

**Review Article**

Production, Analysis, and Impact of Bimetallic Nanoparticles Generated Through Environmentally-Friendly Methods

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Abstract: Nanotechnology has become an increasingly popular topic in recent years, with exciting advancements capturing the imagination of scientists and researchers. However, traditional methods of synthesizing nanoparticles using chemical and physical processes have raised concerns about potential environmental risks. To address these concerns, sustainable approaches to nanoparticle synthesis have been developed, utilizing safe and eco-friendly secondary metabolites from plants as reducing and capping agents. This environmentally friendly process is not only simple and cost-effective but also minimizes ecological impact. Nanoparticles, often hailed as "tiny heroes," offer a wide range of technological applications, revolutionizing various industries. Bimetallic nanoparticles (BMNPs) are of particular interest due to their unique blending patterns and synergistic effects when different metal nanoparticles are combined. Compared to metallic nanoparticles, BMNPs exhibit superior performance in optical, electrical, and medical applications. Moreover, synthesizing BMNPs from plant and microbial sources is environmentally benign, cost-effective, and time-efficient. One significant advantage of BMNPs is their large surface area and small size, which make them highly effective catalysts. Their exceptional catalytic properties have found applications in diverse industries, enabling enhanced chemical reactions. Additionally, BMNPs have shown promise as biosensors, antimicrobials, and in groundwater remediation efforts. They have also demonstrated potential in drug delivery systems, offering improved therapeutic outcomes. This review focuses on the preparation, properties, and bio-applications of BMNPs, highlighting recent advancements in the field. By exploring the latest research, we aim to provide a comprehensive overview of BMNP technology and its potential impact. In summary, sustainable methods of nanoparticle synthesis utilizing plant-derived secondary metabolites have emerged as a solution to environmental concerns. BMNPs, with their unique characteristics and broad applications, have become a focal point in nanotechnology research. With ongoing advancements and research, BMNPs hold great promise in shaping a sustainable and technologically advanced future.

Keywords: Nanotechnology, Bimetallic Nanoparticles, Green Synthesis, Characterization

1. Introduction

The field of "Nanoscience" deals with the study of objects that are intermediate in size between the largest molecules and the smallest structures that can be fabricated using modern photolithography. This technology focuses on objects with dimensions ranging from a few nanometers to less than one hundred nanometers. In chemistry, this size range has typically been associated with colloids, micelles, polymer

molecules, phase-separated areas in block copolymers, and other similar structures. These structures are usually very large molecules or aggregates of many molecules. However, in recent times, nanostructures such as buckytubes, silicon nanorods, and compound semiconductor quantum dots have emerged as particularly exciting classes of structures. [1]. Nanotechnology is the manipulation of matter at a nanometer scale to create new materials and devices with unique properties and functionalities [2].

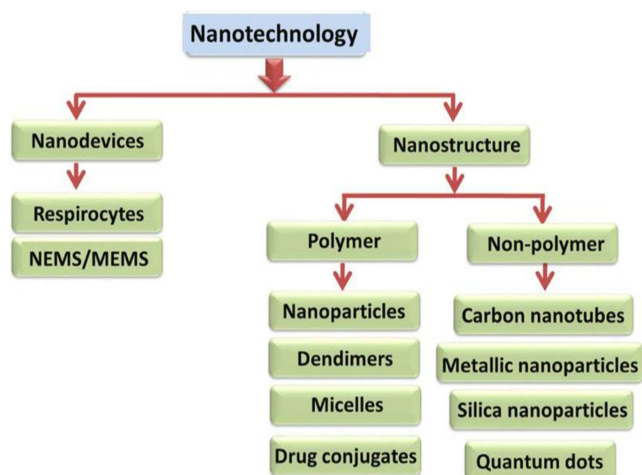


Figure 1. Classification of nanotechnology.

Nanomaterials (NMs) are versatile and have diverse applications in energy, water, medical, agriculture, material science, and cosmetics. Nanotechnology involves creating materials at the atomic, molecular, and supermolecular levels, mainly using NMs with dimensions of 1-100 nm. "Nano" comes from the Greek word "nanos," which means dwarf, representing an estimated one billionth of a meter in diameter and includes frameworks above and below typical measurements (i.e., >1 nm and 100 nm). [3].

Nanoparticles (NPs) have a wide range of applications in various fields, including electrical and thermal conductivity, catalysis, health, food, aerospace, and agriculture. Their high surface-to-volume ratio and increased optical activities make them highly useful compared to their mass state. The importance of nanotechnology in biology, medicine, and industry is increasingly recognized by scientists, technologists, and researchers. Nanotechnology involves fabricating materials at the atomic, molecular, and supermolecular levels, mainly using nanomaterials (NMs) with diameters between one and one hundred nanometers. [4]

Nanomaterials (NMs) are gaining significance in technological advancement due to their distinct, flexible, and adaptive optical, mechanical, electrical, biological catalytic, and magnetic properties that are superior to those of their bulk form. Their unique features are a result of their high surface area and size, which can be tuned to fit their application. The size, shape, and structure of NMs also impact their reactivity, toughness, and other characteristics. NMs have a wide range of applications in various fields, including environmental, imaging, medical, therapeutic, energetic, catalytic, aesthetic, and therapeutic purposes. Metal oxide nanoparticles (NPs) like copper oxide (CuO), tin oxide (SnO₂), zinc oxide (ZnO), and titanium dioxide (TiO₂) are among the most studied NMs due to their wide range of unique features and applications. [5]

Green nanotechnology is a branch of green chemistry that is based on the twelve principles of green chemistry and offers a viable alternative to traditional nanotechnology. It employs safe and clean methods for the synthesis of eco-friendly nanoproducts, using nontoxic chemicals, low temperatures, benign solvents, and producing innocuous waste [6].

Synthetic nanotechnology is being avoided due to the use of harmful chemicals and the production of adverse byproducts that are not eco-friendly.

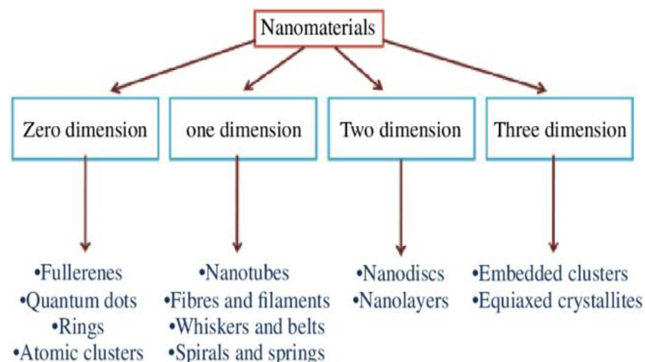


Figure 2. Classification of Nanomaterials.

As a result, green nano-chemistry is replacing synthetic nano-chemistry due to the use of safe chemicals that are not toxic to human health and the production of eco-friendly byproducts. [7]. Three methods are used to synthesize NPs: chemical, physical, and biological. Chemical synthesis requires specific temperature and pressure maintenance and produces toxic by-products, making it not eco-friendly. Physical synthesis does not cause solvent pollution but requires high temperature and pressure for the furnace, polluting the surrounding environment. Biological synthesis, on the other hand, uses biological agents such as microorganisms and plants, making it a more eco-friendly and sustainable method [8]. The biological method is the best approach for preparing NPs. This method involves using plant extracts to reduce metal ions into metal atoms, with biomolecules from the extract acting as both capping and reducing agents. It is a simple process carried out at normal temperature and pressure. Fungi and different types of algae can also be used to produce metallic NPs with protein stabilization. However, maintaining a culture medium for fungi and algae can be difficult. Therefore, the preferred method for producing metal NPs is using metal ions with plant extract [9].

2. Literature Review

2.1. Metal Nanoparticles

Faraday was the first to recognize the existence of metal nanoparticles, while Mie provided a quantitative explanation of their color. Metal nanoparticles are widely used in catalysis due to their unique physical and chemical properties, such as high surface area, catalytic activity, and selectivity, sensing, and optoelectronics. Metal nanoparticles such as copper, zinc, gold, magnesium, silver, and titanium exhibit unique properties based on their size, shape, and structure, making them useful in various fields such as electrical, medicinal, magnetic, and catalytic applications. They have antibacterial properties and are used in medicine, dental materials, water treatment, sunscreen lotions, and coatings. Nanoparticles

made of metal, metal oxides, ceramics, silicates, and polymers have been synthesized and used in several applications due to their small size and high surface area. Their properties are size-dependent and are influenced by the surrounding medium. By changing the environment of nanoparticles, the desired properties can be obtained. [10].

2.2. Monometallic Nanoparticles

Monometallic nanoparticles (MNPs) are composed of a single metal atom, which determines their properties. They can be classified into different types based on the metal atom present, such as magnetic, metallic, and transition metal nanoparticles. The chemical method is the most common route for their synthesis, and their structure can be stabilized using various functional groups. In recent decades, there has been increasing interest in metallic nanoparticles due to their enhanced physical and chemical properties [11]. Due to their enhanced physical and chemical properties, monometallic nanoparticles (MNPs) find applications in various fields such as electronics, optics, and catalysis. They are also used as antimicrobial agents against different microorganisms including *Escherichia coli*, *Streptococcus mutans*, and *Streptococcus pyogenes* [12].

2.3. Bimetallic Nanoparticles

Since the last decade, there has been a growing interest in the synthesis of monometallic nanoparticles using green methodologies. Now a day's many researchers have started synthesizing bimetallic nanoparticles using plant and microbial origin products due to their advantages over monometallic nanoparticles [13].

Bimetallic nanoparticles, composed of two different metals, offer enhanced properties compared to monometallic nanoparticles, making them attractive for various applications. They can be synthesized using green methodologies, which are more sustainable and eco-friendly [14]. The properties of bimetallic nanoparticles are determined by the constituent metals and their nanometric size. These nanoparticles are synthesized by combining different architectures of metallic nanoparticles.

Bimetallic nanoparticles offer the ability to optimize the energy of Plasmon absorption bands, making them useful for biosensing. They exhibit unique size-dependent properties such as optical, electronic, thermal, and catalytic effects, which differ from those of pure elemental particles. Extensive studies on bimetallic nanoparticles began just a decade ago, and different methods have been proposed for their preparation and characterization. Researchers are focusing on selectively preparing new bimetallic nanoparticles in different forms, such as alloys, core-shell, and contact aggregate. Bimetalization can greatly improve the catalytic properties of resulting nanoparticles beyond what is achievable with monometallic catalysts.

Bimetallic catalysts exhibit enhanced catalytic activity and selectivity due to synergistic effects between two metals. The electronic interaction between the metals influences catalyst

stability, reactant adsorption, and activation. Alloying the constituent elements can result in structural changes in bimetallic nanoparticles [15]. The catalytic activities of various bimetallic nanoparticles have been compared using different methods and correlations developed through physical and spectroscopic measurements. The structure and miscibility of the two metals in bimetallic nanoparticles are determined by the preparation conditions.

Metal nanoparticles can be categorized based on their origin, size, and structural characteristics, and can be synthesized using physical, chemical, or biological methods, with the latter being more environmentally friendly. Bimetallic nanoparticles are more interesting than metal nanoparticles because of their unusual mixing patterns and superior optical, electrical, and medicinal applications. When two metal nanoparticles combine to produce a bimetallic, they exhibit synergistic effects. Synthesizing bimetallic nanoparticles from plants and bacteria has become a hot topic due to their environmentally friendly, less expensive, and quicker synthesis. Because of their large surface area and small size, bimetallic nanoparticles are particularly useful as catalysts, but they can also be used as biosensors, antimicrobials, in groundwater remediation, and for medication delivery [16].

Bimetallic oxide nanoparticles, such as CuO-ZnO, have been used in various fields due to their unique chemical and physical properties. These properties include higher conductivity, high sensitivity, thermal stability, and hetero-contact between p-type CuO and n-type ZnO. CuO-ZnO nanoparticles have been used in photocatalysis, H₂ production, dye-sensitized solar cells, supercapacitor applications, sensors, and more [17].

2.3.1. Types of Bimetallic Nanoparticles

The review focuses on different architectures of bimetallic nanoparticles, including the Crown Jewel structure, Hollow structure, Heterostructure, Core Shell structure, Alloyed structure, and Porous structure. In the Crown Jewel structure, a single metal atom, the "jewel" atom, is carefully placed on the surface of another metal, typically expensive materials like Au, Ag, or Pt. The Hollow structure has a large pore volume and high surface-to-volume ratio, making it useful in nanoreactors. In the Heterostructure, one metal grows branches over another's nanocrystalline form. The Core Shell structure has one metal serving as the active shell and another as the core, showing promise as efficient catalysts. Bimetallic alloys are created when two different metal atoms are evenly distributed within a single particle, resulting in the Alloyed structure. Lastly, Porous structure bimetallic alloys have increased surface area, low density, and high gas permeability, making them better catalysts than their solid counterparts [18].

The atomic ordering in bimetallic structures, with similar atomic ratios and compositions, plays an important role in determining the overall properties of the materials. Mixed structures can have a random or ordered atomic arrangement. Alloyed nanoparticles have a random arrangement, while intermetallic structures have an ordered arrangement. Solid solution structures occur when one metal dissolves in a

different metal in the solid state, and can be substitutional or interstitial depending on the size difference between the metal atoms. Segregated structures include subcluster and core-shell structures, where two metals are separate or a metal core is surrounded by a second metal. Subcluster structures with a Janus configuration have two metals exposed to the nanoparticle's environment.

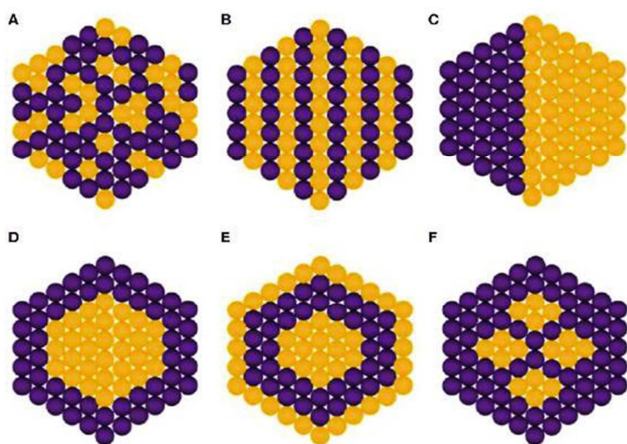


Figure 3. Shows different types of bimetallic nanoparticles, including (a) alloyed, (b) intermetallic, (c) subclusters, (d) core-shell, (e) multi-shell core-shell, and (f) multiple core materials coated by a single shell material. The yellow and purple spheres represent two different kinds of metal atoms.

Unlike mixed structures, segregated structures are achieved in multi-step reactions in which the second metal is added once the first metal has formed an initial structure. Examples of segregated structures include the core-shell structure, the multi-shell core-shell structure, in which the shells are in an alternating arrangement, similar to onion rings, or the multiple small cores coated by a single shell. Various synthetic strategies used for the formation of all types of atomically ordered structures are discussed in the following section [18].

2.3.2. Advantages of Bimetallic Nanoparticles over Monometallic Nanoparticles

Bimetallic nanoparticles have several advantages over monometallic nanoparticles. They exhibit distinctive properties and enhanced functionality through the combination of patterns and geometrical architecture. Compared to monometallic nanoparticles, they exhibit greater stability, selectivity, and catalytic activity. Bimetallic nanoparticles can accomplish certain chemical transformations that were previously impossible with monometallic nanoparticles as catalysts. This is because bimetallic nanoparticles contain a specific combination of two metals that each perform a specific function, allowing for the entire reaction mechanism to take place [19].

The combination of two metal nanoparticles in bimetallic nanoparticles can exhibit novel features that expand their functionality and range of applications. The physical and chemical characteristics of bimetallic nanoparticles can be altered by changing their component parts and geometrical layouts. Bimetallic nanoparticles synthesized from natural extracts have several advantages, including synthesis at room

temperature and the absence of toxic solvents, making the technique cost-effective and environmentally friendly. [20]. Bimetallic nanoparticles have unique mixing patterns and geometrical architecture, which enhances their functionality. Natural extracts are commonly used as a reducing and stabilizing agent for bimetallic nanoparticles. These nanoparticles have significant potential in medicine and pharmacy due to their proven biological activities, including catalytic, antioxidant, antibacterial, antidiabetic, antitumor, hepatoprotective, and regenerative effects. The synergistic effect between the two metals in bimetallic nanoparticles contributes to their enhanced biological activity [21].

Metallic nanoparticles have the ability to remove free radicals, but bimetallic nanoparticles synthesized at low reactant concentrations are even more active in this regard (98). Bimetallic nanoparticles containing noble transition metals such as silver, gold, platinum, and palladium are of great interest for biomedical applications because they offer safety and stability in the biological environment, with low toxicity. [22]

2.3.3. Properties of Bimetallic Nanoparticles

The properties of bimetallic nanoparticles can be significantly different from those of the elemental monometallic nanoparticles. Some of these properties are discussed below:

Catalytic properties

When different metal nanoparticles are combined, bimetallic nanoparticles with higher catalytic properties can be formed. These properties have significant advantages for scientific and technological developments. For instance, combining equal atomic concentrations of gold and silver nanoparticles can enhance surface area and reduce density, making them useful in various catalytic reactions [23].

Optical properties

The plasmonic coupling between nanoparticles results in interesting optical properties. Excited surface electrons exhibit characteristic absorption that leads to a colored nanoparticle dispersion. The absorption wavelength of the surface plasmon resonance (SPR) is dependent on the individual elements. In bimetallic nanoparticles, the SPR reveals the internal distribution of the elements [23].

Antibacterial properties

One of the most significant characteristics of nanoparticles is their ability to penetrate cells and release bioactive ions. Gold-silver bimetallic nanoparticles have been found to exhibit superior antibacterial activity against pathogenic bacteria such as *Staphylococcus aureus* and *Klebsiella pneumoniae*. [24].

Thermal conductivity

Nanofluids that contain silver-gold bimetallic nanoparticles in water have been found to enhance the thermal conductivity of fluids [23].

2.4. Examples of Bimetallic Nanoparticles

Scientists are developing novel bimetallic nanoparticles of desired properties. Some of the examples of such

nanoparticles are discussed below:

2.4.1. Platinum-Based Bimetallic Nanoparticles

Platinum (Pt) nanoparticles are important catalysts that are widely used commercially, particularly in automotive catalytic converters due to their high surface to volume ratio. However, to improve the efficiency of Pt-based electrodes, researchers have developed Pt-based bimetallic nanoparticle catalysts. Pt-X alloys, where X can be gold, silver, copper, or other metals, have been found to exhibit very high catalytic efficiency [25].

2.4.2. Nickel-Based Bimetallic Nanoparticles

Nickel nanoparticles are valued for their low cost, high stability, and magnetic and catalytic properties. When nickel is combined with other metals, fascinating properties can be obtained. Researchers have found that the efficiency of various reactions can be significantly improved by varying the stoichiometric ratios of nickel and other metals in a nickel-based bimetallic nanoparticle, such as nickel-tin and nickel-copper [25].

2.4.3. Gold-Based Bimetallic Nanoparticles

Gold nanoparticles are commonly used as biosensors and catalysts. Recently, gold-palladium bimetallic nanoparticles have shown promising electrochemical, catalytic, and structural properties. Gold-copper nanoparticles have been used to develop various medical sensors and biomedicines.

Gold-silver bimetallic nanoparticles are utilized in detecting glucose levels in the blood and exhibit chemiluminescence properties [25].

2.4.4. Iron-Based Bimetallic Nanoparticles

Iron-copper bimetallic catalyst shows high catalytic activity [25].

2.5. Methods for the Synthesis of Bimetallic Nanoparticles

2.5.1. Synthesis of Bimetallic Nanoparticles

There are two main methods for preparing nanoparticles: bottom-up and top-down. The bottom-up method involves using a soluble source of metals, such as metal cations in the form of soluble salts or coordinated with ligands. A reducing agent is then added to the solution, which influences the particle properties. Various reducing agents, such as glucose, citrate, or sodium borohydride, can be used. In the polyol method, a high-boiling alcohol serves as both the solvent and reducing agent. The growth and stability of the nanoparticles are controlled by capping agents like surfactants, polymers, and polyelectrolytes. The resulting nanoparticles' properties depend on factors like temperature, time, and reagent concentrations, and obtaining monodisperse nanoparticle populations can be challenging due to the complex and not fully understood processes of nucleation and crystal growth [26].

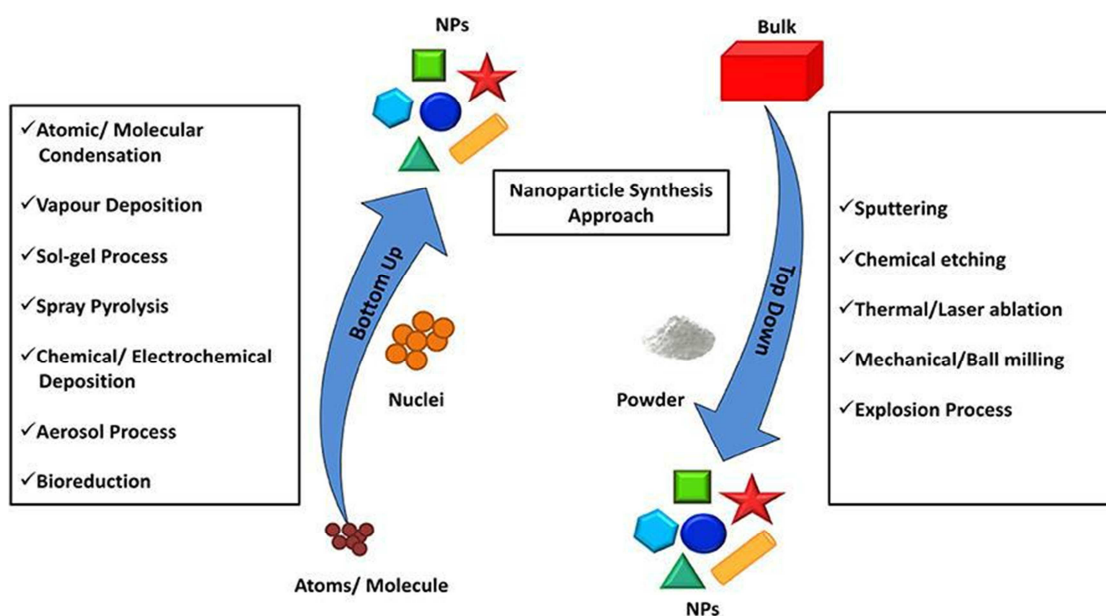


Figure 4. Top-down and bottom-up approaches exploiting different physical, chemical, and biological methods for the synthesis of NPs. Redrawn from the data in [27].

In the synthesis of bimetallic nanoparticles, at least two metal sources must be used (example: Ag^+ and Au^{3+}). [28]. The presence of both metal precursors during reduction can influence the nature of the resulting bimetallic nanoparticles. Simultaneous reduction of both metals can lead to alloyed nanoparticles where both metals (e.g., Ag, Au) are present in a statistical mixture. On the other hand, a sequential reduction

or "seeded growth" can lead to core-shell particles [29]. Sequential reduction of Au^{3+} followed by Ag^+ in the presence of a reducing agent can lead to bimetallic particles with a gold core and a silver shell.

However, the reverse case of Ag^+ reduction followed by Au^{3+} addition can lead to a hollow gold nanoparticle due to silver dissolution. Adding an excess of stronger reducing

agents with the nobler metal ion can prevent this. The top-down method of laser ablation is a controllable way of obtaining well-dispersed bimetallic nanoparticles from a solid target. Another method is a two-step synthesis involving laser

irradiation of a mixture of silver and gold nanoparticles. However, controlling redox chemistry in the complex reaction mixture of bottom-up methods can be challenging [23].

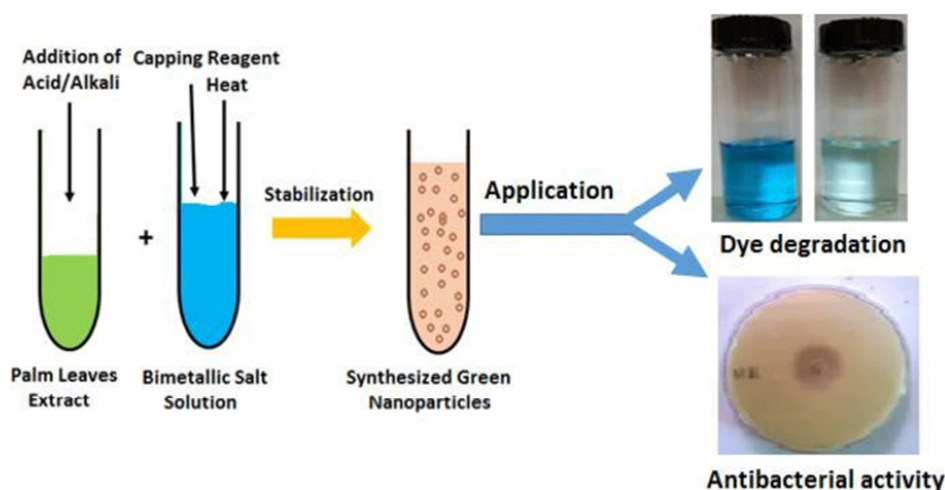


Figure 5. Schematic representation for the synthesis of bimetallic nanoparticles [30].

Plant-mediated synthesis of bimetallic nanoparticles is a low-energy process with minimal operating costs and low toxicity waste. The general protocol involves a sequential reduction process to form core-shell nanoparticles, where a metal salt is reduced by the plant extract followed by the addition of a second metal ion. The formation of bimetallic nanoparticles can be monitored by spectral changes in the UV-visible region due to the plasmonic absorption band. Core-shell nanoparticles are the most common type of composite nanoparticles that offer integrated functionality of the core and coating. The physical and chemical properties of nanostructured composites, including optical, magnetic behavior, and chemical reactivity, can be adjusted by changing the synthesis conditions in correlation with thermodynamic parameters [31]. (b) To synthesize bimetallic nanoparticles using plant extract, inorganic salts such as silver nitrate and gold chloride are dissolved in water, and a plant extract is added while stirring continuously at room temperature. If both metal precursors are present simultaneously, simultaneous reduction can lead to the formation of alloy nanoparticles such as Au-Ag bimetallic nanoparticles. These alloy nanoparticles have shown a synergistic effect on carbon monoxide (CO) oxidation [23].

2.5.2. Green Methods of Synthesis

For the production of metallic nanoparticles, the green synthesis approach has proven to be one of the most effective and environmentally benign techniques.

2.5.3. Plant Synthesis

Plants have been increasingly used for the synthesis of metallic nanoparticles due to their availability, cost-effectiveness, environmental friendliness, and nonhazardous by-products. For example, extracts from the bark of *Terminalia arjuna* have been used for the synthesis of copper nanoparticles with a size of 23 nm. Green synthesis

using plant extracts is simple, economical, and safe for the environment. Chemical synthesis, on the other hand, often requires stabilizing and protective agents that can be toxic and expensive. Developing simple, accessible, and scalable methods for obtaining nanoparticles at low costs is crucial. Plant extracts can meet these goals as they are easy to grow at a large scale, renewable, and environmentally friendly. Phytochemicals in the synthesis process of metallic nanoparticles play two roles: (1) as a reducing agent and (2) as a stabilizing agent for the nanoparticles [32]. Plant-mediated BNPs are more stable and varied in shape and size. Thus, the reducing agent in the plant extract (flavonoids, terpenoids, phenolic acid) is essential for the synthesis of BNPs [33].

Flavonoids, phenolic acids, and lignans are plant-derived polyphenols with strong reducing properties. They are commonly found in fruits, vegetables, and cereals. These compounds are useful as reducing agents in the synthesis of bimetallic nanoparticles and can also act as stabilizing agents to prevent aggregation and maintain stability [34]. Plant-mediated bimetallic nanoparticles have a wide range of applications due to the synergistic effect of metal ions. Various systems such as Ag-Au, Au-Pt, Ag-Pd, Ag-Cu, Ag-Ne, and Fe-Ag BNPs have been obtained using different plant extracts. They have shown potential antibacterial, antifungal, antidiabetic, and anticancer efficacy. Biosynthesis of AuAg bimetallic alloys from fungal biomass and single-cell protein *Spirulina plantesis* has also been reported. These studies highlight the potential of plant-mediated BNPs in various fields, including medicine and environmental remediation [35].

The synthesized nanoparticles were found to have a face-centered cubic (fcc) core-shell structure with nickel (Ni) as the shell component. The dimensions of the nanoparticles were found to be within the range of 1-4 nm. In 2006, Schabes-Retchkiman et al. reported the biosynthesis of Fe-Pd

bimetallic nanoparticles using green tea extracts. The polyphenols in the extract acted as reducing, chelating, and capping agents for the synthesis of the nanoparticles. The reactive nanoparticles were immobilized on a PVDF membrane. The study showed that the nanoparticles retained their reactivity even after three months of repeated usage. Plant extracts offer a promising route for the synthesis of bimetallic nanoparticles for various applications, including catalysis and environmental remediation. Further research in this field could lead to the development of more sustainable and eco-friendly technologies [36]. *Cacumen Platycladi* leaf extracts in an aqueous medium were used for the biosynthesis of Au-Pd bimetallic nanoparticles. The synthesized nanoparticles had an average size of ~7nm, and their properties were characterized using several techniques such as UV, XRD, and FTIR. The study demonstrates the potential of plant extracts as eco-friendly and non-toxic agents for the synthesis of bimetallic nanoparticles [37].

2.6. Factors Influencing BNPs Biosynthesis

The biosynthesis of bimetallic nanoparticles is influenced by factors such as pH, temperature, reaction time, and metal ion concentration. These factors affect the size and shape of the nanoparticles. Optimal metal ion concentration, temperature, and pH are critical for controlling the properties of the synthesized nanoparticles [38]. Centrifugation of the synthesized bimetallic nanoparticles can be used to obtain pure nanoparticles and avoid the interference of other compounds, such as organic dyes, used in analysis methods. By centrifuging the nanoparticle solution at 10,000-15,000 rpm for 15-20 minutes, the nanoparticles can be isolated and redispersed in distilled water. This method is useful for obtaining pure bimetallic nanoparticles and can be an important step in their characterization and subsequent use in applications [39].

2.6.1. The pH of the Solution

The pH value is a crucial factor in controlling the growth dynamics of nanoparticles. It can influence the equilibria involved in the process, and the isoelectric point is an important parameter at which the surface charge can be reduced to zero. Several studies have shown that pH plays a critical role in controlling the size and formation of nanoparticles. For instance, Ganaie et al. (2016) reported that the synthesis of Ag-Au BNPs was completed within minutes at pH 10. Akilandaeswari et al. (2021) found that pH 7 was the optimal condition for synthesizing Au-Ag BNPs from *Lawsonia inermis* extract for high catalytic activity. Therefore, controlling the pH is crucial for efficient and reproducible nanoparticle synthesis with desired properties [40].

2.6.2. Reaction Temperature

Temperature is a crucial parameter that plays a significant role in the biosynthesis of nanoparticles. It affects the rate of reduction of metal ions and can influence the spatial and dimensional distribution of nanoparticles. Various studies have investigated the effect of temperature on the biosynthesis

of different types of nanoparticles, such as Au-Ag, Ag-Pt, and Au-Pd BNPs. For example, Akinsiku et al. (2018) found that smaller nanoparticles were formed at 70°C with *Canna indica* extracts. In another study, Olawale et al. (2022) observed Ag₂Se BNPs synthesis at high temperatures (110°C) with a large volume of extract (500–1700 µL).

Increasing the reaction temperature can accelerate the rate of reduction of metal ions, leading to the formation of smaller nanoparticles. Furthermore, an increase in the amount of extract can improve the yield of nanoparticles by providing more reducing agents necessary for complete reduction. In the case of extracts from leaves of *Ocimum tenuiflorum*, the rate of nanoparticle synthesis increased at 95°C (Roopan and Surendra 2014). The high temperature favors the fast conversion of the metallic solution into nanoparticles, with the synthesis being longer at room temperature.

The increase in temperature catalyzes the formation of nucleation centers and leads to the formation of stable and smaller nanoparticles. However, it is essential to maintain the temperature range between 30-100°C as higher temperatures can cause the decomposition of phytochemicals, which can interfere with the reduction process. Therefore, controlling the temperature is crucial for efficient and reproducible synthesis of nanoparticles with desired properties [41].

2.6.3. Reaction Time

Biosynthesis time is a crucial parameter to consider in the synthesis of nanoparticles, as the reduction reaction cannot occur instantly, regardless of the extract used. The color transformation during biosynthesis is dependent on the time and temperature at which the process takes place. For instance, in the synthesis of Au-Ag BNPs, the reaction process was monitored using UV-VIS spectroscopy for different time intervals. The plasmonic bands in the bimetallic nanoparticles were observed, after 15 minutes of reaction and increased over time. The absorption was observed after 30 minutes at around 534 nm, which changed to red after 45 minutes at 542 nm. The formation of two bands in the bimetallic nanoparticles reflects the core-shell, but in this case, a band from the Au region showing more Au than Ag was reduced, and there was possible nanoalloy formation.

Increasing the reaction time can accelerate the ion reduction rate until the reaction reaches the end. Therefore, monitoring the reaction process during biosynthesis is crucial to achieving the desired properties of the nanoparticles. UV-VIS spectroscopy is a commonly used technique for monitoring the progress of nanoparticle synthesis and determining the optimal biosynthesis time.

2.6.4. Metal Ion Concentration

The initial concentration of metal ions in the reaction mixture is another crucial factor that can influence the yield and properties of nanoparticles. In the synthesis of Ag-Se BNPs, different dilutions of quercetin and gallic acid were used as a bioreduction and coating agent. The solutions were reacted with a mixture of silver and selenium salt at different concentrations, and the effect of AgNO₃ and Na₂SeO₄ concentrations were studied. The study found that a mixture of

quercetin and gallic acid (50 μ M, each) gave a maximum yield of bimetallic nanoparticles, and an increase in the yield of Ag-Se NPs was observed when the metal salt concentration increased from 0.5 mM to 1 mM.

Therefore, determining the optimal concentration of metal ions in the reaction mixture is crucial for achieving the desired properties of nanoparticles. The concentration of the metal salt can affect the size, shape, and composition of the nanoparticles, and can also influence the yield and stability of the nanoparticles. Therefore, it is important to carefully consider the initial concentration of metal ions when designing a nanoparticle synthesis protocol [42]. The nanostructures were characterised by using the UV–VIS spectrum, which displayed a surface plasmon absorption band at 450 nm.

2.6.5. Plant Extract/Biomass Dosage

The plant extract used in nanoparticle synthesis is dependent on several factors, including the plant species, solvent used for extraction, and extraction temperature. Different plants have unique phytochemical compositions that can influence the size and properties of the nanoparticles produced. For instance, the synthesis of Ag-Au BNPs using *Gloriosa superba* leaves extract was completed after 10 minutes at room temperature, indicating the rapid formation of nanoparticles [43]. Moreover, the presence of reducing agents in larger quantities in the *Lawsonia inermis* seed extract led to the formation of smaller and spherical nanoparticles (Akilandaewari and Muthu 2021). The concentration of the plant extract can also influence the properties of the nanoparticles. [43]. demonstrated that the intensity of absorbance increased with an increase in the concentration of pomegranate extract (*Punica granatum*), indicating the formation of nanoparticles with different properties.

Therefore, selecting the appropriate plant extract and optimizing the extraction conditions is crucial for achieving the desired properties of the nanoparticles. Careful consideration of these factors can help to improve the reproducibility and efficiency of nanoparticle synthesis protocols [43].

3. Characterization Techniques

Characterization of nanoparticles is crucial for their evaluation, synthesis control, and future applications. Some of the most commonly used methods for nanoparticle characterization include UV–VIS (ultraviolet-visible spectroscopy), FT-IR (Fourier transform infrared spectroscopy), UHPLC (ultra-high-performance liquid chromatography), TEM (transmission electron microscopy), SEM (scanning electron microscopy), EDX (energy-dispersive X-ray spectroscopy), DLS (dynamic light scattering), zeta potential, and XRD (powder X-ray diffraction). These techniques can provide valuable information about the physical, chemical, and structural properties of nanoparticles, such as their size, shape, surface charge, crystal structure, and elemental composition. By using

these characterization methods, researchers can better understand the behavior of nanoparticles and optimize their properties for specific applications [44].

3.1. UV–VIS (Ultraviolet-Visible Spectroscopy)

UV-VIS spectroscopy is based on the detection of surface plasmon resonance (SPR), which is an interaction between light and matter. In nanoparticle synthesis, UV-VIS spectroscopy can be used to monitor the progress of the reaction by observing changes in absorption spectra. For example, during the synthesis of Au-Ag BNPs, the absorption spectra were observed around 534 nm after 30 minutes and shifted to 542 nm after 45 minutes, indicating the formation of nanoparticles. The presence of the SPR band in the UV-VIS spectra confirmed the formation of Au-Ag BNPs and the change in color to red (due to the SPR effect). UV-VIS spectroscopy is a powerful tool for monitoring the formation and properties of nanoparticles in real-time during synthesis [45]. The synthesis of Au-Ag BNPs with an alloy structure using leaf extract from golden stem (*Solidago canadensis*) was carried out, and the nanoparticles were monitored and characterized using various techniques. UV-VIS spectroscopy was used to monitor the synthesis progress, and the absorption peak was observed at 530 nm, indicating the formation of nanoparticles. Particle size and diffraction patterns were also used to characterize the synthesized nanoparticles. The morphology of the nanoparticles was further characterized using SEM and TEM. These characterization techniques provided valuable information about the properties of the synthesized Au-Ag BNPs, such as their size, shape, crystal structure, and elemental composition. Overall, these results demonstrate the effectiveness of using plant extracts for the green synthesis of bimetallic nanoparticles and highlight the importance of thorough characterization of synthesized nanoparticles for their successful applications.

3.2. FT-IR Spectroscopy

FT-IR spectroscopy is a valuable tool for elucidating the structure and identifying chemical compounds. The technique is widely used to determine the functional groups in a sample by detecting their characteristic frequencies of IR radiation [45]. In nanoparticle synthesis, FT-IR spectroscopy can be used to confirm the bioreduction of metal ions to nanoparticles, which is a crucial step in the green synthesis of nanoparticles using plant extracts. For example, in a study involving the synthesis of Au-Ag BNPs using aqueous *Plumbago zeylanica* root extract, FT-IR spectroscopy was used to confirm the bioreduction process. The results showed characteristic absorption peaks, indicating the presence of functional groups in the plant extract that were responsible for the reduction of metal ions to nanoparticles. FT-IR spectroscopy is a powerful analytical technique that provides valuable information about the chemical composition of samples, making it an important tool in nanoparticle synthesis and characterization.

3.3. TEM and SEM Analysis

Characterization of the size and morphology of synthesized BNPs is crucial for understanding their properties and potential applications. TEM images of Au-Ag BNPs synthesized using plant extracts have shown that they have predominantly spherical shapes, but occasional aggregations can result in rod-like triangular shapes [45]. In another study, Pd-Ag BNPs were synthesized using an aqueous fruit extract of *Terminalia chebula*, which is rich in antioxidant agents such as polyphenols. The characterization of synthesized BNPs using various techniques, including TEM, provides important information about their size, shape, and structure, which can be correlated with their biological and physical properties. These results demonstrate the potential of green synthesis approaches for the production of bimetallic nanoparticles with various morphologies and highlight the importance of thorough characterization for understanding their properties.

3.4. XRD Data

XRD is a powerful analytical technique that is used to identify the crystallinity of BNPs. On the other hand, EDX spectroscopy is used to determine the purity of synthesized BNPs, while DLS is used to identify the agglomeration of BNPs and determine their size distribution in solution [45]. In the case of Pd-Ag BNPs, XRD and DLS analyses confirmed the formation of cubic crystal structure with an average size of 20 nm, while EDS and XRD analyses confirmed the crystalline nature and purity of the NPs, respectively. TEM and DLS micrographs showed that AgNPs were spherical with a size of 60 nm, while AuNPs were anisotropic in nature, with spheres, triangles, and hexagons with dimensions of approximately 20-30 nm. Au-Ag BNPs were found to be polygonal in shape with a blunt hexagonal end with a size of around 90 nm. The unique features of these synthesized BNPs are due to the presence of various compounds in the plant extracts used, such as flavonoids, sugars, organic acids, phenols, starch, and citric acid.

Other bimetallic nanoparticles synthesized using plant extracts include Ag-Pt BNPs, Ag-Pd BNPs, Au-Pt BNPs, Au-Pd BNPs, Pt-Pd BNPs, among others. Studies have shown that these synthesized nanoparticles have different shapes and sizes, ranging from 3-80 nm for Au-Ag BNPs and 7-70 nm for Ag-Pd BNPs. SEM and TEM analysis have shown that the synthesized BNPs are uniform and spherical in shape. Unlike plasmonic NPs, non-plasmonic NPs do not show significant SPR peaks, and their optical properties depend on their size and concentration. UV-VIS, FTIR, electron microscopy, and X-ray diffraction analysis have been used to characterize Ag-Pt BNPs [45]. and other bimetallic nanoparticles synthesized using plant extracts.

4. Applications of BNPs Mediated by Plant Extracts

Biomedical applications

Controlled drug delivery systems are essential for the effective treatment of various diseases, including cancer. The primary goal of such drug delivery processes is to maintain biocompatibility during therapy and improve specificity, which can be achieved through the use of magnetic bimetallic nanoparticles. Magnetic bimetallic nanoparticles are highly specific drug delivery agents due to their unique properties such as magnetic responsiveness, high surface area, and biocompatibility. These nanoparticles can be functionalized with specific targeting moieties such as antibodies, peptides, or aptamers to enhance their specificity towards targeted cells or tissues. The magnetic properties of these nanoparticles also allow for their controlled movement within the body using an external magnetic field, providing a highly precise and localized drug delivery system [46].

In addition to their drug delivery capabilities, magnetic bimetallic nanoparticles also offer other potential biomedical applications such as magnetic resonance imaging (MRI) contrast agents, hyperthermia agents for cancer therapy, and biosensors for disease diagnosis. Overall, the use of magnetic bimetallic nanoparticles for controlled drug delivery holds great promise for improving the efficacy and specificity of therapeutic treatments while minimizing side effects.

4.1. Antioxidant Activity

Phytochemicals, including polyphenols, such as flavonoids and phenolic acids, are compounds found in plants that have been extensively studied for their potential health benefits. These compounds are known for their antioxidant, anti-inflammatory, and anticancer properties. Flavonoids are a diverse group of polyphenolic compounds found in many fruits, vegetables, and herbs, and they are known to have a wide range of biological activities, including anti-inflammatory, antimicrobial, and antitumor effects. Phenolic acids, on the other hand, are found in various plant-based foods, including fruits, vegetables, and whole grains, and have been shown to have antioxidant and anti-inflammatory properties.

Research has shown that phytochemicals, including flavonoids and phenolic acids, have the potential to prevent and treat various chronic diseases, including cancer, cardiovascular disease, and neurodegenerative disorders. These compounds are also being investigated for their potential to modulate the gut microbiota and improve gut health [46].

Overall, the study of phytochemicals and their potential health benefits is an active area of research, and ongoing studies are exploring the use of these compounds in the prevention and treatment of various diseases.) (Lomeli-Marroquín et al. 2019). Flavonoids protect the body from free radicals, help strengthen the immune system, and reduce inflammation. They are responsible for the antioxidant activities of plants. These natural compounds contain one or more hydroxyl groups related to the carbon atoms of the aromatic ring [47]. You are correct that many phytochemicals are specific to different parts of plants, including the bark and outer leaves. These compounds can have a variety of

biological activities, including antioxidant properties, which can help protect against the damaging effects of free radicals on cells and tissues. Some research suggests that phytochemicals, when combined with noble metal nanoparticles, may be effective in reducing the risk of certain types of cancer, including lung, breast, and colon cancer.

The antioxidant activity of phytochemicals can be classified into two mechanisms: hydrogen atom transfer (HAT) and single electron transfer (SET). In the HAT mechanism, an antioxidant molecule donates a hydrogen ion from a stable molecule, allowing it to neutralize reactive oxygen species (ROS). In the SET mechanism, the antioxidant reduces certain molecules and compounds by transferring an electron.

The presence of antioxidants in plants is also important for the green synthesis of metal nanoparticles or metal oxides. Phytochemicals can reduce or chelate metal ions and act as stabilizers for the nanoparticles produced. This has led to the development of green synthesis methods for producing metal nanoparticles using plant extracts as reducing and stabilizing agents. These green synthesis methods do not require toxic chemicals or high temperatures, making them more environmentally friendly and potentially safer for use in biomedical applications [47].

4.2. Antibacterial Activity

Bimetallic nanoparticles have shown promising antimicrobial activity against various bacterial and fungal pathogens. The use of bimetallic nanoparticles as antimicrobials is an active area of research, and ongoing studies are exploring their potential applications in the field of nanomedicine. Cu-Ni bimetallic nanoparticles have shown bacteriostatic activity against certain bacterial strains, including *Escherichia coli*, *Staphylococcus aureus*, and *Staphylococcus mutans*. Fe-Ag magnetic bimetallic nanoparticles have demonstrated excellent antifungal and antibacterial activity against several pathogenic microorganisms. Ag-Au bimetallic nanoparticles synthesized from *Gracilaria* sp. and Au-Ag bimetallic nanoparticles synthesized from *Oscimum basilicum* (Basil) flower and leaf extracts have also shown antibacterial activity against various bacterial strains [47].

The antimicrobial activity of bimetallic nanoparticles can be attributed to their ability to interfere with bacterial growth by disrupting their membrane or generating reactive oxygen species (ROS) that cause destruction of DNA and impede protein functioning. Bimetallic nanoparticles can also work synergistically with antibiotics and sulfa drugs to combat bacterial infections.

Ag-doped ZnO nanoparticles have also shown antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis* by changing the minimum inhibition concentration (MIC), particularly in the case of *S. aureus*. Overall, the use of bimetallic nanoparticles as antimicrobials holds great promise for the development of novel nanodrugs against human pathogens, especially for cases where antibiotics fail to provide satisfactory results.

4.3. Anticancer Activity

You are correct that bimetallic nanoparticles have potential applications in cancer diagnosis and treatment. Iron-based bimetallic nanoparticles, for example, can be used to enhance magnetic resonance imaging (MRI) of cancerous cells, allowing for more accurate detection and diagnosis of cancer. Bimetallic nanoparticles containing gold and silver have also shown promise in photothermal cancer therapy, both in vitro and in vivo. These nanoparticles can be selectively targeted to cancer cells and then activated by a laser to generate heat, which destroys the cancer cells. This approach has the advantage of being minimally invasive and potentially more effective than traditional cancer treatments.

In addition to their potential applications in cancer diagnosis and treatment, bimetallic nanoparticles have a wide range of other potential biomedical applications. These include drug delivery, biosensors, and tissue engineering, among others. Overall, the study of bimetallic nanoparticles and their potential applications in biomedicine is an active area of research, and ongoing studies are exploring the use of these advanced materials in various biomedical applications. [48]. cancer is a time period used to outline malignancies in which atypical cells multiply uncontrollably and can invade the surrounding healthy tissues. peculiar cells come from any tissue within the human frame and might occur everywhere within the body. The BNPs act as chemotherapeutic dealers in treating tumour cells and show a synergistic effect with drug chemotherapy. Many studies display that BNPs have anticancer hobby [49]. It was reported that BNPs have a cytotoxic effect towards numerous varieties of cancer cells, inclusive of the breast most cancers cellular line. The anticancer pastime is due to the synergistic impact between the two metals used as compared to the monometallic NPs. some researchers have cautioned that cancer cells are greater susceptible to electron transfer among BNPs that release reactive oxygen species (ROS) and as a consequence ruin most cancers cells inclusive of MCF-7 (breast most cancers cellular line), HeLa (breast cancer cells), Jurkat (human T lymphocyte cells), HT29 (human colon cancer cell line), HEK 293 (human embryonic renal cells), and T24 (human bladder cancer cells) [50]. Given the size of the metallic NPs and the concentration of the sample, researchers observed that BNPs could cause damage to a cancer cell.

4.4. Antidiabetic Activity

Diabetes is a metabolic disorder characterized by high levels of glucose in the blood (hyperglycemia), often resulting from insufficient insulin secretion or insulin resistance. Diabetes is a chronic disease that affects millions of people worldwide and is associated with a range of complications, including cardiovascular disease, nerve damage, kidney disease, and blindness.

According to the World Health Organization, diabetes is the seventh leading cause of death worldwide. However, diabetes is a major risk factor for other diseases, such as cardiovascular disease and cancer, and is estimated to be the third leading

cause of death globally [51].

Diabetes can be managed through lifestyle modifications, such as healthy eating and regular physical activity, as well as through medications, including insulin and oral hypoglycemic agents. Ongoing research is also exploring the potential of various therapies, including stem cell therapy and gene therapy, to treat diabetes and its complications.

Prevention is also an important aspect of diabetes management. This includes lifestyle modifications, such as maintaining a healthy weight, eating a balanced diet, and engaging in regular physical activity, as well as regular screening for diabetes and related conditions. [51]. α -Glucosidase and α -amylase (porcine) enzymes are considered the important thing antidiabetic enzymes that metabolise carbohydrates. The enzymatic interest of human pancreatic α -amylase and α -glucosidase inside the small gut correlates with postprandial accelerated glucose tiers.

Consequently, amylase and glucosidase inhibitors prevent the breakdown of carbohydrates into monosaccharides, the principle motive for the increased stage of glucose in the blood. Alpha-glucosidase is an enzyme that participates inside the technique of digesting carbohydrates. It mediates the cleavage of polysaccharides and disaccharides to glucose. therefore by means of inhibiting α -glucosidase, it delays the digestion and absorption of carbohydrates [52]. Synthesised BNPs, such as Au–Ag BNPs and Pt–Pd BNPs, using various plant resources, show antidiabetic activity.

Futhermore, Bimetallic nanoparticles hold numerous applications including electrical, optical, catalytic and biomedical. Bimetallic nanoparticles serve as very good catalysts. Reduction reaction between Mohr's salt and $[\text{Co}(\text{NH}_3)_5\text{Br}](\text{NO}_3)_2$ can be effectively catalyzed by Au–Ag nanoparticles which is basically due to transfer of charge from Ag ions to Au ions [53]. Another example is Pt–Ru bimetallic nanoparticles that act as anode catalyst for methanol oxidation done at low temperature in methanol fuel cells [54]. reduction reaction of aromatic nitro compounds by means of sodium borohydride is catalyzed with the aid of Pt–Ni bimetallic nanoparticles [54]. CO oxidation, result in 100% conversion of CO at room temperature. The reaction was catalyzed by Ni–Pt bimetallic hollow spheres [55]. Bimetallic nanoparticles have promising applications in the field of nanomedicine. Chitosan-capped spiky urchin-like Au–Ag bimetallic nanoparticles have shown promising results in photothermal cancer therapy, effectively ablating cancer cells. Ag–Se bimetallic nanoparticles synthesized using gallic acid and quercetin have also been shown to be toxic against cancer cells, suggesting their potential use in preventing or reducing oxidative stress associated with degenerative diseases.

Bimetallic nanoparticles also have potential applications in drug delivery, as their high surface area to volume ratio allows them to cross the blood-brain barrier and epithelial cell junctions to reach the target site. Additionally, their electrical and optical properties make them suitable for use as biosensors. Platinum-based bimetallic nanoparticles, such as Pt–Co, Pt–Ni, and Pt–Fe, have been shown to have excellent catalytic activity, which can increase the detection limits of

biosensors.

Bimetallic nanoparticles are also important in environmental remediation. They have been shown to be effective in decontaminating surface water and in the in-situ remediation of groundwater contaminated with nitrates, using Fe–Cu bimetallic nanoparticles. Pd–Au bimetallic nanoparticles have also shown excellent deactivation resistance and hydrodechlorination for removing chlorinated ethenes from groundwater.

Overall, the study of bimetallic nanoparticles and their potential applications in various fields is an active area of research, and ongoing studies are exploring the use of these advanced materials in a range of biomedical and environmental applications. [11].

You are correct that nanofluids have the potential to enhance thermal conductivity when suspended in a heat-transfer liquid. Ag–Au bimetallic nanoparticles in nanofluids have shown promising results in this regard. Nanoparticles can also be used as electrodes for electrical conductivity, with Au–Ag alloys exhibiting high performance due to reduced inertness.

Bimetallic nanoparticles are also useful for electroplating and fabrication, with alloys of Au with Ag, Pt, and Pd showing promising results in Microelectromechanical Systems (MEMS) transfer fabrication. In addition, bimetallic nanoparticles can be used as substrates for surface-enhanced spectroscopy, providing significant electromagnetic enhancements.

Overall, the study of bimetallic nanoparticles and their potential applications in various fields is an active area of research, and ongoing studies are exploring the use of these advanced materials in a range of technological applications.

5. Conclusion

The synthesis and characterization of bimetallic nanoparticles (BNPs) is an active area of research, with many researchers preferring the biological method over physical or chemical methods due to its potential advantages. Plant extracts are an excellent source of phytoconstituents, and various functional groups found in plants can be involved in the reduction, synthesis, and stabilization of BNPs. BNPs containing noble transition metals such as silver, gold, platinum, and palladium are of particular interest for biomedical applications due to their biological stability, low toxicity, and protective properties. Researchers have focused on the antioxidant, antimicrobial, and anticancer effects of BNPs synthesized from natural extracts.

BNPs have potential applications in various fields, including chemotherapy, diagnostics, drug delivery systems, cosmetics, and the chemical, energy, electronics, and aerospace industries. Further research is needed to develop newer strategies for reducing drug-resistant microorganisms using polyphenolic extracts. In vivo studies are also necessary to evaluate issues such as excretion and non-targeted distribution of BNPs.

Additionally, more research is needed to establish the link

between the biosynthesis of BNPs and their bioactivity and to understand their mechanisms of action. While natural extracts are generally safe, more toxicological data are needed to ensure the safety of BNPs in various applications. Overall, the study of BNPs and their potential applications is an active area of research, and ongoing studies are exploring the use of these advanced materials in a range of fields.

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Conflicts of Interest

The authors declare no conflicts of interest.

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