Review Article

Current Research on Silver Nanoparticles: Synthesis, Characterization, and Applications

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Over the past couple of decades, nanomaterials have advanced the research in materials; biomedical, biological, and chemical sciences; etc., owing to their peculiar properties at the nanoregime compared to their bulk composition. Applications of nanoparticles in the fields like medicine and agriculture have been boosted due to the development of different methodologies developed to synthesize specific shapes and sizes. Silver nanoparticles have tunable physical and chemical properties, so it has been studied widely to improve its applicability. The antimicrobial properties of Ag NPs are finding their application in enhancing the activity of drugs (like Amphotericin B, Nystatin, Fluconazole) and composite scaffolds for controlled release of drugs and targeted delivery of drugs due to their low toxicity and biocompatibility. Similarly, their surface plasmon resonance property makes Ag NPs a top-notch material for developing (bio)sensors, for instance, in surface-enhanced Raman spectroscopy, for detecting biomarkers, diseases, pollutants, and higher catalytic activity in photochemical reactions. Besides these, highly conducting Ag NPs are used in wearable and flexible sensors to generate electrocardiographs. Physicochemical or biological approaches are used to prepare Ag NPs; however, each method has its pros and cons. The prohibitive cost and use of hazardous chemicals hinder the application of physicochemical synthesis. Likewise, biological synthesis is not always reproducible for extensive use but can be a suitable candidate for therapeutic activities like cancer therapy. Excess use of Ag NPs is cytotoxic, and their unregulated discharge in the environment may have effects on both aquatic and terrestrial biota. The research in Ag NPs has always been driven by the need to develop a technology with potential benefits and minimal risk to environmental and human health. In this review, we have attempted to provide an insight into the application of Ag NPs in various sectors along with the recent synthetic and characterization techniques used for Ag NPs.

1. Introduction

The famous visionary lecture given by the American physicist Richard Feynman, “There’s plenty of room at the bottom” in 1959, is believed to have given a conceptual birth to the field of nanotechnology. At present, it is inevitable to almost every area of sciences, including medicine, environment, agriculture, and engineering. Organic and inorganic, as well as hybrid materials, are utilized in nanotechnology. Humankind has been using metals such as silver and copper for ages due to their antimicrobial characteristics, and at present, their applications are being explored in consumer products such as textiles, shampoo, hygiene products, and contraceptives.

Owing to surface plasmon resonance properties, Ag NPs find application in the sensors like colorimetric sensors, surface-enhanced Raman spectrometry sensors, fluorescence sensors, and chemiluminescence sensors [1]. These sensors
detect pollutants discharged in the environment like ammonia, heavy metals, and pesticides. Similarly, treating diseases like cancer is prolonged because of the late identification of the illness; Ag NPs have helped in early identification [2-4]. Moreover, Ag NPs can be applied for pollution remediation as their high surface-to-volume ratio makes the Ag NPs a better catalytic agent for the degradation of pollutants like nitroarenes and organic dyes [5, 6]. Besides, Ag NPs are less toxic to mammalian cells than other metal nanoparticles [7], and because of their relatively small size, they can easily penetrate the cell through the cell membrane and serve as a potential antimicrobial agent. Due to considerable surface energy, Ag NPs tend to agglomerate, decreasing antimicrobial potential, which can be solved by loading Ag NPs on a solid support system [8]. The high electrical conductivity of Ag NPs finds its application as an electronic device imitating ink. It improves the conductivity of the 2D sensors like graphene oxide when coated on the compound's surface. Additionally, this property helps in the detection of heavy metals.

Depending on the reducing agents used, Ag NPs can be synthesized following physical-chemical and biological approaches. The previous approach uses chemicals, light, laser, electricity, sound, microwave, etc., into the substrate to synthesize Ag NPs [9]. However, secondary metabolites or enzymes from microorganisms, fungi, and mostly polyphenol-rich natural products are used for the latter one. The physicochemical approach of nanoparticle synthesis is not economical and environmentally friendly [10]. On the other hand, biological synthesis overcomes such drawbacks [11], and Ag NPs with various structures and crystallinity can be obtained [12]. The materials chemistry of Ag NPs is driven by their shape, size, surface charge, agglomerating tendency, and dissolution rate [13].

When discharged into the environment, the toxicity of Ag NPs may increase, which will affect communities of microbes, nematodes, insects, plants, animals, etc. [11]. Similarly, inhalation of Ag NPs is also unpleasant to humans; it may damage organs like the liver, spleen, lungs, and kidney [14, 15]. Moreover, microbial resistance to the Ag NPs may damage organs like the liver, spleen, lungs, and kidney [16]. Stabilizing/capping agents may enhance Ag NPs ability to synthesize Ag NPs (Table 1) and recent characterization techniques used in identifying the physical and chemical structure of the NPs (Figure 1).

1.1. Chemical Synthesis of Silver Nanoparticles. The most popular method for synthesizing Ag NPs is the chemical reduction of silver precursors (usually silver salts) by different organic and inorganic reducing agents, often accompanied by stabilization using various capping or stabilizing agents. Numerous weak or strong reducing agents such as sodium citrate, ascorbate, sodium borohydride, elemental hydrogen, polyol cycle, Tollens’ reagent, N,N-dimethylformamide, poly(ethylene glycol)-block copolymers, hydrazine, ammonium formate, and gallic acid are used to reduce silver ions (Ag⁺) [17-19]. Stabilizing/capping agents for the nanoparticles are usually surfactants like alkanethiols, long-chain carboxylic acids, or alkylamines that stabilize Ag NPs [20]. The shape, size, stability, and dispersity of the Ag NPs are influenced mainly by the reaction conditions and the nature of reducing and capping agents used. Teodor et al. demonstrated that when a boiling solution of aqueous silver nitrate (AgNO₃) was reduced with a potent reducing agent, e.g., sodium borohydride (NaBH₄) in the presence of sodium citrate, spherical Ag NPs with an average diameter of 15 nm were formed as revealed by FESEM analysis [13]. Zannotti et al. synthesized Ag NPs of different sizes and morphologies by the reduction of AgNO₃ with NaBH₄ in the presence of trisodium citrate (TSC) and polyvinyl pyrrolidone (PVP) as capping and dispersing agents, respectively [21].

Similarly, Ag NPs with different sizes and morphologies can be obtained by adding different concentrations of oxidizing agents such as hydrogen peroxide (H₂O₂). Here, different concentrations of H₂O₂ were employed to etch the synthesized Ag NPs to produce different morphologies ranging from spherical to triangular and quasispherical shapes with the sizes ranging from 15 to 18 nm as revealed by SEM, TEM, and DLS analysis. The reduction due to potent reducing agents like NaBH₄ is rapid but often leads to the particles with heterogeneous size distribution [22]. However, mild reducing agents like ascorbic acid leads to the controlled growth of the Ag NPs. Gao et al. reported the development of the Ag NPs through two steps: first, the Ag⁺ ions were pre-reduced by NaBH₄ to give Ag NPs-Ag⁺, and then ascorbic acid was added to reduce the remaining Ag⁺ ions [23]. The growth of Ag NPs can be enhanced by increasing the concentration of ascorbic acid. However, the use of sodium citrate, a weaker reducing agent compared to ascorbic acid, generates Ag NPs of a slightly larger average diameter of 30 nm, in the presence of glycerin at a temperature of 100°C [24]. SEM and TEM analysis revealed the formation of roughly spherical Ag NPs. When AgNO₃ was reduced with TSC at a slightly higher temperature (110°C) in the absence of any other reducing or capping agents, comparatively larger (40 nm) Ag NPs were formed [25]. Raza et al. conducted a synthetic experiment to demonstrate the effect of a reagent’s type/concentration and the reaction conditions on the shape and size of Ag NPs, where Ag NPs of different shapes and sizes were synthesized by varying the type or concentration of silver precursors (AgNO₃), reducing agents (TSC/NaBH₄), capping agents (TSC/PVP), oxidizing agents (H₂O₂), and reaction conditions (temperature/stirring) [26]. Characterization by UV-vis spectroscopy, XRD, and SEM revealed the formation of Ag NPs of different shapes (spherical and triangular) and sizes (15-200 nm). It was reported that the stirring time also plays a crucial role in determining the morphology of the Ag NPs; the continuously stirred solution resulted in a triangular shape, while the unstirred one resulted in the agglomeration of Ag NPs into clusters. Vorobyev et al. synthesized uniform spherical Ag NPs with diameters ranging from 5 to 15 nm by reducing aqueous AgNO₃ with ferrous sulfate (FeSO₄) in the presence of citrate ions. An extremely concentrated and stable silver hydrosol was produced by this method, the concentration being more than 1000 g/L. XPS and thermogravimetric analysis revealed the capping ability of citrate-derived capping ligands [27]. The mixture of capping agents may enhance Ag NPs’ protection and facilitate control over the reaction time. Chen et al. reported a facile
synthesis of monodisperse spherical Ag NPs with diameters ranging from 16 to 18 nm in ethylene glycol using a mixture of capping agents: tannic acid (TA) and polyvinyl pyrrolidone (PVP) [28]. The enhanced stability may be attributed to the complexation between TA and PVP, leading to a stable particle coating and a fast Ag⁺ reduction rate. In such polyol synthesis, reaction time and temperature also play a vital role in determining the size and stability of synthesized Ag NPs. A short reaction time and a low temperature favor kinetically controlled products yielding anisotropic platelet-like structures, while a longer reaction time and a higher temperature favor thermodynamically controlled products yielding symmetrical spherical Ag NPs [29]. Blanco-Formoso et al. demonstrated the use of iron nitrate Fe(NO₃)₃ as an etching agent to synthesize monodisperse spherical Ag NPs with diameters ranging from 10 to 30 nm by the reduction of AgNO₃ with ascorbic acid and trisodium citrate [30]. It was reported that the size of Ag NPs was directly related to the

Table 1: Applications of Ag NPs generated by various methods.

| Represented nanomaterials                                      | Synthetic method | Application                        | Reference |
|---------------------------------------------------------------|------------------|------------------------------------|-----------|
| Biogenic Ag NPs                                               | Biological       | Antimicrobial                      | [219]     |
| Diosmin-capped Ag NPs                                         | Chemical         | Fiber optic sensor                 | [220]     |
| Curcumin-Ag NPs                                               | Chemical         | Antibiofilm                        | [221]     |
| Microgel-stabilized Ag NPs                                    | Chemical         | Catalyst                           | [28]      |
| Ag NPs incorporated in the Ceiba pentandra fiber              | Biological       | Catalyst                           | [51]      |
| Ag NPs                                                        | Biological       | Therapeutic                        | [222]     |
| Ag NP-embedded hydrogel                                        | Biological       | Antimicrobial                      | [17]      |
| Ag NPs decorated with thiol-terminated photolabile DNA oligonucleotides | Chemical | Therapeutic (drug delivery)       | [223]     |
| HAuCl₄/Ag NPs                                                 | Chemical         | Sensors                            | [24]      |
| Bovine serum albumin-Ag NPs                                   | Chemical         | Therapeutic (drug delivery)        | [224]     |
| Ag NPs on chitosan-grafted (cetyl-alcohol-maleic anhydride-pyrazinamide) | Chemical | Therapeutic (drug delivery)        | [225]     |
| Noscapinoid-bearing Ag NPs                                    | Chemical         | Therapeutic (drug delivery)        | [226]     |

Figure 1: Schematic diagrams of the synthesis, application, and challenges of Ag NPs.
Fe(III) content in the solution. An increase in Fe(III) content decreases the size of the Ag NPs up to a threshold limit. But once the Fe(III) content exceeds the threshold limit, a new nanoparticle’s nucleation occurs, and Ag NPs become large and polydispersed.

Thus, the shape and size of the Ag NPs are greatly affected by reducing agents, capping agents, and reaction conditions, and hence the required conditions can be maintained to obtain the desired shape and size of Ag NPs. The size of Ag NPs can also be controlled by employing specific growth modifiers like polyacrylic acid (PAA), which influences both nucleation processes and the subsequent stages of nanoparticle growth [6].

Chemical synthesis has also been widely used for coating drugs on the surface of Ag NPs. For instance, Masri et al. synthesized stable Vildaglipin-conjugated Ag NPs by stirring the 1:1 mixture of Vildaglipin and AgNO₃ for 10 minutes, followed by the addition of aqueous NaBH₄ solution [31]. The stability of the prepared drug-functionalized Ag NPs depends on the drug’s ratio to the precursor AgNO₃. For example, the most stable conjugates nystatin, fluconazole, and rifampicin were formed when the ratio of drug to metals was 4:1, and 1:1 for the latter one [22, 32]. The carboxyl, hydroxy, triazole, phenolic, etc., functionalities are responsible for the drug’s adherence to the surface of Ag NPs [22, 32]. It has been found that the conjugation of the drug (rifampicin) having an acidic functional group (phenol) leads to the aggregation of NPs; in the acidic condition, they accept protons from the surrounding environment as a result of which interactions between ligands and the Ag NPs’ surface decreases [32]. Similarly, the sizes of the nonfunctionalized Ag NPs are larger than the functionalized one; Li et al. found that a coating of oseltamivir decreases the size of Ag NPs from 3 nm to 2 nm.

Although the chemical reduction methods are widely used for synthesizing Ag NPs, the yield is often too low. Their production can be scaled up using some flow reactors capable of scaled-up production of Ag NPs with the narrow-sized distribution. Lin et al. synthesized Ag NPs in a continuous flow tubular microreactor by reducing silver pentafluoropropionate with isoamyl ether in the presence of trioctylamine as a stabilizer [33]. The flow rate, flow pattern, and temperature of the reactor play a crucial role in controlling the size and size distribution of Ag NPs. TEM analysis revealed that at a lower flow rate (0.08 mL min⁻¹), monodisperse Ag NPs with sizes ranging from 7.4 to 8.7 nm were formed, while at a higher flow rate (0.6 mL min⁻¹), polydisperse Ag NPs with sizes ranging from 3 to 12 nm predominated. Similarly, when the reactor’s temperature is raised, polydisperse Ag NPs predominate over monodisperse Ag NPs, which may be due to the stabilizing agent’s inefficacy to work at higher temperatures.

1.2. Electrochemical and Sonochemical Methods of Synthesis of Silver Nanoparticles. In addition to chemical methods, there is a rapidly growing interest in electrochemical and sonochemical methods for synthesizing Ag NPs. These methods do not require chemical oxidants, which benefit from curtailing the purification route of synthesis and the benefit of getting pure materials. Sanchez et al. employed an electrochemical method based on the dissolution of a silver anode in an acetonitrile solution containing tetrabutylammonium salt to obtain Ag NPs with sizes ranging from 2 to 7 nm as confirmed by TEM [34]. One attractive feature of this method is that the size of the Ag NPs can be tuned simply by varying the current density; when the current density is increased, the overpotential increases, and the size of the Ag NPs decreases [35]. Similarly, Wadkar et al. synthesized Ag NPs with sizes ranging from 2 to 10 nm by electrochemical dissolution of the silver anode in dimethyl sulfoxide (DMSO) without using any stabilizing agent [36]. The exceptional stability of Ag NPs, even in the absence of stabilizing agents, was due to the formation of the Ag(I) DMSO complex on particle surfaces as confirmed by FTIR, thermal analysis, and fluorescence spectroscopy. Reicha et al. synthesized Ag NPs (2-16 nm) by an electrochemical oxidation-complexation method followed by UV-irradiation reduction in the presence of a polysaccharide, chitosan, as a stabilizing (capping) agent [37]. The silver anode was oxidized electrochemically into Ag²⁺ and complexed with chitosan dissolved in an acidic electrolyte solution followed by a UV-irradiation reduction. Spectroscopic analyses revealed the formation of uniform, spherical, and densely dispersed chitosan-Ag NPs. Li et al. showed a new template-less and surfactant-free, digitally controlled, optically induced electrochemical deposition (OEID) method for synthesizing silver nanostructures like silver polyhedral nanoparticles, triangular and hexagonal nanoplates, and nanobelts in a specialized microfluidic device [38]. Here, the electrochemical reaction was induced using the projected light patterns in a microfluidic device consisting of an indium tin oxide (ITO) glass electrode and an optically sensitive layer coated ITO electrode. Such photoinduced methods are versatile, have a high spatial resolution, and can also be used to convert the shapes and sizes of the synthesized Ag NPs [39]. Jiang et al. presented a novel electrochemical method for synthesizing Ag NPs by reducing silver ions at a hemoglobin-modified boron-doped diamond electrode (HB-BDDE) [40]. Here, a Ag/AgCl electrode was used as a reference electrode, a platinum wire was used as a counter electrode, HB-BDDE was used as a working electrode, and the electrolyte solution that was composed of acetate buffer, silver nitrate, and poly(N-vinylpyrrolidone) was used as a stabilizer. SEM and TEM analysis showed stable, monodisperse, crystalline Ag NPs (10 nm).

Besides electrochemical methods, several sonochemical methods for synthesizing Ag NPs have been reported. Sarkar et al. reported a sonochemical method for synthesizing Ag NPs by decomposing AgNO₃ [41]. In this work, an aqueous AgNO₃ solution was ultrasonicated for an hour in an argon-hydrogen atmosphere to generate amorphous Ag NPs (20 nm) as revealed by powder X-ray diffraction, TEM, differential scanning calorimetry (DSC), and EPR spectroscopy. Similarly, Zhu et al. employed a pulse sono-electrochemical method to synthesize Ag NPs (10-20 nm) from an aqueous solution of AgNO₃ in the presence of nitritolriacetate (NTA) [42]. The study further showed that the sonic time has a decisive role in controlling the shape of Ag NPs; a short sonic time generated spherical Ag NPs.
predominantly, while rod- and dendritic-shaped Ag NPs predominated at a longer sonic time.

Moreover, Kumar et al. reported a novel approach for synthesizing colloidal Ag NPs using a polysaccharide, starch, as both a reducing and a stabilizing agent under ultrasound irradiation (sonication) [43]. Spectroscopic analyses revealed the formation of spherical, polydisperse, and partially crystalline Ag NPs (23-97 nm). Thus, the sonochemical methods offer a nontoxic, environmentally friendly, and economical approach for synthesizing Ag NPs at room temperature, but their size-controlling ability is usually less than that of electrochemical methods.

1.3. Nanocage-Assisted Synthesis of Silver Nanoparticles. The polymeric matrices, nanocages, and supercages present in certain compounds can offer reactive sites to reduce silver ions and impose confinement and protection to the nanoparticles, ultimately leading to size control and stability. Therefore, nanocage-assisted synthesis can be a better strategy to obtain small-sized stable silver nanocomposite materials. Zhang et al. demonstrated that the ordered arrangement of micropores and nanocages in the zeolites could serve as a sound barrier against the agglomeration of the synthesized Ag NPs through an electrochemical reduction method on a compact faujasite zeolite film-modified electrodes (CZFMEs-FAU) by an ion-exchange mechanism in an AgNO₃ solution [44]. It was revealed that the size of the Ag NPs and the site of formation, inside or outside of the zeolite, can be controlled by changing the extent of silver exchange in CZFMEs-FAU. By this method, Zhang et al. could synthesize Ag NPs of 1-2 nm, and this dimension is ensured from TEM analysis.

Zhang et al. fabricated a new class of silver nanohybrid particles based on reactive polymeric molecular cages (nanocages) where silver nanohybrid particles were synthesized by the reduction of AgNO₃ with polyamide network polymers (PNP) with an average diameter of 4.34 nm as revealed by TEM [45]. FTIR analysis confirmed the interaction between Ag NPs and polymeric molecular cages, and other characterization tools like EDX, XRD, and XPS verified the formation of stable, polycrystalline Ag NPs. In any synthetic method, after forming nanoparticles, it is essential to transfer them to different physicochemical environments for their practical applications. But since the Ag NPs formed by such nanocage-assisted methods are confined within the polymeric nanocages, it limits their applications usually for catalysis and electronics only.

1.4. Solid Support-Assisted Synthesis of Silver Nanoparticles. In recent years, the synthesis of Ag NPs mediated by some solid support is emerging as a promising strategy for synthesizing uniformly distributed functionalized Ag NPs. Zhao et al. reported a facile synthesis of Ag NPs on graphene oxide as solid support by microwave-assisted reduction of AgNO₃ in the presence of polyethylene glycol as a stabilizing agent [46]. Graphene oxide provides a fixed platform and anchoring sites for the in situ reductions of silver ions and prevents the aggregation of synthesized Ag NPs. Characterization by UV-vis spectroscopy, TEM, zeta potential, and XRD revealed stable, spherical, and polycrystalline Ag NPs (5-10 nm) on the surface of graphene oxide. Similarly, the sol-gel method has been used to develop the highly porous Ag NPs by entrapping Ag inside the silica matrix. Here, the reduction of silver salt (AgNO₃) was made after forming the solid matrix. It has been found that the catalyst has a high surface area and high inner porosity. SEM analysis revealed that the NPs were entrapped within the inner pore of a mesoporous matrix with a diameter of 20 nm [47]. Chiu et al. demonstrated the clay-mediated synthesis of Ag NPs by in situ reductions of AgNO₃ in the presence of exfoliated mica plates having polyoxymethylene-amine (POP-amine) salt tethered on the surface [18]. Here, the POP-amine served both as a reducing agent and as a stabilizer, and the minimal size of the Ag NPs obtained was 8.1 nm, as confirmed by TEM and FESEM analysis.

Similarly, Kumar et al. reported cellulose nanocrystal- (CNC-) mediated synthesis of Ag NPs with diameters ranging from 1 to 20 nm as indicated by TEM by reducing AgNO₃ with NaBH₄ in an aqueous medium [48]. The study further showed that the cellulose nanocrystals in their modified or unmodified form possess fascinating nucleation-controlling and nanoparticle-stabilizing properties. Cai et al. reported a facile synthesis of Ag NPs on silica spheres reducing AgNO₃ with ethanol in the presence of sodium hydroxide without any stabilizing agents at room temperature [49]. The synthesized Ag NPs were characterized by XRD, FESEM, TEM, and XPS, which revealed the formation of uniformly distributed, spherical, crystalline Ag NPs with an average size of 10 nm on the silica surface. It was reported that the composition of the Ag NPs could be tuned by controlling the molar ratio of sodium hydroxide to AgNO₃.

Kubasheva et al. employed a natural mineral diatomite as a support substrate to synthesize (AgCl/Ag) NP diatomite hybrid composite nanoparticle’s size ranging from 1 to 10 nm. The elemental composition of diatomite and the size distribution of synthesized Ag NPs were determined by SEM, EDX, XRD, FTIR, and thermogravimetric analysis [50]. Here, the AgNO₃ solution was reduced with H₂O₂ to synthesize Ag NPs, while the silver chloride nanoparticles were formed due to the exchange reaction of the AgNO₃ solution with the halite impurity present in the diatomite matrix. Similarly, in situ reduction of Ag⁺ ions using NaBH₄ was carried out in PNA-BIS-2 microgels to obtain Ag NP-loaded poly(N-isopropylacrylamide-co-acrylamide) (Ag-PNA-BIS-2) microgels. XRD analysis revealed the Ag NPs as crystalline, and the EDX analysis confirmed the nanoscale Ag’s presence on the surface of the polymer [51].

1.5. Biological Synthesis of Silver Nanoparticles. The biological approaches follow the principle of green chemistry utilizing various natural products like phytochemicals, enzymes, and biodegradable polymerase reducing and capping agents to replace potentially hazardous chemicals like sodium borohydride during the synthesis of Ag NPs, and utilizing water as solvent [52, 53]. Reducing agents for the synthesis can be obtained from extracting different parts of the plants (roots, leaf, and flower), microorganisms (extracellular and intracellular enzymes, metabolites), and biodegradable polymers that are described subsequently.
1.6. Synthesis Using Plant Extracts. Extracts from various plant sources resulted in Ag NPs with different sizes; these plant sources included the bark of *Salacia chinensis* (40-80 nm), the leaf extract of *Brassica rapa* L. (5.7-24.4 nm), the leaf extracts of the medicinal plants *Semecarpus anacardium*, *Glochidion lanceolarium*, *Bridelia retusa*, and *Enicostemma axillare* (Lam.) (15-20 nm), and the extract of *Cannabis sativa* (20-40 nm), which were used as reducing, stabilizing, and capping agents [54-57]. Plant extracts are rich in antioxidant components like polyphenols, triterpenoids, alkaloids, steroids, and tannins [54, 58]. It has been speculated that polyphenols such as alkaloids and polysaccharides in plant extracts might be responsible for reducing AgNO₃ to Ag-NPs [59]. The natural fibers act as a solid surface for the synthesis of Ag NPs using plant extracts as reducing and stabilizing agents [60]. Similarly, *Syzygium cumini* leaf extract acts as reducing and capping agents for the impregnation of Ag NPs on the surface of cellulose fibers with sonication [61, 62].

Coffee extract can be a better alternative for controlling the particles’ size to produce nanoparticles with smaller sizes: 20 nm from the coffee extract and 60 nm from tea extract [56, 63, 64]. Hydroxy and carboxy functionalities are responsible for encapsulating Ag NPs to increase the stability of Ag NPs. Synthesis of Ag NPs using the leaf extract of *E. axillare* is one of the most promising cost-efficient techniques that produced agglomerated sizes ranging from 25 to 80 nm, a polydispersity index of 0.412, and a -24.6 mV zeta potential value as reported from dynamic light scattering (DLS). The obtained zeta potential value specifies a high electrical charge on the surface of NPs that can cause a strong repellent force among the particles to avoid agglomeration [65]. TEM analysis showed smaller-sized NPs: 10 ± 2 nm from the root and 5 ± 1.5 nm from the *Ocimum sanctum* stem [66]. The outer peel of two varieties of sweet potatoes, the Korean red skin (lb1) and the Korean pumpkin (lb2), has many proteins and flavonoids and reducing and stabilizing agents for preparing NPs. The synthesis is faster (rapid, within 1 h) with the lb2 extract, whereas the synthesis is slower (rapid, within 12 h) with the lb1 extract; in lb1-Ag NPs, the Ag element accounted for 22.45% of the total composition, and the corresponding content in the lb2-Ag NPs was 34.92% [67].

Similarly, using plant extract, the average size of Ag NPs can be controlled by changing the extract concentration (extract: salt solution ratio), temperature, and pH of the reaction mixture. Tea leaf extract can be used to synthesize Ag NPs, and the size could be controlled by concentration variation of both extract solution and salt solution; thus, the Ag NPs formed are nontoxic to human beings [68]. The absorption peak got sharper with an increase in the concentration of the extract, and a shifting of color (blue) was observed, indicating a reduction in the diameter of Ag NPs. A low concentration of extract can reduce silver ions but cannot protect quasispherical NPs from agglomeration due to the deficiency of biomolecules to act as capping agents. In the case of a higher concentration, the biomolecules act as both reducing and capping agents, and a higher concentration of extract is also responsible for the formation of symmetrical Ag NPs. In the case of a fast reduction of Ag ions, usually, the further growth of NPs by the Ostwald ripening phenomenon leads to an increase in the size of Ag NPs; however, a low concentration of PEs of *Origanum vulgare* has produced small-sized Ag NPs. The NPs are quite polydisperse and range in size from 2 to 25 nm with an average size of 12 nm, as revealed by TEM analysis [69].

Moreover, the slow rate of reduction of Ag NPs at room temperature can be accelerated by increasing the mixture’s temperature [70]. The temperature-dependent approach was exercised to fabricate nanoparticles by reducing silver nitrate (AgNO₃) solution using aqueous leaf extracts of *O. vulgare*; the conversion rate of silver ions to silver nanoparticles at 60°C was 45%, while the conversion rate at 90°C was 100% [69]. The higher the reaction temperature, the faster the Ag NPs grow, and with increasing temperature, the triangular silver nanoplates and spheres grow more extensively due to the phenomenon of the particle fusion mechanism [71].

Similarly, the shape and size of biosynthesized Ag NPs could be altered by varying the reaction mixture’s pH. The pH facilitates changing the electrical charges of biomolecules, which directly affects the reducing/capping abilities and the subsequent growth of Ag NPs. So, due to the synergistic effect of reducing and capping agents due to variations in pH, the shape and size of the Ag NPs are altered. In most cases, particle size is larger in the acidic medium than in the alkaline medium. The alkaline environment enhanced the reducing and stabilizing capacity of antioxidants in hot water olive leaf extract (OLE), as confirmed by TEM [72].

Moreover, particles synthesized using an aqueous extract are smaller than the particles prepared using the methanolic extract; for example, TEM analysis of Ag NPs of *Phaseolus coccineus* aqueous and hexane nanoextracts showed spherical nanoparticles with mean size ranges between 10.12 and 27.89 nm, and for *Micrococcus lutea*, the mean size ranges between 8.91 and 14.48 nm [73].

Purification utilizing ethanol precipitation is much more useful than centrifugation. Leaf extract of *Taraxacum officinale* for Ag NP synthesis revealed the average size of 33.6 nm when employing centrifugation and 16.8 nm through ethanol precipitation from TEM analysis [74]. The extract from *Nyctanthes arbor-tristis* can serve as a good capping agent for preparing an advantageously smaller size (13 nm) revealed by TEM [75]. Similarly, Ag NPs synthesized from the aqueous root of *Berberis asiatica* were approximately 9.8 nm in size and spherical [76].

1.7. Synthesis Using Microorganisms. In Ag NPs synthesis, the microorganisms such as bacteria and fungi utilize the redox membrane proteins for surface synthesis and extracellular enzymes for extracellular synthesis [77]. The fungal-mediated mechanism of Ag NP synthesis where cell wall, cell membrane, and other biomolecules like proteins and enzymes play vital roles for NPs formation is linked to the cell wall (electron shuttle quinones). Both intra- and extracellular synthesis can be seen in *Fusarium* spp., where the electrostatic interaction in the cell surface traps the silver ion, and the silver ions are reduced by the catalytic activity of the enzymes present in the cell wall. Due to the electrostatic
interaction between the free cysteine residues or amino groups, the fungal proteins and peptides stabilized the formed Ag NPs [78, 79]. The naphthoquinones and anthraquinones act as electron shuffling compounds and redox centers during the bioreduction of *Fusarium* spp. (*F. oxysporum*, *F. semitectum*) by silver ion where NADPH dependent nitrate reductase enzyme catalyzed the reduction of silver ions to Ag NPs [80–82]. Supernatants of *Candida albicans* and *Saccharomyces* sp. are also efficient for Ag NPs synthesis with sizes ranging from 2 to 7.3 nm [12]. Ag NPs of different sizes have been obtained from extracellular synthesis using different fungi: *Aspergillus niger*, Ag NPs-Asp (40 ± 5); *Cryptococcus laurentii*, Ag NPs-Cry (35 ± 10); and *Rhodotorula glutinis*, Ag NPs-Rho (15 ± 8) [83].

Cell-free extract of *Penicillium oxalicum* generated Ag NPs with sizes from 60 to 80 nm, *Penicillium aculeatum* Su1 generated NPs with sizes from 4 to 55 nm, and *Penicillium polonicum* generated NPs with sizes from 10 to 15 nm [84–86]. To avoid harsh toxic chemicals and conserve immense energy, microbial nanosynthesis is one of the exponential possibilities driven by nature’s divergence. Microbes facilitate the synthesis of NPs with small size distribution, less polydispersity, and contain reductase enzymes that detoxify metals like silver [87]. Xue et al. reported that Ag NPs from *Arthrodema fulvum* in alkaline conditions and with an incubation period of 10 hrs were crystalline with a size of 15.5 ± 2.5 nm [88].

Both active and inactive cells of *Corynebacterium glutamicum* are efficient nanofactories; however, dormant cells are much more efficient in the uptake of silver ions; and subsequent reduction leads to nanocrystals with sizes of 5–50 nm (irregular size and shape) [89], which confirms that Ag NPs can be generated even in the absence of reducing enzymes. TEM images detected the quasispherical shape of Ag NPs with diverse sizes ranging within 3.30–17.97 nm synthesized using the aqueous extract of *Oscillatoria limnetica* [90].

Similarly, in *Bacillus licheniformis*, the core idea behind synthesizing Ag NPs was to utilize a cell-free supernatant generated after separating *B. licheniformis* MO9 cells in the form of a metabolite (arginase) produced by the fermentation process. The mean particle hydrodynamic size of the Ag NPs is 20 nm with a zeta potential of −11.37 ± 0.04 mV, which indicates the moderate stability of the Ag NPs in the colloidal suspension as revealed by dynamic light scattering (DLS), TEM, and potential zeta study [91]. As a stabilizing agent for the biosynthesis of Ag NPs, cell-free supernatants of *Bacillus amylophilicaciens* MRS5 can be used. The spherical shapes of biosynthesized Ag NPs were 29.5 nm in size with a 33.4 mV zeta potential as revealed by DLS and zeta potential studies; a nitrate reductase enzyme can transport electrons from nitrates to the metal group. Thus, in this study, the nitrate reductase enzyme might have been responsible for silver nanoparticle synthesis; the Ag NPs were examined after 3 hrs of reaction time, but there was no aggregation [92]. Similarly, using a cell-free extract of *Bacillus stamensis*, Ag NPs can be synthesized; TEM and SEM results indicated that the biosynthesized Ag NPs were spherical with an average of 34 ± 3 nm [93].

Using red algae *Gracilaria birdiae*, Ag NPs were synthesized in a simple, rapid, and single-step process with the appropriate spherical shape, small size, and negative zeta potential [94]. The aqueous extract of *Nostoc linckia* can also be utilized to synthesize Ag NPs of efficiently controlled sizes, with sizes ranging from 5 to 60 nm, and with a spherical shape as revealed by TEM analysis [95].

1.8. Synthesis Using Biodegradable Polymers. A linear polysaccharide (sugar) obtained from the hard outer skeleton of different marine organisms, chitosan, is the most useful for the biosynthesis of Ag NPs because it acts as both a reductant and a stabilizer [96]. Similarly, when preparing Ag NPs using starch, a paste of starch with NaOH may be used as it dissolves starch without degradation and the unmodified starch with high molecular weight prevents the agglomeration of particles for a longer time (six months) [97].

Moreover, NaOH used in the preparation of Ag NPs using casein hydrolytic peptides (CHPs) improves the reducing activity of CHPs [98]. In the absence of NaOH, the CHPs were unable to reduce the Ag ions into Ag NPs. When synthesizing NPs using the oil in water (O/W) microemulsion and nanoemulsion synthesis method for Ag NPs synthesis using *Geranium* (*Pelargonium hortorum*) leaf, it is suggested that low contents of a metallic precursor form NPs with a smaller size of 8.6 ± 4.3 nm with an 80 wt% of Geranium leaf extract (GLE) (nanoemulsion) [99]. The extract of *Azadirachta indica* acts as a reducing agent inside the hydrogel templates containing carboxymethylcellulose (CMC) [100]. Hydrogel networks act as a reservoir for metal ions; the ions are anchored through a carboxylic, amide, hydroxyl groups of carbohydrate polymer (CMC), and acrylamide (AM). Large amounts of metal ions in the network facilitate the reduction and stabilization. DLS data showed the diameter of the silver nanoparticles as 32 nm. Water-soluble *Glycyrrhiza* polysaccharides (GPs) act as reducing and stabilizing agents in the photoreduction for obtaining Ag NPs of varied shapes and sizes, which are determined by the concentration variation of GPs and silver nitrate solution [101]; an increase in UV-irradiation time increases the size as well as the size distribution of the prepared nanoparticle. Xylan, from corn cob, containing mainly xylose (50%), glucose (20%), arabinose (15%), and galactose (10%), as well as some amount of mannose (2.5%) and gluconic acid (2.5%) residues, has been used as a bioreactor to synthesize Ag NPs. The prepared xylan Ag NPs contain xylan (81%) and silver (19%), as revealed by ICP-OES data [102].

1.9. Characterization of Silver Nanoparticles. Different analytical and spectroscopic techniques are used to characterize the nature, size, shape, distribution, state of stability or aggregation, morphology, elemental composition, and dispersity (monodisperse or polydisperse) of nanoparticles [103, 104]. Some of the essential techniques commonly used for silver nanoparticle characterizations are described below with their merits and limitations.

UV-vis spectroscopy is the primary but crucial tool that explains silver nanoparticles’ formation at the initial synthesis stage. It is very characteristic depending upon the shape,
size, and distribution of Ag NPs due to surface plasmon resonance. The smaller and spherical silver nanoparticles absorb near 400 nm and have narrow peaks, while larger Ag NPs have redshift (absorb at longer wavelength) with broad peaks. It is also indicative of the stability of Ag NPs since the peaks start to decrease in intensity and broaden with the appearance of secondary peaks at higher wavelengths, as particles aggregate [105]. Similarly, the broadening of peaks also provides information about the distribution of Ag NPs. Generally, broad peaks indicate the formation of broader size range distribution (wide dispersity) of Ag NPs in solution [74, 106]. The colloidal Ag NPs show the size- and shape-dependent optical properties, i.e., the color of the solution during synthesis is also characteristic of size and shape, and it is possible to perceive and track the progress of synthesis due to the peculiar color of the solution [107, 108]. However, UV-vis alone is not sufficient enough to give the overall picture of synthesized Ag NPs.

Similarly, Fourier transform infrared spectroscopy (FTIR) characterizes the role of chemicals, metabolites involved in reducing and capping of Ag NPs, and the presence of chemical residue on the surface of Ag NPs, which involves the interaction of infrared (electromagnetic radiation) with bonding in molecules in the form of stretching and bending vibrations (in the 4000–400 cm⁻¹ region) [104, 109]. It is fast, inexpensive, nondestructive (when used with the attenuated total reflection accessory), and highly reproducible, but it is less sensitive to nanoscale analysis and gives only qualitative information [103]. The FTIR spectral analysis shows the involvement of amide (–CO-NH₂), carbonyl (–CO), and hydroxyl (–OH) functional groups responsible for the reduction, capping, and stability of Ag NPs [67, 73, 90, 110].

Since every crystalline material has a unique and characteristic X-ray diffraction (XRD) pattern, it is used as a fingerprint to identify the material and determine the crystallinity. It is a nondestructive technique. The Joint Committee on Powder Diffraction Standards (JCPDS) has the databases of card number standards, which are used to identify the crystalline material. For example, the characteristic powder diffraction pattern for face-centered cubic (fcc) silver lines is identified by JCPDS no. 04-0783, whereas the silver oxide is identified by JCPDS no. 01-076-1489. It also gives the information of phase impurity in the material. For example, the presence of silver oxide in the synthesized Ag NPs could be formed due to the oxidation of Ag NPs on hot air oven drying of synthesized Ag NPs that are mainly performed by vacuum freeze-drying [111]. These diffraction peaks are used to calculate the crystallite size of Ag NPs by measuring the line broadening as full width at half maximum (FWHM) using the Scherrer equation (not the Debye-Scherrer equation) [112]. Since line broadening (β) is inversely proportional to crystallite size (t), the broadening of diffraction peaks also indicates the formation of smaller size Ag NPs. The “crystallite size” term should be used instead of “particle size” while calculating the equation’s size:

\[ t = \frac{(K \cdot \lambda)}{(\beta \cos \theta)}, \]

where \( t \) = crystallite or grain size; \( K \) = Scherrer constant, and the value varies from 0.62 to 2.08 (0.94 for FWHM for spherical crystal with cubic symmetry); \( \lambda \) = wavelength of X-ray (0.154 nm for Cu Kα); \( \theta \) = Bragg’s angle (the 2θ value of the chosen peak); and \( \beta \) = line broadening as the full width at half maximum (FWHM) (in radian).

Sometimes, the observed line broadening of diffraction peaks from crystallite is contributed by instrumental broadening; in such cases, the instrumental contribution should be removed before using the Scherrer equation. However, the very smaller size crystallites dominate over instrumental broadening [112, 113]. It is also possible to determine the crystallite size more accurately using the modified Scherrer equation [114]. There are always differences among researchers while calculating the size of Ag NPs using the Scherrer equation. Some have focused on using only the 111 diffraction line, while others calculate the average using all the diffraction planes [106, 111]. However, there are discrepancies in the size of Ag NPs measured from XRD data, TEM, and DLS.

Likewise, energy-dispersive X-ray spectroscopy (EDX) is used to characterize the elemental composition, relative abundance, and impurities of nanoparticles, which involves X-ray interaction with the sample. The presence of emission peaks at 3 keV reveals the presence of silver crystallites, and the absence of any other peaks confirms the impurity-free Ag NPs [90, 115], while the presence of other peaks like carbon and oxygen could be the interaction of metabolites with Ag NPs on the surface or due to oxidation of Ag NPs.

Moreover, dynamic light scattering (DLS) is another short, easy, and nondestructive technique that is used to measure the particle size at the micrometer and nanometer regimes where a monochromatic source of light, a laser, is passed through the suspension of nanoparticles which scatter light at different intensities due to the Brownian motion and size is measured using the Stokes-Einstein relationship. It usually measures the hydrodynamic diameter of nanoparticles affected by the surfactant/stabilizer/capping agents and the presence of an electrical double layer adsorbed on the surface of nanoparticles and works best with monodisperse nanoparticles [116]. Thus, the nanoparticle’s measured size is somewhat larger than the actual size measured from XRD and TEM [104, 106, 116–118]. The DLS also measures the zeta potential of Ag NPs where the large magnitude value indicates the electrostatically stabilized nanoparticles (i.e., no aggregation) [57, 119, 120].

In addition to that, scanning electron microscopy (SEM) is used to characterize the surface morphology, size, aggregation, distribution of nanoparticles where electron beams are utilized as imaging probes. It provides high-resolution images (Figure 2) at the nanometer scale, the role of biomatrix, or encapsulation of Ag NPs and used to measure nanoparticle’s size [75, 109, 121]. However, it requires a dry and conductive sample, and sometimes the size measurements from SEM are not as accurate as TEM due to Van der Waals clusters of small entities [122].

Furthermore, atomic force microscopy (AFM) is an advanced 3D technology that can characterize nanoparticles’ topology at a nanometer scale where it uses a cantilever with a mechanical probe. It has a unique advantage over other
techniques like XRD, DLS, SEM, and TEM: it can provide the height and volume; it can analyze the 3D topology of nanoparticles; and it can envision hydrated nanoparticles, distribution (dispersity), and variability in shapes and sizes of nanoparticles [32, 57, 109, 120]. However, it is a time-consuming process and gives the exterior structural information only [103].

Correspondingly, transmission electron microscopy (TEM) provides the most accurate and high-resolution imaging information about the size, shape, morphology, state of aggregation, and distribution of nanoparticles at nanometer resolution, which also utilizes a beam of electrons for the interaction with the ultrathin sample [103, 109, 123, 124]. It also helps to visualize the role of capping agents and metabolite encapsulation of Ag NPs (Figure 3). However, it is also a time-consuming process, requiring high vacuum, skill, and time to prepare the ultrathin sample, and results are more reliable for homogeneous samples [104, 109, 125]. Also, high-resolution transmission electron microscopy is a phase-contrast microscopy mode of TEM that provides the highest resolution and helps to visualize atomic packing (atomic lattice) in nanoparticles; thus, it provides an image of the crystal defects [104, 126, 127]. Similarly, selected area electron diffraction (SAED), also known as electron diffraction, is a technique that is performed in TEM to characterize the crystallinity, crystal structure, and lattice parameters of nanoparticles from the diffraction pattern where a thin sample is targeted with a parallel beam of high-energy electrons [104]. It can also validate the XRD results by calculating the d-spacing from the circular rings from the diffraction patterns (Figure 3) and correlating it with d-spacing from the JCPDS file [75].

A significant difference in the size of spherical Ag NPs of the same sample has been observed when measured with different techniques. DLS measurements show the size of Ag NPs as 108 nm, AFM shows it as 145 nm in diameter and 48 nm in height, whereas SEM shows the presence of 40-70 nm sized Ag NPs [120]. It indicates the discrepancies from the different instrumental techniques. However, each characterization technique in Ag NP synthesis has its significance as they provide an overall picture of the properties, which is the framework for the applications. The data and results obtained from these characterization techniques complement each other because they validate each other and provide the different aspects of the results, which are very important in scientific study.

2. Applications of Silver Nanoparticles

2.1. Health

2.1.1. Antimicrobial Activity. Silver metal has been used as an antiseptic for wound healing, which prompted researchers to find the role of Ag NPs as antimicrobial agents against both fungi and bacteria. Ag NPs have great potential against the multi-drug-resistant bacteria and fungi [128], and the antimicrobial mechanism of Ag NPs is different for different species due to which susceptibility is also different. The most potent antimicrobial activity has been observed against methicillin-resistant Gram-positive Staphylococcus aureus, followed by methicillin-resistant Staphylococcus epidermidis and Streptococcus pyogenes. However, mild antimicrobial activity was seen against Gram-negative Salmonella typhi and Klebsiella pneumoniae [129], which may be due to the plasmolysis of the bacteria’s cell wall Ag NPs. It has been noted that the chitosan-Ag colloid has higher bactericidal efficiency against Escherichia coli while it has relatively weak activity on C. albicans due to the differences in the cell wall structure and abundance of the functional groups on the cell surface of the different bacteria [96]. The oxidized pullulan-mediated Ag NPs, due to their higher negative values of zeta potential, acquire increased stability due to repulsion among the particles and exhibit potent antibacterial activity against Gram-positive bacteria [130]. The mode of action of Ag NPs on the microbial cells is not fully understood. However, it is speculated that the mechanism is similar to the silver ions, and it involves adhesion and destruction of the cell wall or microbial membrane, interaction and disruption of biomolecules (nucleic acid, enzymes), and generation of ROS and free radicals which trigger the oxidative stress of cells.
Ag NPs function on the bacterial cells and the bacterial biofilms, effectively preventing biofilms and destroying bacteria in existing biofilms. The Ag NPs derived using the extract from S. anacardium and B. retusa exhibited intense antibiofilm activity against numerous clinically relevant human pathogens and modulated a biofilm’s development and structure [55], which is attributed to the biosorption of Ag NPs to the biofilm matrix inhibiting cell-cell adhesion [133]. The biofilm formation for Pseudomonas aeruginosa, E. coli, S. epidermidis, and Gram-negative bacteria (E. coli and P. aeruginosa) is more susceptible to NPs than that of Gram-positive bacteria (S. epidermidis) due to differences in the composition of the cell envelope [57]. It has been found that quercetin has synergistic effects on the antimicrobial activity toward E. coli and S. aureus of Ag NPs; the combination is found to be safe as no cytotoxicity is seen in the in vivo antimicrobial assay [134]. The antimicrobial activity of first-generation antibacterial drugs is improved when conjugated with the Ag NPs.

Ag NPs have enhanced the effect of commonly used drugs (Amphotericin B, Nystatin, and Fluconazole) to treat amoebic meningoencephalitis caused by Naegleria fowleri.

**Figure 3:** Transmission electron micrographs of representative Ag NPs synthesized from N. arbor-tristis through ethanol precipitation at different scale bars: (a, b) 50 nm, (c) 20 nm, (d) 10 nm, and (e, f) 5 nm. (g) A selected area electron diffraction pattern of the Ag NPs [75] (open access and reprinted under CC BY-NC-ND 3.0).
The effectiveness of conjugation is due to increased bioavailability and increased concentration of drugs at the target. Ag NPs have cytotoxicity against the host cells, which can be minimized by the slow release of Ag⁺ ions in the cells. Hydrogel developed from silver nanoparticles—immobilized cellulose nanofibers (CNFs) and alginate—have shown Ag⁺ controlled release of Ag⁺ without showing cytotoxicity for wound dressing [136]. The antiviral activity of Ag NPs against H1N1 influenza has been amplified when the surface of Ag NPs is decorated with oseltamivir; the antiviral activity is due to the generation of reactive oxygen species (ROS) [19]. The spherical-shaped Ag NPs, synthesized using xylan as reducing agents, effectively oppose the Chagas disease-causing protozoan, Trypanosoma cruzi, through necrosis [102]. It has been found that drugs loaded on Ag NPs are more effective than the drugs used against the infection caused by Acanthamoeba castellanii. Moreover, the drug NP conjugate inhibited the host cell cytotoxicity caused by the parasite [22]. The biosynthesized Ag NPs from P. coccineus hexane extract enhanced the antiviral activity against HAV-10, HSV-1, and CoxB4 viruses due to the viral envelope preventing viral penetration into the host cell due to Ag NPs [73].

2.1.2. Sensing and Therapeutic Application. Portable and wearable medical devices can be a better alternative for monitoring patients. Ag NPs, due to their highly conductive property, find application in stretchable sensors [137]. These sensors are prepared by dispersing or coating Ag NPs on the carbon-based nanomaterials' surface like fragmented graphene sponges, multiwalled carbon nanotubes onto polydimethylsiloxane, reduced graphene oxide, and carbon black [138–140]. The prepared sensors have been used for generating electrocardiograms [140] for showing exceptional temperature-sensing properties, etc. [137].

Ag NPs are easy to prepare, and different batches produce Ag NPs with the same shape, size, and dispersion. So, they are suitable for SERS sensors; it has been found that SERS intensity decreases with an increase in the interparticle distances [141, 142]. Plasmonic and cytotoxic properties of Ag NPs are exploited for the diagnosis and treatment of diseases. The plasmonic scattering property and nuclear-targeted silver nanoparticles' ability to cause programmed cell death have been utilized to image the real-time behavior of human oral squamous carcinoma (HSC-3) cell communities during and after the induction of apoptosis [143]. Similarly, Ag NPs are used as a substrate for surface-enhanced Raman spectroscopy (SERS) for the detection of sialic acid (correlated with breast cancer), prostate-specific antigen (a biomarker for prostate cancer), and infertility detection [2–4].

Moreover, a Ag NPs-enhanced immunofluorescence assay detected the biomarkers α-fetoprotein (AFP) and C-reactive protein (CRP), which helps in the clinical diagnosis of hepatocarcinoma [144]. Ag NPs are also used as colorimetric sensors to detect carbohydrate antigen 125 (CA125), found on many ovarian cancer cells [23]. Iodine-131 radionuclide has a therapeutic (therapeutics and diagnostics) application, and polyethylene glycol-decorated Ag NPs act as a delivery vehicle for I-131 to the targeted tissue without affecting the nontargeted one [145]. Similarly, Ag NPs chitosan nanobiocomposite hydrogel beads can be used for prolonged and controlled drug delivery; release of the drug (Ibuprofen) was decreased by increasing the Ag NPs' content [146].

Ag NPs-modified electrode analyzed chloride ion concentration in sweat, which is useful for preliminary screening of cystic fibrosis [25]. Multicolor silver nanoparticles have been used in multiplex point-of-care detection of viral diseases like dengue, yellow fever, and Ebola viruses [147]. Multiplexed colorimetric detection is one solution or method for detecting Kaposi's sarcoma-associated herpesvirus and Bartonella spp. DNA uses Ag NPs [148]. Ag NPs ensemble with a Zn(II) complex of α-lipoic acid-conjugated terpyridine has been developed for arginine’s selective colorimetric detection, helping in disease diagnosis [149]. Streptavidin-labeled fluorescent silver nanoparticles have detected HIV-1 p24 antigen in clinical specimens with improved sensitivity and specificity [150].

Silver nanoparticles have antidiabetic, anti-inflammatory, and anticancer properties, which may improve the treatment of those diseases [14, 151, 152]. Advanced glycation end-products (AGEs) resulting from nonenzymatic glycation are among the significant factors involved in diabetes and its secondary complications and diseases. Ag NPs inhibit AGE formation in a concentration-dependent manner and decrease the toxic reactive oxygen species (ROS) formed during the glycation process [153]. Additionally, the synthesized Ag NPs showed noticeable inhibition of carbohydrate hydrolyzing enzymes, mainly, α-amylase (IC₅₀, 60.2 ± 2.15 μg/mL) and α-glucosidase (IC₅₀, 62.5 ± 2.75 μg/mL), which may be due to polyphenols acting as capping agents or due to protein corona formation which unfolds the protein inhibiting the activity of the enzyme [14, 151, 152]. Similarly, diabetes patients have slower wound-healing capacity; nanocomposite hydrogels containing bamboo cellulose nanocrystals and silver nanoparticles have been found to accelerate wound healing in diabetic rats [61, 62]. The antifouling property enhances the immune response in diabetic wound healing. The antifouling property was obtained by adjusting the cationic chitosan and anionic dextran’s composition to approach zero charges. After hydrogel treatment, the rapid wound contraction suggested its superior healing ability to promote fibroblast migration, granulation tissue formation, and angiogenesis [154]. Ag NPs coated with capsacin show an antiamyloid property (the product from various diseases, including neurodegenerative disease); however, Ag NPs and capsacin alone do not affect amyloid formation [155].

Ag NPs can act as anticancer agents [156, 157]. The biocompatible Ag NPs are found to be effective against liver (Hep G2), lung (L-132), pancreas (MIA-Pa-Ca-2), breast (MDA-MB-231), oral (KB cells), prostate (PC-3), and cervical (HeLa) cancer cell lines, which confirms its potent anticancer action [54, 158, 159]. Similarly, Ag NPs embedded with graphene oxide with conjugated methotrexate enhanced the total ROS production in the treated cells improving cellular apoptosis [160]. Also, they are potent toward other cell lines [58, 71]. The combination of Ag NPs with the broad-spectrum anticancer agent, camptothecin, showed improved dispersion, drug dissolution rate, drug stability, and cellular uptake rate [161]. Hybrid
hydrogels made of silver nanoparticles (Ag NPs) embedded in the carboxymethylcellulose (CMC) polymer cross-linked networks conjugated with doxorubicin (DOX), an anticancer drug, show good activity against melanoma skin cancer cells. The gel shows improved in vitro kinetics of DOX, demonstrating a synergistic effect with Ag NPs killing cancer cells [162]. The proposed antimicrobial mechanism of action of Ag NPs is shown in Figure 4.

2.2. Environment

2.2.1. Sensors. The colorimetric sensing property of Ag NPs can detect the heavy metal ions Ni, Co, and Hg (II) and sulfide anions [13, 59, 163, 164]. Triangular silver nanoplates have higher anisotropy and lightning rod effect. Due to this, they have been used in the plasmon sensors to detect Hg2+ ions in the solution; the regular blue shift is seen to be increasing the concentration of the Hg2+ ions [21]. Ag NPs doped with tris(4,7-diphenyl-1,10-phenanthroline)ruthenium(II) dichloride complex (Ru(dpp)3Cl2) encapsulated in plasticized polymethyl methacrylate (PMMA) has been used to prepare the film, which acts as ratiometric sensors for the measurement of dissolved oxygen in the aqueous solutions [165]. The common herbicide atrazine (Atz) has been detected through an electrochemical sensor developed by Ag NPs [166]. SERS sensors developed by in situ growth of Ag NPs on polydopamine- (PDA-) templated filter papers (FPs) are highly flexible and can quickly collect and detect malachite green residues [167].

2.2.2. Pollution Degradation. The catalytic activity of Ag NPs occurs when the released electrons of Ag ions are exchanged between a reducing agent (electron donor) and a dye (electron acceptor) [168], which rely on their size, shape, and surface structure, as well as bulk and surface composition. Tubular nanocomposite catalysts containing small Ag NPs had higher catalytic activities than those containing large ones [169]. Ag NPs maintain outstanding catalytic efficiency in reducing the nitroarene series irrespective of the substituents’ types and positions [5]. The biosynthesized Ag NPs exhibited a strong chemocatalytic action with comprehensive degradation of 4-nitrophenol into 4-aminophenol [92], methyl orange, and methylene blue (MB) by sodium borohydride [6]. Bleaching organic dyes by applying potassium peroxodisulphate in an aqueous solution at room temperature is enhanced significantly by adding silver-containing nanoparticles [170]. The rate constant increased when the number of Ag NPs used as a catalyst increased. Additionally, Ag NPs as nanocatalysts degraded dyes in wastewater/effluents and have unique properties for carbon dioxide electrolysis that perform a crucial role in converting CO2 into CO [171]. Ag NPs act as a heterogeneous catalyst in reducing halogenated organic pollutants by BH4 [47]. The role of Ag NPs in the photocatalytic degradation of pollutants like crystal violet, methylene blue, and malachite green has been studied [172]. Ag NPs have improved the photocatalytic activity of metal oxide by shifting the visible region’s absorption [173]. It has been found that TiO2/Ag, ZnO/Ag, and NiO/Ag have better photocatalytic activity than the metal oxide along with the degradation of pollutants like colorful methyl orange and colorless toxic 4-chlorophenol [174].

2.2.3. Water Treatment. The bactericidal membrane developed for water purification by impregnating 1 mg/L of biosynthesized Ag NPs on nitrocellulose membrane filters showed the complete inhibition of the microbial community of E. coli, Enterococcus faecalis, Pseudomonas aeruginosa, and S. aureus suspensions, and the inactivation and removal of E. coli and S. aureus reached up to 6 and 5.2 orders of magnitude [83, 175]. Ag NPs, along with zwitterionic sulfobetaine methacrylate (SBMA) grafted on the polyimide (PI) membrane, improved the antifouling and antimicrobial properties of the membrane [176]. The Ag NPs can be easily removed...
from the beads and inhibit microbes’ growth in a real water sample [177, 178]. Multifunctional nanocomposites composed of multiwalled carbon nanotubes (MWCNTs) with embedded iron oxide and silver nanoparticles have potent antimicrobial efficiency [179]. The polycrylonitrile (PAN) sorbent, used in water treatment, stores pathogenic microorganisms on its surface. However, when treated with Ag NPs, biofilm formation was not observed on the surface [180]. The flow of bacterially contaminated water through paper embedded with Ag NPs could be an effective emergency water treatment.

2.2.4. Food and Agriculture. Ag NPs, being an antimicrobial agent, are effective against plant pathogens. The particles influence the colony formation and disease transformation of plant pathogenic fungi (Bipolaris sorokiniana and Magnaporthe grisea) [181]. Synthesis of carcinogenic mycotoxin and secondary metabolites, aflatoxin B1, of Aspergillus parasiticus was decreased using Ag NPs without affecting fungi growth [182]; the citrate-coated Ag NPs show antibacterial effects against rice pathogens, which help in the improvement of rice production [93]. Similarly, fructose-stabilized Ag NPs showed antimicrobial activity against phytopathogens (Erwinia amylovora, Clavibacter michiganensis, Ralstonia solanacearum, Xanthomonas campestris, and Dickeya solani) [183–185]. The graphene oxide-silver nanocomposite (GO-Ag NPs) is applied to treat the leaf spot disease infected by Fusarium graminearum [186]; the GO-Ag NPs are found to inhibit both the fungal hyphae and the spores. Ag NPs found application against bovine herpesvirus due to which agricultural production increased [187]. The action of pesticides can be enhanced when applied with the silver cellulose matrix [183]. Ag NPs as nanofertilizer have been studied, and it is concluded that if used in limited doses of 20 ppm-40 ppm, Ag NPs can improve the agronomic traits of onion without inducing genotoxicity in the plant [97]. Ag NPs have been used to treat early blight of tomato in both in vivo and in vitro experiments in a concentration-dependent manner [188].

For many agriculture-based countries, keeping fruits and vegetables fresh until reaching the market is essential. It is necessary to protect food and vegetables against respiration and microbes, and Ag NPs can solve these problems [189]. Similarly, Ag NP-PVP (polyvinylpyrrolidone) slowed down the weight loss, slowed the microbial degradation, and increased the shelf life of asparagus spears [190].

Nonenzymatic electrochemical glucose sensors have been developed by polyaniline-reduced graphene oxide functionalized with silver nanoparticles for sensing glucose in real samples like juices and milk [191]. Ag NPs as a SERS substrate facilitate on-spot detection of pesticides like thiabendazole (TBZ), thiram (TMTD), methyl parathion (MPT), and malachite green (MG) on the surface of fruits and vegetables [192]. Not only pesticides but also alternariol (AOH, 36 3,7,9-trihydroxy-1-methyl-6H-dibenzo[b,d]pyran-6-one) and mycotoxin that were found in Alternaria-infected plants have also been analyzed applying pyridine-modified silver as SERS substrate [142].

3. Discussion

Using different chemical and photochemical reduction methods, thermal decomposition, UV-radiation, microwave-assisted synthesis, electrochemical and sonochemical processes, and Ag NPs can be synthesized. Although these methods can successfully result in Ag NPs, they usually require environmentally unfriendly chemicals that have many adverse effects on ecology and human health [193]. Further purification steps are needed to obtain the final product, as some of the chemicals/reducing agents/by-products left behind during the process are adsorbed on the surface of Ag NPs, which can cause adverse effects during medical application or treatment. These methods often use costly chemicals, which typically need stabilizers to prevent Ag NP agglomeration.

Despite the propitious properties of Ag NPs, however, there have been some challenges that need to be tackled, namely the ability to synthesize Ag NPs in the aqueous phase with the desired scale, shape, or monodispersity and the ability to associate Ag particles with the desired biomolecules such that the detection can be carried out. Synthesis of Ag NPs capped by thiol molecules in the standard two-phase synthesis [194], sonochemical synthesis [195] using the polyl technique [196], and even synthesizing Ag NPs bordered by oleic acid [20, 197] may enable these techniques to provide good monodispersity and stability for Ag NPs. However, the disadvantage of these techniques is the small size of the resulting particles (which has a low potential for Raman enhancement) and the difficulty of removing the resulting NPs to aqueous media for biological application. Similarly, Ag NPs were successfully synthesized in the aqueous phase using sodium acrylate as a dual reducing-capping agent [198, 199]; the particles displayed minimal monodispersity. Furthermore, aqueous Ag NPs are synthesized using sugar molecules [200], poly(allylamine) [201], polyvinylpyrrolidone [21], ethylenediamine, and cetyltrimethylammonium bromide [202]. All these aqueous synthesis approaches for Ag NPs are useful for biological applications as the resulting particles can also be used directly; however, these methods usually have low monodispersity of Ag particles or sizes, which are difficult to monitor.

Alkali metal borohydrides and amino borane complexes have been used successfully to synthesize metal nanoparticles as reducing agents, although these agents have toxicity issues. To prevent these problems, attempts have been made to create green alternative reducing agents. Noble metals can be easily reduced; even natural materials such as starch [203], glucose [204], plant extracts [205], and polysaccharides [130, 200] can be used as a reducing agent. While these friendly materials were used in the synthesis of nanoparticles, control over the product is weak in terms of size, and therefore physicochemical properties are low [204], possibly because of their weak reducing strength. Thus, chemical methods are the most flexible tools for procuring a vast array of Ag NPs. However, due to related toxicities, use of biologically hazardous chemicals and solvents, impure product formation, and sensitivity to the environmental conditions [206, 207], green approaches are considered essential for Ag NPs synthesis.
Therefore, green routes require the use of biological reducing agents such as the following macromolecules: peptides, polysaccharides, enzymes. These are environmentally friendly and are among the most suitable Ag NPs synthesis approaches. The key problems related to the green synthesis of Ag NPs can be summarized as follows: (i) shortcomings related to scaling up the synthesis process; (ii) improvement in reproducibility of biogenic processes; (iii) the mechanism for the production of nanoparticles is not completely elucidated; (iv) regulation of the size and distribution of nanoparticles needs to be enhanced; (v) the mechanism of action of metal/metal oxide NPs needs to be studied on targets; and (vi) stability of Ag NPs from the green route.

Hence, solvent use has been identified as the most significant challenge among the synthesis of nanoparticles. Biological potency of silver is due to specific morphological and physicochemical parameters, including scale, shape, colloidal stabilization, surface corona, composition, aggregation behavior, surface coating, and surface/volume ratio, which if adequately tuned, may lead to a broad-spectrum inhibitory profile against several pathogenic microbes [208, 209].

Nanosilvers have been used in commercial and biomedical applications as a catalyst and an optical receptor in cosmetics, electronics, bactericidal, wound dressings, operating devices, and disinfectants. In turn, this has increased the potential toxicity to human health and the interactions of Ag NPs with terrestrial and aquatic environments [210]. Although potential applications are proliferating, the environmental effects of biodistribution, accumulation in organs, degradation, possible adverse effects, and toxicity to humans, as well as broader environmental effects are slowly recognized and evaluated [211]. However, a cell-cultured medium suggested that Ag NPs and freshly mixed silver ions were toxic to cells, while aged silver ions were not toxic at all [212]. It is a must to carry out the quantification and detection of Ag NPs in biological systems to understand how it may affect human health.

The main routes of Ag NPs penetration through the body are the respiratory system, gastrointestinal systems, and the skin. Several studies have documented Ag NPs and their adverse effects inside various organisms due to their small size and have shown that Ag NPs can move through biological membranes and infiltrate cells, causing toxicity at different rates [11] with other mechanisms. This is because of their ability to be absorbed by the body, to accumulate in specific tissues, and to produce cytotoxicity, which are likely to be their most essential mechanisms [213]. A study has been conducted on NIH-3T3 (Swiss mouse embryo), HEP-G2 (human hepatocellular carcinoma), A-549 (human lung carcinoma), PC-12 (rat adrenal pheochromocytoma), and Colo-320 (human colon adenocarcinoma) cells, and these were chosen to study different possible absorption paths of nanoparticles into the organism and various areas of particle accumulation in the body using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium-bromide) assay. The study shows that Ag NPs with concentrations of ~1–10 ppm entering the body from the air or liquid suspensions could present a potential risk to human health. Similarly, Ag NPs at a concentration of 0.001 mM are not quite detrimental to environmental microbiota, and this evidence indicates that raising the concentration of Ag NPs has adverse effects on rice seedlings’ germination and growth. Also, Ag NPs concentrations (up to 0.01 mM) modulate the biomass partition, decrease the relative water content in the leaves, increase the damage to the membrane and carotenoid content, and promote chlorosis. Moreover, at a concentration of 0.5 mM, the Ag NPs disrupt rice seedlings’ growth and production [214].

Animal experiments have demonstrated that Ag NPs may accumulate throughout the body and have toxic effects when absorbed at relatively large rates, although it is unknown if those amounts are similar to those currently achievable through food intake [213]. Moreover, a study showed that Ag NPs cause histopathological abnormalities in the spleen, liver, skin, and muscles, which are also targeted organs of Ag NPs toxicity [215]. Also, the entry of Ag NPs into the brain is known to induce neuronal death. There is, however, a gap of experiments investigating the impact of Ag NPs on the brain’s native immune cells, microglia [216]. In turn, because of a significant function of glia in homeostasis management of the central nervous system, exposure to Ag NPs may eventually contribute to the central nervous system [217]. Similarly, Ag NPs have been extensively used and are considered a common contaminant in the ocean, and it is anticipated as marine pollution. Moreover, Ag NPs’ dose-response evidence on cetacean leukocytes is vital for evaluating cetaceans’ adverse health consequences and implementing a marine mammal management strategy [218].

Hence, the above studies regarding human health and the environmental effect of Ag NPs mainly depend on its large amount of production and its unpredictable uses. Mainly, possible harmful effects have been observed because of its size and different factors associated with it. We should understand the fate of Ag NPs, first for a detailed analysis of it and the need to find out the gap of appropriate identification, evaluation, and disposal methods of Ag NPs.

4. Conclusions

Ag NPs have been the topic of interest for researchers for a long time because of its tunable properties. The nanoparticles are usually synthesized by reducing silver salts with appropriate reducing agents like sodium citrate, ascorbate, and sodium borohydride, and metabolites from biological sources. The antimicrobial properties made the Ag NPs appealing for controlling infectious diseases, water purification, and elimination of plant pathogens. However, more research is needed to use the particles beyond the laboratory. Synthesizing cost-effective, evenly distributed, well-dispersed nanoparticles is always challenging as not a single synthesis method is perfect. Similarly, the impact of silver nanoparticles on the environment and health may pose a problem in its widespread applications, so investigation on the accumulation and mechanism of action of Ag NPs inside the human body is required.
Abbreviations

NPs: Nanoparticles  
CNC: Cellulose nanocrystal  
POP: Polyoxypolyethylene-amine  
PNP: Polyamide network polymers  
NTA: Nitrilotriacetate  
UV-vis: Ultraviolet visible  
XRD: X-ray diffraction  
ATR-FTIR: Attenuated total reflectance Fourier transform infrared  
SEM: Scanning electron microscopy  
TEM: Transmission electron microscopy  
DPP-IV: Dipeptidyl peptidase  
AChE: Acetylcholinesterase  
BChE: Butyrylcholinesterase  
DPPH: 2,2-Diphenyl-1-picryl-hydrazyl-hydrate  
DNA: Deoxyribonucleic acid  
ROS: Reactive oxygen species  
CMC: Carboxymethylcellulose  
GLE: Geranium leaf extract  
NADPH: Nicotinamide adenine dinucleotide phosphate  
NSP: Nanoscale silica platelets  
SBMA: Sulfobetaine methacrylate  
NAD: Nicotinamide adenine dinucleotide  
AGEs: Advanced glycation end-products  
PVA: Polyvinyl alcohol  
PVP: Poly(N-vinylpyrrolidone)  
DMSO: Dimethyl sulfoxide  
OED: Optically induced electrochemical deposition  
bio-ITO: Indium tin oxide  
bio-Hb-BDDE: Hemoglobin-modified boron-doped diamond electrode  
DSC: Differential scanning calorimetry  
EPR: Electron paramagnetic resonance  
PVP: Poly(N-vinylpyrrolidone)  
DLS: Dynamic light scattering  
CZFMeS-FAU: Compact zeolite film-modified electrodes-faujasite  
EDX: Energy-dispersive X-ray  
XPS: X-ray photoelectron spectroscopy  
POP: Polyoxypropylene  
CNC: Cellulose nanocrystal  
FESEM: Field emission scanning electron microscopy.

Data Availability

Not available.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Authors’ Contributions

All authors have equally contributed to this project.

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