UNIVERSIDAD DE INVESTIGACIÓN DE TECNOLOGÍAEEE EXPERIMENTAL YACHAY

Escuela de Ciencias Biológicas e Ingeniería

Overview of the Synthesis Methods of Bimetallic Nanoparticles for the Formation of Silver-Palladium as a Potential Antibacterial Agent against *Staphylococcus aureus*

Trabajo de integración curricular presentado como
requisito para la obtención del título de Ingeniera
Biomédica.

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Urcuquí, Julio del 2021.

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ACKNOWLEDGEMENTS

I would like to thank **God** for giving me faith in myself and helping us out even when we felt hopeless. Thanks for letting me get here.

I acknowledge my beloved family for being the air of my existence. Thanks to my dear parents, **Heidy** and **Roberto**, for giving me the strongest, sweetest love; for always putting us first, and for teaching me that I must make my way; thanks for believing in me, and for helping me out every day: from a nice meal to signal theory reports. Thanks to my dear brother, **Robertito,** for taking such good care: of my parents when I could not, of us during the pandemic, and of me, all my life.

I would like to thank **Yachay Tech University** for aiming for scientific excellence in a small country like ours, Ecuador. Thanks to all who believe in the project and the students.

A special thanks to my thesis tutor, **Edward Ávila**, who was always open to my ideas and routed them positively into this project. Thanks for supporting me, for sharing your knowledge, and make a great effort in the development of the present thesis. Thanks to my thesis court professors **Federico S.** and **Marco G.** for your contributions, commentaries and time invested in the present document.

Thanks to **Si Amar** for being my mentor ever since I learned about the nanotechnology field; thanks for guiding me throughout the development of this project with patience and faith. And thanks to all the kind **teachers** who taught and guided me during my career.

Finally, I would like to thank the dear friends who have been with me all these years. Thanks always, **Dayita**, for your advice, food, and kindness. Thanks, **Mabe,** for always being the nicest roommate and friend. Thanks, **Lupe,** for always being on my team. Thanks, **Micky,** for always being that true friend I needed. Thanks, **Carlos**, for your help, teach, and company. Thanks to all of you for being the best adventure companions and my support throughout this stage. Moreover, thanks to all the dear **friends** who, in due time supported me.

I dedicate the present work to my beloved family:

To my mom, Heidy, and my father, Roberto, for your humble working life that has given me everything in this life. To my brother, Robertito, for being motivation in every step of my life and for letting me take some more time to myself, just to get back on my feet.

Emily Daniela Gallardo Andrade

Resumen

Durante las últimas décadas, las propiedades antibacterianas de las nanopartículas han sido el centro de estudio de muchas investigaciones a nivel mundial, sin embargo, no siempre se obtiene la adecuada morfología, tamaño ni química de la superficie, parámetros que son elementales para sus propiedades antibacterianas. Es por esto por lo que las nanopartículas deben ser desarrolladas con una síntesis controlada, cuyos principales parámetros nos ayuden a obtener un tipo de agente antibacteriano eficaz, duradero, estable y sobre todo que no conlleve más problemas a la naturaleza como lo son los químicos peligrosos.

La resistencia de la bacteria *Staphylococcus aureus* a los antibióticos es un asunto alarmante a nivel mundial debido a la rápida habilidad que tiene para adaptarse, estudios recientes demuestran que tanto la penicilina como la meticilina han perdido su efecto contra esta bacteria en más del 80% de los casos; por lo cual, la búsqueda de un agente antibacteriano cuya eficacia no se vea afectada es urgente. Varios estudios han demostrado que las bacterias no pueden tan rápidamente adquirir resistencia a las nanopartículas como a los fármacos, por lo que el desarrollo de nuevos estudios para mejorar la producción de nanopartículas es necesario.

En el presente trabajo se hace una revisión bibliográfica en la que se estudian diversas técnicas de síntesis de nanopartículas bimetálicas; síntesis que tiene el principal objetivo de desarrollar una formación controlada de nanopartículas de Ag-Pd como potencial agente antibacteriano para su uso en la bacteria *Staphylococcus aureus*.

Palabras Clave: *Antibacterial, Nanoparticulas Bimetalicas, Staphylococcus aureus, Síntesis Verde.*

Abstract

During the last decades, the antibacterial properties of inorganic nanoparticles have been the focus of very intense and prolific research worldwide. However, it is challenging to obtain good morphology, size, and surface chemistry that constitute basic parameters for their antibacterial properties are not always achieved. For this reason, nanoparticles must be developed with a controlled synthesis, whose main parameters help us to obtain a type of practical, durable, stable antibacterial agent that does not lead to more problems for nature, such as dangerous chemicals.

The resistance of *Staphylococcus aureus* to antibiotics is an alarming worldwide issue due to the rapid ability this bacterium has to adapt to new pharmaceutics. Recent studies show that both penicillin and methicillin have lost their effect against it in more than 80% of the cases; therefore, the search for an antibacterial agent whose efficacy is not affected is urgent. Several studies have shown that bacteria cannot acquire resistance to nanoparticles as quickly as to drugs; therefore, new studies to improve the production of inorganic nanoparticles with desired antibacterial properties are necessary.

In the present work, a bibliographic review is developed to overview various techniques for synthesizing bimetallic nanoparticles and their characterization along with their unique properties. This synthesis offers the main objective of the controlled formation of bimetallic Ag-Pd alloy nanoparticles, making them a potential antibacterial agent against Staphylococcus aureus.

Keywords: *Bimetallic Nanoparticles, Staphylococcus aureus, Green Synthesis, Antibacterial.*

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Abbreviations

NP = Nanoparticle

BMNP = Bimetallic Nanoparticle

SPR = Surface Plasmon Resonance

MRI = Magnetic Resonance Imaging

ROS = Reactive Oxygen Species

GF = Growth Factor

GI = Gastrointestinal

NS = Nervous System

IV = Intravenous

ISO = International Standardization Organization

WHO = World Health Organization

PVP = Polyvinylpyrrolidone

PEG = Polyethylene Glycol

PAA = Polyacrylic Acid

CHAPTER I

1. INTRODUCTION

Staphylococcus aureus has been an essential participant in nature for millennia. This Gram-positive bacterium infections, like bacteremia has a mortality rate of ~20% worldwide; this percentage represents a more significant number of deaths than combined with acquired Immunodeficiency syndromes (AIDS), like tuberculosis, and viral hepatitis;[1] this mortality rate have been shown to be directly affected by many factors like age, making the elderly the most susceptible group. Its infection is associated with virulence factors like exoproteins, which favor surface adhesion, invasion, and evasive immunity leading to the toxic response of the patient. *Staphylococcus aureus* bacterium can trigger an infection, directly affect organ functions, and provoke death.

Multiple efforts are continuously performed to prevent, treat, and heal *S. aureus* infections. However, this bacterium has continuously developed resistance to the new classes of antibiotics. This bacterium remains and will remain a hazardous, challenging pathogen for nature, patients, and medical personnel until an effective antibiotic is developed.[2] Antibacterial resistance of *S. aureus* has specific mechanisms by which it can acquire immunity. Some of these are the production of enzymes like β-lactamase to degrade the drug or the cell wall adaptability to express or inhibit efflux pumps.[3] Despite these, none known mechanism can stop the interaction of NPs with the bacterial cell wall, raising a relevant source of investigation to develop potential antibacterial agents that could become more efficient than regular antibiotics.

The synthesis of nanoparticles has been an ever-growing field in science and engineering. Their functionalization and application are critical aspects to investigate. These particles are crucial for the creation and improvement of innovative structures for medical and material sciences. As mentioned above, one of their promising applications is their bactericidal activity to act as "inorganic antibiotics". Many studies have presented the utilization of nanoparticles as a potential antibacterial agent, especially those made of metals, such as silver.[4] Kim et al. demonstrated that tiny silver-based NPs presented an increased ability to attach and penetrate the cell wall of bacteria; as a result, this provokes an irreversible membrane damage by pore enlargement that leads to cell death.[5]

The application of these nanoparticles has also risen studies to enhance their functionality and reproducibility to form efficient, easy to use and reliable antibacterial agents. Hence, the implementation of multiple compositions and combinations of nanoparticles has been widely discussed in the literature. One of these potential combinations lies in bimetallic systems, whose bacterial activity is greatly dependent of the geometrical structure and size. Bimetallic nanoparticles (BMNPs) accomplish chemical, physical, and catalytic processes that their monometallic counterparts cannot. Therefore, the introduction of other metals may improve the antibacterial activity of the silver nanoparticles, for instance.

Silver-Palladium bimetallic nanoparticles have proven enhanced antibacterial yield. These can be synthesized by two main approaches of nanotechnology, which are the top-down and bottom-up methods. However, their nanofabrication has not yet allowed to control their features that would be suitable for the antibacterial applications. Size and morphology, for example, may be altered from minimal variations in the system. The unreacted organic surfactants may adsorb to the NP surface, thus decreasing their functionality.[6] For these reasons, a fully controlled synthesis process is required to enable the industry to develop cost-effective, greener, scalable, and optimal nanoparticle-based antibacterial.

In this bibliographic review, the methods for the synthesis and characterization of nanoparticles are discussed to support the development of bimetallic nanoparticles as an enhanced agent to use in antibacterial applications. The present work aims at overviewing the controlled synthesis via green chemistry, yielding bimetallic Ag-Pd nanoparticles of desired properties, such as size and morphology, along with their outstanding activity against *Staphylococcus aureus*.

1.1 Problem Approach

The acute development of antibiotic resistance has created a risk to public human health. There are many questions about the increasing bacterial ability to resist bactericidal agents. A reasonable answer to that is the rapidity by which bacteria mutate and multiplicate; on the other hand, patient’s compliance is questionable too. These lead bacteria to adapt to their changing environment. This problem has caused many developed antibiotics to be no longer capable of fulfilling their purposes effectively. Hence the need to produce innovative, facile, and efficient antibacterial agents has become a crucial issue for public health to contain and combat cross-contamination outbreaks.

For the past decades, nanoscience and nanotechnology have offered multiple, innovative applications in different areas, like engineering, material sciences, and biomedical field. Furthermore, numerous studies highlight the promise of nanoparticles as efficient, novel bactericidal agents, making these potential complements to traditional antibiotics. However, the scalable synthesis of nanoparticles with controlled properties requires further investigations towards their efficient, facile, rapid, and green production. The present study provides alternative methods to traditional synthesis techniques by highlighting some recent investigations that utilized greener agents and combined methods to achieve controlled properties.

1.2 Objectives

1.2.1 General Objective

To overview the best synthesis methods to obtain bimetallic palladium-silver nanoparticles according to the green synthesis criteria, size, and morphology to optimize their antibacterial activity.

1.2.2 Specific Objectives

i. To compare the various synthesis techniques.
ii. To achieve an analysis considering green synthesis vs controlled nanoparticle formation.
iii. To overview the potential of bimetallic Ag-Pd nanoparticle as antibacterial agent against *Staphylococcus aureus.*
iv. To analyze the impact of NPs’ size and morphology on their antibacterial activity.
v. To sum-up the information regarding their antibacterial properties.

CHAPTER II

2. NANOPARTICLES

The prefix ‘nano’ comes from the Greek for dwarf and represents 10^{-9} or one over 1’000 000 000. Therefore, a nanometer (nm) is the billionth part of a meter. According to the International Standardization Organization (ISO), the nanoscale ranges between 1 to 100 nm.[7] Nanoscience brings together the study of all nanostructures. Hence, nanoscience studies the manipulation of atomic matter at molecular and macromolecular levels; at this scale, physical and chemical properties vary significantly depending on minimum variations. Nanotechnology is the field that deals with the design, synthesis, and characterization of NMs and aims to improve nanoscience to produce nanoparticles for potential novel applications.[8] Nanoparticles are particles with a nanoscale structure and present enhanced optical, electrical, chemical, magnetic, and thermal properties.[9] These nanoparticles occur naturally, or humans have produced them due to the combustion or milling of ordinary things for thousands of years.

2.1 History

Evidence supports the first civilizations using nanoparticles due to their enhanced optical and medicinal properties, such as Romans and Egyptians in the IVe and Ve centuries BC. For example, colloidal dissolutions were used in Egypt as eternal youth elixirs or medicine. The Maya civilization used nanomaterials and the production of dyes, which resulted in highly perdurable colorants. The Chinese civilization used various nanoparticles for medical and ornamental purposes. The father of modern medicine, Hippocrates, thought of silver for disease prevention and special healing properties[10]. The Romans used colloidal gold (31%), silver (66%) and copper (3%) nanoparticles in the *Lycurgus Cup*,[11] for decorative purposes. As it is observed in Figure 1, it presents an interesting optical phenomenon: when illuminated from the outside or the inside, it displays different colorations. This cup is recognized as one of the oldest synthetic nanocomposites, explaining scattering and absorption of spherical particles.

Figure 1. Lycurgus Cup.

Obtained from the online British Museum's Collection.

Another relevant example took place in the 18th century by applying silver nanoparticles to photography; they triggered the development of photographic films due to their light-sensitive properties, which served as pixels of an image.[12] In 1960, the physicist and father of the nanoscience Richard Feynman said at a scientific conference: "There is plenty of room at the bottom":[7] with these words, he promoted and predicted the advances that the scientific community would achieve in developing and manipulating nanostructures over decades to come. However, the term nanotechnology was not used until 1974, when the Japanese scientist N. Taniguchi referred to the possibility of performing engineered materials at a nanoscale level.[8] Nowadays, nanoparticles are exploited in many industrial and medical fields to enhance traditional processes or innovate and provide solutions.

2.2 Properties

The potential applications of nanoparticles result from their unique properties; these give them special characteristics that are highly differentiated from their bulk or atomic characteristics. Some of the fundamental properties that make the NPs an excellent source for investigation are mechanical, optical, magnetic, physical and chemical properties:

2.2.1 Mechanical properties

Mechanical properties of the NPs can be defined as the properties that these possess to resist a physical force applied to the particle. The mechanical properties will determine the efficacy for some applications like enhanced nanomaterials. These mechanical properties of NPs are divided into three main categories: a) hardness, which can be measured by the analysis of the particles' deformation with Atomic Force Microscopy (AFM), and its comparison with bulk material to determine size particle dependence. b) Adhesion or friction can be measured by attaching the particle to the AFM tip to assess the force between them. Finally, c) movement, in which it can track the particle motion without stimuli by fluorescence microscopy or under mechanical forces by Transmission Electron Microscopy observations.[13]

2.2.2 Optical

One of the most relevant properties of metallic NPs is the localized Surface Plasmon Resonance (SPR), which occurs as the frequency of the incident light matches the oscillation of electrons at the surface of a metal.[14] Noble metal bimetallic nanoparticles exhibit strong plasmon resonances, which could be controlled by varying their composition. This unique property is attributed to the collective excitation of the particles' electrons, which periodic change in electron density can be seen on the surface.[15] As shown in Figure 2, when the particles are smaller than the visible light wavelength, the solution acquires an element-dependent coloration and absorbance band. The interaction between the incident light and the free electrons of the material exhibit a resonance effect, this interaction depends on the size and shape of the particles. Surface plasmon of the NP can be used to determine the charges required for the correct interaction with other surfaces or macromolecules. For example, silver NPs may present a yellow/brown color while palladium NPs may present a brownish/black color; both have different colors compared to their bulk material.

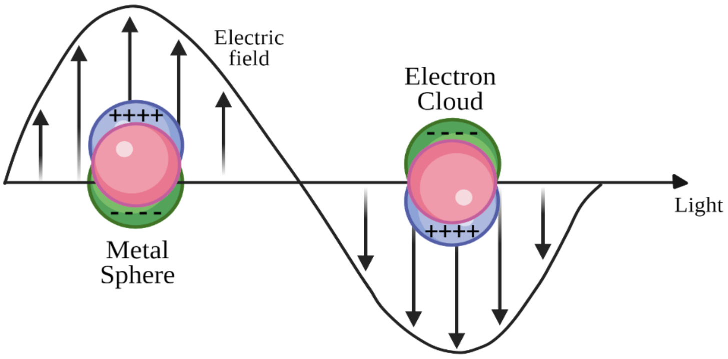

Figure 2. Surface Plasmon Resonance of noble metal NPs.

Created with BioRender. Adapted from Ref. [14].

2.2.3 Magnetic

Magnetic properties of NPs allow the control of the motion of the particles. Bimetallic alloys have exhibited high magnetic moment and are reported to have the highest saturation magnetization value. Therefore, the combination of metals enhances magnetic moments and enlarges anisotropy.[16]

Supreparamagnetism is a form of magnetism which appears in ferromagnetic NPs, this property depends on the size of the NPs, in which under special circumstances (temperature), the magnetic forces can randomly flip direction. Supramagnetism offers enhanced magnetic spin behaviors; this phenomenon is fundamental for characterization techniques, bioimaging like MRI or fluorescence, therapeutics like thermal processes or drug release, and cell control angiogenesis.[17] Magnetic nanoparticles are of great interest in several biomedical applications, such as catalysis, magnetic resonance imagining, environmental decontamination, biosensing, and drug delivery systems. The literature presents a more extraordinary performance of NPs when the size is under a critical value.[18]

2.2.4 Physical

i. Size

Size is the spatial dimension, proportion, or magnitude that an object occupies in space. As explained before, nanoscale may vary from 1 nm to 100 nm; however, multiple parameters depend on the size that the synthesis produces. NPs’ properties present dependence mainly on the size by which it was synthesized. At the nanoscale, minimal changes in the size may present huge, varied optical, chemical, catalytical, and antibacterial properties. The high surface area to volume ratio is directly related with the size of a NP. In general, a material with higher surface area to volume ratio, shows faster interaction with the environment, than a plain material. When the size of the NP is reduced, the surface area to volume ratio is increased. However, smaller NP size tend to be less stable.[19]

The study of this physical property can be divided into three main aspects: first, the physical dimensions of its atomic structure; then, the study of the size on a specific matrix, depending on the diffusion/sedimentation ability of the NP; and the size weighted by its mass/electron distribution.[20] High-resolution microscopy techniques can perform size analysis.

ii. Morphology

Structural properties may vary into different geometries and shapes. NPs that have distinct morphologies can present similar nanometric sizes, dimensions, and compositions. However, similar nanoparticles could differ drastically on their behaviors, like surface binding abilities, cellular interactions, optical and plasmonic properties.[21] According to the geometry, nanomaterials can be divided into Zero-dimensional nanoparticles prepared by chemical syntheses and produce nanospheres, nanopolyhedrons, nanoframes, and concavity parameters; these nanoparticles show any dimension outside the nanoscale. One-dimensional nanoparticles, present one dimension outside the nanoscale and could be presented as nanowires, nanorods, or nanotubes. Moreover, Two-dimensional nanoparticles are commonly nanosheets, nanoplates, or nanoribbons. Three-dimensional nanomaterials are not limited by the nanoscale in any dimension; for example, multi-nanolayers. In general, the properties of a material are the consequence of the forces over it; however, at the nanoscale, an important effect over the size and geometry of the NPs is the Quantum effect. This event is the interaction of the atoms and individual molecules as the size of a particle is reduced to nanometric; consequently, the NPs present different properties, altering their behavior.[22]

As observed in Figure 3, taken from Dykman et al., gold plasmon-resonance NPs were characterized to compare the various types of morphologies: a) spherical, b) nanorods, c) bipyramids, d) nanorods with silver core, e) nanorods with gold core, f) nanosheets, g) nanobowls, h) spike nanoshells, i) octahedra, j) nanocubes, k) nanocages and l) nanonecklaces. The different synthesis methods displayed various morphologies; And, for the varied applications, it is noticed that smaller nanoparticles presented less stability, tending to form clusters.[23]

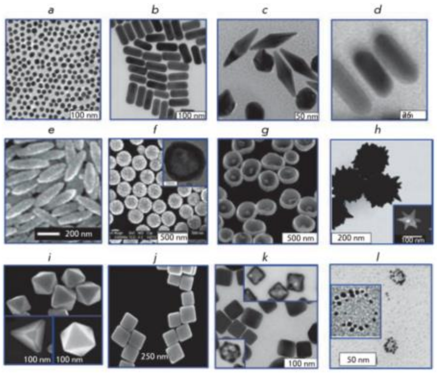

Figure 3. Nanoparticle Shapes.

Adapted from Ref. [24].

iii. Crystallinity

The crystallinity of nanoparticles refers to the atomic-structural order inside a particle. Most solids are crystalline as their atoms possess three dimensions. This order can determine different properties depending on a long or short-range order, determining a solid to be a crystal or an amorphous material.[12] At the nanoscale, crystallinity can be studied through the analysis of the disorder inside a system. The crystallinity of a nanoparticle can help in the development and controlled synthesis of porous nanomaterials; literature shows that crystal lattice can control the pore size and the porosity when the crystal structure is known.[20] Due to enhanced characteristics that deviate from respective bulk material in a size-dependent manner, nanocrystalline materials provide exciting properties for multiple applications, such as drug delivery technology for controlled release of the drug.[25]

2.2.5 Chemical Properties

i. Surface chemistry

The surface of a nanoparticle is a fundamental parameter for many applications, like antibacterial processes and catalysis. The performance of a NP nanoparticle highly depends on its surface chemistry as this constitutes the interface by which the NPs interact with other particles, molecules, and biological entities. The surface presents a specific charge that primarily affects the NP behavior in different environments and controls the NPs aggregation and stability.[26] The surface chemistry of the nanoparticles is a strong parameter to consider, especially on porous, concave, or hollow morphologies in which NPs exhibit a very high surface area.[27] The surface charge of a particle is related to the surface area; the charge distributes over the surface, hence, a smaller surface area would “pileup” the charge, provoking a stronger repulsion or attraction to other particle. Zeta potential technique is the study of the surface charge, in which the difference of the electric potential between the stationary layer and the surrounding charges is calculated. Therefore, it has been established that NP suspensions that present a zeta potential higher or equal to 15 mV are considered stable; to keep colloidal stability: lower attractive wan der Waals forces imply that the zeta potential should be significantly lower.[28] Hence, surface chemistry is an important property to control during the synthesis of nanoparticles to ensure their correct stability and functionality.

ii. Hydrophobicity or hydrophilicity

The surface chemistry discussed above, influence the exchange reactions depending on the chemical ligand bonded to the metal surface. Furthermore, the stabilization of the NPs is regularly influenced by the ligands; this process will lead to Coulombic repulsions depending on the chemical structure of the ligands.[29] Hence, water dispersion (wettability) of nanoparticles can be studied to understand many criteria like hydrophobicity or hydrophilicity. In the first case, water repulsion can determine toxicity levels against biological entities and in the second case, water affinity could determine a homogenous water dispersion. These properties are essential for the dispersion in the media and the prevention of aggregation as these are critical for biological purposes like drug delivery technology for direct interactions with the cell wall, like antibacterial property.[29] Besides, hydrophobicity and hydrophilicity can determine the potential toxicity of NPs; then, these are relevant parameters to prevent, for example, organic antifouling coatings materials,[30] especially against biofilm formation, is an enclosed matrix that bacteria generate to survive a hostile environment or chemical stress.

2.3 Characterization techniques applied to NPs

In 1981, the International Business Machines (IBM) team created an instrument named scanning tunneling microscope that allowed to capture of an image of the atomic structure of the matter.[7] This characterization technique led to discovering some properties at the nanoscale and presented the necessity of creating new screening techniques to see and analyze new physicochemical attributes for the NPs. As mentioned in section 2.2, metallic NPs present unique physicochemical, electrical, catalytic, and antibacterial properties. However, to provide evidence of the NP properties and their applicability, characterization is required to determine surface chemistry, size, morphology, and dispersion of the NPs. Some of the most used characterization techniques reported in the literature are discussed in the following sections:

i. X-Ray Diffraction

Diffraction results from the radiation dispersion regularly from dispersive centers, of which space is equal to the radiation wave.[31] In 1912, Von Laue postulated that if X-rays are waves and atoms are comparable to a wavelength, the atoms can diffract them. Therefore, X-rays are scattered by the electron atoms.[32] However, as the atom is heavier than the electrons, the atom will not be scattering the X-rays. With this principle, the X-ray Diffraction (XRD), as a characterization technique, analyzes parameter changes, such as the crystalline structure and nature of the medium phase, and the relationship between these parameters and the varying shape and size of the nanoparticles.[33] In nanoscience, XRD can be used to determine the composition of the particles by comparing the position and the intensity of the peaks. As it is presented in Figure 4, for the characterization, the source is pointed towards the sample, the incident beam (with known angle) will be refracted in the nanoparticle's surface. Therefore, the detector will be obtaining the angle in which the incident beam was refracted, and this information will be driven to a computer that will compare these two angles to give a predictive representation of the nanoparticle's size and atomic structure.[31]

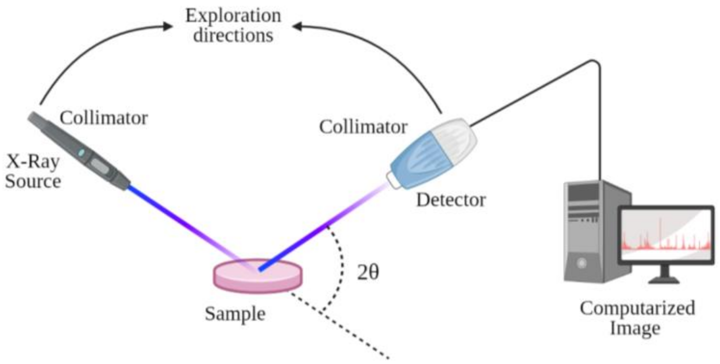

Figure 4. XRD graphic process.

Created with BioRender. Adapted from Ref. [31].

ii. UV-Visible Spectroscopy

Ultraviolet-visible spectroscopy (UV-Vis) is based on the capacity of a sample to absorb light when illuminated with electromagnetic rays in the visible and adjacent ranges of the light spectrum.[34] UV-Vis determines the concentration or number of particles in a sample; Through a calculation based on the wavelength of the materials of the NPs, the amount of NPs is determined. Hence, it provides an advantageous and reliable technique to analyze the successful synthesis and stability; also, to provide quality control of the as-synthesized NPs, by the measurement of the light intensity that passes through the sample and comparing them with the intensity of light that passes through a blank.

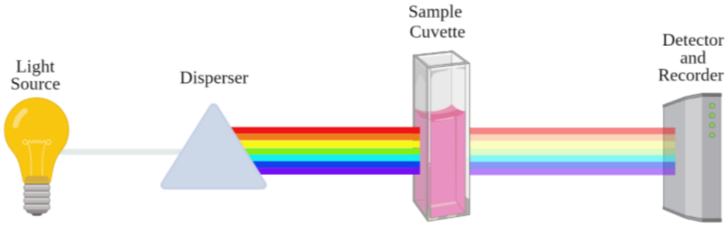

Figure 5. Scheme of a UV/VIS Spectrophotometer.

Created with BioRender. Adapted from Ref. [34].

As observed in Figure 5, the light source presents a specific direction towards the detection system, and the sample is placed in the middle. During the light emission, the light passes through a disperser, which will scatter the white light into its components; the sample cuvette serves as a filter, and only some wavelengths will cross it. These will get caught by the detector and transmitted to the recorder. For example, silver NPs show their conductivity band close to the valence band; consequently, the free electrons in resonance with the light wave led to an absorption band of SPR. The absorption depends on the particle size, dielectric medium, and surface chemistry.[35]

iii. Transmission Electron Microscopy

This characterization technique generates micrographs of NPs with a high lateral spatial resolution, and the same resolution can go to a high resolution down to 0.05 nm. In Transmission electron microscopy (TEM), an electron beam is used to develop an image of a nanoparticle structure surface, by the interaction of transmitted electrons with the atoms of the NPs. This characterization technique produces a visual crystallographic description of the structure, size, and shape single-particle level or a more significant scale to determine size distribution.[36] As displayed in Figure 6, electrons are projected to form an incident light source, then they are transmitted through the sample and scattered. Later, the electrons are focused on an objective lens, and finally, this lens will amplify and produce a signal which will be transformed by a display to create an image.[12] In this process, the sample preparation is needed to be as thin as possible to obtain a clear image; therefore, this part of the characterization can be time-consuming and expensive.

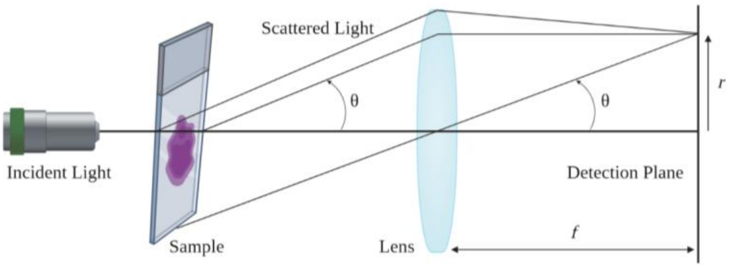

Figure 6. Transmission Electron Microscopy Technique.

Created with BioRender. Adapted from Ref. [12].

iv. Scanning Electron Microscopy

This characterization technique aims to obtain information about the surface or topography of the NP; this process is carried out by the identification of the secondary electrons emitted from the sample as it interacts with the electron beam.[37] Scanning Electron Microscopy (SEM) is a widely used characterization technique; it can provide a digital resolution of ~10 nm and display a chemical analysis. As shown in Figure 7, the sample surface study is carried out by measuring the amount of elastically backscattered electrons, which the detector will obtain. The interaction between the focused electrons and the NP will present the information for the micrographs interpretation.[20] SEM micrographs take place at low pressure, making viable the characterization of nonconductive NPs. Working at lower pressures reduces electron scattering, improves the signal-noise ratio, and achieves a more focused beam current. The control over these parameters will present an enhanced image and effective X-ray analysis.[38]

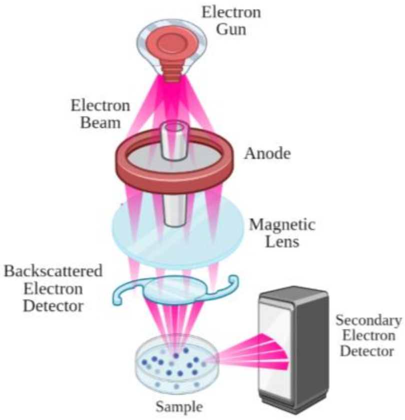

Figure 7. Scanning Electron Microscopy.

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v. *X-Ray Photoelectron Spectroscopy*

This characterization technique is based on detecting photoelectrons from a nanoparticle; the emitted signal results from the X-ray single-energy photons irradiated on the sample. X-ray photoelectron spectroscopy (XPS) is due to the photoelectric effect discovered by Hertz in 1887.[39] As shown in Figure 8; the incident X-ray photons can penetrate a few micrometers into the nanoparticle. Thus, XPS is a sensitive surface characterization technique, owing to the short path the photons pass through. The free path range is around 0.5 nm to 3 nm; therefore, the short-range of these photons into the sample will prevent inelastic scattering.[40] The photons' emission is the characteristic energy of each metal; the binding energy is measured from the electron ejected from the kinetic energy. This synthesis technique is used for various NPs, as it presents a non-disruptive penetration, it can identify all elements except for H or He and can determine the surface chemistry.[39]

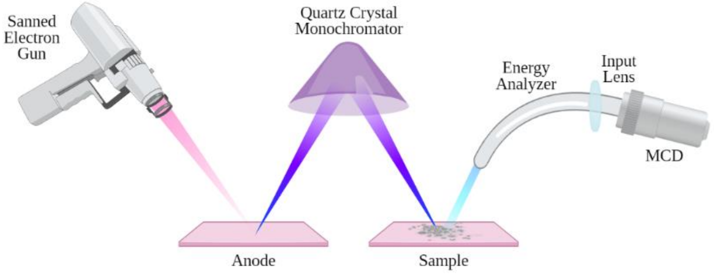

Figure 8. X-Ray Photoelectron Spectroscopy.

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vi. *Dynamic Light Scattering*

This technique is used to determine the hydrodynamic size and particle distribution on a solution; it depends on the interaction between the light and the NP. Dynamic light scattering (DLS) can characterize a nanoparticle from 1 nm up to 500 nm.[41] Particles can be determined from a suspended liquid, and light is scattered off the particles in suspension. The variation in the intensity is produced by the NPs and collected for the normalized analysis.[42] Dynamic range goes from 1 nm to 10 µm, and it measures the movement of nanomaterials due to Brownian motion. This technique can be carried out on clear suspensions with a known kinematic viscosity of the solvent for optical reasons.[43] As observed in Figure 9, the laser is first focused on the sample, and then a thermal motion produces fluctuations that pass through a lens; and the detector will sense these changes. The rest of the light that passes linearly through the suspension is transmitted.[20] The time variation between these fluctuations depends on the scattering of the NP's surface.

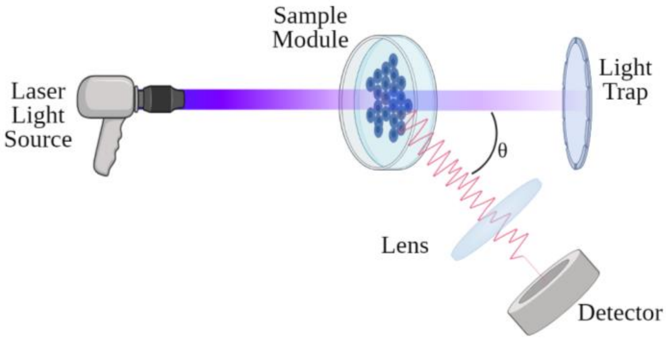

Figure 9. Dynamic Light Scattering.

Created with BioRender. Adapted from Ref. [44].

vii. Atomic Force Microscopy

Physicist Binnig developed this characterization technique in 1986 in order to determine the solid surface of the particles and provide a three-dimensional image. Atomic force microscopy (AFM) utilizes a sharp tip that deflects upward in the case of a net repulsive force or downward in a net attractive force. The contact of the tip can be modified to prevent the disruption of alteration of the surface. Besides NP surface, some other properties can be analyzed with this characterization technique, such as magnetic forces, chemical forces, or surface potential. AFM is usually used in a high vacuum to achieve very high resolutions, down to the atomic level to the microscale.[45] As observed in Figure 10, in AFM, the cantilever serves as a laser reflector energy between the diode laser and the sample surface; this produces a force measurement as the tiny probe pushes the surface, the particle resistance elevates the cantilever, and therefore the reflex is detected and processed on a computer for feedback control of the sample.[46]

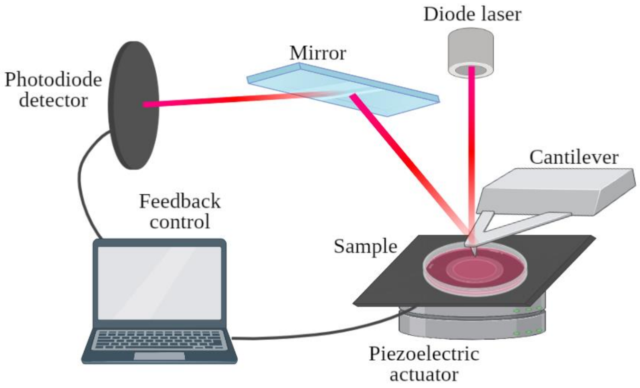

Figure 10. Atomic Force Microscopy

Created with BioRender. Adapted from Ref. [39].

viii. Fourier Transform Infrared Spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR) uses the interaction between infrared radiation with materials to obtain information about molecular structure, absorbance variations, and chemical species for redox reactions.[47] This characterization technique uses infrared spectrum to excite vibrational or rotational states of the atoms or particles. Data collection can be carried out in less time because of the use of low-intensive infrared spectroscopy.[39] The mathematical method of the Fourier transform is utilized to develop a curve obtained from the signals; this transform, constituted by the summation of sines and cosines functions, will work the different optical frequencies into a easy to interpret signal. This mechanism requires an adequate quantification of film thickness, and composition can be acquired and compared with other nanostructures.[48]

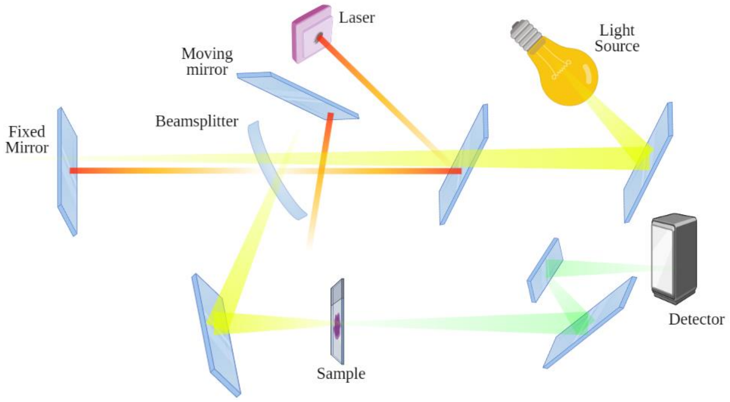

Figure 11. Fourier Transform Infra-Red Spectrometer.

Created with BioRender. Adapted from Ref. [49].

As observed in Figure 11, the FTIR consists of a laser, a light source, and multiple optical components, like a beam splitter and adjustable mirrors. The detector can obtain information about surface chemistry properties to study or prevent other organic/inorganic compounds from aggregating to the surface. FTIR also facilitates the analysis of the outermost layer coated on the element and alters this phase.

2.4 Applications

Nanotechnology has risen many applications, mainly in industrial and medical fields. Furthermore, bimetallic nanoparticles like Ag-Pd NPs are being exploited by their enhanced properties presented in 2.2. Some of the most studied and promising applications of nanoparticles are the following:

i. Catalysis

NPs are used in the industry for catalytic processes, thanks to excellent surface area values that make them more inexhaustible in the remedial field.[50] Catalysis is the process in which the rate or velocity is directly influenced by a molecule or particle (catalyst); this substance is not naturally part of the reaction and could be added or removed to improve the quality and ease of the process. Multiple agents can serve as catalysts, like biomolecules or chemical reagents; however, since 1950, the term nanocatalyst was considered from nanoscience studies. On the nanoscale, NPs present a higher surface to volume ratio to enhance the catalyst to be more accessible to reactants and other substances.[51]

Recent studies have presented excellent catalytical properties of NPs, especially bimetallic. Bimetallic NPs have been used as catalytic agents since they present a more active surface to provide efficient contact between the reactant and the catalyst; this property increases its robustness and stability.[52] A nanocatalyst can also improve environmental problems like sustainable processes with lower energy consumption and, therefore, less environmental impact to aim for a greener industrial field.

ii. Material Science

This Physics field comes from: first, the scientific view about function and classification of structures; second, from materials and the four primary structural materials with electric and magnetic properties; and the engineering view, regarding the processing, degradation, and the correct selection of the materials.[31] Governments keep implanting public norms to reduce the increasing contamination, even though this could represent economic and efficiency losses to industries. Accordingly, the creation of enhanced materials and processes is urgently required.

NPs open to novel electronic, chemical, thermal, and mechanical properties into regular manufactured materials. For example, NPs' utilization to develop nanostructured materials to obtain resistant surfaces to scratches or make them sterile and waterproof.[7] Therefore, a novel engineered nanomaterial (NM) can obtain enhanced properties that could help it endure extreme conditions, such as electronic devices and aerospace sciences, or replace traditional materials harmful to nature, human health, and the economy.

iii. Environmental Engineering

This bioengineering is a field which scope is to process analytical designs for the water supply, waste disposal, and control all risks of pollution. Moreover, environmental engineering materials must be planned to stand the challenging conditions when operated, controlled, and enforced.[53] Therefore, engineered materials like nanoparticles are a potential application in this field. For instance, bimetallic NPs are also being used for hydrogen storage and as environmental catalysts. New technologies are developed to improve the efficiency and cost of standard environmental devices, such as solar panels and photovoltaic cells. This field is helping save energy on food, transport, and illumination industries worldwide.[7] One of the most relevant NPs' applications is to help with decontamination processes, for example, waste water or on polluted rivers. NPs have the ability to interact with contaminants as they absorb them into their surface; therefore, the controlled synthesis of these NPs is fundamental to lean for a specific size, composition, and porosity to adjust a specific contaminant.[25]

iv. Mechanical Sciences

Mechanical sciences are branches that study the generation, transmission, and utilization of heat and mechanical power for industrial and medical purposes; this field designs and produces tools, machinery, and products to conduct this energy.[54] Among multiple technologies being studied for mechanical sciences, nanotechnology has attached much attention because of its remarkable features, like high sensitivity and heat response.[55]

For mechanical purposes, the nanoparticles are designed to present specific properties such as stress and strain resistance. NPs offer sliding and delamination properties that can affect materials that suffer stress and friction; hence, these could help with lubrication[13]. In the medical field, Al, Ti, and carbon-based NPs coatings are applied to enhance mechanical properties and avoid microfilm formation. Therefore, NP coatings may serve in multiple applications, as it improves toughness and resistance; it may also develop a smoother surface and resistance to corrosion, which are fundamental features for the engineering of mechanical applications. [56]

2.5 Silver Nanoparticles

Silver's properties were found as an effective antimicrobial agent for almost all unicellular organisms by Vonnaegele in 1890.[6] Since then, the search for the applicability of silver has been exploited in several fields, including medical, industrial, material coatings, cosmetics, orthopedics, food industry, diagnostics, and anticancer treatments. Biomedical applications of Ag NPs are emphasized, as these specific nanostructures presented marvelous results as listed following: Antibacterial activity against *S. aureus*,[57,58] antifungal activity against various types of *candida*,[59] enhanced antiviral activity against human immunodeficiency virus (HIV) and hepatitis B virus (HBV),[60] anti-inflammatory processes into diminishing inflammation chain reactions on would increase healing processes,[61] anti-angiogenic activity, tested on bovine endothelial cells, inhibiting vascular growth factor (GF),[62] anticancer activity producing increased alterations of metabolism of cancer cells, and increasing production of reactive oxygen species, leading to mitochondrial damage and cancer reduction.[63]

AgNPs can be obtained via three main methods: physical, chemical, and biological methods. The chemical methods are the most used thanks to easing efficiency to perform a controlled synthesis. However, the chemical method can use or result in reactive compounds that are very hazardous for nature and human health. [64] Thus, recent studies propose new synthesis techniques based on the utilization of organic compounds to aim for a greener nanofabrication method.

2.6 Palladium Nanoparticles

Palladium is a precious metal with extraordinary catalytic, mechanical, and electroanalytical properties. Diverse studies have reported improved catalytic systems when supported on Pd NPs; this property can be explained thanks to Palladium's increased affinity to aliphatic compounds.[65] Despite the remarkable properties of Pd NPs, it has not been deeply studied until recent years, especially in the biomedical field, for example, on anticancer treatments such as photothermal therapy, dental applications, needles, and melanoma brachytherapy.[66] Besides, Pd NPs have proven enhanced antibacterial and cytotoxic-pharmacological activity against the growth of Gram-positive microbes such as *S. aureus*.[50] However, PdNPs present a safe toxicologic profile against other Pd species. While Pd is an expensive material to work with, new approaches focus on reducing Pd's amount by using a second metal. Taking the benefit of metallic catalytic and optical features, the creation of a bimetallic NP is required to improve engineered nanostructures.

CHAPTER III

3. BIMETALLIC NANOPARTICLES

The use of multi-metallic NPs came from the necessity to solve various monometallic applications' limitations, such as reducing the amount of an expensive metal such Pd and enhancing specific processes' catalytic activity. Hence, many studies proved that bimetallic nanoparticles presented distinct properties compared to a nanoparticle of the metal alone; these features can get enhanced, modified, or impaired. Therefore, metallic nanostructures' remarkable properties would provide enhanced systems and capability of multiple effects when used as bimetallic NPs. The modification in the structural and electronic properties may directly affect their catalytic properties improving selectivity and durability.[67]

The morphology of bimetallic NPs can be varied; for example, an alloy NP presents a structure that can be a homogeneous particle of the two metals. A core-shell and super core-shell morphology present a different phase for each metal and can be built multiple times. A particle-in-particle NPs are a metal's particle inside the second metal. A particle-on-particle, particles of metal are attached to the second metal's surface. An aggregate nanoparticle, in which a particle is formed out of the aggregation of metallic domains or sub-particles. Moreover, individual nanoparticles present separated particles of each metal. To summarize, possible morphologies of bimetallic nanoparticles are represented in Figure 12.

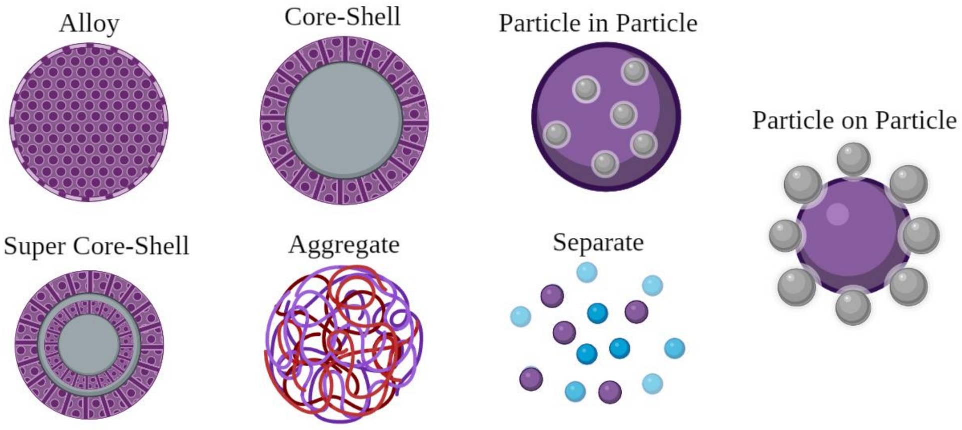

Figure 12. Morphologies of bimetallic nanoparticles.

Created with BioRender. Adapted from Ref. [68].

In the synthesis, when both metals are present simultaneously, their simultaneous nucleation leads to an alloy structure, in which both metals are mixed in the product. SPR can analyze the internal distribution of bimetallic NPs; as for alloyed NPs, the maximum absorption band shifts linearly with the composition, and for core-shell nanoparticles, only the shell can be analyzed.[69] As observed in Figure 12, the alloy NPs present a new phase visibly different from either of the two metal phases out of all morphologies. Therefore, the properties of bimetallic NPs are distinctive from the monometallic NPs, depending on the size, atomic order, and continuity.[68]

3.1 Synthesis of Bimetallic Nanoparticles

The NPs synthesis aims to build well-defined structures at a nano level. This part of nanotechnology studies the design and development of NPs that meet relevant criteria such as cost-effectiveness, controlled size, shape and composition, viability for large-scale production, facile or single stepped processes, and benign to nature and human health. Nanofabrication methods for NMs can be divided into two main approaches: Top-down and bottom-up. As observed in Figure 13, the top-down method, minimization of the raw material (bulk), is the pathway to obtain the nanoparticle, in contrast to the bottom-up method in which the self-organization of the atoms is required to produce the nanoparticles. The top-down approach is a faster method; therefore, control over the shape and size of the BMNPs is more challenging. On the other hand, in the bottom-up approach, the size and shape can be controlled by the management of synthesis parameters; hence it is a slower method.[70]

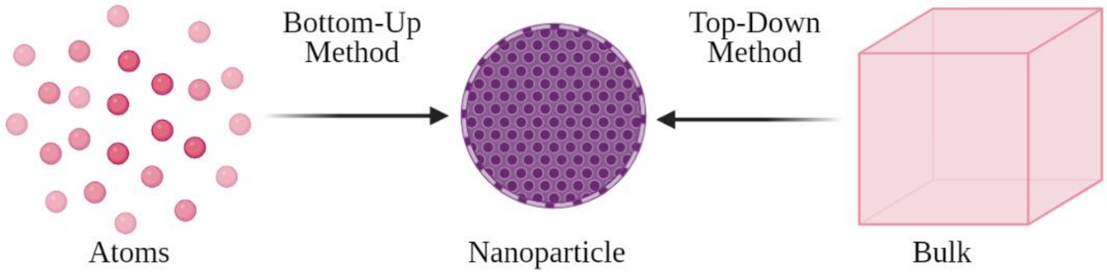

Figure 13. Nanofabrication Methods.

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3.1.1 Top-down Approach

This nanofabrication method consists of the division of bulk materials into more petite proportions. This fabrication type can be physical methods like milling, chemical methods, attrition methods, and a material's volatilization for its condensation into a solid.[71] Some of the most appropriate top-down methods are:

i. Thermal decomposition method

Thermal evaporation refers to the vaporization of a specific material by increasing a solution's temperature; hence, the material's vapor pressure gets significant, and atoms are loosened from the surface thanks to the vacuum applied[72] or deposited onto a cold surface. This method is driven by the decomposition temperature, defined as the specific temperature at which the element is chemically decomposed. This endothermic process produces a chemical decomposition by the introduction of heat[73]. Thermal evaporation is usually used to fabricate thin films of materials; however, multiple types of NPs such as bimetallic are fabricated. This method is one of the most used to obtain stable monodisperse suspensions with self-assembly ability. Due to size reduction, the high surface area to volume ratio can lead to enhanced catalytic activity[74]; besides, the metal oxidation can be reduced, and contamination can be avoided.

Hao et al. presented the synthesis of bimetallic NPs via thermal evaporation, obtaining an average size NP of ~7 nm and a spherical shape.[75] This synthesis technique is known for the resulting deposition on a surface for nucleation and growth; hence, the applications can provide a valuable deposition of thin coatings for multiple purposes like antibacterial and antifouling surfaces. Although the thermal evaporation technique does not require the use of hazardous chemicals, at commercial/industrial levels, the electric furnace needed to generate enough temperature rise is significant, implying an increase in energy and costs.[76]

ii. Laser ablation

This method is a facile technique for the synthesis in a different solvent. The laser ablation method can prepare bimetallic nanoparticles without using any chemical agent; thanks to the high potency laser, it produces vapors carried out by inert gas and deposited into a substrate.

The irradiation on metal submerged in the solution condenses a plasma to produce the nanoparticles[73]. Beyth et al. presented the synthesis of bimetallic nanoparticles with laser irradiation; silver-based BMNPs presented enhanced antibacterial applications. The synthesis method chosen was given thanks to the photosensitivity the metal presents[77].

Literature has presented the laser ablation method as a process for successful control of bimetallic nanoparticle synthesis; hence, the formation of Ag-based bimetallic NPs presented a spherical shape and presented average sizes of 13 to 30 nm when utilized with acetone as solvent.[78] In this process, the stabilization and agglomeration can be controlled with the temperature by maintaining the solution at 3 °C as presented by Censabella et al. on the fabrication of Pd-based bimetallic NPs. Loza et al. propose laser ablation as the most controllable top-down method,[69] in which it is suitable to obtain well-dispersed bimetallic nanoparticles from a solid target (bimetallic alloy).

iii. Lithography

Lithography is based on utilizing a pre-designed patterning substrate to create similar or equal structures; the principle is based on the interaction of beams of photons or particles with a mask. Nanolithography starts where microlithography stops; this synthesis method can be presented as two types of nanofabrication methods: primary, based on radiation, photons or charges particles can drive, and secondary, non-radiation based which can be nanoimprint, dip-pen-polymer pen or directed by the self-assembly. The nanofabricated material should be susceptible to radiation and must be able to keep stable during the lithographic process, to optimize these methods.[79] Hence, the literature presents lithography as a correct nanoparticle synthesis method. For example, Cha et al. presented the lithographic patterning of Fe-Pt NPs with an average size of ~11.5 nm and a crystalline domain with a core-shell shape.[80]

The patterns are designed to present a specific spacing to gain control over the NPs' properties; this synthesis method can modify or implement additional parameters such as unidirectional shear or strain on the mold or substrate.[81] The green synthesis can be aimed in this method depending on the technique required; for example, hazardous chemicals can be replaced for organic solvents in nano-imprint lithography. Pattern replication techniques like nanolithography can generate copies for rapid, efficient production of copies with low cost and high yield.[82] However, some lithographic techniques may require slower processing time to achieve a high-resolution pattern and product. Hence, scale-up processes can get difficult.

iv. Milling

This physical method is performed by the utilization of heavy balls for the milling; these metallic balls will reduce the size of the particles and attach the NPs for the correct distribution. The required material is loaded together with many steel or tungsten carbide balls in a container during the process. For the mechanical drops of the NPs, a mechanical mill has been stated; finally, it will be observed an alteration of the physicochemical properties and structure of the nanoparticle[83]. During this synthesis, kinetic energy will be transferred to the material, which is under reduction. This mechanical method can produce nanoparticles and is preferred due to its inexpensive operation from bulk. Many nanoparticles can be produced with this method, such as Ti and Ag Oxide powders can be transformed into 14 to 22 nm nanoparticles in 22 hours [73]. Ban and team members presented the synthesis of bimetallic nanoparticles for hyperthermia therapy. This study obtained a nanocrystalline CuNi alloy with an average crystallite size of around 10 nm platelet-like, at Curie temperature of 45 °C, and used NaCl to stabilize the nanoparticles and avoid agreggation[84].

The homogeneity of the NPs by milling must constantly improve the parameters to enhance scale-up processes and ensure quality. The literature presents for this method that the coating of these BMNPs with surfactant can prevent agglomeration and are dispersed in a polar medium which can be water. The few or scarce utilization of reducing and stabilizing chemicals converts the process into an unexpensive reaction compared to other top-down methods. Hence, the method is extensively used for industrial processes and nanoparticle formation, and its application can eliminate or dramatically reduce the usage of hazardous agents. However, one of the main limitations is the high energy required for the mechanism, converting the ball milling method in a prolonged-time reaction to synthesize BMNPs.

3.1.2 Bottom-Up approach

Unlike top-down methods, bottom-up ones do not require advanced or complex instrumentations, making these less expensive than top-down approaches. This synthesis technique is characterized by its fundamental self-assembly processes, which significantly alter particle structures and sizes[85]. The nanoparticles' growth can be controlled through multiple parameters, such as concentration, solvent, stabilizing agents, and temperature. The short time nucleation reinforces monodispersed particles' synthesis; this occurs thanks to the atoms' catch on an existing nuclei[86]. The large particles will be reduced in size, and the smaller particles will grow. Therefore, any new particle will be created, and the size distribution will be controlled.

i. Emulsion and Precipitation

This nanofabrication method is designed with three main factors: a dispersed phase, a minor droplet, then a continuous phase, an immiscible solvent, and finally, a surfactant to cover the minor droplet. The system modification of these three parameters will depend on the emulsion type, like water-oil or water-Triton x-100.[70] The synthesized metallic NPs will be deposited inside the droplets; these will control size and composition during growth. The process is driven by the aqueous droplets colliding during the reaction and consequently breaking apart, resulting in the modification of the physicochemical properties of the particles from the solution. As the concentration reaches a soluble state, the nucleation will go by, and finally, the growth of each particle will stop once the concentration decreases.[87] The synthesis of bimetallic nanoparticles by emulsion and precipitation has been presented in the literature by Zhang and Chan. They prepared a homogeneous alloy of Pt-Ru with an average size of 4.5 nm;[88] furthermore, they tested the dependency of the nanoparticles' size regarding the concentration of the precursor. The results presented the NPs to be limited by the collisions during the reaction and mixing with thousands of hydrazine droplets, and at higher concentrations, the NP size gets increased.

The synthesis is commonly performed with organic solvents that will overcome the reduction and start nucleation. The use of stabilizers is not usually required, thanks to the promoter that stabilizes the nanoparticles' growth to prevent or regulate precipitation. Among all the nanofabrication methods, emulsion and precipitation method is a versatile technique. With the right design of this system, we can obtain a controlled synthesis of parameters like size, geometry, morphology, homogeneity, and surface area of the NPs[89]. However, the nucleation is limited by the precursor's collision, decreasing the yield of the synthesis method on scale-up processes.

ii. Chemical Vapor Deposition

Chemical Vapor Deposition (CVD) is the chemical process in which a gas transports a precursor to be decomposed and form a solid; this precursor interacts with other gases and products[90]. This method is based on the chemical reaction of combining gas phase in contact with a heated surface. Here, compounds get degraded inside the reactor or near the solid surface, resulting in a thin layer-shaped material or a NPs[71]. The CVD method can synthesize NP that can tolerate high temperatures such as metal oxide NPs for coatings and bimetallic nanoparticles for electrical conductivity enhancement.

Choi et al. presented bimetallic nanoparticles synthesized by CVD; when dissolved in hexane, it served as the stabilizing agent and helped with the shape and size control of the NPs[91]. On the other hand, this process requires expensive instrumentation and may produce toxic by-products as a consequence. Khiriya et al. developed bimetallic NPs by CVD; these presented a size range of 5-11 nm and spherical[92]. In the direct chemical vapor depositions, a controlled synthesis can be carried out by modifying some parameters, for example, the direction of the gas flow and pressure; these can influence the crystallinity and morphology[90]. Studies have reported pre-patterned sources to control the nucleation of materials during the chemical deposition in the vapor phase. However, the lack of control in the domain could be a limiting factor for more significant applications such as graphene-based devices with more reliable performances[93].

iii. Pyrolysis

The NPs' formation through pyrolysis is driven as an anaerobic process. First, the decomposition of the precursor on the metallic surface, then ionic diffusion through the metal particle and its precipitation, and finally, the diffusion through the bulk[94]. Ijaz et al. suggested a mechanism to explain the synthesis via pyrolysis. By the fusion of the molecule or atom due to a specific mechanism like spinning, then the precipitation of the material will occur, leading to nucleation; finally, the collected particles will be dried. Commonly, laser or plasma can be implemented to increase the temperature rise needed for the synthesis; hence the temperature levels will produce evaporation of the particles[73].

The synthesis of bimetallic NPs by pyrolysis has also been studied; Zhao et al. developed a Pd-based BMNP that was presented as a uniformly dispersed nanoparticle with an average crystalline size of ~7 nm for catalytical purposes[95]. They utilized trisodium citrate as a reducing agent and sodium borohydride as the stabilizer, chemicals that are highly hazardous for human health and the environment. On the other hand, recent studies have proven the successful synthesis of metallic NPs by replacing deionized water as solvent. Due to high levels of efficiency, inexpensiveness, simplicity, and high yield, Pyrolysis is the most used synthesis method in the industry. However, this process is still conditioned by the heat transfer limitation and needs further studies.

iv. Chemical Reduction

This synthesis method can be divided into two phases: reduction and growth. The first, a metal precursor precipitate in a redox reaction and forms a core; then, a second precursor is deposited as a shell[70]. The chemical reduction method uses chemical reducing agents such as hydroquinone, sodium borohydride (as a strong reductant), alkaline solutions, ascorbic acid, and dimethylamine borane reduction of metal ions.[64] Potent reducing agents yield smaller NPs compared to weak reducing agents; however, smaller NPs tend to be more unstable than others of the same metal but bigger size. Ammonia can be used to solve low stability. Huang et al. developed a synthesis of nanocrystalline Ag-Pd alloy by chemical reduction; the bimetallic nanoparticles were obtained from a range of 2.46 to 6.6 nm, in which a 50-50 rate obtained a crystallite size of 4.16 nm cluster. Huang et al. also state that this chemical procedure is suitable for scale-up procedures, but more investigation is required[96]. To reduce the use of hazardous chemicals, for example, silver ammoniacal solution, we could use biological agents like saccharides and polyols, and the temperature of the process can be increased to prevent the yield from diminishing.

a. Galvanic replacement

Galvanic Replacement can be considered a modification of a Chemical Reduction method. This synthesis method replaces the atoms of the first metal that react with ions of a second metal with a higher electrochemical potential; these atoms get oxidized, and the second's metal ions get reduced into the surface of the metal template[97]. This method provides a facile way to convert more noble metals into well-structured nanoparticles; The reducing agent on galvanic replacements presents enhanced properties such as remarkable tunability and catalytical activity[98]. Wu et al. presented a continuous synthesis of hollow Ag-Pd NPs with an average size of ~15 nm through galvanic replacement, in which the method can be significantly enhanced in the presence of a mild reducing agent such as hydroquinone. However, catalytical properties of the alloy get improved, and by-products could get controlled[93]. The main limitation of this methodology is the search for a capping agent that stops the dealloying or agglomeration process of the NPs, as the utilization of these agents can significantly reduce the reaction yield[99].

v. Solvothermal

The metallic precursor is dissolved in a hermetic reactor, in which temperature will be risen above the metal's boiling point, consequently increasing temperature. Liquid agents like ammonia, hydrazine, and organic solvents are commonly used[71]. The solvent of the reaction can also be water; hence the technique is named hydrothermal synthesis; as the reaction takes place over 100 °C and 1 atm, it is viable to use some materials that can be hard to dissolve. Most solvothermal techniques are employed below the critical temperature; hence batch processes can make the production on scale-up levels be cost-effective and less environmental impact; however, unexpensive solvents can cause problems due to limitations caused by low boiling point [100], for example, ethylenediamine.

The synthesis of NPs can be driven thanks to the use of solvents above their boiling point and atmospheric pressure. Bimetallic NPs are seldom studied in literature; however, Wang and Gengyu presented the synthesis of bimetallic Pd/Ag nanoparticles by solvothermal method. Here they obtained spherical alloys of an NP with an average size of 10 nm and core-shell structures with an average size of ~40 nm, suggesting optimal conditions of 180 °C.[101] This method is presented as an energy-efficient, environmentally benign, and versatile process for multiple materials.

vi. Hydrothermal method

The hydrothermal method is processed at high temperatures and pressure values; this method takes place in a sealed reaction vessel, producing nucleation and growth of the NPs. Polyethylene glycol can be used as the surfactant; this polymer is highly recommended as it is non-toxic, non-flammable, and easy to handle. In the hydrothermal process, the materials are carefully weighed and dissolved in water, then dried and repeated multiple times.[102] Ultrasonic and centrifugation can also be used to enhance the reaction. The nucleation of nanoparticles is enhanced by the rate and supersaturation, with the aim to lower the solubility of a material to induce particle formation. This nanofabrication technique is based on supercritical water preparation, which will give the reaction-specific parameters for the correct synthesis of nanoparticles; these parameters will also determine particle size and stabilization.[103]

A bimetallic alloy prepared by the hydrothermal method was reported, in which an average spherical NP of 22 nm was successfully obtained and presenting optimal conditions at 95 °C.[104] Two metals are added to citric acid solutions with continuous magnetic stirring. Then ammonia is used to maintain the pH. The reactant's concentration, the reaction temperatures, and times can be adjusted to reduce the size and control the shape of the synthesized NPs. With rapid heating, uniform size, and faster reaction time, the yield and homogeneity of NPs can be scaled up[102]. However, since supercritical water can be obtained under high temperature and high-pressure conditions, there are still limitations for in-situ measurements to elucidate NP formation.

vii. Sonochemical

This technique is produced by sound waves that propagate through the media in low- and high-pressure cycles, generally from 20 kHz to 40 MHz. Ultrasonic cavitation will form, and bubbles will create, grow, and collapse, consequently, break chemical bonds. This process will produce changes in the particle's physical and chemical properties, such as high temperature, pressure, and cooling rates, which are significant parameters to keep under extreme conditions. NPs can be formed due to the fast kinetics that does not allow the growth of the nuclei or due to the non-volatile precursor that surrounds the bubble. The temperature in the outer ring is lower than inside the collapsing bubble but higher than the bulk's temperature. The morphology depends on the ultrasound process; when simultaneous sonication is chosen, an alloy particle could be produced, or when successive sonication is applied, a core-shell structure could be obtained. The literature presents that the dilution of the reaction's precursor will directly affect the nanoparticle size by decreasing it.[94] The particles' size is dose-dependent; as the sonication intensity increases, smaller particles will be obtained.

Bimetallic Au-Pd NPs presented a dispersive distribution and average size of 8 nm of a gold core and palladium shell. This technique is presented as an inexpensive, facile, quick method to obtain NPs; it does not need the excessive use of reducing agents and hence will not exhibit hazardous chemicals as secondary products. This procedure can be taken at room temperature and assemble multiple shapes, like nanobelts, nanotubes, core-shell, nanorings, and other bimetallic morphologies[105]. The design of different parameters like frequencies, power, pulse, stabilizers, the temperature can directly enhance the synthesis yield and the NPs produced. However, ultrasound synthesis is a highly energetic process, and scale-up processes can get complicated.

viii. Microwave

Microwaves are a form of electromagnetic radiation; they have operation frequencies from 900 to 2450 MHz range and vibrate close to radiofrequencies[106], as observed in Fig 14. Microwave irradiation causes energy transference by directed molecular interactions; therefore, heating is a conversion of electromagnetic energy into thermal. This energy causes volumetric heating producing a faster temperature rise compared to other methods[107]. Microwave synthesis has two main mechanisms: First, the dipolar mechanism occurs under a very high-frequency electric field, and a polar molecule attempts to follow the field in the exact alignment. Second, the high temperature of the molecules drives the reaction forward. Then, the irradiated sample, in which a component conducts electrical charges (ions and electrons), moves through the material within the sample[108], increasing its temperature.

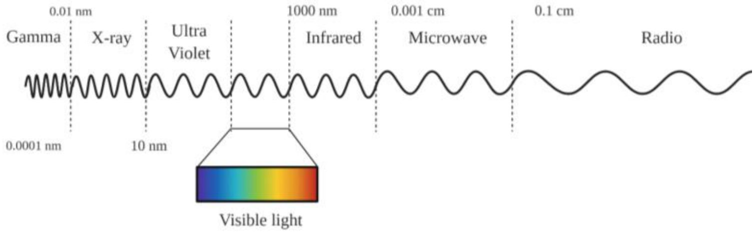

Figure 14. Electromagnetic Spectrum

Created with BioRender. Retrieved from [109].

This NPs Synthesis method can direct action the materials, as these enhance heating and allow quick cooling. In the microwave method, ethylene glycol can be replaced with water, which is the most suitable solvent, as ions move through it and are influenced by electric and magnetic fields[110]. Any hazardous chemicals are needed for this high-temperature procedure. Literature conducted a synthesis of metallic NPs at different pH values and obtained various shapes, such as cubic or spherical particles with an average size of ~ 25-40 nm. The preparation of BMNPs has also been reported by microwave technique, for example, for Ag-based nanostructures. Nadagouda et al. produced spherical NPs and with average sizes of ~ 10 to 11 nm diameter[108]. Nanoparticle formation can be given just after 300 s of total microwave heating. This synthesis can increase the energy yield, present short-time reaction, produce small-size NPs, and minimize the production of side products and impurities that could risk nature or human health. Despite being an excellent alternative green method, on the scale-up synthesis, the microwave method presents some limitations like the variable physicochemical properties of the NPs, which may vary from one batch to another, and that frequent purification may be needed.

ix. Sol-Gel

The Sol-Gel method is a process in which solid materials are obtained from small molecules, in which a solution (Sol) gradually grows on the solid macromolecule (Gel); hence, a biphasic system is produced. The synthesis of NPs is usually carried out with the support of physical methods like stirring and shaking. After the synthesis, a separation process will be required for the obtention is performed to obtain the NPs. For bimetallic NP synthesis, it is carried out hydrolysis and reaction condensation form a stable sol system. Citric acid and ethylene glycol are some of the agents needed for this method. The pH of the synthesis can be adjusted by adding ammonia to the sol, and this will convert the sol from homogenous transparent to viscous brown.[111] Supriya et al. presented the synthesis of noble bimetallic nanoparticles by sol-gel technique; TEM images indicated an average particle size of particle ~3.5 nm and the particles' stability at the nearly spherical structure.[112] The BMNPs synthesized through the Sol-Gel method presented great purity, controlled porosity, and easy preparation of varied sizes.

A Sol-Gel synthesis for the obtention of metallic NPs with good dispersity was obtained by Hu et al.; they utilized ferric nitrate and citric acid with ammonia to adjust the pH of the reaction to control the parameters like size and avoid agglomeration of the synthesized NPs.[113] Due to simplicity, the sol-gel method is the most used synthesis method; however, the use of organic solvents could be hazardous for the environment and human safety,[105] especially on scale-up processes.

x. Electrochemical

This synthesis method utilizes the electrical energy of a source and produces a chain reaction. The closeness of two electrodes can produce this electrical field. This chemical reduction process of the NPs will be driven thanks to the conductivity of the metallic material, which can serve as an anode and a solvent with the metallic precursor as the cathode.[70] The electrochemical synthesis method has also been studied for multi-metallic NPs widely. Ren et al. designed Pt/Au BMNPs for biosensing applications; the synthesized particles presented a polycrystalline structure with a flower-like morphology.[114] Yang et al. presented a Pt-based BMNP with organic solvents as reducing agents and hexa-chloroplatinate as the stabilizer and obtained a plate NP with an average size of ~ 20 nm.[115]

This synthesis method allows the obtention of particles with high purity, and the process is regularly fast and straightforward. The electrochemical synthesis method is generally preferred in industrial applications thanks to the viability of the process to control the size and purity of the NPs by adjusting the current density, besides being a cost-effective and ecological alternative for the nanofabrication of bimetallic NPs. On the other hand, scale-up processes can be complicated with this synthesis technique, as literature presents that the reaction varies depending on the current density produced.[116] Moreover, depending on the materials required, it may utilize hazardous chemicals for the operator.

3.1.3 Green Synthesis

This novel field aims to use renewable, biodegradable, and optimized materials that do not persist in the environment[117]. Green Chemistry is a set of principles based on the design, development, creation, and enhancement of processes that use chemical products that could not harm the economy, nature, and human health. The principles are as follows: prevent waste, atom economy, less hazardous synthesis, design benign chemicals, benign solvents, energy efficiency, renewable feedstocks, reduce derivatives, catalyze, plan degradation, analysis of pollution prevention, and benign chemistry for accident prevention[118]. These principles of green chemistry aim for all scientific, industrial, and medical processes to be more environmentally conscient and, therefore, use less hazardous chemicals to prevent contamination and human health issues. The green synthesis of NPs aims for the nanofabrication methods to innovate, modify, or replace the traditional methodologies that utilize potent chemicals or produce them as a product of the synthesis.

i. Biological

Biological methods rely on biological reactants as synthesis agents; these can originate from plants such as roots, leaves, flowers, or from microorganisms such as algae, bacterial resources, fungus extracts, and from biochemical products or mammalian cells. The particles are deposited in the microbial culture supernatant; the parameters like size and morphology depend on the biological substrate[15]. Kalishwaralal et al. suggested that an enzymatic reduction is a mechanism to obtain the NPs, such as silver nitrate, by nitrate reductase with other compounds that carry electrons[119].

There has been reported the synthesis of Pd NPs from *Pseudomona*s and *Escherichia coli*. Macaskie et al. suggested that even though further investigation is required to understand the mechanisms, NPs are synthesized by the bacterial hydrogenases of the cell envelope[120]; this helped ease access to the NPs. Another study presents the synthesis by *Mirabilis Jalapa* leaf, obtaining bimetallic nanoparticles with an average size of ~15.2 nm and presented as plates, sheets, and spherical structured, depending on the concentration of the precursors used.[121] The as-synthesized nanoparticles were also used against *Klebsiella pneumonia* and *S. aureus*. Despite biological methods have excellent stability, being unexpensive, and accomplishing green chemistry, it produces monodispersed nanoparticles. The concentration of macromolecules that directly act on the synthesis of NPs is low; hence, the nucleation takes a more extended period, and the synthesis rate is diminished[122].

Table 1. Review of the top-down nanofabrication methods for bimetallic Pd-based and Ag-based alloy NPs.

Method	BMNP	Reducing Agents	Stabilizing Agents	Size and Morphology	Ref.
Thermal decomposition	Ag-Cu	Hydrazine hydrate, SDS	Oleic acid	~7 nm, core-shell.	75
Laser ablation	Ag-Cu	Ethanol, acetone	None	~20 nm, spherical.	78
Nanolithography	Fe-Pt	Hydroquinone	Cetyltrimethylammonium chloride	~11.5 nm, core-shell.	80
Milling	Cu-Ni	Sodium Hydroxide	None	~12 nm, blades.	84

Table 2. Review of the bottom-up nanofabrication methods.

Method	**BMNP**	**Reducing Agents**	**Stabilizing Agents**	**Size and Morphology**	**Ref**
Emulsion and precipitation	Pt-Ru	H2, Sodium Borohydride, Hydrazine	Surfactant, Triton X	~4.5 nm, cubic.	88
Chemical Vapor Deposition	Fe-Ru	Sodium hydroxide	Polyacrylic acid (PAA)	~10 nm, spherical.	92
Pyrolysis	Pd-Ni	Sodium Borohydride	Trisodium Citrate	~7.2 nm, cubic.	95
Chemical Reduction	Ag-Pd	Sodium borohydride, citrate,	Trimethyl ammonium bromide, triton x100	~ 6 nm, clusters.	70
Galvanic Replacement	Ag-Pd	Hydroquinone, formaldehyde	Organic Ligands	~15nm, hollow	123
Solvothermal	Ag-Pd	Ethylene glycol, ammonia, hydrazine.	Organic cations, Polyvinylpyrrolidone (PVP)	~10 nm, spherical.	101
Hydrothermal	Ni-Cu	Sodium Dodecyl sulfate, Hydrazine.	Triton X.	~22 nm, spherical.	25
Sonochemical	Au-Pd	Ethylene Glycol.	PVP, Polyethylene glycol (PEG)	~ 10 nm, triangular.	124
Microwave	Au-Ag	Polyol solvents, ethylene glycol.	PVP, Trisodium Citrate	~10 nm, spherical.	108
Sol-Gel	Au-Pd	Citric Acid, Ethylene glycol	Silicates	~3.5 nm, spherical	112
Electrochemical	Au-Pt	None	PVP, hexachloroplatinate	~20nm, rectangular	115
Biological	ZnO-Ag	Mirabilis Jalapa leaves.	Biomolecules	~15.2 nm, spherical	121

3.2 Controlled Synthesis

The minimal modification of the nanoscale parameters leads to the alteration of characteristics like size and morphology. The control of the synthesis of NPs is significant for the design of biomedical applications like antibacterial agents to ensure the quality and efficacy of the as-synthesized products. Furthermore, biomedical complexes require characteristics to fulfill their purposes effectively. For example, a bimetallic nanoparticle as a nanozyme needs a controlled pH to achieve hydroxyl radicals' catalytic production for enhanced antitumor effects.[125] Or a bimetallic NP as a biosensor for detecting antimetabolites, the size and morphology depend on the nucleation rate, and its minimal alteration can lead to poor selectivity.[126] Hence, the synthesis of bimetallic NPs is designed to have a controlled production; and some of the most influencing factors are as follows:

3.2.1 Temperature

The reaction temperature is controlled in every synthesis method; the nucleation and NP growth design are developed depending on the resistance and susceptibility of the metal to the temperature. The temperature can be modified to minimize the activation energy required to start a reaction; therefore, this parameter is fundamental to achieve cost-effective synthesis. Ali et al. 2020, state that temperature optimization is essential because its increase can accelerate the deposition of metallic atoms, enhancing bimetallic NP' formation.[127] For example, the temperature utilized for the synthesis of Rh atoms on the surface of Pd NPs is less in comparison to Pt atoms on the same surface due to lower bonding energy.

3.2.2 Concentration

The concentration of the specific parameters described below, interferes in all the steps of the synthesis. Controlled nucleation, growth, and stabilization of the bimetallic NPs are given by the design of the concentration of:

i. Precursors

Most of the parameters for the synthesis of NPs depend on the nature of the precursors; for example, the reaction temperature depends on the composition of the metal. Generally, for bimetallic NP synthesis, the precursors' ratio gets modified to optimize the materials and their application. The concentration of the metal precursors influences the size and shape of the NPs. For example, the synthesis of bimetallic NPs of Pt-Au and Pt-Ru was driven with the variation of the salts: $HAuCl_4$ and $RuCl_3$, respectively;[128] the as-synthesized NPs presented variated morphology, aggregation, and surface area.

ii. Reducing agents

The reducing agent is the compound that donates an electron to the oxidation agent in a chemical reduction, in this case required for the synthesis; this agent can change accordingly to the reaction conditions. For example, with a low pH, some reducing agents can enhance or diminish the reduction rate; or, at high pH, these agents can play an essential role in a controlled synthesis.[127] Jing and Wang, 2010, presented the synthesis of bimetallic Ag-Pd NPs, in which the surface area and morphology were directly influenced by the mild reducing agents.[98] The slow reduction of the reaction is the key to aim control over the products' shape and morphology; hence, the concentration and reduction agents are modified to optimize the controlled synthesis. Furthermore, the selection of these agents is essential to avoid a negative impact on the designed system.

iii. Stabilizers

The stabilization of the NPs is fundamental to prevent undesired aggregation. The literature presents the stabilizers as agents added to the reaction to specifically attach to the as-synthesized NP's surface to reduce its energy and obtain a stable shape[127]. However, the concentration of the stabilizer agents can modify the surface chemistry of a bimetallic NP due to chemisorption. PVP is the widest stabilizer for BMNPs, but literature has presented it to affect their morphology and shape. Yang et al. adjusted a slow titration with PVP and obtained triangular Ag-Pd NPs with high practical and suitable applications.[129]

3.2.3 pH

The pH of a solution interferes with the yield of the chemical agents essential for forming the desired NPs. For example, at a lower pH, the reducing agent losses capacity to induce reduction; on the other hand, it can reduce the agent's ability to become more assertive at high pH.[130] the synthesis agents like stabilizers can modify a solution's pH. The reaction's pH should be controlled in every reaction during the synthesis of bimetallic nanoparticles, as it helps in the control of the size and shape of BMNPs. The literature conducted the synthesis of bimetallic nanoparticles at different pH values; with this, they obtained a wide variety of shapes, including circular cubic and spherical particles, all in the nanometer range.[94] With a higher pH, the reaction increases the nucleation rate in the initial stages, and smaller nanoparticles could be obtained.

3.2.4 Reaction Time

The synthesis of bimetallic NPs requires more time for its controlled formation, in contrast with their monometallic counterparts. The synthesis is taken in separate forms, nucleation of each particle or simultaneous nucleation; for each case, the reaction time must be optimized and controlled to aim for reliability and cost-effectiveness. Unlike the parameters listed above, the reaction time generally does not affect the final properties of the BMNPs; the reason for this statement is that when the growth and stabilization occur, the reaction time does not result in any change for the NPs[131]. However, the reaction time should be optimized to aim for an energy-effective design, especially for scale-up processes.

CHAPTER IV

4. PALLADIUM-SILVER NANOPARTICLES

The preparation of bimetallic NPs can be performed by two main procedures; successive reduction of two metals, which is mainly used to prepare core-shell structured nanoparticles. Alternatively, simultaneous reduction of two metals by which alloy nanoparticles can be obtained. Generally, bimetallic Pd/Ag nanoparticles can be prepared by chemical and physical methodologies, such as reduction, solvothermal, irradiation. Nonetheless, these synthesis techniques could present multiple limitations and use undesired subproducts that could be harmful to the environment and human safety. In contrast, co-reduction is a simple method for nanofabrication: the same performance as monometallic NPs, but with two or more metals.

The ease of characterization chooses the bimetallic combination of Ag and Pd, thanks to solid surface plasmon absorption of silver needed for UV-Vis mentioned in section 2.2.2, in contrast with Pd, which presents a broad absorption tail only, facilitating the characterization.[68] Some of the most common techniques used to characterize bimetallic nanoparticles are based on electron microscopies and some based on Brownian motion of particles, such as DLS, analytical ultracentrifugation, disc centrifugal sedimentation, and nanoparticle tracking analysis.[69]

Material coatings with noble metals (Ag, Au, Pd) were recently tested on animal models; in this study, scientists found that these presented lower inflammatory responses and induced just a thin fibrous capsule after a few weeks. Therefore, demonstrating outstanding biocompatibility and performance.[132] This investigation supported earlier conclusions about the low toxicity of noble metal coatings. Pd/Ag NPs exhibit the highest permeability for hydrogen and are continuously studied for industrial applications such as catalysis and electronics.[133,96] The applicability of these nanoparticles is broad; hence, the bimetallic Pd/Ag nanoparticle synthesis is aimed at a more controlled reaction. Alloying Pd with a second element has been studied to lower the CO adsorption strength and enhance the CO production rate. The use of Ag benefits from the ease of synthesizing atomically precise AgPd BMNPs as well as the higher efficiency and earth abundance of Ag.[6]

The bimetallic Ag/Pd NPs present enhanced properties compared with their monometallic counterparts. The system of Ag/Pd NPs has been utilized in many applications, especially as a potential catalyst for direct ethanol fuel cells. The literature states the enhanced electrocatalytic activity and stability towards ethanol oxidation reaction in alkaline solutions due to nanoporous structuring. This alloy is frequently presented to have the best performance with high activity, selectivity, and durability at low overpotentials, making it an enhanced catalyzer.[134] However, the aggregation of bimetallic Ag-Pd NPs still requires further studies to be controlled without decreasing the mass activity of the BMNPs.

4.1 Biomedical Applications

Nanotechnology has been proven as an expanding science whose applications are at the service of the biomedical field. Thomas Webster has worked on the design, synthesis, safety, and evaluation of NMs for multiple biomedical applications since 1998; he has conducted multiple studies of the toxicology of NPs, included antibacterial and catalytical advances[135], presenting compelling evidence that raises nanomedicine as a highly proficient alternative and improvement to existing treatments, diagnoses, and therapeutic devices. Novel technologies, such as the following, are studied continuously in the field of nanotechnology to aim for better results.

i. Biocatalysts

Biocatalysis is the field of chemistry that aims at the use of catalysts for biochemical purposes. Industrial processes commonly are made at high temperatures, creating the necessity of temperature-resistant catalytical agents. Hence, nanotechnology has been developed as an essential, inexpensive, stable, reproducible, and resistant support[136]. NPs' catalytical activity is a widely studied field aimed to enhance the selectivity, stability, and oxidation velocity in glucose, carbon monoxide, alcohols, and toluene. Pd NPs have gained special attention from catalytical activities because these present a high surface area to volume ratio[137]. Li et al. recently presented a study showing that Ag NPs' catalytic activity was significantly reduced at certain pH, but Ag-Pd NPS performed efficiently on every pH[125]. It is expected that the existing challenges will be addressed soon to advance in the development and commercialization of efficient catalysts for medical and industrial processes.

ii. Drug delivery technology

Drug delivery technology is the biomedical field that seeks a controlled delivery system of a drug to enhance bioavailability and improve health. Nanotechnology on drug delivery is aimed to enhance targeted delivery of medicines and improve the permeability of cells to absorb the drug[138]. NPS can be designed to present controlled pharmacokinetics ADME processes: Adsorption, Distribution, Metabolism, and Excretion. NPs can enter the human body through inhalation, oral intake, and direct injection; the first interaction rapidly occurs between the particle and a protein15; as nanoparticles cross the blood barrier, it can then produce a sustained delivery of medication could usually be hard to control.

Some aspects can be limiting for the delivery of the drug, like NPs, commonly being recognized by the lymphatic system. However, the particles' coatings have been presented as solutions, as these make the nanoparticle less hydrophobic to prevent it from binding with blood components[139]. Many factors are taken into account to release the loading (drug), such as pH, temperature, and solubility[140]. In pharmaceutical sciences, bimetallic NPs are also being used to reduce toxic activity and side effects of drugs[141]. Therefore, the NPs' structure can be designed to release under specific conditions; this bioavailability will be increased, and the treatment will be more efficient.

iii. Cancer Treatments

Cancer treatments can significantly deteriorate the patients' health; for this reason, new technologies aim to target a single hallmark or pathway[142]. Nanoparticles for cancer are used in a varied range of applications, from diagnostics to enhanced therapeutics. NPs are designed to work as biomarkers to enlighten specific parts of an organism that recurrent methods cannot. Pd and Ag NPs have taken beneficial catalytic and optical features that are the base for multiple therapeutic applications. Upon improved cancer treatments, the self-lighting photodynamic therapy (PDT) has presented many advantages over traditional PDT, as the need for only one single energy source, the application of lower radiation doses, and a cost-effective solution[143]. While NPS has a value of persistent luminescence, emissions can be prolonged when photo-synthesizers are active, significantly improving the treatment's effectiveness.

iv. Biosensors

A biosensor is a biological sensing material with a transducer; this associates a reaction and transduces it to a signal interpretation. However, bioactive molecules can get prematurely deactivated thanks to unstable materials[144]. Nanotechnology uses electrochemical deposition of biomolecules; As Ag-Pd NPs present a relatively low cost, high stability of the structure, and sensitivity, these metals are commonly used for molecules biosensing[126]. Cao et al. presented a bimetallic biosensor for the effective detection of cholesterol using a vitreous electrode; this new sensor presented higher stability and sensitivity toward the molecule tested in comparison to pure noble metals' biosensor[145]. These enhanced properties could be controlled by the alloy's electro-catalytic properties, influencing charge transfer for the efficient detection of the analyte.

CHAPTER V

5. ANTIBACTERIAL APPLICATION

Nanotechnology presents innovative, promising applications in the biomedical field for antibacterial applications due to their capability to adhere and accumulate in the cellular membrane, inducing cellular death (disruption). Nowadays, leading solutions to antibacterial resistance and rapid growth/spread of bacteria are the NPs' bactericidal properties and their multiple mechanisms to combat antibiotic resistance and the NPS as bio carriers.

Recent experiments have found out that bimetallic nanoparticles' bioconjugation has improved bactericidal activity against many bacterial strains, especially *Escherichia coli* and *Staphylococcus aureus*.[127] Another study suggested that the biocompatible composites of chitosan added with Ag-Pd nanoparticle presented a better antibacterial activity against Gram-positive and Gram-negative bacteria, in contrast with a single metal nanoparticle.

5.1 Staphylococcus Aureus

S. aureus is a member of the *Micrococcaceae* family. It is a Gram-positive coccus that consists of a circular chromosome of around 2800 bp; this bacterium presents phages, plasmids, and transposons. *S. aureus* can be found single or clustered in pairs, chains, and grape-like shapes. Staphylococcus can be found commonly on the nostrils and skin of healthy children and adults. Its spread is given by direct contact with a contaminated surface or airborne transmission; the portion of this bacterium is higher in nostrils of people who work at hospitals and patients. [147] This facultative anaerobe bacteria can produce catalases, which can trigger the rupture of water and oxygen molecules. The cell wall of *S. aureus* is mainly composed of peptidoglycan, which is a polysaccharide of N-acetylglucosamine and N-acetylmuramic acid (Figure 15). This peptidoglycan may be the macromolecule resulting in a toxic activity by simulating the macrophages releasing cytokines, activating the complement, and forming platelet aggregation.[146] More than eleven serotypes are known, and by which are the main causing agents for infections like endocarditis, meningitis, osteomyelitis, and mastitis, among others listed in Table 3.

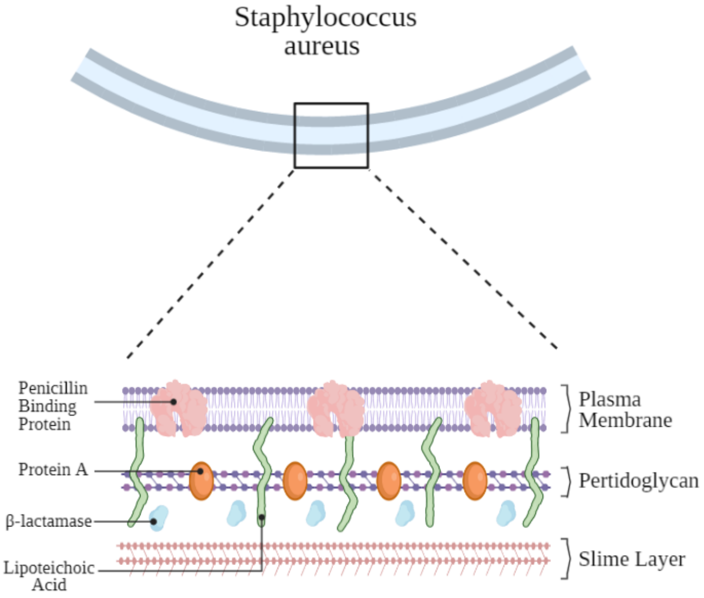

Figure 15. Staphylococcus aureus cellular membrane

Created with Biorender. Adapted from Ref. [146].

5.1.1 *Staphylococcus Aureus* effects on Human Health

Clinical reports on *S. aureus* have been presented since 1880; however, this remains a warning issue for human health, thanks to their outstanding ability to gain resistance.[148] *S. aureus* is the promotor to multiple types of infections, going on a wide range of illnesses from minor affections like furuncles or folliculitis to potentially lethal diseases such as pneumonia/ meningitis.[149] As observed in Table 3, each illness's mortality rate is presented as a direct death-risking factor that affects mainly the elderly population, and if it is correctly diagnosed, it can be treated with antibiotics. It is essential to mention that the incidence of these illnesses caused by *S. aureus* can also lead to other concerning diseases such as Cancer.[150] Moreover, a study developed in 2018 in the city of Quito presented that the prevalence rate of *S. aureus* methicillin-resistant is ~ 30% in Ecuador and ~ 20 % worldwide.[151]

Table 3. Illnesses and their mortality rate caused by S. aureus.

Type	Illness	Brief Description	Mortality Rate (%)	Ref
Cutaneous	Folliculitis	Follicle gets infected, causing redness and pain on the base of each hair.	0,12	152
	Toxic Shock Syndrome	Severe rash. It May lead to hypotension, affects the kidneys, Gastrointestinal, Nervous System.	3,93	153
	Impetigo	Series of Pustules and yellow crusty sores that are itchy and hurtful.	1,60	154
	Cellulitis dermatitis	Infection of the skin and the underneath tissue. Cause redness and extended pain.	1,10	155
	Necrotizing fasciitis	On newborns may be complicated infections. Cause painful detachment of large amounts of skin.	>1	156
	Mastitis	Mammary glands infections. The nipple can include cellulitis and abscesses. It can be infectious for the baby.	3,08	150
Respiratory	Pneumonia	Air sacks infection. Cause high fever and breathing difficulty. May provoke accumulation of pus.	32,61	157
Gastrointestinal System	Intoxication	Cause severe nausea, vomiting, and diarrhea.	2,32	158
	Urinary	Fever, burning, pain. May lead to leukocyturia.	8,00	159
Circulatory System	Bacteriemia	Blood Stream infection. High fever may cause shock syndrome.	12,46	160
	Endocarditis	Endocardium inflammation may cause cardiac insufficiency and death.	25,31	161
	Meningitis	Meninges inflammation may cause seizures and death.	32,43	162
Osseous	Osteomyelitis	Bone inflammation and destruction. Cause chills, fever, and pain.	11,70	163
	Arthritis	Joint inflammation. May produce fever and pain.	17,60	164

*The mortality rate is presented as the percentage number of cause-specific deaths by each illness. The rate was calculated from the cause-related mortal cases from different studies which measured population in general and specific cases. Each reference presents variated sample and mathematical analyses normalized for the present study (see Appendix 1).

5.1.2 Treatments for *Staphylococcus aureus* infections

For Gram-positive bacteria such as *S. aureus*, the most widespread class of human antibacterials is the β-lactam antibiotics, these have a bactericidal effect by inhibiting the synthesis of the peptidoglycan layer of the wall, which is a fundamental part for cellular integrity.[165] Depending on the type of infection and severity of the case, the doctor will prescribe the antibiotic; in different presentations like IV, oral, dermal, and even surgery in the case of arthritis by protheses[166]. However, *S. aureus* has presented an outstanding ability to develop antibacterial resistance. As a result, penicillin is no longer an effective antibiotic to treat *S. aureus* infections in more than 80% of the cases[2]. The difficulty of a resistant *S. aureus* is an example of the warning diminishing efficacy of antimicrobial agents for bacterial infections.[167]

5.2 Antibiotic Resistance

Antibiotic resistance can be defined as the evolutionary changes of bacteria that have generated the loss of antibacterial agents' effectiveness on these organisms. This resistance has become a severe problem due to the massive application of antibiotics in an inappropriate and discontinued way. Resistance can be classified as intrinsic resistance, in which a sudden mutation of genes can develop and acquired resistance by the obtention of resistance genes from other organisms by transposons or plasmids.[3] Hence, a single bacterium can achieve resistance to multiple antibacterial agents, and health organizations are worried about this warning antibiotic resistance. The bacteria can develop resistance by changing proteins, enzymes, and surface targets. These mechanisms can alter targets, inactivate enzymes, activate pump systems, obstacles to permeation, and the formation of biofilms.[168] Some bacteria could present more than one of these mechanisms listed before, hence led to multidrug-resistant bacteria. For example, β-lactam resistant bacteria can develop ability to degrade β-lactamases, could target modify the penicillin-binding proteins to produce lack/insufficiency of β-lactam binding sites, and could regulate β-lactam entry and efflux.[165]

According to the World Health Organization (WHO), the development of new and more powerful antibacterial agents has exponentially grown; however, bacteria's ability to mutate and adapt has proven higher rates.[169] This event would lead to the outbreak of hazardous diseases for the public since these organisms will not respond to any medicine available, causing epidemics and increasing the global mortality rate. Therefore, the development of new antibacterial agents effective against rapid adaptation and acquired resistance is increasingly needed. Some studies have associated the acquired resistance to antibiotics with the resistance to heavy metals, proving these two to be correlated. However, since nanoparticles have presented multiple mechanisms to provoke bacterial death, it is challenging for bacterial strains to develop resistance to nanoparticles.

Also, Zhang et al. 2019, presented an analysis of the sub-inhibitory concentrations of metallic NPs. In this study, they concluded that sub-inhibitory doses can induce Reactive Oxygen Species favorable for the antibacterial effect; However, sub-inhibitory exposure could also increase expression of SOS response, which is responsible of the main mutagenicity that induces antibiotic/ metal resistance.[170]

5.2.1 Antibacterial mechanism of NPs

Nanotechnology presents multiple mechanisms for its application as a bactericidal agent, using NPs to disrupt bacterial membranes and block biofilm formation. For the past years, the search for enhanced development of nanoparticles has noted that bimetallic NPs present better antibacterial abilities than their monometallic counterparts. This differentiation is given thanks to the smaller volume and larger surface area of the bimetallic NPs, increasing their ability to penetrate the membranes into bacterial cells.[171] Multiple studies presented NPs' bactericidal properties as more efficient for Gram-positive bacteria, such as *Bacillus* or *Staphylococcus aureus*.[50] Baptista et al. presented multiple studies on the synergetic effect of the bimetallic nanoparticle with the antibiotic drugs.[172] Hence, the study of the potential antibacterial application is mainly given by drug delivery technologies for more significant bactericidal activity. However, metal-based NPs may present different mechanisms to be developed as antibacterial agents. These can degrade the cellular membrane through electrostatic interactions depending on the surface of the nanoparticle. Another mechanism is triggering reactive oxygen species caused by producing oxidative stress to disrupt the bacterial membrane's proteins and enzymes. Finally, the homeostasis disturbance through the nanoparticles' binding to some proteins of the cellular wall.[171] Bimetallic nanoparticles have been reported as a great bactericidal agent by the utilization of the following mechanisms:

i. Cell Wall Disruption

It is well known that cell walls and membranes are the fundamental barrier for bacterial survival to external factors such as antibiotics; a cell membrane classification could be presented as two main differences: Gram(+) and Gram(-) bacteria.[173] Gram-negative cell membranes are composed of lipidic complexes, therefore allowing the entrance of macromolecules only. Gram-positive cell walls express teichoic acid and a significant number of pores so that particles can be distributed along the molecular phosphate chain. Gram (+) bacteria like *S. aureus* have a high negative charge on the cell wall surface, which will be able to attract and bind to NPs.[174] The interaction of the cell wall with the NPs modifies the structure and permeability of the cell, consequently leading to oxidative stress. The primary mechanism presented regulation of several antioxidant genes and genes for metal transport, reduction, and ATPase pumps, and the concentration of silver ions needed for an optimal antibiotic effect range between 10 nM to 10 μM.[175] Therefore, the antibacterial mechanism of these NPs is related to the exhaustion of antioxidant capacity via the action of the metal ions.

ii. Reactive Oxygen Species

The oxidative stress induced by reactive oxygen species (ROS) is one of the most effective antibacterial mechanisms of engineered nanoparticles. These hazardous species for the bacteria can be superoxide anions, hydroxyl radicals, hydrogen peroxide, and organic hydroperoxides.[176] The concentration of ROS is directly related to their antibacterial activity, due to the reactive species that induce the formation of more ROS. The production of these species can be controlled by the reducing of the particle size and surface properties of the synthesis; in this way, ROS will be induced over the surface of the nanoparticles.

Wang, et al. 2017, states that silver ions produce a catalytic activity to activate the oxygen of the media, leading to producing hydrogen peroxide (oxidative stress). These metal ions are released in the surrounding area of the space that the NP gets attached.[3] Biomolecules such as carboxyl and phosphate groups will become dispersed, destroying the membrane's functionalization and, consequently, leading to bacterial death. This mechanism produces multiple consequences over the bacterial stability: First, the membrane will be injured and trigger a direct malfunction due to ions flow; therefore, the oxidation of lipids, unfolding of proteins, and eventual modification of RNA will lead to cell death.[171]

iii. Homeostasis Disturbance

Bacterial exposure to metallic ions can induce abnormal metabolic functions. NPs with positive charges that lead to cell wall erosion and disruption also provoke the accumulation of metallic ions inside the cell wall. Hence, the bacterial cell membrane becomes disordered, and metal ions can interact with cytosolic proteins; consequently, the bacteria lose respiratory and metabolic pathways.[5] Besides these mechanisms, the bimetallic alloy of Ag-Pd nanoparticles can also induce genotoxicity and signal transduction inhibition by modifying chromosomal duplication. These can produce photocatalytic degradation mechanisms thanks to enhanced catalytic properties to attack nucleic acids, lipid, peroxidation processes, and degradation of cell membranes.[171] Thus, the combination of more than one mechanism that BMNPs may produce over the bacteria will achieve an enhanced antibacterial effect.

5.3 Physicochemical Properties for Antibacterial Applications

Physicochemical properties of the bimetallic nanoparticles may directly affect their efficiency towards the bactericidal application. Some properties of BMNPs, such as activity, selectivity, and stability, are determined depending on the dimensions of the bimetallic nanoparticle.[177] As presented in section 3.2, size and morphology can be controlled by the variation of parameters such as temperature, various reducing agents, and the presence of ions.[127] However, large specific surface area, high surface energy, and atomic ligand deficiency may lead to unwanted aggregation of metal NPs. Therefore, it is essential to discuss the main physicochemical parameters to perform an overview of the best factors to optimize their antibacterial activity.

i. Size:

Slonberg et al. 2013, shown that anodic oxidation adjustment of particle length resulted in a prolonged antibacterial activity; particle size and shape played fundamental role in biofilm eradication, more effectively with smaller sizes.[178,179] Therefore, smaller NPs have larger specific surface areas; this characteristic would enhance the ability to attach or enter the bacterial cell membrane compared to more significant NPs. On the other hand, smaller nanoparticles may present excessive surface energy and high thermodynamic stability, undergoing agglomeration, cluster, and precipitates.[180] Hence, it is essential to control the synthesis of bimetallic nanoparticles to achieve the desired size for an enhanced antibacterial application efficiently.

ii. Surface Chemistry

The surface of the nanoparticle is the main factor by which bimetallic nanoparticles will get attached or repelled to other material surfaces or biological structures like a cell wall; this process is determined by the ligands attached to the surface of the NP, which will determine physical or chemical adsorption. The outermost layer of the BMNPs can vary thanks to the adsorption onto the surface and the strength of the interaction with these molecules.[181] Depending on its structure, the surface chemistry of the outermost nanoparticle will accomplish toxic effects on the bacteria. Therefore, it is required to control specific parameters in the synthesis of the BMNPs, to aim for specific surface chemistry that favors the nanoparticle interaction with the bacterial membrane or with antibiotics.

iii. Morphology

NPs can interact at different levels, depending on the morphology. In the case of core-shell structure, the control of the composition and thickness of the shell is crucial to get great antibacterial activity with different enzymes and provoke enhanced cellular membrane damage, depending on their shape. [127] For example, Hong et al. presented prismatic nanoparticles prismatic-shaped NPs as great antibacterial activity against *S. aureus*; they established that bactericidal activity was enhanced due to the shape's interaction nanoparticles and the bacterial cell membrane.[182] Actis et al. developed cube-shaped Ag NPs, which exhibited more potent antibacterial activity than sphere-shaped and wire-shaped Ag NPs; these morphologies presented the exact sizes and, therefore, explained the shape effect antibacterial activity to the specific surface area.[183]

5.4 Green Synthesis Techniques used for Antibacterial Activity

The controlled nanofabrication of bimetallic NPs for antibacterial applications is not yet fully displayed; however, the study for a green synthesis has been proposed in the literature for other purposes like plasmid formation, colloidal suspensions, among others; Indicating that a green synthesis is possible for reduction and stabilizing effectiveness. [184] As it can be observed in Table 4, all the viable methods for bimetallic nanofabrication can be developed with green chemistry parameters, except lithography, which requires the use of Cetyltrimethylammonium chloride as a stabilizing agent and by which a greener replacement could not be concreted from literature yet. It is essential to mention that the same synthesis methods are widely used with different chemicals for different metals; however, these can be aimed for greener options, as observed below.

Table 4. Classification of the synthesis methods depending on the sizes and shapes of the bimetallic NPs.

Size (nm)	Shape	Synthesis Method	Hazardous Chemical	Greener option	Ref.
>10	Core-shell	Thermal decomposition	Hydrazine	Vitamin C	184
			~	Oleic acid	75
	Core-shell	Lithography	Hydroquinone	Glucose	185
			Cetyltrimethylammonium chloride	~	80
	Cubic	Emulsion and precipitation	Hydrazine	Vitamin C	184
			Triton x100	Aerosol OT	186
	Cubic	Pyrolysis	Sodium Borohydride	Sodium alginate	187
			~	Trisodium Citrate	95
	Clusters	Chemical reduction	Sodium Borohydride	Sodium alginate	187
			Triton x100	Aerosol OT	186
	Spherical	Sol-Gel.	~	Ethylene glycol	112
			~	Silicates	
10-19	Blades	Milling	~	Sodium hydroxide	84
	Spherical	Chemical vapor deposition	~	Sodium hydroxide	92
			~	PAA	
	Clusters	Galvanic replacement	Hydroquinone	Glucose	185
			~	Organic ligands	123
	Triangular	Sonochemical	~	Ethylene glycol	124
			~	PEG	
	Spherical	Microwave	~	Ethylene glycol	108
			~	PVP	
20-30	Spherical	Laser ablation	~	Ethanol	78
	Spherical	Hydrothermal	Hydrazine	Vitamin C	184
			Triton x100	Aerosol OT	186
	Rectangular	Electrochemical	~	PEG	115

5.5 Parameter Analysis for Proper Controlled Synthesis

The study of the synthesis methods presented multiple differences, as presented in Table 1 and Table 2. However, a detailed study of the size, time, temperature, and precursors concentrations obtained from the literature, are presented below with a multivariate graphical design. For better visualization of the data, the parameters were scaled, in which the higher value of the parameter was assigned a value of 1 (see appendix for real values). It is also important to mention that the studies presented in the chart have utilized as-synthesized nanoparticles to prove their antibacterial activity.

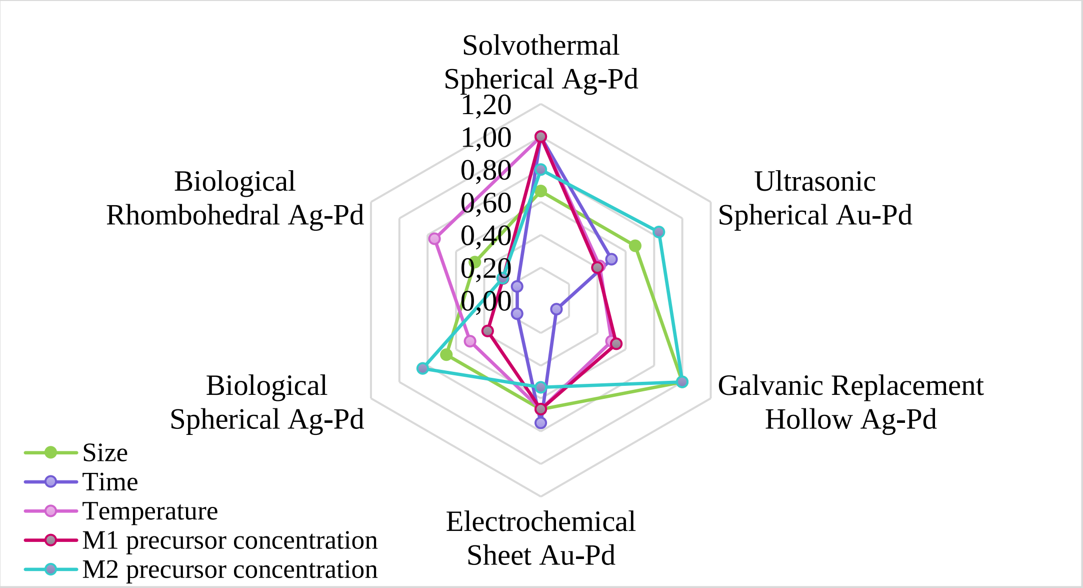

Figure 16. Kiviat/ Radar Chart of some synthesis techniques for proper antibacterial applications.

As observed in Figure 16, biological methods presented particles with smaller sizes; and lower precursor concentrations were required for both metals (M1 and M2) when the temperature levels were high, or the time was prolonged. It is also observed that the Solvothermal method required high rates for the four parameters (time, temperature, metal precursors M1 and M2), producing a large nanoparticle size. On the other hand, galvanic replacement utilized the higher metal precursor concentrations and presented the larger size of bimetallic nanoparticles.

As mentioned in section 3.1.3, it is important to diminish the utilization of hazardous precursor concentrations to reduce sub-products and achieve controlled synthesis. Hence, the present analysis suggests that the investigations for bimetallic Ag-Pd nanoparticles, driven via green methods, present feasible, controlled synthesis for a proper antibacterial application.

CHAPTER VI

6. CONCLUSIONS

Nowadays, with the accelerated ability of bacteria to achieve antibiotic resistance, it is important to aim to improve traditional medicine or to develop new technologies to solve this alarming issue worldwide. In Ecuador, the prevalence rate is ~ 30% for *S. aureus*; this bacterium is currently resistant in more than 80% of methicillin. Hence, it is fundamental to the development of an effective antibacterial agent that bacteria hardly could acquire resistance. NPs are proven as a facile, cost-effective, and efficient antibacterial agent against multiple microorganisms such as Gram-positive bacterium like *S. aureus*. Furthermore, nanotechnology keeps advancing in the potential applicability of these systems to improve their bactericidal properties. A promising complex is the nanofabrication of bimetallic nanoparticles, which presented better antibacterial competence in contrast with their monometallic counterparts. However, the development of controlled synthesis techniques is fundamental to guarantee its bactericidal properties. For this reason, different nanofabrication methods are studied to provide an overview of the techniques that will present the desired output. This is also important to aim for greener solutions that could help scale up processes to obtain similar nanoparticles in every batch.

The study of bimetallic Ag-Pd NPs has shown that their small size, with increased surface interactions and varying properties, present nano-toxicity against *S. aureus*. These, presenting mechanisms that damage the cell wall, producing reactive oxygen species and leading to cell death. Eventhough there is registered resistance to nanoparticles, since nanoparticles have presented multiple mechanisms to provoke bacterial death, it is challenging for bacterial strains to develop resistance to nanoparticles. Hence, as presented, various mechanisms of Ag-Pd NPs state the application of bimetallic NPs as a potential antibacterial agent.

Finally, as observed in the multivariate study the bibliographic review supported biological methods can fully control the synthesis of NPs, with less hazardous chemical concentrations and outcoming stable and small-sized NPs. Hence, it is possible to seek the synthesis of bimetallic Ag-Pd NPs via green synthesis techniques since this process can control the parameters to obtain the desired properties for their application as antibacterial agents.

6.1 Limitations and Outlooks

i. Even though NPs sound like a promising tool for bacterial issues, their scale-up processes are still a challenge that the scientific community must overcome.
ii. To synthesize BMNPs with the correct technique, it is important to keep a clear record of stability assays.
iii. Innovative techniques should be presented to avoid the utilization of hazardous chemicals that produce non-ecological subproducts. It is recommended to screen for safer chemicals to aim for a greener synthesis, especially on scale-up processes.
iv. Ag-Pd NPs have presented remarkable catalytical properties, but recent studies present the importance of their analysis as a bactericidal agent.
v. Bimetallic NPs can be used on coatings to avoid biofilm formation, on hospital surfaces to clear direct contamination, on biomedical instrumentation to prevent infectious diseases caused by catheters, or in laboratories to evade cross-contamination, which regularly contaminates samples and leads to false positive/negative reports.
vi. The utilization of many platforms and organizations that develop studies and databases for the efficient replacement of hazardous chemicals for eco-friendly options. Some of these are PISCTOX, Basta, Subsport, Cleantool, among others.

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APPENDIX

a. Calculation of the mortality rates from the reported cases from the literature.

Type of Infection	Illness	Reported deaths	Total Cases reported	Ref
Cutaneous	Folliculitis	1,15	1000	152
	Toxic Shock Syndrome	208	5296	153
	Impetigo	16	1000	154
	Necrotizing fasciitis	4,8	1000000	155
	Mastitis	4,4	143	156
Respiratory	Pneumonia	15	46	157
Gastrointestinal System	Intoxication	1300	56000	158
	Urinary	10	125	159
Circulatory System	Bacteriemia	81	650	160
	Endocarditis	41	162	160
	Meningitis	12	37	162

b. Analysis without scale of controlled synthesis for KIVIAT/Radar chart

	Ag-Pd	Au-Pd	Ag-Pd	Au-Pd	Ag-Pd	Ag-Pd
Method	Solvothermal	Ultrasonic	Galvanic Replacement	Electrochemical	Biological	Biological
Shape	Spherical	Spherical	Hollow	Sheet	Spherical	Rhombohedral
Size (nm)	10	10	15	10	10	7
Time (h)	6	3	0,66	4,5	1	1
Temperature (°C)	120	50	60	80	60	90
M1 precursor concentration (mM)	3,75	1,50	2	2,50	1,41	1,00
M2 precursor concentration (mM)	3,75	3,92	4,7	2,50	3,92	0,00
Ref.	101	124	123	115	188	189