Chapter 5

Processes of Synthesis and Characterization of Silver Nanoparticles with Antimicrobial Action and their Future Prospective

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Abstract

The discovery of novel therapies is required due to the stark rise in microbial resistance to currently available conventional antibiotics, which poses a significant obstacle to the effective management of infectious diseases. Nanomaterials between 1 and 100 nm in size have recently become effective antibacterial agents. In particular, several classes of antimicrobial nanomaterials and nanosized carriers for antibiotic delivery have demonstrated their efficacy for treating infectious diseases, including antibiotic-resistant ones, in vitro and in animal models. Because of their high surface area-to-volume ratios, these materials can provide better therapy than conventional drugs and have new mechanical, chemical, electrical, optical, magnetic, electro-optical, and magneto-optical properties. So, nanoparticles have been proven to be fascinating in the fight against bacteria. In this chapter, we will go into detail about the various characteristics of microorganisms and how they differ across each strain. The toxicity mechanisms change depending on the stain. Even the effectiveness of nanomaterials to treat different bacteria and their defence mechanisms varies depending on strains, particularly the composition of cell walls, the makeup of the enzymes, and other factors. As a result, a perspective on nanomaterials in the microbial world, a method to combat drug resistance by tagging antibiotics in nanomaterials, as well as predictions for their future in science.

Keywords
Nanoparticles, Antibacterial Action, Microbial Resistance, NP-Assisted Drug Delivery, Nanoparticle-Assisted Therapy
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### 1. Introduction

The frequent occurrence of infectious disease outbreaks and illnesses brought on by disease-carrying microorganisms has always been a major concern. These outbreaks effects include substantial remediation expenses, interruption of everyday life, regional and
national economic downturns, and in the worst cases, fatalities. Some of these contagious diseases have been wiped off. However, certain diseases that were believed to have completely wiped out have made a comeback due to drug-resistant variants [1]. Antibiotics and antibiotic-resistant microbes (also known as "superbugs") are caused by the overuse, improper dosage, and widespread misuse of antimicrobials like antibiotics, antifungals, antivirals, and antiparasitic, which causes these pathogens to develop resistance to the antimicrobials. By boosting people's immunity to certain diseases, vaccinations have been designed to stop the spread of diseases. Bacterial infections can cause serious illnesses including endocarditis, meningitis, and pneumonia that are a serious threat to human health globally [2-5]. One of the main obstacles to treating diseases caused by bacteria is antimicrobial resistance (AMR), particularly from biofilm formation. According to a World Health Organization (WHO) report, antibiotic-resistant (MDR) bacteria kill about 7.5 million people annually worldwide and are expected to kill 12 million people by the year 2050 [6-8]. Antibacterial activity is defined as a substance's capacity to either kill or slow down bacterial growth. Currently available antibacterial products are primarily either chemically produced or naturally derived [9]. Both organic substances, like aminoglycosides, and wholly synthetic antibiotics, such sulfonamides, are frequently used. Broad spectrum drugs can have mediator molecules that are either bacteriostatic (or) bactericidal.

However, a substantial portion of the populace either has a negative reaction to these vaccinations or refuses to take them because of personal beliefs and/or preconceptions. Therefore, decreasing widespread use puts the general public and efficiency at risk [10,11]. This has developed into a significant issue for public health, resulting in expensive pharmaceuticals that do not work. The spread of these infections is also widespread, particularly in view of the inadequate antimicrobials available to address the issue. An avenue for the potential spread of microbes through goods and products contaminated especially at manufacturing facilities can be problematic currently of widespread advanced industrialization, manufacturing, and commercialization of all kinds of products with the potential to reach all corners of the globe. To curb the development of diseases and bacterial strains resistant to antibiotics, researchers and pharmaceutical corporations have been motivated to look for new, powerful antibacterial medicines.

Recently, the use of antimicrobial substances like nanoparticles to control bacteria has attracted the interest of researchers all around the world. These materials' morphological and physicochemical characteristics, such as their high surface area to volume ratio and other physical and chemical characteristics that have been effectively applied in other fields, have generated interest in their utilization [12,13]. Additionally, the surface charge of these nanoparticles can make it easier for them to bind to bacteria's opposing surface charges, resulting in efficient antibacterial actions [14]. Moreover, due to their insolubility and intimate interaction with microbial membranes, the lifespan and endurance of these antimicrobial nanoparticles when used in antimicrobial applications seem promising [15,16]. Antibiotics can be directed at an infection site and nanoparticle carriers can reduce systemic adverse effects.
By using a carrier, we can promote high-dose drug absorption at the targeted region while reducing side effects, such as drug toxicity. Systems for delivering antibacterial medications based on nanoparticles reach the drug's target site, minimizing side effects. Active or passive targeting are involved in targeted nanoparticle-based medication delivery. Passive targeting is accomplished through better penetration and retention at the infection site, whereas active targeting is accomplished through nanoparticle surface modification, enabling the nanoparticle-based drug delivery system to selectively identify exact ligands on the cells at the site of infection. Receptor, temperature, and magnetic targeting are all included in active targeting [17].

Researchers are shifting their attention to investigating alternate antibacterial techniques with reduced risk of developing antimicrobial resistance due to the rising expense and difficulties in generating new antibiotics. One of the most well-known techniques in the antibacterial therapeutic field is the use of nanomaterials. Examples of this approach include the use of antibacterial polymers, photothermal therapy (PTT), photodynamic therapy (PDT), stimuli-triggered antibiotic release using nanomaterials, catalytic bacterial killing using nanozymes, and anti-virulence therapy [18]. The transmission of diseases caused by pathogens, particularly bacteria and viruses, and their prevention are two of the biggest issues facing the health sector. When pathogenic contamination is discovered, it can sometimes be lethal and difficult to stop. Due to the disease-causing bacteria' size in the micron and nanoscales, contamination or disease transmission through various routes is always a possibility, whether it be in medical facilities like hospitals, laboratories, or pharmaceutical businesses. Due to the nature of the environment, hospitals and laboratories are more at risk of contamination. As a result, there is a considerable risk of blood sample exposure in laboratories and cross contamination of numerous disease-causing, drug-resistant organisms due to the high patient traffic. It is unavoidable for diseases to spread through contact with people, objects, and equipment as well as through liquids, vapours, or airborne mists. Therefore, it is essential to create antimicrobial technologies to stop the progression and spread of illnesses right where they start in the healthcare industry. Recently, nanoparticles with antimicrobial properties have gained popularity and are vigorously being explored as an effective substance against a wide spectrum of infections. These particles can impair the growth and, in some cases, eradicate the pathogens. Due to their extensive antibacterial properties against a broad range of diseases, silver (Ag) and its compounds are among the nanoparticles that have been studied [19-21].

Nanomaterials may quickly cross cell membranes compared to bulk materials, which has a toxic effect on bacterial cells. To boost the antibacterial effects of the agents, many medicines can be combined within a single nanoparticle or with help from additional constructions. Due to the co-ordinated action of numerous mechanisms, the simultaneous combination of medications with different effects helps to develop efficiency. On the other hand, combining two or more nanoparticle types can enhance their antibacterial activities and prevent the development of resistance [22]. Recent research has shown that the size and shape of nanoparticles significantly affects their bio and antimicrobial activities [23,
24]. Roughness [25], doping modification [26], and environmental issues are all the factors that displayed significant differences in antimicrobial activities.

2. Silver nanoparticle synthesis:

Researchers have focused on silver nanoparticles (NPs) because of their distinctive features, including size- and shape-dependent, optical, antibacterial, and electrical capabilities. For the synthesis of silver NPs, a variety of preparation methods have been documented; significant examples include laser ablation, gamma radiation, electron radiation, chemical reduction, photochemical procedures, microwave processing, and biological synthetic approaches. This chapter gives a general overview of the physical, chemical, and biological production of silver nanoparticles, therefore, to consider the present situation and probable outcomes, particularly the advantages and disadvantages of the industrial practices.

2.1 Physical methods

The most significant physical methods are laser ablation and evaporation-condensation. The produced thin films were free of solvent contamination, in comparison to chemical processes, the uniformity of NPs dispersion is a benefit of physical synthesis techniques. Atmospheric pressure physical synthesis of silver NPs has some drawbacks. For instance, the tube furnace takes up a lot of room, uses a lot of energy when raising the temperature around the source material, and takes a long time to reach thermal stability. According to Lee and Kang's research, monodispersed silver nano crystallites are produced as a result of the thermal decomposition of Ag⁺-oleate complexes [27]. A tiny ceramic heater was employed in a work by Jung et al. to create metal nanoparticles by evaporation/condensation processes. It was discovered that polydisperse nanoparticles were produced over time as the heater surface maintained a steady temperature. These silver nanoparticles were round and not clumped together [28]. To vaporize the raw materials, a little ceramic heater was used. As a result of the temperature gradient being far greater at the heater surface than it would be in a tube furnace, the evaporated vapor can cool at a suitable rapid rate. Because of this, highly concentrated tiny NPs can arise. Due to the heater surface's constant temperature, the particle creation is extremely stable. This physical technique can serve as a calibration device for nanoparticle measurement equipment as well as a nanoparticle generator for long-term investigations for inhalation toxicity studies [28]. Silver nanoparticles' geometric mean diameter and geometric standard deviation, respectively, ranged from 6.2-21.5 nm and 1.23-1.88 nm. Recent research has shown that the polyol method yields spherical nanoparticles of various sizes when laser ablation is used [29, 30]. Silver nanoparticles were created using laser ablation using various wavelengths to explore the effects of the wavelength on particle size. It was discovered that the average particle diameter decreased from ~ 29 to ~12 nm as the laser wavelength decreased [31]. Through a direct physical deposition of metal into the glycerol, Seigal et.al. investigated the creation of silver nanoparticles. It was discovered that this strategy works well in place of laborious chemical methods. Furthermore, the consequent
nanoparticles had a restricted size distribution and were resistant to aggregation [32]. The benefits of physical methods of manufacturing silver NPs include speed, the lack of hazardous reagents, and the use of radiation as a reducing agent. Physical methods' drawbacks include solvent contamination, low yield, uneven distribution, and significant energy consumption (Table 1).

<table>
<thead>
<tr>
<th>Silver nanoparticle type</th>
<th>Reducing agent</th>
<th>Biological activity</th>
<th>Method</th>
<th>Size (nm)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polydiallyldimethylammonium chloride and polymethacrylic acid capped silver nanoparticles</td>
<td>Methacrylic acid polymers</td>
<td>Antimicrobial</td>
<td>Laser ablation</td>
<td>10-50</td>
<td>[33]</td>
</tr>
<tr>
<td>AgNO₃</td>
<td>Sodium citrate</td>
<td>Antimicrobial</td>
<td>Electrical arc discharge</td>
<td>14-27</td>
<td>[34]</td>
</tr>
</tbody>
</table>

2.2 Chemical methods

Chemical reduction using reducing chemicals that are both organic and inorganic is the most used method for creating silver nanoparticles. Chemical procedures are advantageous because the necessary equipment is more practical and straightforward than that employed in biological methods. For the reduction of silver ions (Ag⁺) in aqueous or non-aqueous solutions, a variety of reducing agents are typically used, including sodium citrate, ascorbate, sodium borohydride (NaBH₄), elemental hydrogen, polyol process, Tollén’s reagent, N, N-dimethylformamide (DMF), and poly (ethylene glycol)-block copolymers. AgNO₃ is one of the most often utilized silver salts in chemical production of silver nanoparticles because of features like low cost. Monodispersed silver nanocubes were created by Sun and Xia by reducing nitrate [35]. These reducing chemicals cause the reduction of Ag⁺ to metallic silver (Ag⁰), which then aggregates into oligomeric clusters. Eventually, these clusters cause the emergence of metallic colloidal silver particles [36 - 38]. AgNO₃ was used as a precursor, sodium borohydride and trisodium citrate as stabilizing agents, and silver nanoparticles were synthesized by Mukherji and Agnihotri. For the synthesis of silver nanoparticles with a size range of 5–20 nm, NaBH₄ has reportedly been found to be an effective reducing agent. Comparatively, trisodium citrate is the most efficient reducing agent for the synthesis of silver nanoparticles in the 60-100 nm size range [39]. According to reports, silver nanoparticles with an average size of less
than 10 nm can be produced using ethylene glycol as a solvent and a reducing agent together with polyvinylpyrrolidone (PVP) as a size controller and capping agent [40].

By employing polyvinyl alcohol as the stabilizing agent and hydrazine hydrate as the reducing agent, Patil et al. were able to successfully create silver nanoparticles. According to their findings, the resulting nanoparticles had a spherical form and had important uses in biotechnology and biomedical science [41]. When compared to physical procedures, the main benefit of chemical approaches is high yield. To synthesize silver nanoparticles chemically, substances like borohydride, 2-mercaptoethanol, citrate, and thiol-glycerol are noxious, and chemical approaches are very expensive. Obtaining silver nanoparticles of a specific size is highly challenging, and a further step is needed to prevent particle aggregation (Table 2) [42].

<table>
<thead>
<tr>
<th>Silver nanoparticle type</th>
<th>Reducing agent</th>
<th>Biological activity</th>
<th>Method</th>
<th>Size (nm)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>AgNO₃</td>
<td>NaBH₄</td>
<td>Antimicrobial</td>
<td>Chemical</td>
<td>5-20</td>
<td>[39]</td>
</tr>
<tr>
<td>AgNO₃</td>
<td>DMF</td>
<td>Antimicrobial</td>
<td>Chemical</td>
<td>&lt; 20</td>
<td>[43]</td>
</tr>
<tr>
<td>AgNO₃</td>
<td>Trisodium citrate</td>
<td>Antimicrobial</td>
<td>Chemical</td>
<td>&lt; 50</td>
<td>[39]</td>
</tr>
<tr>
<td>AgNO₃</td>
<td>PVP/Ethylene glycol</td>
<td>Antimicrobial</td>
<td>Chemical</td>
<td>30-50</td>
<td>[40]</td>
</tr>
<tr>
<td>AgNO₃</td>
<td>Hydrazine hydrate</td>
<td>Antimicrobial</td>
<td>Chemical</td>
<td>2-5</td>
<td>[41]</td>
</tr>
</tbody>
</table>

3. Biological Methods

Physical and chemical methods for synthesizing silver nanoparticles are costly, time-consuming, and environmentally harmful. Therefore, it is essential to develop a system that is both inexpensive and environmentally benign, eliminates the use of harmful substances [44] and other issues related to both physical and chemical means of production. By controlling a variety of biological activities, biological approaches close these gaps and have numerous applications in the management of health. Fungi, bacteria, and yeasts are used in biological production techniques in addition to plant sources. These sources make this method highly well-liked for using silver nanoparticles in medicinal applications.

When important factors, such as the types of organisms, inheritable and genetic characteristics of organisms, ideal conditions for cell growth and enzyme activity, ideal reaction conditions, and selection of the biocatalyst state, have been taken into consideration, bio-based protocols could be used to synthesize highly stable and well-
characterized silver NPs. Changes to several crucial factors, such as substrate concentration, pH, light, temperature, buffer strength, an electron donor (such as glucose or fructose), biomass and substrate concentration, mixing speed, and exposure time, can affect the sizes and morphologies of the NPs.

3.1 Bacteria

It has been investigated how bacteria can produce silver nanoparticles. According to a study, Bacillus licheniformis, a non-pathogenic bacterium, might be used to bio reduce aqueous silver ions into very stable silver nanoparticles of size ~ 40 nm [45]. Additionally, utilizing the bacteria B. licheniformis, well-dispersed silver nanocrystals of size 50 nm were synthesized [46]. By combining B. subtilis culture supernatant with microwave irradiation in water, Saifuddin and colleagues have developed a unique combinational synthesis technique to produce silver nanoparticles of size ranging from 5-50 nm. [47]. Likewise variety of silver nanoparticles have been demonstrated using Aeromonas sp. SH10, Klebsiella pneumonia, Lactobacillus strains, Pseudomonas stutzeri AG259, Corynebacterium sp. SH09 and Enterobacter cloacae.

3.2 Plants

Because plant-based synthesis of NPs is so inexpensive, it can be employed as a practical and profitable alternative to the large-scale manufacturing of NPs [48]. As a reducing and stabilizing agent to produce silver NPs, camellia sinensis (green tea) and black tea leaf extracts has been utilized [49,50]. These NPs' production appeared to be mediated by polyphenols and flavonoids. Alfalfa (Medicago sativa), lemongrass (Cymbopogon flexuosus), and geranium (Pelargonium graveolens) plant extracts have been used as green reactants in the manufacture of silver nanoparticles. Additionally, by subjecting silver ions to Datura metel leaf extract, a large density of exceptionally stable silver NPs (16–40 nm) was quickly synthesized [51]. The leaf broths of Pinus desiflora, Diospyros kaki, Ginkgo biloba, Magnolia kobus, and Platanus orientalis all produced stable silver NPs with average particle sizes ranging from 15 to 500 nm extracellularly, according to research by Song et.al. When the reaction temperature was raised in the case of M. kobus and D. kaki leaf broths, the rate of synthesis and ultimate conversion to silver NPs was faster. However, when the temperature was raised from 25°C to 95°C, the average particle sizes produced by the D. kaki leaf broth fell from 50 nm to 16 nm [52]. Likewise, a variety of silver NPs have been synthesized using various plant extracts such as Aloe vera, Azadirachta indica, Cinnamomum camphora, Emblica Officinalis, Pelargonium graveolens, Pelargonium graveolens, Pinus eldarica leaf extracts.

3.3 Algae

There are a few studies on the accumulation of gold employing algae species, especially cyanobacteria, as a biological reagent. For the reduction of silver ions and subsequent production of Ag NPs, marine algae such as Chaetoceros calcitrans, Chlorella salina, Isochrysis galbana, and Tetraselmis gracilis can also be employed [53]. Oscillatoria willei
(NTDM01), a marine cyanobacterium, was used to manufacture silver nanoparticles of size 100–200 nm. After 72 hours of incubation with washed marine cyanobacteria, the colour of the silver nitrate solution changed to yellow, signifying the production of silver nanoparticles (NPs). Others like Spirulina platensis, Oscillatoria willei, and Gelidiella acerosa were also used to synthesize silver NPs. It was demonstrated that extracellular nanoparticles were created when Humicola sp. interacted with Ag⁺ ions to reduce the precursor solution.

3.4 Fungi

There have been reports that several fungi are involved in the synthesis of silver nanoparticles [54]. It has been discovered that fungi may produce silver nanoparticles quite quickly. Numerous researchers have thoroughly investigated how fungus produce silver nanoparticles [55]. According to one study, Fusarium solani and silver nitrate interact to produce spherical silver nanoparticles outside of cells [56]. It was demonstrated that extracellular nanoparticles were synthesized when Humicola sp. interacted with Ag⁺ ions to decrease a precursor solution [57]. According to Owaid et. al., the extract of Pleurotus cornucopiae caused the bioreduction of silver nitrate, which led to the creation of silver nanoparticles [58]. According to reports, silver nanoparticles accumulated on the surface of the Aspergillus flavus fungus's cell wall as a result of silver nitrate solution's interaction with it [59]. Bhainsa and D'Souza also looked at Aspergillus fumigatus's role in the extracellular production of silver nanoparticles [60]. The outcomes showed that silver nanoparticles were produced quickly by the interaction of silver ions with the cell filtrate. Silver nanoparticles between 5 to 50 nm in size are produced extracellularly when Fusarium oxysporum is used [61]. Furthermore, Phanerochaete chrysosporium mycelium is incubated with a silver nitrate solution to yield silver nanoparticles [62]. Fusarium oxysporum were employed by Korbekandi and colleagues to demonstrate the bio reductive synthesis of silver nanoparticles [63].

4. Characterization of silver nanoparticles

Nanoparticles need to be characterized to identify and determine their behaviour, efficacy, and safety. Characterization of silver nanoparticles will evaluate the functional attributes and efficiency of the particle. Various analytical techniques like UV-Vis spectroscopy, Fourier transform infrared spectroscopy (FTIR), dynamic light scattering (DLS), Transmission electron microscopy (TEM), High resolution transmission electron microscopy (HR-TEM), Field emission scanning electron microscopy (FESEM), scanning electron microscopy, atomic force microscopy (AFM), Gas chromatography mass spectroscopy (GC-MS), etc. Many renowned articles and books have given extensive reviews on the techniques used to characterize their principles. Some of the inevitable techniques that need to be considered or characterization of silver nanoparticles are listed and discussed below.
4.1 Ultra-violet spectroscopy

This is a primary, simple, selective, quick, and sensitive technique that is quite reliable and used to analyse the stability of silver nanoparticles [64]. Optical properties of silver interact quite strongly with wavelengths of light [65]. Conduction and valence bands lie very adjacent to each other. Hence movement of electrons seems to be quite free. This gives rise to surface resonance (SPR). Oscillations at the conduction band are seen when exposed to light waves [66,67]. Green synthesis of silver from natural sources is generally preferred by many scientists and the nanoparticles yielded in the process have shown stability for about a year with SPR peaks at ranges around 400-500 nm.

4.2 X-ray diffraction (XRD)

XRD is a non-destructive technique opted to determine the orientation, purity, imperfections as well as size of crystalline and metallic structures [64]. This can identify catalysts, compounds, superconductors, chemical groups, isomorphous structures, etc. [68]. X-ray exposed on crystals gets reflected and patterns of diffraction are generated onto a film. These patterns are found to be characteristic for a given metal at a particular orientation. Patterns are compared to a reference database from the joint committee on powder diffraction also known as JCPDS. This test is one of the main tests for confirming a nanoparticle's size and orientation [69]. It works on the principle of bragg's law. The cons associated with this technique is its ability to assess only single state and low diffraction intensity when compared to electron probing [70]. General orientation and peaks that confirm the presence of silver nanoparticles are 38°, 54° and 46°. This relates to JCPDS file number 04-0783 [71]. Indices of these peaks are (200), (311), (111) and (220). Mostly crystalline face centred cubic lattice is observed in silver nanoparticles [72].

4.3 Dynamic light scattering (DLS)

Radiation scattering ability of nanoparticles determine their biological activities and size distribution. This technique gives a narrow size distribution of particles ranging from 2nm to 500 nm. DLS analysis non-destructively the laser light scattering ability of particles as they pass through a colloidal suspension. It relies on Rayleigh scattering [73]. Modulation in the intensity of scattered light is calculated in terms of time and hydrodynamic size is calibrated [74]. Toxicity of silver nanoparticles is quite dependent on the size of the particle. Size devised by DLS (due to brownian motion) is larger when compared to TEM. DLS probes large particles in liquid suspension but limitation of sample specific interaction that influences the result [75].

4.4 Fourier transform Infrared spectroscopy (FTIR)

FTIR provides reproducible, non-invasive, economical, accurate, and favourable signals that can detect small changes in absorbance due interaction with light and particles, especially to analyse the reduction of silver to its nanoparticle form. Changes in the range of 0.001 can be distinguished with ease using FTIR. Biomolecules used in the fabrication of nanoparticles are detected well using FTIR. They study the conformation of molecules
that provide additional functionality to silver molecules [76]. FTIR collects data rapidly with less sample damage as well as strong signal and large ratio between signal and ratio [77]. Functional groups found in silver nanoparticles are amines (both primary and secondary), ketones, alkynes, aldehydes, and carboxylic acid [78].

### 4.5 X-ray photoelectron dpectroscopy (XPS)

XPS is a surface analysis electron spectroscopic method that can be used to quantify the chemical composition of a metal nanoparticle and devise its empirical formulae [79]. It is performed at vacuum conditions where interaction of the sample with laser emits electrons from the sample. The kinetic energy of the emitted electrons is measured in comparison to the number of electrons released from the surface. Combined data generates spectra specific for the given metal nanoparticle [80].

### 4.6 Scanning electron microscopy (SEM)

High energy beams of electrons have been subjected to samples under study to generate high resolution images [81]. Its raster scans the surface to generate three dimensional images of living and non-living samples. Non-conductive samples need to be sputtered using gold to make the scanning resolution more accurate. It can resolve particles of various sizes, shapes, morphology, and scales [82]. Combination of SEM with energy-dispersive X-ray spectroscopy (EDX) has been used to infer silver structures along with compositional analysis. Internal structures cannot be identified and analysed using SEM, but the morphology images generated with purity check are quite high in SEM.

### 4.7 Transmission electron microscope (TEM)

TEM is used to measure quantitatively the size of the grain, particle, morphology as well as internal components of a nanoparticle. The ratio of the distances between sample as well as objective lens and to its image plane determines the magnification of TEM [83]. Along with advanced spatial resolution, TEM provides extra measurements on the sample analytically [84]. The cons involved in using TEM is the tedious sample preparation, sectioning and working conditions like high vacuum [85].

### 4.8 Atomic force microscope (AFM)

Aggregation and dispersion of particles on the surface, size, sorption, shape, and topology of the samples have been analysed at different rates of scanning by AFM. Three general modes are used namely contact, tapping and non-contact using Vander Waals attraction as the main mode of interaction between the probe and sample surface. Interaction of nanoparticles with layers of biomolecules can be easily studied by AFM. Specifications of the sample to be oxide free, conductive and abrasion free are not required to be analysed using AFM. Probe used in AFM does not damage the sample but measures even the nanometre scale structures in liquids [86]. But the cantilever dimension influences the lateral size of the sample [87].
4.9 Localized surface plasmon resonance (LSPR)

Collective oscillations of spatial electrons on a metallic nanoparticle are known as LSPR. It generally occurs in the visible light region. LSPR depends on shape, size, temperature, and dielectric nature of the particle. Refractive index of the nanoparticle influences the frequency of spectra generated by LSPR [88]. This technique forms the basis to evaluate molecular, thermodynamic, kinetic as well as imaging properties of nanoparticles. Table 3 depicts few of the recent studies done for biological source-based silver nanoparticle synthesis and the characterization used by authors to confirm the fabrication of silver in them.

Table 3. Characterization used by researchers recently to confirm the presence of silver nanoparticles

<table>
<thead>
<tr>
<th>Source</th>
<th>Synthesis technique</th>
<th>Techniques used for characterization</th>
<th>Size</th>
<th>UV-Vis spectroscopy peak</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acacia nilotica</td>
<td>Reduction</td>
<td>SEM, EDX, XRD, UV-Vis spectroscopy</td>
<td>50 nm</td>
<td>380-420 nm</td>
<td>[89]</td>
</tr>
<tr>
<td>Pomelo peel waste</td>
<td>Ultrasound</td>
<td>SEM, DLS, Zeta, EDX, FTIR, XRD, UV-Vis spectroscopy</td>
<td>40 nm</td>
<td>420 nm</td>
<td>[90]</td>
</tr>
<tr>
<td>Tricholoma ustale/Agaricus arvensis</td>
<td>microwave</td>
<td>STEM, DLS, FTIR, XRD, UV-Vis spectroscopy</td>
<td>20 nm</td>
<td>400 nm</td>
<td>[91]</td>
</tr>
<tr>
<td>Bryophyllum pinnatum</td>
<td>Reduction</td>
<td>FESEM, DLS, FTIR, XRD, UV-Vis spectroscopy</td>
<td>35 nm</td>
<td>465 nm</td>
<td>[92]</td>
</tr>
<tr>
<td>Acremonium borodinense</td>
<td>Reduction</td>
<td>FESEM, DLS, FTIR, XRD, HR-TEM, UV-Vis spectroscopy</td>
<td>0.19 nm</td>
<td>420-450 nm</td>
<td>[93]</td>
</tr>
<tr>
<td>Psidium guajava</td>
<td>Reduction</td>
<td>Zeta, FTIR, UV-Vis spectroscopy, SEM</td>
<td>65 nm</td>
<td>420 nm</td>
<td>[94]</td>
</tr>
</tbody>
</table>
5. **Mechanism of Action in silver nanoparticles**

The exact mechanism opted by silver nanoparticles on cells is still under study. But literature holds many prospective suggestions as to how silver works on cells and pathogens. These nanoparticles can interact with cells either physically or chemically. Silver seems to physically accumulate on surfaces of the pathogen especially their cell membranes. Internalizations by porins on the cell membranes lead to penetration of silver into the cytoplasm of the microorganisms. Gram negative bacteria often internalize silver nanoparticles much faster than their gram-positive counterparts [99]. Porins enable hydrophilic molecules of varying sizes as well as charges to move across the membrane. Gram positive strains have thicker peptidoglycan layers that make penetration quite tedious in comparison to gram negative bacteria [99]. Lipopolysaccharide making up the cellular integrity of gram-negative bacteria makes it more sensitive to silver nanoparticles thus causing easy internalization [14]. Interaction between the phosphate, amino and carboxyl groups present on the bacterial membrane and positively charged silver leads to successful penetration into the pathogen. These electrostatic interactions lead to changes in the structural components of cell membrane resulting in enhanced permeability and dissipation of hydrogen ions from the surface. Thus, the membrane disrupts and kills the pathogen [100]. As the hydrogen ion gets released in the form of reduced proton motive force, the pH decreases and releases silver ion concentration from the nanoparticle [102].

Chemically silver nanoparticles can generate free radicals on contact with microbes and damage cellular membranes by creating pores [103]. Interaction with thiol moieties, proteins and complexes in the membrane can lead to dissolution of the structural layer [104]. These reactions are possible as donors of electrons from molecules of nitrogen, oxygen, sulphur, and phosphorus [105]. Membrane bound enzymes and proteins are inactivated as silver nanoparticles interact with their di-sulphide bonds and block their
active sites. Orientations of lipids are altered from cis to trans and vice versa by silver nanoparticles resulting in alterations in membrane fluidity and thus the overall composition of the layer. Biofilm components are neutralized by adhesion of silver nanoparticles on them [23]. Cellular apoptosis is also initiated in microbes as actin cytoskeleton (MreB) is disrupted and alters the fluidity as well as integrity of the outer membrane by these particles [106]. Reactive oxygen species (ROS) or oxidation of cellular components of pathogens by silver nanoparticles has been previously reported in *Pseudomonas aeruginosa* [107]. Double bonds in lipid bilayers are oxidized to generate radical species that cause subsequent damage to organelles and components of the microbial cell [96].

Enzymes are affected structurally and functionally by silver nanoparticles as it alters the shape and morphology of enzymes. 900 parts per billion administration of silver nanoparticles seem to affect expressions of some important cellular enzymes like maltose transporter, ribosomal units (30S), fructose bisphosphate aldolase and succinyl coenzyme A synthetase. These nanoparticles deactivate the enzyme or protein complexes and hinder the functions of the respective cell component. Cellular metabolism is disrupted by silver nanoparticle’s interaction with an important citric acid cycle enzyme called succinyl coenzyme A synthetase [108]. Suppression or manipulation of cellular metabolism creates oxidative stress and hinders gene expression, nutrition, energy production and blocking enzymes of the electron transport chain like cytochrome oxidase.

DNA damage by silver nanoparticles is also reported by many articles in past few years [109]. Replication process is interrupted by complex formation by silver nanoparticles by breaking hydrogen bonds between the complementary strands. Change in the relaxed to condensed state of the DNA decreases its ability to multiply, subsequently suppressing transcription and eventually translation [110]. Modulation of tyrosine residues by dephosphorylation is supported by silver nanoparticles, thus inhibiting pathogen multiplication [111]. Size plays a major role in determining the overall effect of the nanoparticle on the pathogen. Smaller the nanoparticle, easier is its penetration in the cytoplasm through the membranes [112]. Hence the link between synthesis and mode of action in silver nanoparticles on target cells for better practical applications is indisputable. Table 4 gives a summary of the perspective mode of action by silver nanoparticles on microorganisms.
Table 4. Overview of the prospective mode of action by silver nanoparticles on pathogens

<table>
<thead>
<tr>
<th>Interaction</th>
<th>Mode</th>
<th>Cells/Component affected</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical</td>
<td>Adhesion</td>
<td>Gram negative Bacteria</td>
<td>[98]</td>
</tr>
<tr>
<td></td>
<td>Penetration</td>
<td>Gram negative Bacteria, Fungi</td>
<td>[98]</td>
</tr>
<tr>
<td></td>
<td>Disruption of organelles</td>
<td>Bacteria</td>
<td>[99]</td>
</tr>
<tr>
<td></td>
<td>Disruption of biomolecules</td>
<td>Bacteria</td>
<td>[99]</td>
</tr>
<tr>
<td></td>
<td>Electrostatic attraction</td>
<td>Gram negative bacteria</td>
<td>[14,100,101]</td>
</tr>
<tr>
<td></td>
<td>Reduced Proton motive force</td>
<td>Gram negative bacteria</td>
<td>[102]</td>
</tr>
<tr>
<td></td>
<td>Apoptosis/ MreB disruption</td>
<td>Bacteria</td>
<td>[106]</td>
</tr>
<tr>
<td>Chemical</td>
<td>Oxidative stress</td>
<td>Enzymes</td>
<td>[108]</td>
</tr>
<tr>
<td></td>
<td>Signal modulation</td>
<td>Dephosphorylation of cellular signals</td>
<td>[113]</td>
</tr>
<tr>
<td></td>
<td>Free radicals</td>
<td>Bacteria</td>
<td>[103]</td>
</tr>
<tr>
<td></td>
<td>Inactivation of disulfide bonds</td>
<td>Proteins</td>
<td>[105]</td>
</tr>
<tr>
<td></td>
<td>Alter Cis/trans orientation and fluidity</td>
<td>Lipids</td>
<td>[105]</td>
</tr>
<tr>
<td></td>
<td>Neutralization</td>
<td>Biofilms</td>
<td>[23]</td>
</tr>
<tr>
<td></td>
<td>Alter relaxed state to condensed state by breaking hydrogen bond</td>
<td>DNA</td>
<td>[110]</td>
</tr>
</tbody>
</table>
6. **Role of silver nanoparticles in antimicrobial action**

The biggest challenge of the century in the healthcare sector is the global rise of antimicrobial resistance. Though development of antimicrobial resistance is an evolutionary mechanism, it has accelerated immensely due to the indiscriminate use and misuse of antimicrobials. This has resulted in the development of drug resistance amongst the microbial pathogens that is potentially irreversible and untreatable with the drugs currently in use. Prevalence of such disease pathogens may result in increased morbidity and mortality amongst the patient population, increased hospital stays and thereby higher healthcare costs. This may ultimately impact the country’s economic burden. Irrational use of antimicrobials further escalated during the COVID-19 pandemic which has resulted in adverse effects. Antibiotic resistance superbugs are strains of microbial pathogens that are resistant to most of the antimicrobial agents used for treatment of infectious diseases that they cause. Some of the important superbugs complicating treatment strategies include methicillin resistant staphylococcus aureus (MRSA), carbapenem resistant enterobacteriaceae including escherichia coli and klebsiella pneumonae, vancomycin resistant enterococci (VRE) and the hospital bug, acinetobacter baumannii. In the current scenario, it becomes a necessity to look for alternative approaches to combat the silent pandemic of antimicrobial resistance.

Nanotechnology provides huge potential applications in the field of infection biology and its processes. Nanomedicine is one of the important applications of nanotechnology that involves the use of nanomaterials for therapeutics, drug delivery, vaccine development, implants, diagnostics, and imaging tools and in screening platforms. Nanomaterials that are used for therapeutic antimicrobial action have distinctive properties over other chemical components which increases the efficacy of being used in disease control [114]. The antimicrobial action of metals, metallic salts and metallic oxides have been known since time immemorial for the treatment of various bacterial and fungal infections. Silver nanoparticles are one of the most promising antimicrobials amongst the metal and metal oxides. Apart from silver nanoparticles, gold and other metallic oxide nanoparticles like copper oxide, zinc oxide, iron oxide, magnesium oxide and titanium dioxide have been extensively studied for antimicrobial efficacies. Gold nanoparticles possess excellent photothermal properties that produces heat to efficiently disrupt biofilm and kill microbes. The metallic oxides are highly biocompatible and are effective against both gram positive and gram-negative bacteria including drug resistant strains. Their mode of action involves generation of ROS production in bacteria thereby killing by creating oxidative stress [115].

Silver has been used since ancient times for curing various ailments. Silver nanoparticles in the dimension of 1-100 nm have strong capacity and higher surface area to volume ratio that enables it to be used as a promising therapeutic agent. The unique properties of AgNPs are its ability to kill almost all the drug resistant bacteria and potentiality in targeting different sites of a single bacteria in any given time. AgNPs have exhibited antimicrobial, antibiofilm and wound healing properties that makes it the most extensively researched metal oxide nanoparticle [116].
The action mechanisms of AgNPs on bacteria are multiple, the most common being direct interaction of AgNPs with the plasma membrane to create pores causing bacterial cell lysis or inhibition of cell wall synthesis. Silver ions are released on silver oxidation which binds to thiol containing key enzymes thereby preventing their functions. AgNPs can also trigger the formation of ROS that kills bacteria by creating oxidative stress [117]. The action mechanism of AgNPs is attributed to the release of Ag⁺ ions which is responsible for its antibacterial activity. AgNPs may release the Ag⁺ ions which interact with the nucleosides of the nucleic acids thereby inhibiting DNA replication. Due to the electrostatic attraction and affinity with the sulphur proteins, Ag⁺ ions bind to the cytoplasmic membrane and cell wall causing increased permeability leading to cell lysis. Cytoplasmic membrane can also be denatured due to accumulation of Ag⁺ ions by modification of the cell membrane arrangements. Ag⁺ ions can also cause protein synthesis inhibition by denaturation of the cell’s ribosomal components. Another mechanism is the disruption of microbial signal transduction by phosphorylation of protein substrates like tyrosine which may ultimately cause apoptosis of the cell and inhibition of cell proliferation. AgNPs also deactivates the respiratory enzymes leading to the formation of ROS and interruptions in ATP release. Gram negative bacteria is significantly affected by AgNPs than gram positive bacteria owing to its cell wall components. Studies suggest that as the gram-positive cell wall is made up of a thick layer of peptidoglycan when compared to the thin layered peptidoglycan in the gram-negative bacteria, the AgNPs are not be to penetrate and therefore produce the desired effect [118].

The main limitation of using AgNPs for microbial inhibition is the cytotoxic effects. Research studies in mammals, especially in rabbits and rats have shown that use of AgNPs have a direct effect on the organs of the animals thereby resulting in irreversible damage to the reproduction and growth processes. The cytotoxicity of the AgNps depends on its physical and chemical properties and it is now convenient to synthesize silver nanoparticles using green technology which produces nanoparticles which are less toxic compared to other synthesis methods [118].

7. Applications of silver nanoparticles with antimicrobial action in healthcare

The antimicrobial properties of silver nanoparticles are widely exploited in the health care sector and medicine because of its many advantages such as wide functionality, biocompatibility, high infusibility across the tissue barriers, solubility, and multiple antimicrobial mechanisms. Face masks have become an indispensable tool in the prevention of airborne infections especially during times of the COVID-19 pandemic. Facemasks incorporated with AgNPs have been widely studied to prevent infection spread in hospital settings where the most drug resistant microbes exist. About 100% reduction of E. coli and staphylococcus aureus CFU were observed in facemasks which were incorporated with silver nitrate and titanium dioxide. In another similar study, commercial masks treated with AgNPs were shown to inhibit E. coli and staphylococcus aureus at 50 and 100ppm concentrations. AgNPs were also used along with disinfectants to
decontaminate surgical masks with an high potential of antibacterial activity against E.coli, klebsiella pneumoniae and staphylococcus aureus [116].

Catheters associated with urinary tract infection can cause serious complications like urosepsis and septicaemia. Therefore, AgNPs are coated in catheters to significantly reduce the growth and prevent biofilm formation in catheters. AgNPs catheters are shown to prevent and inhibit biofilm producing bacteria such as E.coli, staphylococcus aureus, pseudomonas aeruginosa, enterococcus and also the opportunistic yeast, candida albicans. Catheters coated with AgNPs also did not induce toxic effects or inflammation in animal models making it an efficient and safe for long term use. The concentrations of AgNPs coated and the release rate needs to be validated for commercial applications of such catheters. However, commercially available catheters coated with AgNPs in different countries include ON-Q Silver Soaker™, SilverlineR, and AgTive.

The healing and antimicrobial characteristics of AgNPs have progressed its use in wound dressings for faster curing and skin regeneration. It is assumed that use of AgNPs has an immunomodulatory effect on promodulatory cytokines by decreasing the inflammation period and hence allow the regeneration to happen quickly. AgNPs when combined with organic molecules show enhanced wound healing properties, thereby making its applications as hydrogels in topical applications for better wound repair. Silver ions also destroy the pathogenic bacteria found in wound exudates. Today, many drugs containing AgNPs in combination with other biopolymeric compounds are used commercially as wound dressings. Acticoat™ and Bactigras™, Aquacel™, PolyMem Silver™, and Tegaderm™ are some bio composites modified with ionic silver and approved by the FDA for applications as wound dressings [117].

Silver nanoparticle technology finds extensive use in the field of orthopaedics for its antimicrobial action. Contamination of implants from opportunistic pathogens may result in loss of implants and may hinder the restoration and repair of bone function. AgNPs with antimicrobial activity is extensively used in trauma implants, tumour prosthesis, bone cement, and in combination with hydroxyapatite coatings in implants. Silver nanoparticles have a unique ability to improve the differentiation process of MC3T3-1 pre-osteoblast cells and subsequently bone-like tissue mineralization. Silver nanoparticles combined with hydroxyapatite coatings in trauma implants aids osseo-integration. Human bone, dentin and dental enamel made with hydroxyapatite and nano silver is evidenced to be effective against both gram positive and gram-negative bacteria. Electrically generated silver ions have been investigated to be effective in treatment of osteomyelitis and in non-union of bones. Nano silver loaded bone cement had shown good antimicrobial activity against Staphylococcus.

The antimicrobial and biofilm inhibiting properties of AgNPs are exploited in the dental field by incorporating it in dental materials and implants. Silver nanoparticles are either used alone or in combination with other biomaterials as major components in adhesive resins, orthodontic cements, antimicrobial filling agents, dental composites, and as biocidal coatings in titanium-based implants. AgNPs based nano systems are evaluated as
performance enhanced drug delivery systems for therapeutic molecules based on their biocompatibility and functionality with tremendous antioxidant, antimicrobial and anti-inflammatory properties in current healthcare practices. Because of its intrinsic anticancer properties, AgNP based nanocarriers for anticancer drugs are extensively investigated as efficient antitumor based drug delivery systems. The broad-spectrum bioactivity of AgNPs makes them potential and promising agents for widespread applications in biomedicine and healthcare settings [118].

8. Limitations

Worldwide, the usage of silver nanoparticles is expanding quickly across various industries, including health care. However, it is crucial to reduce the risk of silver nanoparticles' negative effects on both human patients and the environment. It should be noted that many of NPs' antibacterial processes are still mostly unknown, which may surprise some. For instance, a lot of research links oxidative stress and ROS for antibacterial activity, while for other NPs, like MgO NPs, the antibacterial mechanism might not be connected to the control of bacterial strains' metabolism. Therefore, future research should pay close attention to the antibacterial processes of NPs.

Nanoparticles have shown significant potential for antibacterial activities and applications due to their enormous surface area and size, which increases contact with bacteria. The research that has been done so far on the antimicrobial mechanisms of NPs are constrained by the absence of unifying criteria. Because each type of NP has a unique microbial impact, no single method can be used to collect evidence about the antimicrobial processes of NPs. To assess the toxicity of silver nanoparticles and their impact on physiology and tissue architecture, numerous research using animal models have been carried out. The mitochondrial inner membrane's permeability increases non classically as a result of Ag+. Additionally, there was a higher degree of permeability in the rat liver mitochondria, which led to swelling in the mitochondria, aberrant metabolism, and ultimately cellular death [119]. The smaller silver nanoparticles (10 nm) caused the highest level of congestion, single cell necrosis, and focal necrosis in the liver and congestion in the spleen in a study on female mice exposed to different sizes of silver nanoparticles (10, 60, and 100 nm). This suggests that the smaller-sized particles caused greater acute toxicity in mice [120]. An additional investigation discovered a large reduction in glutathione levels, a reduction in mitochondrial membrane potential, and an increase in reactive oxygen species. These findings imply that oxidative stress likely facilitates the cytotoxicity of Ag particles between 15 and 100 nm in liver cells [121].

The development of an adaptive NP medication for antibacterial therapy is extremely challenging, as was already mentioned in above reports. In order to alleviate the multidrug resistance (MDR) and associated negative effects, researchers came up with the idea of antibiotic-tagged NPs. The synergistic effects of silver NPs with eight antibiotics against harmful microorganisms were therefore examined by Kumar et. al. in 2016. Apart from B. cereus, which experienced a 6.1-fold increase, the synergistic interaction of AgNPs with
streptomycin resulted in a minute increase in the inhibitory zone against seven harmful bacteria in the range of 0.1 to 0.9. Additionally, this research offers valuable information on the development of innovative antimicrobial drugs. A new formulation of NPs that work in synergy with antibiotics can be developed since the combination of antibiotics and NPs will make it more difficult for pathogenic bacteria to acquire resistance, which would otherwise render the present antibiotics ineffective.

Future prospective

Multiple scientific domains, including electronics, probes, illness diagnosis and therapy, cleanup, imaging, and cellular transportation, have numerous uses for silver nanoparticles. Also, due to their numerous uses in food packaging, agriculture, the healthcare industry, and as antibacterial and antitumor agents, silver nanoparticles have a substantial impact on health management. Moreover, it is generally recognised that most empirical antibiotic use results in resistance, which renders the drugs ineffective. Alternative therapy approaches are receiving more attention globally as a means of combating the issue of antibiotic resistance. Among these alternate procedures is the possible application of silver nanoparticles as antioxidant and antimicrobial agents, as well as chemical surface or copulation of nanomaterials. Due to their potential use in the fight against multidrug-resistant pathogens, nanomaterials can be associated to various cell processes as opposed to antibiotics, which may only have one mechanism of action. When fully understood, it will transform both laboratory and industrial microbiology. The entire manuscript must be in English. Please use the Time New Roman font with 13 font size.

References


