemicals in the process is a serious concern for living beings and the environment (Tran et al., 2018). Photochemical methods involve the use of photochemically generated intermediates. It is a clean and convenient process but the high cost and requirement of environment-controlled experimental conditions reduce its frequency of use. Therefore, the greener routes for the synthesis of AgNPs have gained wider attention (Philip et al., 2009).

For the biological synthesis of AgNPs, the reducing and stabilizing agents have been reported from bacteria, fungi, algae, amino acids, sugars, vitamins, plants, and animals (Huang et al., 2006; Khan et al., 2010; Jacob et al., 2011; Rafey et al., 2011; Nidya et al., 2015; deMatos and Courrol, 2017). The reduction of $AgNO_3$ using biologically derived chemicals has advantages like non-toxicity, an abundance of raw material, renewability, avoidance of hazardous waste generation, cost-effective production in an ambient environment, and eco-friendly way of synthesizing AgNPs for various applications (Mocanu et al., 2009). Out of the biological sources, used to derive reducing agents, the plant parts like leaves, roots, flowers, bark, and fruits do not require special isolation techniques, cultures, and culture conditions that are needed for microbial and animal-based sources. In addition, synthesis of plant based AgNPs is highly economic and easier for bulk synthesis of AgNPs (Iravani, 2011; Sharifi-Rad et al., 2021).

AgNPs have a wide range of applications in various fields. The present chapter focuses on the applications of AgNPs in solving environmental issues such as the removal of organic pollutants from water, removal of toxic metal ions, use in bioplastic for food packaging, an alternative to chemical pesticides and fertilizers, biosensors, solar cells, etc. AgNPs-based water filters are used to disinfect the usable water. The catalytic activity of AgNPs has also been reported for the degradation of dye contamination in water. Removal of heavy metals (like Hg^{2+}, Cr^{3+}, Co^{2+}, Pb^{2+}, and Ni^{2+}) from the water has also been accomplished using AgNPs and their conjugates (Pradeep, 2009). Biosynthesized AgNPs have also been used in producing food-grade bioplastic for providing antimicrobial and mechanical strength to it (Youssef et al., 2014; Ediyilyam et al., 2021). For biosensing devices, AgNPs have also proved their environmental importance in detecting toxic substances in the environment (Remya et al., 2022). The properties of nanopesticides like target specificity, slow and effective release, improved adhesion, and dispersion on plant surface make them a better choice compared to conventional pesticides. Now a days, the use of biosynthesized AgNPs as nanopesticides is much more eco-friendly and user-friendly (Deka et al., 2021).

Solar energy generated by solar cells is the most potential, green, and eco-friendly alternative to nonrenewable sources of energy like coal and petroleum. Silicon solar cells are commercial solar cells but research in different other types of solar cells such as heterojunction polymer solar cells, dye-sensitized solar cells, and perovskite solar cells is still in progress. In these solar cells AgNPs are used to generate plasmonic effects, to improve the optical and photovoltaic properties, and efficiency of solar cells (Prasad et al. 2013). Various researchers have used biosynthesized AgNPs for use in solar cells, observing the importance of their eco-friendly method of generation. In this chapter, we will focus on the synthesis and properties of biosynthesized AgNPs and discuss their applications for the environment followed by future prospects of the research in this area.

Methods of Biosynthesis of AgNPs

The biosynthesis of metal NPs has the advantage of being eco-friendly, non-toxic, and low-cost compared to the synthesis by chemical and physical methods. In general, the biological extracts are mixed with a metal precursor solution and the color change indicates the formation of nanoparticles. The biological extract source can be plants, animals, fungi, bacteria, algae, or biomolecules like vitamins, polysaccharides, and amino acids (Mittal et al., 2013). Here the biological sources are the sources of polyphenols, biomolecules, or metabolites that act as reducing (reduces the metal ion from positive to zero oxidation state), capping, and stabilizing agents for the synthesis of metal nanoparticles. In common, the parameters that decide the shape, size and amount of metal nanoparticles using biological sources are the concentration of biological extract, reaction time, temperature, pH, salt, etc. (Roy et al., 2019). Here we will summarize the biosynthesis methods of AgNPs from the mentioned sources. The studies related to the use of biomolecules directly (as brought from the market) are mainly concentrated to produce functionalized AgNPs and using them as biosensors rather than their direct involvement in the synthesis of AgNPs.

Synthesis of AgNPs Using the Following

Plants

Synthesis of AgNPs using plant parts like leaves, roots, stem, flowers, and bark help in easy scale-up, eco-friendly, non-pathogenic, and inexpensive production as compared to physical and chemical methods (Huang et al., 2007). In 2003, the production of AgNPs using alfa-alfa was the first study reporting the use of plant material for the synthesis of AgNPs (Gardea-Torresdey et al. 2003). The common protocol for the synthesis of AgNPs from plants includes the collection of required plant parts and washing with double distilled water 2-3 times. Washed plant parts are dried and boiled in double distilled water for 10-15 min followed by filtering the obtained solution. In the collected filtrate $AgNO_3$ solution is added and preserved at room temperature. Change in the color from pale yellow to dark brownish green indicates the formation of AgNPs which is further confirmed by UV-Vis spectrophotometer (Rajeshkumar, 2016). The plants used for the synthesis of AgNPs are mostly medicinal plants due to their richness in phytochemicals. These plants majorly include *Azadirachta indica*, *Ocimum species*, *Viburnum nervosum*, *Cinnamon*, *Cocos nucifera*, *Catharanthus roseus*, *Eucluptus*, *Tinospora*, *Camellia*, etc. (Mukunthan et al., 2011; Ramteke et al., 2013; Mariselvam et al., 2014; Gauthami, 2015; Mohammed, 2015; Ahmed et al., 2016; Selvam et al., 2017; Zahoor et al. 2021). In the majority of AgNPs synthesis using plant extracts, the phytochemicals act as reducing, capping, and stabilizing agents. Proteins present in the plant extracts are reported to trap Ag^+ ions on their surface due to electrostatic interactions leading to a change in the secondary structure of proteins and the formation of Ag nuclei. On the other hand, some studies have also reported the role of phytochemicals like amides, aldehydes, ketones, terpenes, flavonoids, alkaloids, and flavones as reducing agents of Ag^+ ions. In xerophytes, anthraquinone and emodins are reported to undergo tautomerization to form AgNPs (Li et al., 2007).

Microbes

Microorganisms are proven source for the biosynthesis of AgNPs. The two modes of microbial biosynthesis are intra and extracellular (Das et al., 2014). In intracellular mode, the silver ions accumulate inside the cell and nucleate the formation of AgNPs which increases with the growth of the microbe. Nanoparticles are harvested from the live bacterial cells with special treatments at optimum temperature. In an extracellular mode of synthesis, the secretory products of bacteria like enzymes, vitamins, amino acids, and sugars are used to synthesize AgNPs (Roy et al., 2019). In most accepted mechanisms it is shown that nitrate reductase (NADH- dependent enzyme which is a part of the electron transport chain) converts nitrate (NO_3^-) into nitrite (NO_2^-). The electrons released in the process are used to reduce Ag^+ to Ag^0 (Kumar et al., 2007). Besides this bacterial cell wall is also reported to reduce the Ag^+ to make AgNPs. Majorly used bacterial species are *Streptomyces* spp., *Pseudomonas* spp., and *Pantoea* spp., (Slawson et al., 1992; Fayaz et al., 2010).

Fungi

In comparison to bacteria, the production of AgNPs from fungi have the advantage of over-production due to the large availability of enzymes, proteins, and reducing agents on its cell surface required to synthesize AgNPs (Xu et al., 2020). Fungi have unique properties like metal tolerance, bio-concentration, fast growth, and extracellular secretion of enzymes, which make them fit for the production of AgNPs. Depending upon the location, the fungi-mediated synthesis of AgNPs can be of two types' intracellular and extracellular (Tiwari et al., 2015; Majeed et al., 2015). Intracellularly, AgNPs can be obtained from mycelia while extracellularly AgNPs can be obtained from cell-free filtrate. In comparison to intracellular, extracellular synthesis is preferred due to the easy collection and downstream processing. The most used fungi for the synthesis of AgNPs are *Fusarium* spp., *Phomopsis* spp., *Penicillium* spp., *Trichoderma* spp., and *Aspergillus* spp. (Salaheldin et al., 2016; Ottoni et al., 2017; Neethu et al., 2018; Seetharaman et al., 2018). For the extracellular synthesis of AgNPs, the fungal extract is purified by washing off or precipitating the fungal components. Fungi trap the Ag^+ on their cell surface and reduce them to Ag^0 using the enzymes (like xylanase and nitrate reductase), naphthoquinone, and anthraquinone (Devi and Joshi, 2015; Elegbede et al., 2018). In addition to these enzymes, fungal proteins are also reported to act as a capping agent in the synthesis of AgNPs. Various factors like temperature, nitrate source and light source provided to fungus act to control the synthesis, shape, and size of AgNPs (Hamedi et al., 2017).

Animals

There are some reports which have shown the synthesis of AgNPs using animal parts like cockroach wings, honey from honeybees, cow milk, cobweb, goat fur, paper wasp net, etc. (Lee et al., 2013; Lateef et al., 2016a; Lateef et al., 2016b; Balasooriya et al., 2017; Khatami et al., 2019). The biomolecules, especially proteins from these animal parts have been reported to act as reducing and capping agent in the synthesis of AgNPs. In general, the animal parts used are first hydrolyzed with alkali, centrifuged and the extract thus obtained is used for the synthesis of AgNPs (Morones et al., 2005).

Algae

Algae are one of the important sources for AgNPs biosynthesis due to fast growth, metal accumulation ability, and presence of various biologically active molecules. The various active biomolecules like pigments, enzymes polysaccharides, proteins, secondary metabolites, sulfated agents, amino acids, oils, fats, antioxidants, phycobilins, etc. are present in algae. These act as reducing and capping agents in the synthesis of eco-friendly, size and shape-controlled AgNPs (Asmathunisha and Kathiresan, 2013; Michalak and Chojnacka, 2015). The most commonly used algae for the synthesis of AgNPs are the members of *Cynophyceae*, *Rhodophyceae*, *Chlorophyceae*, and *Phaeophyceae*. Specific factors like temperature, pH, incubation period, and the ratio of algal extract and $AgNO_3$ solution determine the shape, size, and amount of AgNPs during synthesis. The general process of biosynthesis of AgNPs using a precursor solution is to mix the algal extract with the $AgNO_3$, the specific change in color indicates the formation of AgNPs (Khanna et al., 2019). There are two modes for the synthesis of AgNPs from algae viz., intracellular, and extracellular. The intracellular mode needs no pre-treatment of the algae as it relies on the internal processes going on inside the algae like respiration, photosynthesis, and nitrogen fixation. In this case, the reducing agents which reduce Ag^+ to Ag^0 are NADPH or NADPH-dependent reductase enzymes generated during photosynthesis or respiration (Sicard et al., 2010; Sharma et al., 2016). On the other hand, the extracellular mode refers to the use of algal cell exudates like lipids, proteins, enzymes, metabolites, and other biomolecules for the synthesis of AgNPs. In this, the algal biomass undergoes the pre-treatments like washing and mixing (Vijayan et al., 2014; Dahoumane et al., 2016).

Biomolecules

The direct use of biomolecules like amino acids, polysaccharides, and vitamins has been reported for the biosynthesis of AgNPs. Amino acids like cysteine have been used to produce nanocrystalline silver sol (Roy et al., 2012). Amino acids attached to phenolic compounds were used to synthesize AgNPs with spherical and prism morphologies by Kumar et al. (2013b). Tyrosine and tryptophan have been used by Shankar and Rim (2015) to synthesize AgNPs. These two amino acids worked both as capping and reducing agents. AgNPs are not only synthesized rather functionalized using amino acids for bio-sensing activities. A simple, fast, and cost-effective method of biosynthesis of AgNPs, using twenty one types of amino acids with white light illumination without any other additive was investigated by de Matos and Courrol (2016). Chandra and Singh (2018) used alanine, tryptophan, histidine, glutamic acid, aspartic acid, and methionine to functionalize AgNPs for catalytic and oxygen-sensing activities. Similarly, Khalkho et al. functionalized the AgNPs by using L-cysteine for probing the Vitamin B_1 (Khalkho et al., 2020), while the AgNPs functionalized with Vitamin B_{12} were used for detection of iron (Fe^{+3}) in food and baby products (Harke et al., 2022). The biological activity of AgNPs was modulated by functionalizing with sugars like D-glucose, D-mannose, and D-galactose. These surface modifications reduce the toxicity of synthesized AgNPs (Kennedy et al., 2014). Moreover, AgNPs functionalized with carbohydrate derivative, glutathione-lactose (GSH-Lac) were used to detect thiram pesticide in agricultural samples (Dhavle et al., 2021).

Characterization of AgNPs

Among the various metal nanoparticles, AgNPs due to their unique properties have gained significant interest and found application in various fields of science and technology. The AgNPs can be synthesized by physical, chemical, or biological methods. The different synthesis methods can lead to different properties of the nanoparticles. Therefore, the characteristics of synthesized nanoparticles must be carefully investigated to explore their full potential for application in different fields. A very basic, and usual way is visual inspection i.e., to check the color change of the solution from yellow to brown, indicating the formation of AgNPs. It can be further confirmed with various characterization techniques, which not only reveal the formation of AgNPs but also provide the qualitative and quantitative information of different characteristics in a precise manner. It helps to suggest the best-fit application of the synthesized nanoparticles. The structural, electrical, optical, and compositional properties of the synthesized AgNPs can be studied with different characterization techniques, such as X-ray diffraction, scanning electron microscopy, transmission electron microscopy, EDAX, FTIR, and UV-Vis spectroscopy. We will discuss these characterization techniques in brief, before proceeding for the applications of the AgNPs.

X-ray Diffraction (XRD)

X-ray diffraction is a powerful and versatile tool to study the crystal structure of nanoparticles. The technique works on Bragg's law and can provide qualitative as well as quantitative information of the material. The incoming X-rays after diffraction from atomic planes interfere with each other resulting in a diffraction pattern. The obtained diffraction pattern is fingerprint for a material, which can be compared with the standard diffraction patterns by the Joint Committee on Powder Diffraction Standards (JCPDS) to confirm the identity of the synthesized material. Figure 1a shows the XRD diffraction pattern for the biosynthesized AgNPs obtained from the leaf extract of *Ipomoea aquatica* (Khan et al., 2020).

In the diffraction patterns (Figure 1a) peaks corresponding to 2θ values of 38.01°, 44.18°, 64.26°, 77.16° and 81.29° can be assigned to (111), (200), (220), (311), and (222) crystallographic planes and confirm the formation of face-centered cubic AgNPs (Khan et al., 2020). The additional peaks appearing in the diffraction pattern were supposed to be related to the crystallization of bioorganic phase on the surface of AgNPs, originating from leaf extract. The average crystallite size of the nanoparticles can also be estimated from the broadening of diffraction peaks using Debye-Scherrer's equation.

$$D = (K\lambda)/(\beta\cos\theta) \qquad (1)$$

Here, K (0.94) is a dimensionless constant (Jain et al., 2017), β is the full width at half maximum (FWHM) of the peak and λ is the wavelength of X-ray radiation. The lattice parameter '*a*' of AgNPs can also be calculated with the following equation.

$$d = \frac{a}{\sqrt{h^2 + k^2 + l^2}} \qquad (2)$$

Here, (hkl) represents miller indices of the plane, and 'd' represents interplanar spacing. Moreover, information about the strain present in the material can also be obtained with this technique.

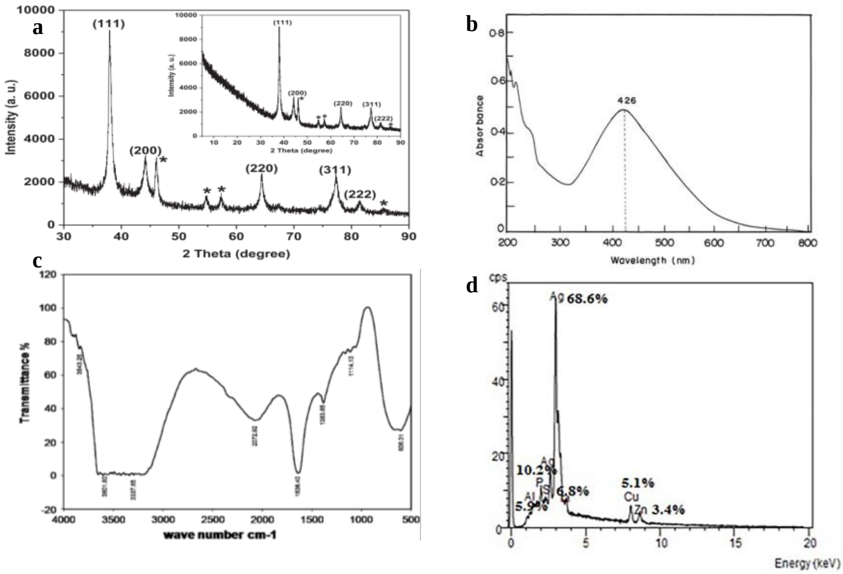

Figure 1. (a) X-ray diffraction spectrum of AgNPs biosynthesized using leaf extract of *Ipomoea aquatica,* (b) UV-Visible spectrum of AgNPs biosynthesized using leaf bud extract of *Rhizophora mucronata,* (c) FTIR spectrum of AgNPs biosynthesized using leaf extract of *Coleus aromaticus,* and (d) EDAX spectrum of AgNPs biosynthesized using *Aspergillus terreus.*

UV-Visible Spectroscopy

UV-Visible spectroscopy is a primary, fast, and reliable technique to confirm the synthesis of AgNPs. The technique also provides the information about the stability of synthesized nanoparticles. In this technique a beam of light is passed through the sample and the intensity of transmitted light is compared with the reference. Usually, a wavelength range of 400 to 800 nm is used for the characterization of AgNPs. Figure 1b shows the UV-visible spectrum obtained for the AgNPs biosynthesized using leaf bud extract of *Rhizophora mucronata* (Umashankari et al., 2012). The optical properties of the nanoparticles are sensitive to their size, shape, and distribution which makes optical characterization an important tool to probe the properties of nanoparticles and suggest their applications. In a certain range of wavelengths AgNPs induce surface plasmon resonance (SPR). From the intensity and position of SPR peaks the quality of synthesized AgNPs can be analyzed (Prathna et al., 2011). A broad absorption peak appearing at a longer wavelength suggests the formation of large-size or aggregated particles, whereas a narrow peak appearing at a low wavelength resembles the small size of the nanoparticles (Smitha et al., 2008). Additionally, the stability of the green synthesized AgNPs can also be estimated with the SPR. The SPR peak appearing at the same wavelength indicates the stability of green synthesized AgNPs maintained for several months (Zhang et al., 2016).

Fourier Transform Infrared (FTIR) Spectroscopy

The role of biological molecules in the green synthesis and stabilization of synthesized AgNPs can be studied with FTIR spectroscopy. It is very valuable and economic technique. The identification of biomolecules involved in the reduction of Ag^+ ions as well as functional groups responsible for the synthesis of AgNPs can be studied with this technique (Anandalakshmi et al., 2016). In the qualitative analysis, the presence of different types of chemical bonds is identified using infrared radiation. The FTIR spectrum exhibits various peaks depending on different kinds of chemical bonds and the interaction of various functional groups (alkanes, ketones, and amines) with infrared radiation (Palithya et al., 2022). A comparative study of the FTIR spectrum of medicinal plant extract and biosynthesized AgNPs can help in the identification of functional groups involved in the surface coating and stabilization of the synthesized nanoparticles (Akintelu et al., 2020). FTIR spectrum obtained for AgNPs biosynthesized using the leaf extract of *Coleus aromaticus* is shown in Figure 1c (Vanaja et al., 2013). The FTIR spectrum shows different peaks resembling the various functional molecules associated with AgNPs.

Scanning Electron Microscopy (SEM)

The morphology of synthesized nanoparticles has a significant effect on the properties and therefore, visualization of the size and shape of nanoparticles is very important. In scanning electron microscopy an intense beam of electrons interacts with the material and produces various signals. After interaction of primary electron beam with the material, the generated secondary and backscattered electrons reveal useful information about the material. SEM imaging can provide information of size, morphology, particle distribution, and particle aggregation. It does not provide information about the internal structure of the material. A typical SEM for AgNPs synthesized