

Green nanotechnology: a review for aurum nanoparticles

Nanotecnología verde: una revisión de nanopartículas áuricas

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ABSTRACT: Green chemistry, based on the principles of Paul Anastas and John Warner, promotes the sustainable synthesis of gold nanoparticles (AuNPs) by reducing the use of toxic substances and minimizing hazardous waste. Traditional methods, which employ reducing agents such as sodium borohydride (NaBH_4), generate harmful by-products, while green approaches use natural agents such as plant extracts and microorganisms that act as reductants and stabilizers, without generating toxic waste. These methods are not only safer, but also improve the biocompatibility of AuNPs, making them much more suitable for biomedical applications, notably drug delivery, targeted therapies and molecular diagnostics. The use of natural sources and biocatalysts, such as enzymes or microorganisms, facilitates synthesis under mild conditions, allowing greater control over the shape and size of AuNPs. These nanoparticles can be designed to specifically target cells, improving the efficacy of cancer treatments and reducing adverse effects. In this paper, we present the main features and advantages of green synthesis of AuNPs for a promising alternative with significant applications in nanomedicine and other technological areas.

KEYWORDS: green nanotechnology, nanotoxicology, bionanocompatibility, gold nanoparticles, green chemistry.

RESUMEN: La química verde, basada en los principios de Paul Anastas y John Warner, promueve la síntesis sostenible de nanopartículas de oro (AuNPs) reduciendo el uso de sustancias tóxicas y minimizando los residuos peligrosos. Los métodos tradicionales, los cuales emplean agentes reductores como el borohidruro de sodio (NaBH_4), generan subproductos nocivos, mientras los enfoques verdes utilizan agentes naturales como extractos de plantas y microorganismos actuando como reductores y estabilizadores, sin generar residuos tóxicos. Estos métodos no solo son más seguros, sino que también mejoran la biocompatibilidad de las AuNPs, haciéndolas mucho más adecuadas para aplicaciones biomédicas, en particular para la administración de fármacos, las terapias dirigidas y los diagnósticos moleculares. El uso de fuentes naturales y biocatalizadores, como enzimas o microorganismos, facilita la síntesis en condiciones suaves, lo cual permite un mayor control sobre la forma y el tamaño de las AuNPs. Estas nanopartículas pueden ser diseñadas para dirigirse específicamente a las células, mejorando la eficacia de los tratamientos contra el cáncer y reduciendo los efectos adversos. En este artículo, presentamos las principales características y ventajas de la síntesis verde de nanopartículas de oro como una alternativa prometedora con importantes aplicaciones en nanomedicina y otras áreas tecnológicas.

PALABRAS CLAVE: nanotecnología verde, nanotoxicología, bionanocompatibilidad, nanopartículas de oro, química verde.

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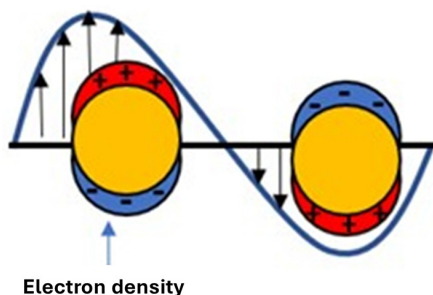


Introduction

Since about the first quarter of the 21st century, the research, development and application of nanoparticles (NPs) has increased significantly (Prakash *et al.*, 2024). These particles range in size from 1 to 100 nm in each of their dimensions, which means that these materials can be of different chemical compositions. Examples include polymeric NPs, carbon-derived NPs, lipids, metal oxides, and metallic NPs, for example aurum nanoparticles (AuNPs). Due to their unique chemical properties and remarkable ability to interact with light, the AuNPs have been extensively studied (Kio *et al.*, 2021).

AuNPs exhibit special properties compared to their macroscale counterparts. They possess a unique combination of physicochemical properties that are crucial for their applications in various fields such as electronics, catalysis and the development of molecular sensors. The latter application is of particular interest in the biomedical field. Some of the most notable properties of AuNPs are their exceptional chemical stability, especially in their colloidal form, their ability to be biocompatible in biological systems, and their remarkable optical properties exemplified by the localized surface plasmon resonance (LSPR) (figure 1) effect (Fan *et al.*, 2020; Bhatia *et al.*, 2023).

FIGURE 1. General scheme of LSPR effect.



Source: Author's elaboration. Image created with BioRender.

However, despite their considerable importance, the conventional synthesis of AuNPs is subject to several limitations, primarily relating to their biocompatibility and the environmental safety of their removal and disposal. The conventional synthesis methods usually use toxic and hazardous chemical reagents such as sodium borohydride (NaBH_4) in conjunction with surfactants and solvents of organic character, which directly affect human health and consequently their biomedical applications (Kimling *et al.*, 2006; Dong *et al.*, 2020; Oliveira *et al.*, 2023).

In addition, they generate by-products and intermediates that require appropriate handling and disposal. Despite their traditional effectiveness in controlling size, morphology and yield of AuNPs, chemical synthesis pro-

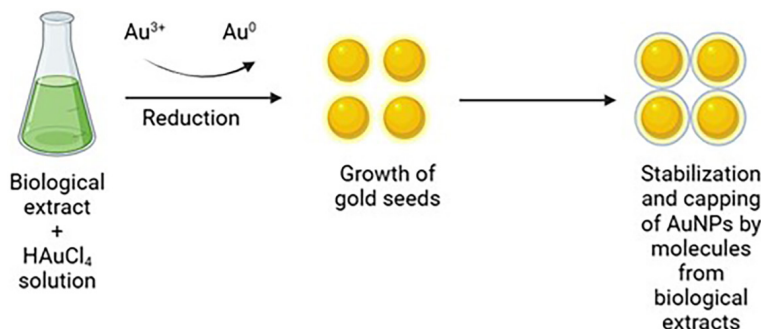
cesses are the subject of studies and have led to various proposed changes due to their negative impact on the use of AuNPs as biomaterials, their environmental impact and the toxicological risks associated with their industrial implementation (Yah *et al.*, 2013; Wuithschick *et al.*, 2015; Dvorakova *et al.*, 2022).

In view of these problems, a branch of chemical synthesis known as “green synthesis” has been proposed as a means of developing AuNPs that offer safer, less toxic and more environmentally friendly alternatives. Green chemistry is the development of chemical processes that minimize or eliminate the use and generation of toxic residues, with the goal of minimizing biological and environmental impacts (Anastas and Warner, 2000 & 2005). This approach is based on the 12 basic principles proposed by Paul Anastas and John Warner in 1998, which advocate the use of safer reagents and chemicals and/or chemicals of natural origin (derived directly from nature) (Anastas and Warner, 1998; Anastas and Eghbali 2010).

The application of the principles of green chemistry to the synthesis of AuNPs has led to the development of novel methods that effectively reduce or virtually eliminate the use of toxic reagents. One notable advance is the introduction of reducing agents and surfactant stabilizers derived from biological and natural sources, including plant extracts, bacteria, fungi and other living organisms. These processes are commonly referred to as biosynthesis or green synthesis as they utilize natural compounds from said extracts, such as flavonoids, terpenoids and polyphenols, to form AuNPs (figure 2) (Panda *et al.*, 2011; Ahmed *et al.*, 2016).

In addition to biosynthesis, there are other green synthesis methods, such as the use of distilled water or Milli-Q water instead of toxic organic media and the use of biocompatible reducing agents such as ascorbic acid or sodium citrate, which can be easily degraded by the human body and the ecosystem (Ojea-Jiménez *et al.*, 2010; Merza *et al.*, 2012).

FIGURE 2. General scheme of AuNPs synthesis using biological extracts.



Source: Author's elaboration. Image created with BioRender.

Nevertheless, the use of green synthesis is not without its drawbacks. These include issues of scalability and reproducibility arising from the lack of precision in optimizing the concentrations used in these methods. This is because different extracts may contain different amounts of the desired substances. In addition, the control of the size and morphology of AuNPs is not uniform in these methods.

Taking these considerations, various research groups have proposed alternative methods in the field of nanoscience, with a particular focus on the application of AuNPs in different areas.

This review explains the principles of green chemistry and the synthesis methods used for AuNPs and describes their benefits and applications in biomedicine. It also highlights the shortcomings of these methods and provides insights into possible ways to analytically optimise AuNP synthesis.

Principles of green chemistry in the synthesis of AuNPs

To apply the principles of green chemistry as outlined by Paul Anastas and John Warner to the synthesis of AuNPs, it is necessary to understand the benefits that can be achieved during the synthesis process. The most important of these benefits are listed below.

Avoiding the generation of toxic waste is one of the main goals of green chemistry. One of the basic tenets of green chemistry is the reduction or even elimination of toxic waste, which requires the development of processes that minimize or eliminate the generation of hazardous by-products. Traditional methods for the synthesis of AuNPs of different shapes and sizes usually involve the use of reducing agents such as NaBH_4 . However, this approach has been shown to be a source of toxic and hazardous by-products that can remain as residues in colloidal dispersions for biological and/or biomedical applications (Altuwayjiri *et al.*, 2022).

Consequently, the application of green chemistry in the synthesis of AuNPs facilitates the development of synthetic routes in which precursors achieve high quantitative yields in the generation of NPs while reducing the generation of waste products from toxic reagents. For example, the use of reducing agents has been explored, including the use of natural or biocompatible stabilizers from plants, microorganisms or biomolecules that do not generate toxic by-products. In addition to facilitating the reduction of gold salt ions to metallic gold, plant extracts can also stabilize AuNPs without the need for additional surfactants, further reducing the generation of toxic waste (Bhattarai *et al.*, 2018; Asiya *et al.*, 2020).

It is important to optimize the efficiency of the chemical equilibrium. Maximizing efficiency is due to increasing the percentage of reactant molecules incorporated into the final NPs. This contrasts with the traditional synthesis processes for AuNPs, where some molecules or atoms cannot be fully incorporated into the desired final product, resulting in waste. Consequently,

the goal of green chemistry is to improve the efficiency of the synthesis pathways to maximize the conversion or integration of these chemical species into the NPs. This approach reduces the quantity of reagents required and therefore the amount of waste produced. Examples of these reactions include methods in aqueous or single-phase media, where postponing the reaction results in the highest possible conversion of the desired product(s), avoiding the formation of unnecessary by-products (Paciotti *et al.*, 2006; Ovais *et al.*, 2017).

The use of biocompatible raw materials is a basic principle of green chemistry in the synthesis of AuNPs. This approach involves the modification of toxic chemical reagents with the aim of replacing them with biocompatible and renewable alternatives. Traditionally, reducing agents are among the most toxic substances used in this process. Therefore, the use of natural substances from plant extracts and/or microorganisms, which act as both reducing agents and stabilizing surfactants, has gained importance (Sharma *et al.*, 2012; Meléndez-Villanueva *et al.*, 2019). For example, extracts of green tea, aloe vera, turmeric, peppermint and neem leaves have been shown to contain reducing substances, including citric acid and tannic acid, which effectively reduce the Au (III) present in the precursor salt to metallic Au(0) during the synthesis of AuNPs, eliminating the need for additional reagents. These extracts not only have a reducing effect, but also the ability to act as stabilizers for the AuNPs formed, eliminating the need for toxic surfactants such as cetyltrimethylammonium salts (Gurunathan *et al.*, 2014; Elia *et al.*, 2014; Fadaka *et al.*, 2021). Conversely, the use of microorganisms, including bacteria, fungi and algae, has been shown to be effective in the production of AuNPs under mild conditions. This approach offers a significant advantage in the form of biocompatible NPs in colloidal dispersion (Zhaleh *et al.*, 2019).

Reducing energy consumption is a major advantage of this approach. Green chemistry strives to reduce energy consumption by using mild reaction conditions such as ambient temperature and pressure. This is a significant advantage over conventional methods of synthesizing AuNPs, which often require high temperatures or long reaction times, resulting in increased energy costs and environmental impact.

Green synthesis methods, in which plant extracts are used as reducing agents, allow reaction rates to be accelerated without the need for an external energy source. In addition, alternative energy sources, such as solar radiation, have been investigated to accelerate the synthesis of AuNPs while reducing energy consumption (Singh *et al.*, 2013; Das *et al.*, 2010).

Biocatalysts: in many chemical processes, including the synthesis of AuNPs, catalysts are essential to increase the efficiency and quantitative part of the reaction. However, many of these catalysts are toxic and have a high market value due to their chemical nature, which includes precious or heavy metals (Zhao *et al.*, 2013; Prielcel *et al.*, 2016). Nevertheless, green chemistry advocates the use of catalysts that are neither toxic nor difficult to remove from the reaction system. An alternative is the use of biocatalysts, such as en-

zymes, which can accelerate the reaction process of AuNPs without generating toxic residues. This can include the use of plant extracts or microorganisms. Certain microorganisms, such as *Rhodopseudomonas capsulata*, can facilitate the reduction of gold ions to AuNPs under mild biological conditions (He *et al.*, 2007 & 2008; Singh *et al.*, 2014; Menon *et al.*, 2017).

The synthesis of AuNPs from plant extracts is of great importance for the reduction of toxicity in the environment and the possibility of large-scale synthesis at lower cost (Yang *et al.*, 2013). These extracts can serve as reducing and stabilizing agents in the synthesis of NPs of different shapes and sizes. Due to their accessibility and physicochemical properties, plant extracts have been used for the conversion of metal salts into NPs, a process that has attracted considerable attention in recent years (Clemente *et al.*, 2017).

The use of these extracts for the biosynthesis of AuNPs has gained importance due to the potential of these particles to be used as biomarkers, sensors and carriers of molecules of interest, as well as their potent antibacterial activity (Ikram *et al.*, 2015). The synthesis of AuNPs using plant extracts is a straightforward, one-step process. During the reduction of gold ions to NPs, the plant extracts themselves act as stabilizers and surfactants and facilitate the formation of the NPs. For example, it has been documented that those medicinal plants such as *Cucurbita pepo* and *Malva crispa* have been used to synthesize spherical AuNPs that are used in the food industry as antibacterial agents against pathogens that cause spoilage of certain fruits (Chandran *et al.*, 2019).

In another documented case, an aqueous extract of *Acalypha indica* leaves was used to synthesize AuNPs with a size of 20 to 30 nm. These NPs were then used in targeted cancer therapy in various cell lines. The synthesis of AuNPs with a triangular shape was achieved using lemon leaf extract, which is mainly composed of ascorbic acid (vitamin C) (Krishnaraj *et al.*, 2014). Similarly, AuNPs ranging in size from 5 to 100 nm were synthesized from extracts of *Syzygium aromaticum* using the flavonoid-like molecules present in this plant (Raghunandan *et al.*, 2010). The reductive-stabilizing effect of agricultural waste products, such as banana peels, was also investigated and led to the synthesis of AuNPs with an average size of 300 nm (Bankar *et al.*, 2010). In addition, extracts of *Mentha piperita*, *Madhuca longifolia*, *Suaeda monoica*, *Stevia rebaudiana*, *Coleus amboinicus* and *Zingiber officinale* were used (Mubarak *et al.*, 2011; Mohammed *et al.*, 2011; Arockiya *et al.*, 2014; Sadeghi *et al.*, 2015; Narayanan *et al.*, 2010; Kumar *et al.*, 2011). In addition, other plants have also been used to synthesize AuNPs in a range of shapes and sizes. The data presented in table 1 illustrates the diversity of plant extracts used in the last five years and reflects the dynamic advances in nanotechnology synthesis.

Most of the molecules contained in these plant extracts that are responsible for reducing the formation of NPs are flavonoids, ascorbic acid, tannic acid and citric acid. However, this approach has some disadvantages. The

concentration of chemical species derived from plant extracts is not fully regulated, leading to the formation of AuNPs with different sizes and shapes. Consequently, the desired physicochemical properties cannot be fully optimized or controlled

TABLE 1. Synthesis of AuNPs using various plant extracts.

Extract	Size and shape of AuNPs	References
<i>Plum peel</i>	8-10 nm, quasi-spherical polyhedra	Vorobyova <i>et al.</i> (2024)
<i>Halimeda macroloba</i>	18-20 nm, quasi-spherical polyhedra	Lavanya <i>et al.</i> (2024)
<i>Acorus calamus</i>	30-50 nm, quasi-spherical polyhedra	Peng <i>et al.</i> (2024)
<i>Vitis vinifera</i> , <i>Buchananialanzan</i> , <i>Phoenix dactylifera</i>	---, quasi-spherical polyhedra, rods	Patil <i>et al.</i> (2023)
<i>Andrographis paniculata</i>	40 nm, quasi-spherical polyhedra, triangles & hexagons	Do-Dat <i>et al.</i> (2023)
<i>Papaya peel</i>	10-15 nm, quasi-spherical polyhedra	Anadozie <i>et al.</i> (2022)
<i>Polianthes tuberosa</i>	50-70 nm, quasi-spherical polyhedra & triangles	Alghuthaymi <i>et al.</i> (2021)
<i>Mentha longifolia</i>	36 nm, quasi-spherical polyhedra	Li, S. <i>et al.</i> (2021)
<i>Pimenta dioica</i>	20-30 nm, quasi-spherical polyhedra	Fadaka <i>et al.</i> (2021)
<i>Sambucus wightiana</i>	10-20 nm, triangles, cubes, hexagons y decaedres	Khuda <i>et al.</i> (2021)
<i>Platycodon grandiflorum</i>	30-80 nm, triangles & octaedres	Anbu <i>et al.</i> (2020)
<i>Jasminum auriculatum</i>	8-37 nm, quasi-spherical polyhedra	Balasubramanian <i>et al.</i> (2020)
<i>Litsea cubeba</i>	8-18 nm, quasi-spherical polyhedra	Doan <i>et al.</i> (2020)
<i>Simarouba glauca</i>	10 nm, quasi-spherical polyhedra	Thangamani <i>et al.</i> (2019)
<i>Annona muricata</i>	25 nm, quasi-spherical polyhedra	Folorunso <i>et al.</i> (2019)

Source: Author's elaboration.

Synthesis through microorganisms

The synthesis of AuNPs was also carried out using microorganisms, as these are easy to handle. In addition, the medium is inexpensive, and the resulting waste is biocompatible and environmentally friendly (Sehgal *et al.*, 2018).

Synthesis can occur intracellularly or extracellularly, depending on the site of formation. In the first process, the specific gold ions originating from the precursor salt are transported to the cell wall or membrane, which has a negative electrical charge. This enables more efficient ion transport through electrostatic attraction and diffusion. In this mechanism, the enzymes present in the cell walls or membranes reduce Au(III) to metallic gold, facilitating the formation of AuNPs (Menon *et al.*, 2017; Shedbalkar *et al.*, 2014). In the extra-

cellular method, enzymes synthesized by prokaryotic microorganisms and/or fungi, such as nitrate reductase, reduce the urea ions present in the NPs to metallic gold (Perotti *et al.*, 2015; Brito *et al.*, 1999).

Both methods have in common that upon contact with heavy metals such as gold, the microorganisms employ metal elimination mechanisms through ion channels, endocytosis, lipid permeation, metallothionein-mediated transport and other means to facilitate the active excretion of these ions from the synthesized AuNPs (Mishra *et al.*, 2013; Nies *et al.*, 1999).

Bacteria-mediated synthesis

Some bacteria can reduce metals such as Fe (III) to Fe (0). This ability was investigated with the aim of reducing Au (III) to Au (0) (Pacioni *et al.*, 2015). The adsorption of these ions occurs via the transport of vesicles, with protein enzymes being responsible for the reduction during this process. This prevents the formation of toxic residues for microorganisms. The synthesis of AuNPs by these methods usually leads to a high degree of uniformity in size and shape and thus to a well-defined crystal structure. Nevertheless, due to the lack of precise control over the synthesis of AuNPs by this method, it is challenging to accurately predict their size and shape (Khandel *et al.*, 2016).

Fungal-mediated synthesis

Another category of microorganisms that has recently been researched with regard to the development of AuNPs are fungi. They are used because they can secrete large quantities of enzymes that are useful for this purpose. This makes them suitable for use on a laboratory scale and they are also easily accessible and relatively inexpensive (Molnar *et al.*, 2018). Some fungi, such as filamentous fungi, exhibit higher tolerance in bioaccumulation of heavy metals and thus facilitate the formation of AuNPs in a faster and more efficient manner.

Specific enzymes are involved in the intracellular biosynthesis method, including phytochelatin synthase and glutathione synthetase (Savi *et al.*, 2012). Most AuNPs synthesized by this method are typically spherical and have a diameter of 10 to 20 nm (Xu *et al.*, 2024).

Prokaryote-mediated synthesis

Prokaryotic microorganisms such as actinomycetes, which have characteristics of both prokaryotes and fungi, can be genetically modified to enable their metabolism to produce AuNPs of specific size and shape by intracellular mechanisms. However, comprehensive modification for preferential synthesis with the physicochemical properties of specific AuNPs has yet to be achieved. Some of these microorganisms, such as mycobacteria and coryneforms, have already been studied for the synthesis of spherical AuNPs (Ahmad *et al.*, 2003).

As mentioned above, the use of these microbial species offers the advantage of accessibility, control and cost efficiency. All these microorganisms produce products that are biocompatible with the environment and biolog-

ical systems, which can be a great advantage in the synthesis of AuNPs. It should be noted that the lack of specificity in controlling the physicochemical properties of these NPs compared to chemical and traditional methods may be a disadvantage that should be considered in future studies. Table 2 shows a compilation of AuNPs obtained with microorganisms in the last five years. It shows that the predominant shape in this synthesis is spherical, although the size varies considerably among the different strains.

TABLE 2. Synthesis of AuNPs using microorganisms.

Extract	Size and shape of AuNPs	References
Bacteria		
<i>Deinococcus radiodurans</i>	30-50 nm, quasi-spherical polyhedra	Velmathi <i>et al.</i> (2024)
<i>Sarcophyton crassocaule</i>	5-50 nm, quasi-spherical polyhedra	Rokkarukala <i>et al.</i> (2023)
<i>Lysinibacillus odyseyi</i>	20-100 nm, quasi-spherical polyhedra	Chowdhury <i>et al.</i> (2022)
<i>Staphylococcus aureus</i>	6-30 nm, quasi-spherical polyhedra	Qiu <i>et al.</i> (2021)
<i>Escherichia coli</i>	13 nm, quasi-spherical polyhedra	El-Shanshoury <i>et al.</i> (2020)
<i>Bacillus subtilis</i>	13 nm, quasi-spherical polyhedra	El-Shanshoury <i>et al.</i> (2020)
<i>Paracoccus haeundaensis</i>	21 nm, quasi-spherical polyhedra	Patil <i>et al.</i> (2019)
<i>Vibrio alginolyticus</i>	100-150 nm, irregulars	Shunmugam <i>et al.</i> (2021)
Fungi		
<i>Trichoderma atroviride</i> , <i>Trichoderma asperellum</i> , <i>Botrytis cinerea</i>	25, 17 y 93 nm, quasi-spherical polyhedra	Olvera-Aripez <i>et al.</i> (2024)
<i>Candida rugopelliculosa</i>	10-30 nm, quasi-spherical polyhedra	Zhao <i>et al.</i> (2024)
<i>Alternaria chlamydospora</i>	12-15 nm, quasi-spherical polyhedra	Ameen <i>et al.</i> (2023)
<i>Penicillium rubens</i>	15 nm, quasi-spherical polyhedra	Bhandari <i>et al.</i> (2023)
<i>Aspergillus terreus</i>	9-14 nm, quasi-spherical polyhedra	Mishra <i>et al.</i> (2022)
<i>Agaricus bisporus</i>	10-15 nm, quasi-spherical polyhedra & poliedrums	Krishnamoorti <i>et al.</i> (2021)
<i>Fusarium solani</i>	40-45 nm, flowers & needles	Clarence <i>et al.</i> (2020)
<i>Cordyceps militaris</i>	15-20 nm, cubes	Ji <i>et al.</i> (2019)

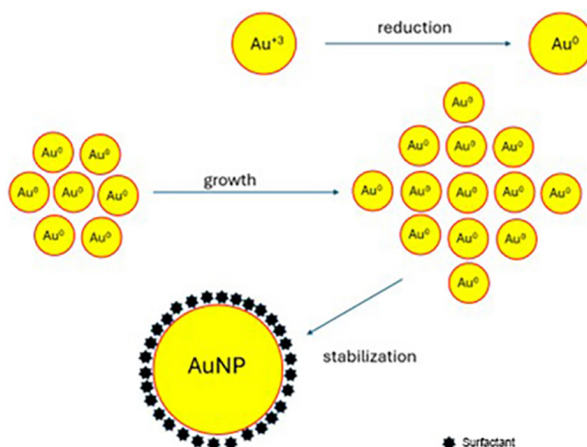
Source: Author's elaboration.

Synthesis of AuNPs by colloidal chemistry

The synthesis of AuNPs by colloidal chemistry involves the reduction of metal salts in colloidal solutions, both in aqueous and non-aqueous media, using traditional reducing agents such as NaBH₄. This process allows control of the physicochemical properties of the NPs, in particular their size and shape. In these cases, the most common starting material is HAuCl₄, chloroauric acid and its hydrated salts. After reduction in a solution, this leads to the forma-

tion of gold nuclei, which subsequently develop into stable anisotropic NPs (figure 3) (Zhou *et al.*, 2009).

FIGURE 3. AuNPs synthesis process using the colloidal method.



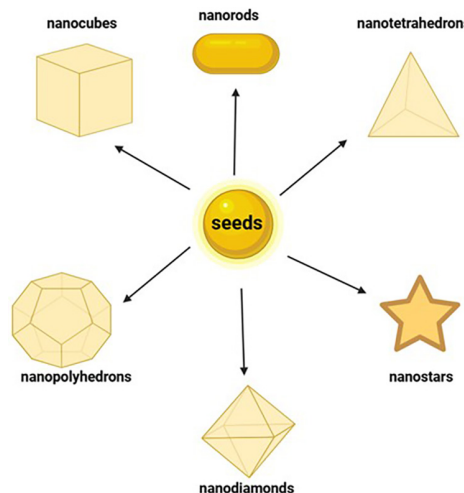
Source: Author's elaboration. Image created with BioRender.

The Turkevich method is a well-known technique for synthesizing AuNPs through colloidal chemistry. Developed by John Turkevich in 1951, this method involves reducing $HAuCl_4$ in an aqueous solution with reducing agents at elevated temperatures. This process results in the formation of spherical AuNPs with sizes typically ranging from 10 to 20 nm (Kimling *et al.*, 2006). Various research groups have utilized this method to refine the size of the NPs by adjusting several parameters, including salt concentration, gold salt concentration, and the amount of stabilizer used. One notable modification to the Turkevich method is the Brust-Schiffrin approach, which uses non-aqueous media and thiols as stabilizing agents, allowing to produce AuNPs with sizes between 1 and 5 nm (Brust-Schiffrin *et al.*, 1994). However, the presence of toxic reducing agents and non-biocompatible solvents in non-aqueous media has prompted investigations into new synthesis methods to improve nanoparticle biocompatibility (Ginzburg *et al.*, 2018).

The choice between aqueous and non-aqueous reaction mediums and surfactants plays a crucial role in these syntheses, directly influencing the size, shape, and stability of NPs throughout their development (figure 4) (Xiao *et al.*, 2011).

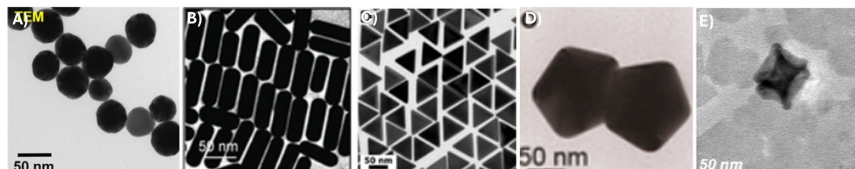
The adsorption of these molecules on the surface of AuNPs allows for the control of their growth and size, preventing aggregation and resulting in particles with a uniform size. Additionally, the chemical nature of the surfactant and the reaction medium can affect the formation of specific shapes, such as quasi-spherical polyhedral, rods, tetrahedrons, cubes, and others (figure 5) (Smith *et al.*, 2008; Hormozi-Nezhad *et al.*, 2013; Sakai *et al.*, 2009).

FIGURE 4. Different shapes of AuNPs from quasi-spherical polyhedral seeds.



Source: Author's elaboration. Image created with BioRender.

FIGURE 5. Compilation of different shapes of AuNPs observed by TEM.



From left to right:

- A) Quasi-spherical polyhedral (Hong, Y. A., Ha, J. W., 2022).
- B) Rods (Zheng, J. *et al.*, 2021).
- C) Tetrahedrons (Scarabelli, L., Liz-Marzán, L. M., 2021).
- D) Polyhedrons (Sánchez-Iglesias *et al.*, 2016).
- E) Cubes (Romo-Herrera *et al.*, 2016).

Source: Author's elaboration.

Notable examples of surfactants used for physicochemical control include cetyltrimethylammonium chloride (CTAC) and cetyltrimethylammonium bromide (CTAB), which are primarily employed to synthesize anisotropic and tip-terminated forms (Li *et al.*, 2014). The chemical compositions of surfactants like sodium citrate, polyvinylpyrrolidone (PVP), and sodium dodecyl sulfate (SDS) are currently under investigation, as these compounds have been shown to enhance the biocompatibility of synthesized AuNPs. These are mainly used for spherical forms, since no optimized method has been identified for producing stable anisotropic forms (Ginzburg *et al.*, 2018). This relates to green synthesis methods mediated by plant extracts, fungi, and bacteria, which predominantly yield spherical forms, like those produced using the biocompatible surfactants.

The research group led by Zhou and colleagues has proposed a ligand exchange method for synthesizing anisotropic AuNPs. They recommend re-

placing (CTAC) or (CTAB), which are commonly used in synthesizing cubic, triangular, cylindrical, and polyhedron-shaped NPs, with sodium citrate. This synthesis method can produce particles with specific shapes and sizes, and the use of citrate helps reduce toxicity and improve biocompatibility. However, the yield of this reaction is not particularly promising (Zhou *et al.*, 2018).

The benefits and applications of green synthesis of AuNPs in biomedical research

The green synthesis of AuNPs in various shapes and sizes offers numerous benefits, especially in the field of biomedical research. The following section outlines these advantages.

Biocompatibility is a significant advantage of these NPs

AuNPs synthesized through environmentally friendly methods demonstrate high biocompatibility, which means they show minimal or no toxicity at the cellular level within biological systems. This property makes them an excellent option for biomedical applications (Gurunathan *et al.*, 2014).

Because these substances are synthesized without creating toxic residues, they are less likely to cause adverse reactions. This characteristic allows for their safe use in medical diagnostics and the development of advanced therapies, including drug delivery systems and targeted treatments (Kus *et al.*, 2021; Kadhim *et al.*, 2021; Fan *et al.*, 2009).

Facilitated functionalization is a process that enables the attachment of functional groups to the surface of NPs.

One of the key advantages of AuNPs is their ease of functionalization. This characteristic allows for the binding of a wide range of biomolecules, including proteins, antibodies, drugs, and nucleic acids. By attaching specific ligands to their surface, these particles can be customized for targeted and specific applications. Additionally, when materials are used without toxic residues or incompatible surfactants, they are safer and free from potential adverse effects (Amina *et al.*, 2020; Tiwari *et al.*, 2011).

Targeted delivery

AuNPs are particles that possess unique properties, making them highly effective for targeted drug delivery. Due to their small size and ability to be modified on their surface, AuNPs can carry specific molecules. These NPs can be engineered to transport therapeutic agents and release drugs in a controlled manner directly at the intended target site. In cancer treatment, functionalized AuNPs can selectively target tumor cells, delivering the drug directly into them while minimizing damage to surrounding healthy tissues. This targeted approach has the potential to reduce the side effects typically associated with conventional therapies and improve the overall efficacy of treatment (Daraee *et al.*, 2016; Dreaden *et al.*, 2012; Pissuwan *et al.*, 2011).

Biomedical diagnostics and photothermal therapy

The localized surface plasmon resonance (LSPR) effect of AuNPs allows them to be used in highly sensitive biosensors, which can detect overexpressed biomarkers in diseases, even at very low concentrations. AuNPs have been particularly useful in diagnostic assays for the quick identification of infectious agents, including viruses and bacteria, as well as the early detection of nascent cancer cells. They can also be functionalized to target specific therapeutic interventions. Afterward, the NPs are irradiated with lasers in the near-infrared spectrum, generating localized heat that can destroy specific cells (Tabish *et al.*, 2020; Zeng *et al.*, 2016; Huang *et al.*, 2010).

Tissue regeneration

Nanomaterials, such as AuNPs, are utilized in the field of tissue regeneration and engineering. Several studies have been conducted, including research by Gutiérrez-Calleja *et al.* (2021), which demonstrated the ability of mast cells to enhance cell proliferation and support tissue regeneration, particularly in bone and dermal tissue. Additionally, integrating AuNPs into biocompatible scaffolds can create a more favorable environment for cellular regeneration (Yadid *et al.*, 2019; Vial *et al.*, 2017; Bodelón *et al.*, 2017).

The main disadvantages of using green chemistry in the synthesis of AuNPs arise from the natural variability in the composition of biological substances, such as plant extracts and microorganisms. This variability is affected by numerous external factors, including geographical location, climate, and seasonal changes.

The composition and concentration of plant extracts and microbial derivatives can vary based on seasonal and regional factors. For example, the use of flavonoids as reducing and stabilizing agents in the synthesis of AuNPs can fluctuate in concentration due to external influences such as climate, temperature, atmospheric pressure, and soil type. This highlights the direct impact that geographical conditions have on the levels of these chemical compounds (Quintero-García *et al.*, 2021; Tolic *et al.*, 2017; López-Orenes *et al.*, 2017).

Genetic variations within the same species of plants or microorganisms can alter their chemical composition, leading to inconsistent synthesis of AuNPs with controlled physicochemical properties (Rao *et al.*, 2009; Santos *et al.*, 2016).

When considering the physicochemical properties of particles, the size and shape are particularly important in their synthesis. Utilizing natural extracts can lead to less precise control over the growth of AuNPs, resulting in a heterogeneous distribution of sizes and shapes. This variability can affect the surface plasmon effect in an unpredictable manner, which may be beneficial for applications such as sensors for biomarkers or in photothermal therapies (Elia *et al.*, 2014; Iravani *et al.*, 2011). Additionally, the use of biological products that contain complex molecules often requires multiple reduction

processes, making their characterization crucial for process optimization (Albrecht *et al.*, 2006).

Compared to traditional methods of synthesizing AuNPs, reactions that utilize green chemistry may have slower reaction rates. This slower pace can lead to limited short-term efficiency improvements. Such a challenge may hinder industrial applications that require fast and high-volume production processes (Sheldon *et al.*, 2012; Anastas *et al.*, 2002).

In conclusion, one major drawback is the inferior colloidal stability when compared to conventional chemical reagents. For example, using biomolecules in the synthesis process leads to their presence on the surfaces of various AuNPs. This occurs because the stabilizing agents used are not as strong as traditional surfactants. It has been established that the aggregation of AuNPs alters their optical and catalytic properties, ultimately reducing their efficiency in applications (Parveen *et al.*, 2016).

Conclusions and perspectives for the development of new green synthesis methods for AuNPs

Green synthesis methods for developing AuNPs have shown significant potential, providing better environmental impact and biocompatibility compared to traditional chemical synthesis methods. Based on this information, we can conclude that green chemistry enables the production of AuNPs with improved biocompatibility and a lower risk of toxicity and adverse effects. This is particularly important for their use in biomedical applications. Moreover, green synthesis can have notable economic benefits, as many of the raw materials used—such as leaves, shells, or agricultural residues—are abundant and inexpensive. This not only reduces costs but also minimizes toxicity risks compared to conventional chemical reagents.

Despite the potential of green synthesis for generating AuNPs, there are still several issues and challenges that need to be addressed before it can be deemed a viable method. Current protocols require optimization to ensure that the physicochemical properties of the NPs such as size and shape are consistent and reproducible. This consistency is crucial for their effectiveness and application in industrial and biomedical fields.

To tackle these challenges, it is crucial to conduct focused research on biological sources for synthesizing AuNPs. Utilizing readily accessible and cost-effective plants or microorganisms, we can achieve this. The goal is to optimize reaction conditions to have better control over the physicochemical properties of AuNPs. Additionally, this approach could enable the synthesis of a wider variety of AuNP shapes and sizes, thereby broadening their potential applications in technology and biomedicine.

Research on AuNPs synthesized using green methods could be a valuable direction for applications in nanomedicine, including controlled drug delivery, targeted therapies, and the development of sensors for medically rele-

vant biomolecules. Future studies should thoroughly investigate the interactions between AuNPs and biological systems to ensure their safety and effectiveness. It is essential to accurately characterize these particles both during the synthesis process and in their interactions within nano-biological systems.

Establishing clear standards and regulations is essential to ensure that the development and production of green NPs focus on sustainability. By doing so, we can ensure that these processes align with sustainability principles. These regulations should address key issues, including the safe production, use, and disposal of AuNPs, with the goal of minimizing potential risks to the environment and public health.

The implementation of these regulations will promote the broader use of green NPs in industrial and medical applications. The benefits to technology and the environment are expected to be realized through this expansion (Manchikanti *et al.*, 2010; Cely-Bautista *et al.*, 2023).

It is very important to highlight that the wide variety of natural sources serves as a basis for further research into the impact of synthesis on AuNPs. As time goes on, we can expect a more comprehensive database and comparative results to develop, providing valuable insights into the best methods for achieving the desired products. Additionally, the integration of artificial intelligence could further enhance this context.

Authors contribution

The authors contributed equally to the search for information, writing and reviewing this article. There is no conflict of interest between them.

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