Vol. 19, No. 2 (2020) 585-592
Revista Mexicana de Ingeniería Química

# Room temperature biosynthesis of gold nanoparticles with *Lycoris aurea* leaf extract for the electrochemical determination of aspirin °

# Biosíntesis a temperatura ambiente de nanopartículas de oro con extractos de hojas *Lycoris aurea* para la determinación electroquímica de la aspirina

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Received: July 23, 2019; Accepted: September 9, 2019

**Abstract**

Nanoparticle synthesis using plants is an alternative to conventional physical and chemical methods. In this paper, we demonstrate the synthesis of AuNPs using *Lycoris aurea* leaf extract. The prepared AuNPs show an average size of 24.1 nm based on transmission electron microscopy characterization. The biosynthesized AuNPs were then applied for electrode surface modification. The modified electrode was successfully used for the electrochemical determination of aspirin. The modified electrode could detect aspirin from 2.6 mM to 100 $\mu$M with a limit of detection of 11.3 $\mu$M.

*Keywords*: biosynthesis; *Lycoris aurea*; aspirin; electrochemical sensor; leaf extract.

**Resumen**

La síntesis de nanopartículas con plantas es una alternativa a los métodos físicos y químicos convencionales. En este artículo, demostramos la síntesis de AuNP utilizando extracto de hoja de *Lycoris aurea*. Los AuNP preparados muestran un tamaño promedio de 24.1 nm basado en la caracterización de microscopía electrónica de transmisión. Los AuNP biosintetizados se aplicaron luego para la modificación de la superficie del electrodo. El electrodo modificado se utilizó con éxito para la determinación electroquímica de aspirina. El electrodo modificado podría detectar aspirina de 2.6 mM a 100 $\mu$M con un límite de detección de 11.3 $\mu$M.

*Palabras clave*: biosíntesis; *Lycoris aurea*; aspirina; sensor electroquímico; extracto de hoja.

## 1 Introduction

From a global perspective, nanomaterials have already reached a certain market scale. Nano-ceramic materials, nano-textile materials, nano-modified coatings and other materials have been widely developed and industrialized. The application of nanoparticles in medical diagnosis and microelectronics is shifting from experimental research results to industrial production. Benefiting from the continuous innovation of nanotechnology, the global market for nanomaterials exceeded $8,970,000 in 2017 (Chen et al., 2017; Chieh et al., 2015; Hoet et al., 2004).

With the development of nanotechnology, the synthetic methods of nanomaterials have been undergoing constant innovation. The traditional synthetic methods include precipitation, sol-gel, ion exchange, and other physical methods including ball milling, sputtering and high-gravity reactive precipitation. However, these traditional methods are generally accompanied by pollution problems and high energy consumption (Chávez-Magdaleno et al., 2018; Izadiyan et al., 2018; Miri et al., 2018; Wadhwani et al., 2018; Zarzuela et al., 2018). Compared with traditional physical and chemical methods, the biosynthesis of nanomaterials is more environmentally friendly in terms of raw material selection, the regulation of reaction conditions and post-treatment. Nanomaterials with different morphologies and properties are prepared by combining nanotechnology with different organisms, which allows for a broad field for development.

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https://doi.org/10.24275/rmiq/Mat741
*issn-e*: 2395-8472

Some organisms have their own delicate morphological characteristics and can therefore be used as templates to prepare nanomaterials with a specific biological morphology without the use of traditional template methods. Some of the constituents of organisms or their extracts contain active ingredients that are excellent reductants and stabilizers, which could reduce the use of toxic chemicals (Annamalai and Nallamuthu, 2015; Gutiérrez-Rebolledo et al., 2018; Keshavarzi et al., 2018; Miri et al., 2018; Qu et al., 2017; Srinath et al., 2018; Vasantharaj et al., 2018). Metal nanomaterials, such as gold, silver and platinum, have broad application prospects in the development of biosensors due to their excellent electron transfer properties. Among them, gold nanoparticles are the most widely used because of their simple preparation, excellent biocompatibility and easy surface modification. Integrating gold nanoparticles into the preparation of biosensors can increase the number of DNA probes, increase the signal response of the biosensor, improve the biosensor's biocompatibility, improve the detection sensitivity, reliability, stability, and shorten the detection time (Gupta et al., 2017; Losada et al., n.d.; Ning et al., 2017; Sánchez-Franco et al., 2019; Zhang et al., 2017; Zou et al., 2017). Aspirin (2-benzoic acid) is a commonly used western medicine due to its antipyretic, analgesic and anti-inflammatory effects. However, the long-term use of aspirin easily leads to an increase in the incidence of adverse drug reactions which can act on the pylorus, leading to pyloric spasm. The long-term use of aspirin stimulates the stomach and other organs which increases the incidence of gastrointestinal bleeding (Chieh et al., 2015; Cohen et al., 2010; Gash et al., 2017). Therefore, the development of a reliable aspirin detection method can provide a basis on which clinical patients can choose the appropriate form of aspirin (Karimi-Maleh et al., 2017; C. Li et al., 2017; J. P. Li et al., 2017; Machado Alencar et al., 2018; Saadat et al., 2018; Sankar et al., 2017).

In this article, we attempted to use *Lycoris aurea* leaf extract as a reducing agent for gold nanoparticle (AuNP) synthesis. Fresh *Lycoris aurea* leaf extract was prepared using a sonication method with methanol as a solvent. Then, the leaf extract was filtered to remove the solid tissue before the addition of the gold precursor solution. The AuNPs were successfully formed at room temperature with stirring. After a series of characterizations, the AuNPs were used for the surface modification of a glassy carbon electrode (GCE) and subsequently applied for the electrochemical determination of aspirin. Due to the excellent electrocatalytic properties of the biosynthesized AuNPs, the proposed electrochemical sensor could linearly detect aspirin between 2.6 mM to 100 $\mu$M with a low limit of detection of 11.3 $\mu$M.

## 2 Materials and methods

### 2.1 Materials

*Lycoris aurea* leaves were harvested from Nanjing Botanical Garden of Jiangsu province, China. The plant was taxonomically identified and authenticated by the botanical survey of Nanjing botanical garden. Chloroauric acid, aspirin, $KH_2PO_4$, $K_2HPO_4$ and a KCl were purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). Milli-Q water (18.2 MΩ cm) was used throughout the experiments.

### 2.2 Methods

Plant leaf pre-treatment: The plant leaves were cleaned with double distilled water. 1 g of *Lycoris aurea* leaves were cut up into small pieces and dispersed in to 20 mL methanol for 1 h sonication. Then, the dispersion was filtered using a filter paper (200 nm pore size) for removing the solid part. The extract was then dried in an oven to form powder for further experiments.

Biosynthesis of AuNPs and electrode surface modification: An aqueous chloroauric acid solution (1%) was prepared as precursor solution. Then 5 mL *Lycoris aurea* leaf extract (1 mg/mL) was added into 20 mL chloroauric acid solution and kept under continuous stirring for overnight. The blue precipitate was collected using centrifugation (1000 rpm for 10 min) and washed with water. The AuNPs were then re-dispersed into water to form a stable dispersion.

For glassy carbon electrode (GCE) modification, a GCE was firstly polished using alumina slurry and washed by water (Zheng et al., 2019). Then, a certain amount of biosynthesized AuNPs dispersion (1 mg/mL) was dropped on the GCE surface and dried under room temperature. The modified GCE was denoted as bAuNPs/GCE.

### 2.3 Characterization

The morphology of AuNPs was observed using a transmission electron micrograph (TEM, JEOL-2011FEF). All electrochemical measurements were

carried out using a CHI760E workstation using a three-electrodes system (Shi et al., 2019). A GCE, Pt electrode and Ag/AgCl(3M KCl) were used as working electrode, counter electrode and reference electrode, respectively.

## 3 Results and discussion

The schematic diagram of AuNP biosynthesis using *Lycoris aurea* leaf extract is shown in figure 1. The antioxidants, alkaloids, polysaccharides and polyols present in the plant tissues could act as reducing agents in the reduction of the target metal precursor (Begum et al., 2009; Huang et al., 2007). In this case, the extract of *Lycoris aurea* leaf acts as the reducing agent in the reduction of chloroauric acid. The presence of these reducing agents can be confirmed using differential pulse voltammetry since they can be easily oxidized in a low potential range (figure 1) (Fu et al., 2019).

The morphology of the biosynthesized AuNPs was characterized using TEM. As shown in figure 2A, the TEM image clearly shows the formation of spherical nanoparticles. The boundary of each particle was very evident under high-resolution characterization (figure 2B), suggesting that the formed AuNPs did not aggregate (Zheng et al., 2017). The particle size distribution of the biosynthesized AuNPs is shown in figure 2C. The average particle size of the formed AuNPs is 24.1 nm.

UV-vis spectroscopy was used to confirm the formation of AuNPs. As shown in figure 3, the spectrum shows a very broad peak located at approximately 560 nm corresponding to Au plasmon resonance, which successfully confirmed the formation of AuNPs (Jayaseelan et al., 2013; Kamyar et al., 2012).

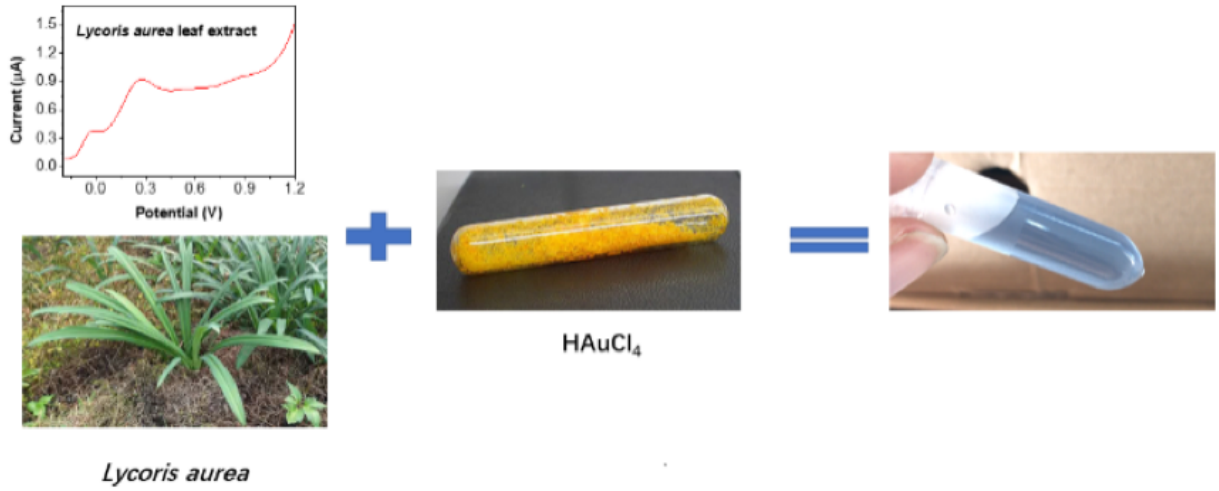


Fig. 1. Scheme of biosynthesis of AuNPs using *Lycoris aurea* leaf extract.

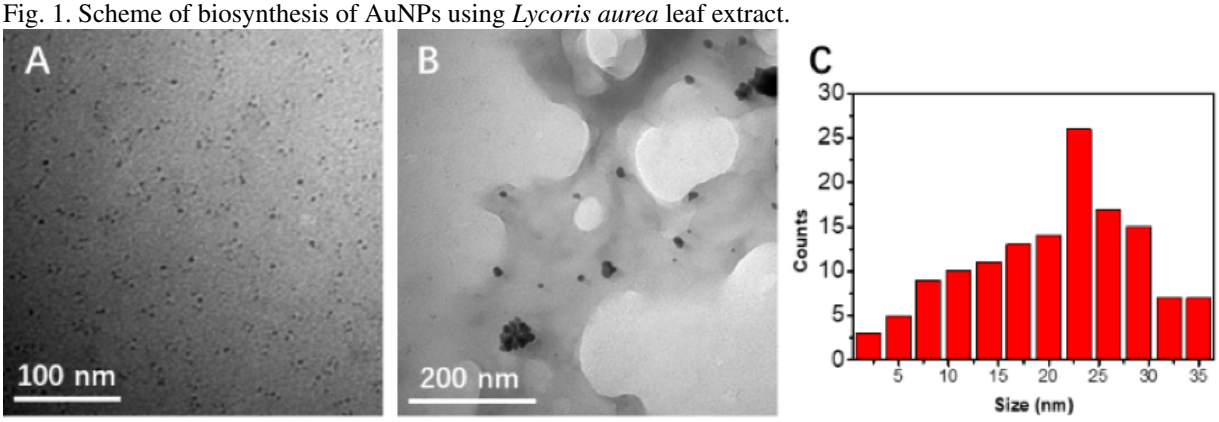


Fig. 2. SEM images of biosynthesized AuNPs at (A) low resolution and (B) high resolution. (C) Particle size distribution of biosynthesized AuNPs.

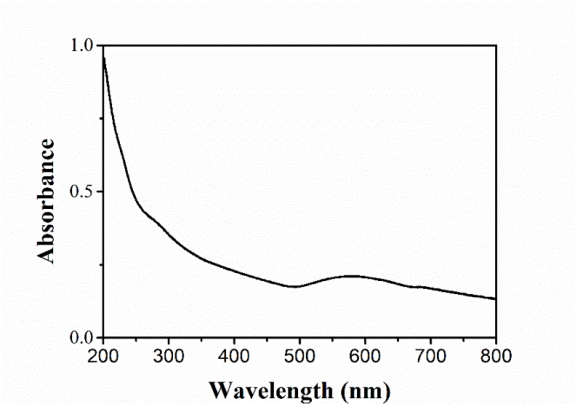


Fig. 3. UV-vis spectrum of biosynthesized AuNPs.

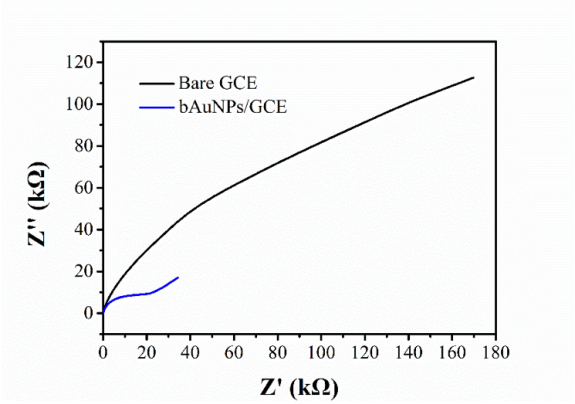


Fig. 4. Nyquist plots of GCE and bAuNPs/GCE in 5 mM $[Fe(CN)_6]^{3-/4-}$.

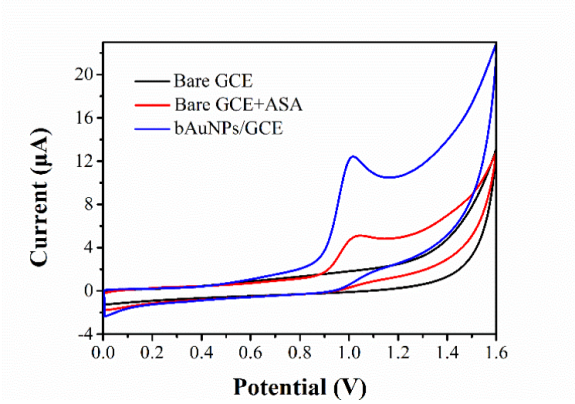


Fig. 5. Cyclic voltammetry of bare GCE and bAuNPs/GCE responding to 1 mM aspirin.

Electrochemical impedance spectroscopy (EIS) is an effective technique for characterizing the internal properties of an electrode before and after modification with biosynthesized AuNPs. Figure 4 shows Nyquist plots of the bare GCE and the bAuNPs/GCE. The plot of the bare GCE shows a larger semicircle, while the bAuNPs/GCE plot contains a small semicircle. The semi-circular part of the bAuNPs/GCE plot represents the electron transfer restriction, while the straight line is due to the diffusion limit. The presence of a small semicircle indicates that the charge transfer resistance between the biosynthesized AuNPs and the electrochemical probe was decreased. Therefore, the modification of the surface of a GCE with biosynthesized AuNPs can promote the electron transfer rate and increase the sensitivity of the electrode (Kamyar et al., 2012).

The electrochemical behaviours of the bare GCE and bAuNPs/GCE toward aspirin were studied using cyclic voltammetry (CV). Figure 5 shows the CV curves of the bare GCE and the bAuNPs/GCE toward the electro-oxidation of 1 mM aspirin. The bare GCE exhibits a clear oxidation peak at 1.05 V with a current of 3.97 $\mu$A. In contrast, the bAuNPs/GCE exhibits an oxidation peak at a similar potential but with a significant current enhancement (13.11 $\mu$A). This enhancement can be ascribed to the improvement in conductivity improvement after modification with biosynthesized AuNPs as well as the high affinity between the AuNPs and the aspirin molecules (Fu et al., 2015).

The surface kinetics of aspirin oxidation at the bAuNPs/GCE surface was studied by scan rate. Figure 6 shows the CV curves of the bAuNPs/GCE towards the electro-oxidation of aspirin at scan rates ranging from 20 mV/s to 200 mV/s. The oxidation peak current increased as the scan rate increases. The inset of figure shows that the peak current is proportional to the scan rate suggesting that the surface reaction is controlled by adsorption (Asadian et al., 2017; Qiu et al., 2016). The linear regression equation is I=32.6281v (V/s)+0.9564 ($R^2$=0.9989).

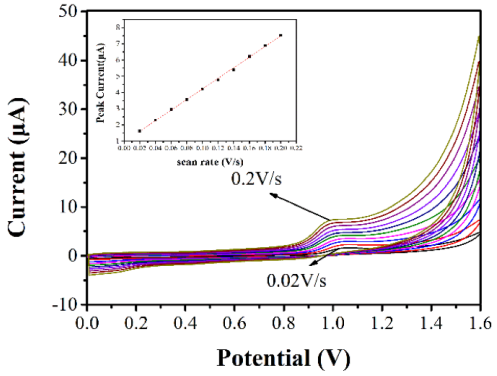


Fig. 6. Cyclic voltammetry of bAuNPs/GCE responding to aspirin from 20 mV/s to 200 mV/s.

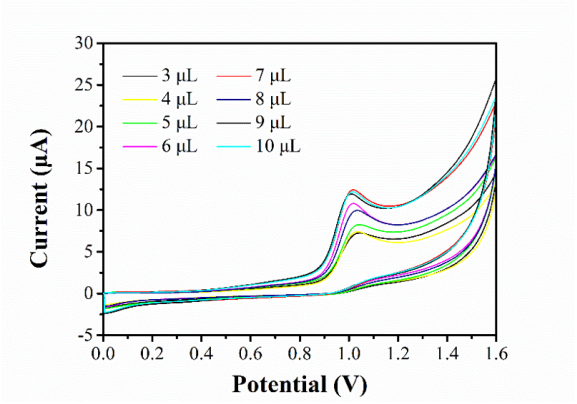


Fig. 7. Influence of amount of modifier for electrochemical oxidation of aspirin.

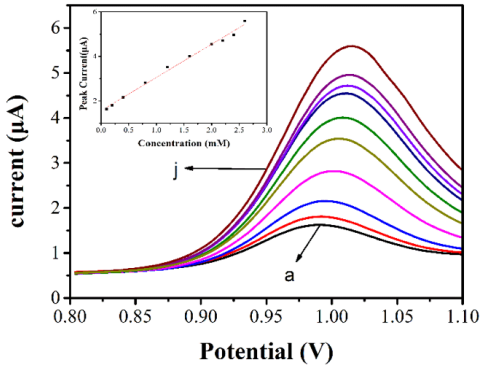


Fig. 8. Differential pulse voltammograms of bAuNPs/GCE towards aspirin in 0.1 M PBS (pH 7). (a to j: 0.1 mM, 0.2 mM, 0.4 mM, 0.8 mM, 1.2 mM, 1.6 mM, 2.0 mM, 2.2 mM, 2.4 mM, 2.6 mM). Inset: Plots of aspirin concentrations against peak currents.

The amount of biosynthesized AuNPs used for GCE surface modification was optimized. As shown in figure 7, 7 $\mu$L of modifier resulted in the highest oxidation peak current. Therefore, 7 $\mu$L of the AuNP dispersion was selected for GCE surface modification. DPV was used to study the linear detection range and feasibility of the bAuNPs/GCE due to the high sensitivity of the method. Figure 8 shows the DPV curves of the bAuNPs/GCE towards aspirin concentrations ranging from 100 $\mu$M to 2.6 mM in 0.1 M phosphate buffer solution (PBS). The anodic peak responses of aspirin were proportional to concentration. As shown in the inset of figure 8, the bAuNPs/GCE showed a linear detection range from 100 $\mu$M to 2.6 mM with a linear regression equation of I=1.4938C (mM)+1.5597 ($R^2$=0.9924). The limit of detection was 11.3 $\mu$M based on a signal-to-noise ratio of 3.

To test for reproducibility, five independent bAuNPs/GCEs were used to measure aspirin. As shown in figure 9A, the five independent bAuNPs/GCEs had very similar detection results. A relative standard deviation (RSD) of 7.4% was observed, suggesting that the proposed biosynthesized AuNPs could result in a reliable reproducibility. Based on the CV characterization, the oxidation of aspirin is an irreversible reaction process. The oxidized product is likely adsorbed on the GCE surface, which results in a poisoning effect. Therefore, degradation can be observed when a bAuNPs/GCE is used for successive detection. As shown in figure 9B, by the 10th detection, half the oxidation current response remains relative to the original detection. Due to the low cost and fast assembly process, the proposed electrochemical showed potential for field application.

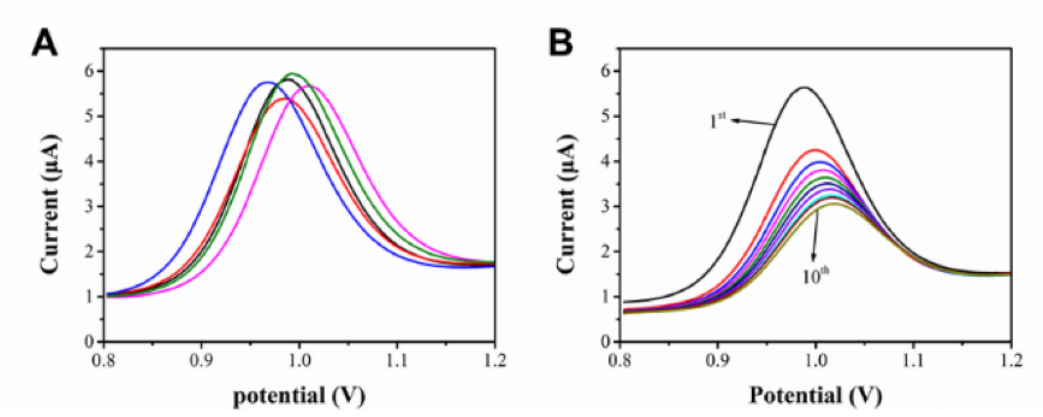


Fig. 9. (A) Five individual bAuNPs/GCE for aspirin detection. (B) A bAuNPs/GCE for ten successive aspirin detection.

## Conclusions

In conclusion, AuNPs were successfully synthesized via a green *Lycoris aurea* leaf extract-assisted bio-reduction technique. The biosynthesized AuNPs were characterized by TEM and UV-vis spectroscopy. A uniform size of AuNPs were formed using the proposed approach. Due to the high electrocatalytica property of the AuNPs, the biosynthesized AuNPs were then used for the modification of GCE surfaces, and the electrocatalytic performance for the detection of aspirin was studied. The surface mdofication of the GCE with AuNPs could significantly enhance the sensing performance. The results show that the biosynthesized AuNP-modified GCE displays a wide linear range of detection (2.6 mM to 100 $\mu$M) and a low detection limit for the electrochemical sensing of aspirin.

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