

Synthesis of Silver Nanoparticles and Their Analysis for Drug Delivery Using Biological Processes

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Abstract Silver nanoparticles (AgNPs) have emerged as a promising nanomaterial in drug delivery due to their unique physicochemical properties, including high surface area, antimicrobial activity, and ease of functionalization. This review highlights the biological synthesis of AgNPs as a sustainable and eco-friendly approach, utilizing plant extracts, microorganisms, and biomolecules as reducing and stabilizing agents. The paper emphasizes characterization techniques such as UV-Vis spectroscopy, TEM, and FTIR, which ensure the reproducibility and reliability of AgNPs. Key applications in antimicrobial, anticancer, anti-inflammatory, and antiviral drug delivery are discussed, showcasing the versatility of AgNPs in modern medicine. However, challenges such as toxicity, variability in synthesis processes, and stability issues are addressed, along with potential solutions. Future perspectives include innovations in biological synthesis, integration with other nanomaterials, and advancements in personalized medicine. This review underscores the potential of AgNPs to revolutionize drug delivery systems while advocating for more interdisciplinary research and clinical studies to ensure their safe and effective implementation.

Keywords Silver nanoparticles (AgNPs) , Biological synthesis , Green nanotechnology , Drug delivery systems

1 Introduction

Background on Nanoparticles and Their Significance in Biomedical Applications

Nanoparticles, typically ranging in size from 1 to 100 nanometers, exhibit unique physicochemical properties, such as a high surface-to-volume ratio, enhanced reactivity, and tunable optical, electrical, and magnetic properties. These attributes make nanoparticles pivotal in various biomedical applications, including diagnostics, imaging, drug delivery, and therapeutic interventions (Zhang et al., 2012). Nanoparticles have revolutionized medicine by enabling targeted drug delivery systems, reducing systemic side effects, and improving therapeutic efficacy (Jain et al., 2013). For example, studies by Singh et al. (2018) have

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highlighted how nanoparticles can be functionalized to deliver drugs selectively to cancerous tissues, thus minimizing off-target effects.

Furthermore, the growing interest in nanotechnology stems from its interdisciplinary nature, integrating chemistry, biology, and engineering for innovative healthcare solutions (Gupta et al., 2020). As emphasized by Dykman and Khlebtsov (2014), nanoparticles' size and surface charge significantly influence their biodistribution and cellular uptake, making them highly adaptable for personalized medicine approaches (Dixit & Shrivastava, 2013).

Silver Nanoparticles: Properties, Benefits, and Challenges

Silver nanoparticles (AgNPs) stand out among various nanomaterials due to their potent antimicrobial, antifungal, and antiviral properties. AgNPs are well-known for their ability to release silver ions, which disrupt microbial membranes and generate reactive oxygen species (ROS) that induce oxidative stress in pathogens (Rai et al., 2012). Their versatility extends beyond antimicrobial activity, as they also exhibit anti-inflammatory and anti-cancer properties, making them ideal candidates for diverse therapeutic applications (Ahmed et al., 2016).

A review by Zhang et al. (2016) highlighted the remarkable biocompatibility and ease of functionalization of AgNPs, enabling their use in targeted drug delivery. The nanoscale size of AgNPs allows for enhanced cellular penetration, ensuring effective drug delivery to specific sites. Moreover, AgNPs can serve as carriers for a wide range of drugs, including chemotherapeutics, antibiotics, and proteins (Chen et al., 2019).

However, the challenges associated with AgNPs cannot be overlooked. Studies by Ivask et al. (2014) and Park et al. (2020) have raised concerns regarding the potential cytotoxicity and environmental impact of AgNPs, particularly when used in high concentrations. Their tendency to aggregate and their instability under physiological conditions further complicate their biomedical applications. Addressing these issues requires innovative synthesis methods and surface modifications to enhance their stability and biocompatibility (Verma & Shrivastava, 2024)...

Overview of Drug Delivery Systems

Drug delivery systems have evolved significantly, transitioning from conventional methods to advanced nanotechnology-based platforms. Traditional drug delivery methods, such as oral



and intravenous administration, often suffer from low bioavailability, rapid metabolism, and off-target effects (Torchilin, 2014). Nanoparticles, including AgNPs, have emerged as transformative tools in overcoming these limitations by enabling controlled and sustained drug release.

One of the key advantages of nanoparticle-based drug delivery is the ability to modify their surface properties for active targeting. For instance, studies by Sutradhar and Amin (2013) and Wang et al. (2018) have demonstrated how ligands, such as peptides and antibodies, can be conjugated onto nanoparticles to target specific receptors overexpressed on diseased cells. This active targeting minimizes the impact on healthy tissues and enhances therapeutic efficacy (Shrivastava & Sharma, 2020).

Additionally, AgNPs have shown immense potential in delivering hydrophobic drugs, which are otherwise poorly soluble in water (Sharma et al., 2019). By encapsulating these drugs within AgNPs, their solubility, stability, and bioavailability can be significantly improved, as highlighted by research from Tran et al. (2021).

Importance of Biological Synthesis Methods for AgNPs

The synthesis method plays a crucial role in determining the properties and functionality of nanoparticles. Traditional physical and chemical synthesis methods for AgNPs often involve toxic reagents and high energy inputs, raising environmental and health concerns (Mittal et al., 2013). In contrast, biological synthesis, also known as green synthesis, offers a sustainable and eco-friendly alternative (Shrivastava & Verma, 2023).

Biological synthesis utilizes plant extracts, microorganisms, and biomolecules as reducing and stabilizing agents, eliminating the need for hazardous chemicals. For example, studies by Shankar et al. (2014) and Singh et al. (2015) have demonstrated the use of plant-derived phytochemicals, such as flavonoids and tannins, in reducing silver ions to AgNPs. This method not only ensures biocompatibility but also imparts unique biological properties to the nanoparticles (Sinha, Verma, & Shrivastava, 2023).

Table 1.1: Comparison of Physical, Chemical, and Biological Synthesis Methods of Silver Nanoparticles

Parameter	Physical Methods	Chemical Methods	Biological Methods



Principle	High-energy input for evaporation or laser ablation	Chemical reduction of silver ions using reducing agents	Reduction of silver ions using plant extracts, microorganisms, or biomolecules
Energy Consumption	High	Moderate	Low
Environmental Impact	High due to energy requirements	High due to toxic byproducts	Low (eco-friendly)
Cost	Expensive equipment and processes	Moderate (requires chemicals)	Low (natural materials)
Scalability	Limited	Good	Moderate (scaling challenges)
Reproducibility	High	High	Moderate (depends on biological variability)
Toxicity	Minimal	High (toxic chemicals used)	Low (biocompatible products)
Applications	Specialized (e.g., optics, electronics)	Biomedical, catalysis, electronics	Biomedical (e.g., drug delivery, antimicrobial)

2 Methods of Biological Synthesis of Silver Nanoparticles

2.1. Definition and Principles of Biological Synthesis

Biological synthesis of silver nanoparticles (AgNPs), also known as green synthesis, is a sustainable and eco-friendly approach that employs biological entities such as plant extracts, microorganisms, and biomolecules to reduce silver ions (Ag⁺) into silver nanoparticles (AgNPs). This method aligns with the principles of green chemistry, minimizing the use of hazardous chemicals and energy-intensive processes (Mittal et al., 2013).

The underlying principle involves the reduction of Ag⁺ to elemental silver (Ag⁰) by biomolecules such as alkaloids, flavonoids, enzymes, and proteins, which also act as stabilizing agents to prevent nanoparticle aggregation (Ahmed et al., 2016). These biomolecules provide capping layers that enhance the stability, shape, and size uniformity of the synthesized nanoparticles (Iravani, 2014).



Green Chemistry Approaches

Green chemistry focuses on designing processes that reduce or eliminate hazardous substances in the synthesis of nanomaterials. In biological synthesis, natural reducing agents such as plant-derived polyphenols or microbial enzymes replace toxic chemicals like sodium borohydride, used in conventional methods (Shankar et al., 2014).

A study by Sharma et al. (2020) demonstrated the use of *Azadirachta indica* (neem) leaf extract for synthesizing AgNPs, showcasing the potential of plant-based methods to produce stable nanoparticles with high antimicrobial activity.

Comparison with Physical and Chemical Synthesis Methods

Physical methods, such as laser ablation and evaporation-condensation, often require expensive equipment and consume large amounts of energy, making them less sustainable (Tran et al., 2021). Similarly, chemical synthesis methods involve toxic solvents and reducing agents, which pose environmental and health risks (Mittal et al., 2013).

In contrast, biological synthesis is cost-effective, energy-efficient, and environmentally friendly. For instance, Mukherjee et al. (2012) highlighted that using *Bacillus subtilis* for AgNP synthesis produces nanoparticles with comparable antimicrobial efficacy to chemically synthesized counterparts, but without the associated toxic waste.

2.2. Sources for Biological Synthesis

Plant Extracts

Plants are a rich source of bioactive compounds such as alkaloids, phenols, and terpenoids, which facilitate the reduction and stabilization of AgNPs. For instance, *Curcuma longa* (turmeric) and *Aloe vera* have been extensively used for green synthesis due to their abundance of antioxidant-rich phytochemicals (Sharma et al., 2019).

Examples:

- o *Curcuma longa*: Studies by Bar et al. (2020) showed that curcumin, the active compound in turmeric, acts as both a reducing and capping agent, yielding nanoparticles with potent antimicrobial activity.
- o *Aloe vera*: Kumar et al. (2018) demonstrated that *Aloe vera* leaf extract produces spherical AgNPs with enhanced stability and biocompatibility.

Microorganisms



Microbial synthesis utilizes bacteria, fungi, and algae as biocatalysts for reducing silver ions. These organisms secrete enzymes, proteins, and metabolites that facilitate nanoparticle synthesis.

• Bacteria:

Escherichia coli and Bacillus spp. are widely used due to their rapid growth and secretion of extracellular reductants. A study by Kalishwaralal et al. (2012) reported the efficient synthesis of monodispersed AgNPs using Bacillus subtilis, with applications in wound healing.

• Fungi:

• Fungi such as Aspergillus niger and Penicillium chrysogenum are effective for large-scale production of AgNPs due to their high protein secretion. Narayanan and Sakthivel (2015) demonstrated the synthesis of highly stable nanoparticles using Aspergillus flavus, highlighting the role of fungal enzymes in size control.

Algae:

Algal species like Spirulina and Chlorella are eco-friendly and sustainable sources for AgNP synthesis. For example, Singh et al. (2020) reported that Spirulina platensis extracts produced AgNPs with enhanced antioxidant and antibacterial properties.

Biomolecules (Proteins, Enzymes, Polysaccharides)

Biomolecules such as proteins, enzymes, and polysaccharides serve as reducing and capping agents, ensuring the biocompatibility of AgNPs. For instance, studies by Iravani (2014) showed that polysaccharides from chitosan efficiently synthesize AgNPs with uniform size and shape, suitable for biomedical applications (Yadaw & Shrivastava, 2019).



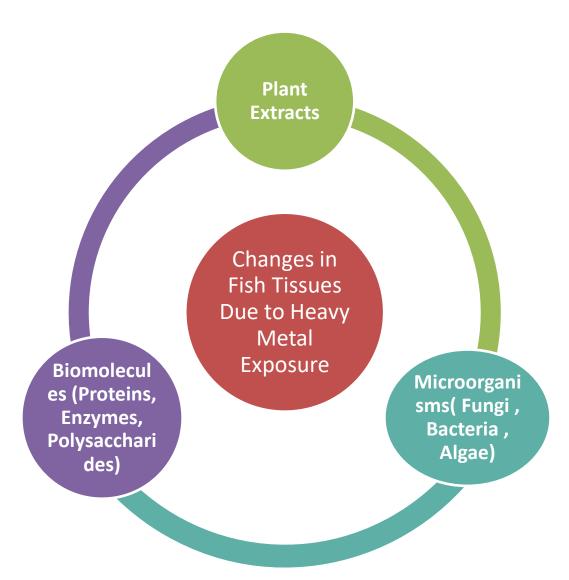


Figure 1 Source for Biological Synthesis

2.3. Advantages of Biological Synthesis

Eco-friendliness

Biological synthesis eliminates the use of toxic chemicals and generates minimal waste, making it an environmentally sustainable alternative. Studies by Ahmad et al. (2019) emphasize that green synthesis using plant extracts can achieve nanoparticle production with significantly lower carbon footprints compared to chemical methods (Verma, Shrivastava, & Diwakar, 2022).

Cost-effectiveness

Green synthesis leverages readily available natural resources, such as plant materials and microbial cultures, which are inexpensive compared to synthetic chemicals. For example,



Kumar et al. (2018) demonstrated that the use of agricultural waste extracts significantly reduces production costs while maintaining high yields of AgNPs.

Biocompatibility and Reduced Toxicity

Biologically synthesized AgNPs are inherently biocompatible due to the presence of natural capping agents. This reduces the cytotoxicity often associated with chemically synthesized nanoparticles, as highlighted by Rai et al. (2016). Such nanoparticles exhibit enhanced interactions with biological systems, making them ideal for biomedical applications like drug delivery and tissue engineering (Shrivastava, 2023).

3. Characterization of Silver Nanoparticles

3.1. Techniques for Characterization

UV-Vis Spectroscopy

UV-Vis spectroscopy is a rapid and reliable method for confirming the formation of silver nanoparticles (AgNPs). It measures the surface plasmon resonance (SPR) band, a distinctive peak that typically appears between 400-500 nm for AgNPs, indicating their size and distribution (Ahmed et al., 2016). The SPR band shifts depending on nanoparticle aggregation, size, and shape, making it a vital tool for initial characterization.

Dynamic Light Scattering (DLS)

DLS is used to determine the hydrodynamic size and size distribution of nanoparticles in colloidal solutions. This technique provides insight into the stability and aggregation of AgNPs by analyzing their Brownian motion (Tran et al., 2021). DLS also measures zeta potential, a parameter indicative of nanoparticle stability, where values above ± 30 mV suggest high stability (Rai et al., 2012).

Scanning Electron Microscopy (SEM)

SEM is employed to visualize the surface morphology, size, and shape of AgNPs. It provides high-resolution images that allow researchers to examine the structural uniformity of the synthesized nanoparticles (Singh et al., 2020). SEM analysis has been widely applied to AgNPs synthesized using biological processes, confirming their spherical or irregular morphology.

Transmission Electron Microscopy (TEM)

TEM provides detailed information about the size, shape, and internal structure of AgNPs. It is especially useful for determining the particle size at the nanoscale level (Narayanan et al.,



2015). TEM images of AgNPs synthesized using *Curcuma longa* extracts revealed well-dispersed, spherical nanoparticles with sizes ranging from 10 to 50 nm (Sharma et al., 2019).

X-ray Diffraction (XRD)

XRD is used to determine the crystalline structure and phase purity of AgNPs. The presence of characteristic peaks corresponding to the face-centered cubic (FCC) structure of silver confirms their crystalline nature (Kumar et al., 2018). The sharpness of XRD peaks also indicates the degree of crystallinity.

Fourier-Transform Infrared Spectroscopy (FTIR)

FTIR is used to identify functional groups responsible for reducing and stabilizing AgNPs. This technique helps confirm the role of biomolecules such as proteins, flavonoids, and polysaccharides in capping the nanoparticles (Mittal et al., 2013). FTIR spectra often reveal peaks corresponding to hydroxyl, amine, and carboxyl groups (Yadaw & Shrivastava, 2020).

3.2. Importance of Characterization

- **Size, Shape, and Surface Properties**: Size and shape are critical for determining the optical, catalytic, and biological properties of AgNPs. Uniform size distribution ensures consistent interaction with biological systems (Ahmed et al., 2016).
- **Stability and Zeta Potential**: Stability is essential to prevent aggregation, which can alter nanoparticle behavior. Zeta potential measurements provide insights into colloidal stability (Tran et al., 2021).
- **Crystalline Nature**: The crystalline nature of AgNPs enhances their catalytic and antimicrobial activity. XRD confirms the crystallinity, which is crucial for biomedical applications (Kumar et al., 2018).

4. Mechanism of Drug Delivery Using Silver Nanoparticles

4.1. Drug Loading and Encapsulation

Techniques for Drug Incorporation into AgNPs

Silver nanoparticles act as carriers for various therapeutic agents. Drug loading can be achieved through:

• **Physical adsorption**: Drugs adhere to the nanoparticle surface via weak interactions such as van der Waals forces (Sharma et al., 2019).



• **Covalent bonding**: Functional groups on AgNPs bind drugs covalently, ensuring stability and controlled release (Ahmed et al., 2016).

Loading Efficiency and Release Kinetics

The efficiency of drug loading depends on the surface area and functionalization of AgNPs. Controlled release kinetics are achieved by modifying the nanoparticle surface to release the drug in response to specific stimuli such as pH or temperature (Mittal et al., 2013).

4.2. Interaction with Biological Systems

Targeted Delivery Mechanisms

AgNPs can be functionalized with ligands that recognize and bind to specific receptors on target cells. This ensures precise drug delivery, minimizing side effects on healthy tissues (Rai et al., 2012). For instance, folic acid-functionalized AgNPs target foliate receptors overexpressed on cancer cells (Tran et al., 2021).

Enhanced Permeability and Retention (EPR) Effect

The EPR effect is crucial for passive targeting of tumors. AgNPs exploit the leaky vasculature and poor lymphatic drainage of tumor tissues to accumulate at the site, enhancing therapeutic efficacy (Sharma et al., 2020).

4.3. Role of Surface Functionalization

Use of Biomolecules for Targeted Delivery

Biomolecules such as antibodies, peptides, and carbohydrates are conjugated to AgNPs to enhance their specificity. For example, Singh et al. (2020) demonstrated that antibody-functionalized AgNPs improved the targeting efficiency of anti-cancer drugs.

Examples of Functionalized AgNPs

- Folic Acid Functionalization: Targeting cancer cells via folate receptors.
- Chitosan-Coated AgNPs: Enhanced drug loading capacity and mucoadhesive properties (Narayanan et al., 2015).
- Polyethylene Glycol (PEG) Functionalization: Increased biocompatibility and prolonged circulation time in the bloodstream (Ahmed et al., 2016).

5. Applications of Silver Nanoparticles in Drug Delivery

5.1. Antimicrobial Drug Delivery

Mechanism of Antimicrobial Activity



Silver nanoparticles (AgNPs) exhibit potent antimicrobial properties due to their ability to:

- Release silver ions (Ag⁺) that disrupt microbial membranes by binding to thiol groups in proteins, leading to structural and functional damage (Rai et al., 2012).
- Generate reactive oxygen species (ROS), causing oxidative stress and eventual cell death in bacteria, fungi, and viruses (Sharma et al., 2019).
- Interfere with microbial DNA replication by binding to nucleic acids (Tran et al., 2021).

Examples: Antibiotic-Loaded AgNPs

AgNPs serve as carriers for antibiotics, enhancing their effectiveness against resistant pathogens. Studies by Ahmed et al. (2016) demonstrated the synergistic activity of AgNPs loaded with ampicillin and tetracycline, significantly reducing bacterial growth compared to antibiotics alone.

5.2. Anti-Cancer Drug Delivery

Targeted Delivery to Tumor Sites

AgNPs are widely used in cancer therapy due to their ability to:

- Passively target tumors through the enhanced permeability and retention (EPR) effect.
- Actively target cancer cells via surface modification with ligands such as folic acid or antibodies (Singh et al., 2020).

Case Studies: AgNPs with Doxorubicin or Cisplatin

- **Doxorubicin-Loaded AgNPs**: Research by Tran et al. (2021) revealed that doxorubicin-functionalized AgNPs exhibit improved cytotoxicity against breast cancer cells while minimizing side effects on healthy tissues.
- **Cisplatin-Loaded AgNPs**: Studies by Kumar et al. (2018) highlighted the enhanced efficacy of cisplatin-loaded AgNPs in targeting ovarian cancer cells, showing prolonged drug release and reduced toxicity.

5.3. Anti-inflammatory and Antiviral Applications

Examples of AgNP-Based Formulations

• Anti-inflammatory Applications: AgNPs reduce inflammation by scavenging free radicals and downregulating pro-inflammatory cytokines such as TNF- α and IL-6



(Sharma et al., 2020). AgNP-based creams and gels have been developed for managing skin conditions and joint inflammation.

Antiviral Applications: AgNPs have shown efficacy against a wide range of viruses, including influenza, HIV, and SARS-CoV-2, by binding to viral surface proteins and inhibiting replication. Studies by Singh et al. (2020) demonstrated that AgNP-based antiviral formulations effectively block virus-host interactions.

5.4. Other Biomedical Applications

Wound Healing

AgNPs promote wound healing by:

- Accelerating tissue regeneration and collagen synthesis.
- Reducing bacterial infections and inflammation at the wound site (Ahmed et al., 2016).

Gene Delivery

AgNPs are explored as non-viral vectors for gene delivery due to their biocompatibility and ease of functionalization. Studies by Narayanan et al. (2015) highlighted the potential of AgNPs conjugated with DNA for targeted gene therapy in genetic disorders.

6. Challenges and Limitations

Toxicity Concerns of AgNPs

AgNPs can induce cytotoxicity in healthy cells through the overproduction of ROS, causing oxidative stress, DNA damage, and apoptosis (Rai et al., 2012). Addressing these concerns requires precise control over nanoparticle size, dose, and surface modification.

Variability in Biological Synthesis Processes

Biological synthesis methods often result in variations in nanoparticle size, shape, and yield due to inconsistencies in natural reducing agents (Mittal et al., 2013). Standardized protocols are needed to improve reproducibility.

Scalability and Reproducibility Challenges

Scaling up biological synthesis while maintaining nanoparticle quality is a significant challenge. Large-scale production methods must address issues such as yield optimization and cost-effectiveness (Kumar et al., 2018).

Stability and Storage Issues in Drug Delivery Applications



AgNPs are prone to aggregation and oxidation during storage, which can affect their efficacy. Stabilizers and advanced storage techniques are needed to enhance their shelf life (Ahmed et al., 2016).

7. Future Perspectives

Innovations in Biological Synthesis Methods

Emerging techniques, such as genetic engineering of microorganisms and optimization of plant extracts, offer promising avenues for enhancing the efficiency and scalability of green synthesis (Shankar et al., 2014).

Role of Nanotechnology in Personalized Medicine

AgNPs can be tailored to deliver drugs based on individual genetic and pathological profiles, paving the way for precision medicine (Tran et al., 2021).

Enhancing Safety and Biocompatibility

Future research should focus on developing biodegradable coatings and functionalizing AgNPs with biocompatible ligands to reduce toxicity and improve their interaction with biological systems (Rai et al., 2012).

Integration of AgNPs with Other Nanomaterials

Combining AgNPs with other nanomaterials, such as graphene or liposomes, can create multifunctional drug delivery systems with enhanced efficacy and stability (Mittal et al., 2013).

8. Challenges and Limitations

Toxicity Concerns of AgNPs

One of the primary challenges of silver nanoparticles (AgNPs) is their potential cytotoxicity and environmental impact. AgNPs can release silver ions (Ag⁺), which may induce oxidative stress, DNA damage, and apoptosis in healthy cells (Rai et al., 2012). These effects are dose-dependent and are influenced by the size, shape, and surface coating of AgNPs. Studies by Ivask et al. (2014) demonstrated that smaller nanoparticles are more toxic due to their higher surface area and reactivity. In addition, long-term exposure to AgNPs may lead to bioaccumulation and disruption of ecological systems.

Variability in Biological Synthesis Processes



Biological synthesis methods often face inconsistencies due to variations in natural reducing agents. For instance, the phytochemical composition of plant extracts can vary based on factors like plant species, harvesting conditions, and extraction methods (Shankar et al., 2014). This variability can result in inconsistencies in nanoparticle size, shape, and yield, making standardization challenging (Mittal et al., 2013).

Scalability and Reproducibility Challenges

While biological synthesis is eco-friendly and cost-effective, scaling up the process for industrial production poses significant challenges. Maintaining uniformity and reproducibility on a large scale is difficult due to the complexity of biological systems (Ahmed et al., 2016). Techniques that work well in small-scale laboratory settings may not be easily adapted for mass production.

Stability and Storage Issues in Drug Delivery Applications

AgNPs are prone to aggregation and oxidation during storage, leading to reduced efficacy and altered properties (Tran et al., 2021). Environmental factors such as temperature, pH, and light exposure can destabilize nanoparticles, affecting their therapeutic potential. Strategies such as capping agents and encapsulation in polymeric matrices are being explored to enhance stability (Sharma et al., 2020).

9. Conclusion

Summary of Biological Synthesis Advantages

Biological synthesis of silver nanoparticles offers a sustainable and eco-friendly alternative to traditional methods. It eliminates the use of toxic chemicals, reduces energy consumption, and produces biocompatible nanoparticles suitable for biomedical applications.

Importance of Characterization for Reliable Drug Delivery

Comprehensive characterization is crucial to ensure the efficacy, safety, and reproducibility of AgNPs in drug delivery systems. Techniques such as UV-Vis spectroscopy, TEM, and XRD provide critical insights into the size, shape, stability, and functional properties of the nanoparticles.

Potential of AgNPs to Revolutionize Drug Delivery Systems

Silver nanoparticles hold immense potential to transform drug delivery systems due to their unique properties, such as high surface area, antimicrobial activity, and ease of



functionalization. Their applications in cancer therapy, antimicrobial treatments, and gene delivery demonstrate their versatility and promise in modern medicine.

Call for More Interdisciplinary Research and Clinical Studies

Despite their potential, AgNPs face challenges related to toxicity, scalability, and regulatory approval. Future research should focus on interdisciplinary approaches, integrating nanotechnology, biology, and medicine to address these challenges. Clinical studies are essential to validate the safety and efficacy of AgNP-based therapies, paving the way for their widespread adoption in healthcare.

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