

# GOLD NANOPARTICLES FOR EMERGING ELECTROCHEMICAL SENSING APPLICATIONS

## Abstract

Nanotechnology is a growing field due to its excellent properties such as the large surface area, high sensitivity, high chemical and thermal stability, decent electrical, magnetic, and optical properties etc. The engineered nanomaterials like gold nanoparticles (AuNPs) plays a crucial role in sensing applications due to their high sensitivity, biocompatibility, and low detection limit. Among various sensing applications of AuNPs, electrochemical sensing has gained substantial interest over the years owing to their electrochemical compatibility with various analytes. In this book chapter, we discuss a range of synthetic methods to synthesize AuNPs followed by their broad application in the field of electrochemical sensing. A variety of important electrochemical sensing applications like biomolecule (protein and DNA) and small molecule (hydrogen peroxides, glucose, and metal ions) sensing have been discussed in great detail.

**Keywords**-Nanoparticles synthesis, electrochemical sensing, gold nanoparticles (AuNPs).

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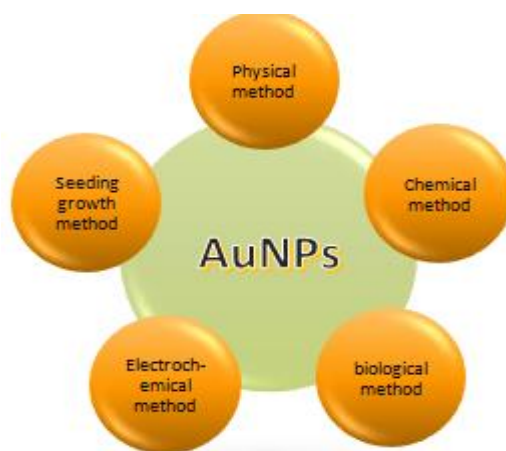
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## I. INTRODUCTION

With the current advancement of nanotechnology, nanomaterials have gained substantial interest in the domain of sensing applications owing to their exquisite sensitivity in chemical and biological sensing. Various class of nanoparticles, including metal nanoparticles, oxide nanoparticles, semiconductor nanoparticles, and nano-dimensional conducting polymers have been utilized in sensing applications. Among various sensing applications, electrochemical sensing which converts the analyte (redox-active small molecules/oligomers/polymers, metal ions, proteins, DNA etc.) binding events to the surface of electrodes into useful electrical signals, have gained substantial recognition in the scientific community lately as they yield quick, easy, and cost-efficient detection potential. These electrical responses have been well analysed by various electrochemical tools, including cyclic voltammetry, chronoamperometry, differential pulse voltammetry, and electrochemical impedance spectroscopy. AuNPs, in general, have been studied considerably and are envisaged to be the building blocks of nanoscience due to their outstanding thermal, magnetic, optical, *and* electrical properties. In addition, AuNPs possess decent biocompatibility, magnificent electrical and heat conductivity, a larger aspect ratio, and higher chemical stability [1]. AuNPs have an ability to facilitate fast and direct electron transfer between a wide range of electroactive species and electrode materials. As a result, these modified electrode surfaces can reduce the overpotential of many electroanalytical reactions and maintain the electrochemical reversibility of the chemical reactions which appear irreversible at the classical electrodes. Owing to these attributes, AuNPs have been extensively utilized for the electrochemical sensing. In addition, AuNPs are considered to be methodical labels in electrochemical sensing mainly because it can modify the surface of the electrodes resulting in a strengthened detection limit of the sensor [2]. These modified electrodes act as an electroanalysis device boosted with sensitivity and selectivity. Thus, it has captured substantial interests in the development of successful methods in the deposition of metal NPs [3].

## II. SYNTHESIS OF AUNPS

The state of the art of the synthesis of AuNPs is very easy and embraces physical, chemical, biological, electrochemical and seeding growth methods [1].



**Figure 1: Various methods of the synthesis of AuNPs**

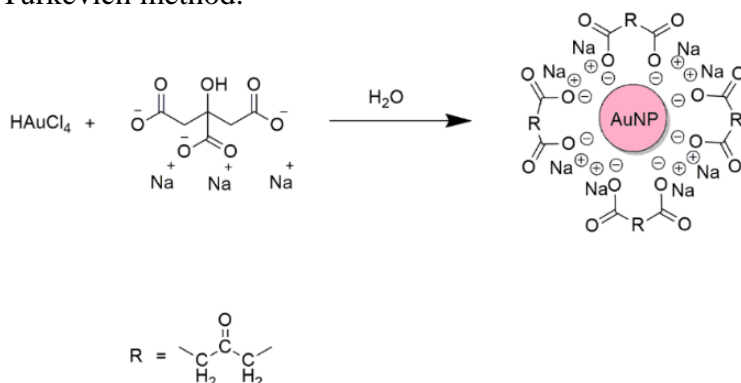
**1. Physical method:** As mentioned above AuNPs can be synthesized using physical methods like vacuum deposition, electrical circuitry dispersion, laser ablation method etc. that can convert huge solid gold to nanoparticles. This method is also useful in controlling the shapes of AuNPs. This method is not preferred as it is a costly and long drawn out. Thus, scientists developed tie-up of ligands by physical absorption or electrostatic interaction of AuNPs in a simple way. Although this does not lead to the synthesis of stable nanoparticles that can hold out against the requisite steps like washing and long-time incubation with the buffer solution in future reactions [4].

**2. Chemical method:** The method follows reduction process mainly in two ways:

- Reduction by chemical reagents like hydrazine, citric and oxalic acids, sugars, hydrogen peroxide, borohydrides, etc.
- Usage of stabilizing agents like trisodium citrate dihydrate, phosphorous-, oxygen-, and nitrogen-based ligands, polymers, surfactants, etc.

Stabilizing agents are added to avoid the accumulation of particles. Some chemical methods for the synthesis of AuNPs are discussed below. These processes are carried out in favourable conditions and are comparatively uncomplicated.

**3. Turkevich method:** This method is a very popular method for the synthesis of AuNPs and AgNPs. The method was proposed by Turkevich in 1951 on the basis of the reduction mechanism of gold hydrochlorate ( $\text{HAuCl}_4$ ) by citrate in water. The citrate ion acts as both reducing as well as stabilizing agent. The solution of  $\text{HAuCl}_4$  was boiled at  $100^\circ\text{C}$  followed by the rapid addition of trisodium citrate dihydrate (reduction initiator) with vigorous stirring using Teflon-coated magnetic bars in a double-walled reactor, which was then heated by a bath thermostat that maintains a uniform temperature of the reaction solution. After some time, the colour of the solution changes from light yellow to wine red indicating the formation of AuNPs. The size of the particle is about 20 nm in diameter. Recent studies show the role of sodium citrate on the pH of the solution and in controlling the size of the AuNPs [5, 6]. Fig. 2 below shows, the scheme for the synthesis of AuNPs by Turkevich method.



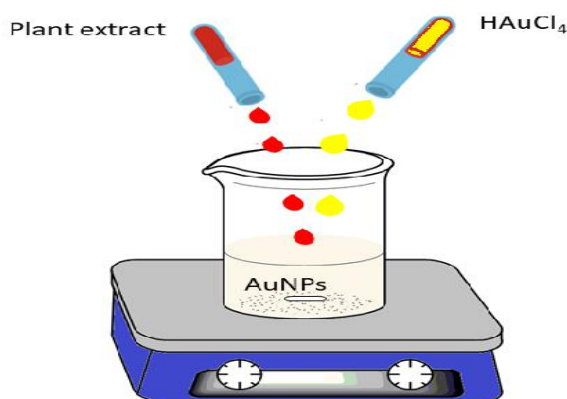
**Figure 2: A schematic route for the synthesis of AuNPs by Turkevich method**

- **The Brust-Schiffrin method:** An electrochemist from the University of Liverpool, David Schiffrin, obtained AuNPs of diameter 50 nm by fast chemical reduction of gold ions at an oil-water interface which is followed by quick absorption of thiolated

molecules. This has made it as easy as handling a simple chemical compound. This method is suitable for gram scale production of AuNPs [7].

- 4. Biological method:** It is a notable fact that plants take up heavy metals from the soil and store it in the plant matrix. This process of accumulation of heavy metals in parts of plants can be dated back to 1855. This is the process of detoxification of soil by plants. The chances of metals to get converted into nanoparticles are high even before they are stored into the plant matrix. This conversion of metals into nanoparticles prevent ionization/chelation of metals thereby preventing the parts of the plants which store it from toxicity. Such process leads to storage of high concentration of different metals by a variety of plant species. Hence, extracts from the plants can be used to synthesize nanoparticles. This method had also been used for the reduction of silver by plant roots [8].

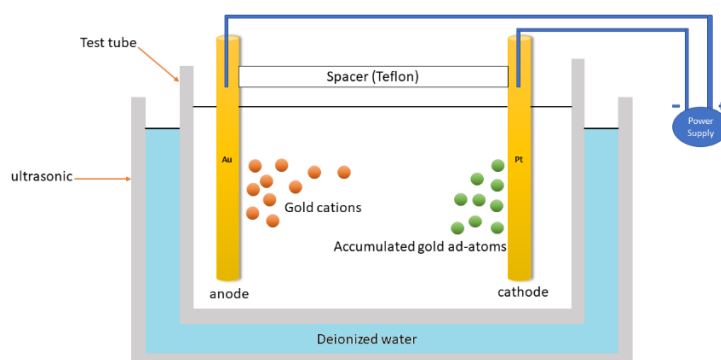
This method can be categorized as the intracellular and extracellular synthesis of nanoparticles from plants. In 2002, Gardea-Torresdey *et al.*, synthesized AuNPs ranging in a size 2-20 nm from the alfalfa (*Medicago sativa*) seedlings through the intracellular process [9]. This process makes the extraction of the nanoparticles obtained from the plant biomass more complicated due to the additional steps including ultrasonication, the cell cracking with detergent, separation of enzymes from undesired cellulosic materials, present on the surface, etc. In comparison to this, extracellular process of synthesis uses plant extracts instead of living plants. This makes the process much simpler, economical, and suitable for the production of the nanoparticles in a large-scale [8, 10]. The first report on the extracellular process of the synthesis of AuNPs was done using plant broth of geranium (*Pelargonium graveolens*) by Sastry and co-workers. Furthermore, the conversion of Au(III) to stable Au nanoparticles was obtained by the reduction process using biomolecules containing amino, sulfhydryl, and carboxylic groups [11]. A recurrent extracellular process for the synthesis involves the initiation by  $\text{HAuCl}_4$  when mixed with green extracts with continuous stirring on a magnetic stirrer. The colour of the solution changes to purple-red when the AuNPs are formed. The next step involves the centrifugation and drying of the NPs [12]. Fig. 3 is a depiction of the synthesis of AuNPs using green extracts under moderate environmental conditions.



**Figure 3: A pictorial demonstration of the synthesis of AuNPs using green extracts under moderate environmental conditions.**

- 5. Electrochemical method:** This method is a bit more advantageous over the chemical methods due to its capability of producing size-selective nanoparticles in organic phase. The anode method was developed by Reetz and Helbig to synthesize metal clusters. In this method, tetraalkylammonium salt was used both as an electrolyte and stabilizer for the metal clusters formed in the nanometer range [13]. This method can also follow direct electroreduction of bulk metal ions in electrolytic solution. This process is a bit complicated as it requires the presence of two completely opposite cathode surface methods, i.e., the formation of metal nanoparticles and the electrodeposition of metal at the cathode. Due to the reason that the latter part is dominant over the former part, the use of a good stabilizer is necessary to accelerate the formation of nanoparticles, prevent the accumulation of nanoparticles, and reduces the rate of deposition of the metal on the surface of the electrode [14].

Huang *et al.* in 2006 synthesized gold nanodumbbells by the electrochemical method. The apparatus for the synthesis consisted of a two-electrode system kept facing opposite to each other inside a cell. Gold and platinum plates (99% pure) were cut with dimensions 30 x 10 x 0.5 mm to form anode and cathode, respectively. The gold plate is washed by water and acetone in ultrasonication. Electrodes in the cell were held together by Teflon at a distance of 5 mm from each other. This whole cell was kept in a standard glass tube which is then kept in the ultra-sonicator bath. During the electrochemical process, the anode (gold plate) gets oxidized to gold cations. These cations migrate to the cathode and form gold ad-atoms on its surface by reduction process. The surfactant added in the solution acts as a stabilizer that traps the gold ad-atoms to form gold nanoparticles. The surfactant used is generally a micelle template that controls the shape and size of the nanoparticle. After obtaining the gold nanoparticles, the platinum plate was cleaned with aqua regia in order to dissolve the leftover Au particles on its surface. Furthermore, the electrodes were blown out with clean nitrogen gas in the end [15]. Fig. 4 shows the set-up of the apparatus for synthesis of gold nanoparticles via electrochemical method.



**Figure 4: A representation of cell diagram for the synthesis of gold nanoparticles via electrochemical method**

- 6. Seeding Growth method:** The process involves chemical reduction of gold salt by a strong reducing agent like sodium borohydride in the presence of citrate that acts as a capping agent. The seeds obtained are then added to a solution that contains more of the gold salt in it along with a weak reducing agent like ascorbic acid and a surfactant. The seeds become a nucleation site and promotes the anisotropic growth of stable gold nanorods [5].

### III. ELECTROCHEMICAL SENSING APPLICATIONS OF AuNPs

The futuristic properties of AuNPs like excellent biocompatibility, unique structures, catalytic, magnetic, and optical properties, etc. makes it different from nanoparticles seen in bulk scale. Its optical and electrochemical sensing methods have made it suitable as labels in sensing applications.

#### 1. Biomolecule sensing

- **Protein sensing:** The distinctive chemical and physical properties of AuNPs have made it attractive for its use as electrochemical sensors. It has been quite a challenge to develop a direct electrochemistry of proteins as it requires facile electrical communication between the surface of the electrode and the active site of the enzyme. It is referred to as direct electron transfer (DET) from the redox protein to the electrode's surface. Due to the presence of an insulated protein shell across the redox center, the reduction or oxidation of the protein at an electrode becomes difficult at any applied potential. To overcome this factor, the surface of the electrodes are redesigned using AuNPs making it possible for the protein molecules to orient in any direction which ultimately results in the reduction of the insulating effect of the protein shell [16]. The method of modifying the electrode by using AuNPs are also effective in lowering ohmic resistance contact values that increases the rate of detection by electrochemical sensing. This can be obtained by the incorporation of gold nanoparticles on the walls of carbon nanotubes (CNTs) making it a highly efficient sensor. This ultrasensitive gold-modified electrodes attaches biomolecules easily. As for example, the binding of ferrocene-peptide conjugates to the electrodes empowers the detection and screening of HIV-1 PR in physiological conditions with detection limit (LOD) as low as 0.1 nM [17].
- **DNA sensors:** Development of highly selective and sensitive DNA sensors play a pivotal role in the area of clinical diagnosis, maintaining the food safety, and environmental contamination studies. The target DNA is encapsulated via hybridization when the single stranded DNA are deactivated on the surface of the electrode [18]. AuNPs provide a robust platform for efficient DNA sensing owing to their capacity to greatly increase the current response of the sensor with decent conductivity and simply immobilize the DNA probes through Au-S bonding [18, 19]. Due to the captivating biocompatibility, AuNPs are simply incorporated with the biomolecules even without interfering with their biochemical reactivity – prerequisite for an efficient biosensor. For instance, Li and coworkers employed the ultra-high charge productivity of AuNPs in order to build a DNA sensor [20]. AuNPs were adsorbed on the surface of single strand DNA (ssDNA) electrode through the interactivity between bases containing gold and nitrogen. In the availability of target DNA, the double strand DNA was created with the exclusion of AuNPs because of the charge transfer hinderance occurring as the DNA contains negatively charged phosphate. On the other hand, without target DNA, there was no exclusion of AuNPs which were identified by electrical signals. Thus, the biocompatibility and efficient charge transfer characteristics of AuNPs have led to the development of biosensors that are extremely sensitive for the diagnosis of breast cancer gene electrochemically

within a low detection limit of 1 pm without the requirement of any signal amplification process or the involvement of other synthesized complex.

2. **Small molecule sensors:** AuNPs can detect various small molecules like glucose, uric acid, dopamine, ascorbic acid etc. through the electrochemical detection at high intensity. Detection of toxic chemicals and drugs are also possible by using AuNP-based electrodes. These electrodes have high-catalytic activity that promotes electrocatalytic oxidation making the detection of harmful gases possible [21].
  - **Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) sensing:** Selective and sensitive sensing of H<sub>2</sub>O<sub>2</sub> holds practical importance in various fields, including bioanalysis, food safety, and environmental protection [22, 23]. The direct electrochemical oxidation or reduction of H<sub>2</sub>O<sub>2</sub> on the surface of the electrode involves the transfer of 2H<sup>+</sup> and 2e<sup>-</sup> to form O<sub>2</sub> or H<sub>2</sub>O, respectively. On the bare Au electrode surface this redox process gives high overpotential and weak current signal [24]. On the contrary, the redox process kinetics is facile on Au nanostructure due to the enormous specific surface area and sufficient stepped sites. For instance, Stanciu and coworkers scrutinized the electrocatalytic properties of AuNPs with varying morphology about H<sub>2</sub>O<sub>2</sub> and found the great dependency of morphology on the sensitivity of the redox process [25].
  - **Glucose sensing:** Accurate track of glucose level in the blood and other food origin, including the pharmaceuticals is vital in monitoring and controlling diabetes mellitus which motivates to develop efficient and highly sensitive sensing of glucose [26, 27]. Owing to their huge electroactivity towards glucose, a range of AuNPs fabricated electrodes have been utilized for the efficient glucose sensing. Hebie *et al.* synthesized globe-shaped AuNPs with a mean diameter of 4-15 nm suitable for the oxidation of glucose. The oxidation process was found to be size-dependent with the small-sized (4.2 nm) AuNPs exhibiting higher electrocatalytic activity due to the enhancement of surface electronic effect [28].
  - **Metal Ion sensing:** The fast and precise sensing of the toxic heavy metal ions is the indispensable necessity to ensure the healthy ecological systems and living resources [29, 30]. Tunable electrochemical compatibility of AuNPs with the redox potential of metal ions plays a pivotal role in developing efficient electrochemical sensors for metal ion detection. On that line, Wang and coworkers reported a screen-printed gold electrode improved with AuNPs for the development of an electrochemical sensor for the concurrent detection of Pb<sup>2+</sup> and Cu<sup>2+</sup> by utilizing their distinct stripping potential (0.03 V for Pb<sup>2+</sup> and 0.4 V for Cu<sup>2+</sup>) [31]. Owing to their larger number of active sites, good electrical conductivity, and decent ability to absorb, the modified electrodes made up of conjugated polymers have gained substantial curiosity for the heavy metal sensing. On that line, Lu and coworkers reported the *in-situ* synthesis of dandelion-like conductive polyaniline modified gold nanoparticle nanocomposite (Au@PANI) to all together spot the Pb<sup>2+</sup> and Cu<sup>2+</sup> [32]. The PANI molecules possess a lot of amine (-NH-) and imine (=N-) that can act as the sites for adsorption for metal ions via quadridentate coordination that enhanced the electrical conductivity and the active surface area leading to the powerful adsorption capability of PANI towards metal ions.

#### IV. CONCLUSION

Nanotechnology is an interdisciplinary area of research and have considerably developed since last few years. The AuNPs are chemically noble and elementary unit of nanoscience and are one of the most attractive nanomaterials that can be easily synthesized and have unique characteristics like good sensitivity, large aspect ratio, good thermal, magnetic, electrical, and optical properties as well as outstanding biocompatibility. There are various methods to synthesize AuNPs amongst which the Turkevich method is the most popular method for the synthesis of AuNPs by citrate reduction. Nevertheless, the electrochemical method is dominant over the chemical methods due to their size-selective production ability of AuNPs in organic phase. However, this process is comparatively more complex than the Turkevich method as it requires a decent stabilizer that can accelerate the formation of AuNPs without getting them agglomerated. The chapter also encloses applications of AuNPs in electrochemical sensing for various applications like biomolecule and small molecule sensing due to their compatibility with the analytes.

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