**Edible gold leaf as a viable modification method for screen-printed sensors**

*Jéssica R. Camargo1,2,4, Sabrina Cleto1, Amanda Neumann1,* *Déborah C. Azzi3, Robert D. Crapnell4, Craig E. Banks4, Bruno C. Janegitz1\**

*1Laboratory of Sensors, Nanomedicine and Nanostructured Materials, Federal University of São Carlos, Araras,13600-970, Brazil*

*2 Department of Physics, Chemistry, and Mathematics, Federal University of São Carlos, Sorocaba, São Paulo, Brazil, 18052-780*

*3 ADB Pesquisa e Desenvolvimento, Araras 13600-140, SP, Brazil*

*4Faculty of Science & Engineering, Manchester Metropolitan University, John Dalton Building, Chester Street, Manchester M1 5GD, UK*

\*Corresponding authors: brunocj@ufscar.br (Bruno Campos Janegitz) and jessica.10.camargo@gmail.com (Jéssica Rocha Camargo)

**Abstract**

The demand for low-cost, efficient, selective, and sensitive analytical methods is increasing globally, making electroanalysis combined with screen-printed electrodes an appealing option. Gold, known for its excellent electrical conductivity and biocompatibility, improves the electrocatalytic activity of the sensors and reduces interference with other substances. Here, we propose the modification of disposable sensors with edible gold leaf, which is a cost-effective alternative to sputtering. The gold leaf was immobilized on the screen-printed electrode surface with chitosan. L-tyrosine, an amino acid, and an important biomarker for the diagnosis of various diseases was detected as a proof-of-concept. Using square-wave voltammetry for the determination of L-tyrosine, the disposable sensor modified with gold leaf showed a linear response in the range between 0.10 to 70 μmol Lwith a limit of detection of 0.09 μmol L. Overall, the use of gold leaf to modify disposable electrochemical sensors for L-tyrosine detection represents a promising approach for electrochemical sensing.

**Keywords:** Gold leaf; conductive ink; screen-printed electrodes; L-tyrosine; electrochemistry

1. **Introduction**

Currently, the search for analytical methods that pollute less, produce easily, and are more accurate is growing at the same pace as scientific progress [1]. In this context, electrochemical sensors have emerged as indispensable instruments for everyday living, performing analysis in various industrial fields with greater efficiency [2, 3]. In recent years, the use of many classical bulky electrodes has been abandoned, making way for small and inexpensive electrodes with enhanced flexibility and easy-to-use systems [4-6]. This makes disposable electrodes an attractive alternative, as their single-use nature overcomes problems with poisoning or adsorption on the electrode surface [7, 8].

Gold is an exceptional electrical conductor and has excellent biocompatibility. Its use as a surface coating on electrochemical sensors can improve the electrocatalytic activity of the electrode and reduce interference from other substances [9-11]. The gold surface can also prevent the oxidation of the conductive graphite ink, which can affect the performance of the sensor [12, 13]. Modification of disposable electrochemical sensors with gold involves applying a thin layer of gold to the surface of the sensor [14] which can be achieved using a sputtering technique, in which gold atoms are ionized and deposited on the sensor surface using an electric field [15, 16]. However, this modification has a high cost due to the need for the metallizer. Gold leaf is a thin layer of gold that is commonly used for decorative and food purposes [17], which can also be used as a method to modify disposable electrochemical sensors [14]. In the literature, there are some works proposed using gold leaves, such as Li et al. [18] and Zhang et al. [19] that developed glassy carbon electrodes modified with these materials.

L-tyrosine is an amino acid used as a nutritional supplement [20] and it can be found in many proteins, and its detection is important for the diagnosis of various diseases [21]. In the body, it has a primary function in the brain as it is involved in the synthesis of dopamine, which is responsible for mood, sleep, and attention [22]. Typically, the concentration of Tyr in healthy individuals is in the range of 30 to 120 μmol L−1; however, in the biological fluids of patients with tyrosine metabolism disorders, it may surpass 200 μmol L−1. In urine, the acceptable level is 11 to 135 μmol per gram of creatinine [23]. In this context, the use of disposable electrochemical sensors allows the easy and rapid detection of L-tyrosine, which may be important in the clinical setting. Also, the use of gold leaf as a method to modify disposable electrochemical sensors for L-tyrosine detection offers a promising approach for the diagnosis of various diseases. Thus, the focus of this work is to demonstrate the simplicity and functionality of the preparation of the edible gold leaf-modified screen-printed electrodes and the application of this system in the electrochemical detection of L-tyrosine.

**2. Experimental**

*2.1 Reagents and solutions*

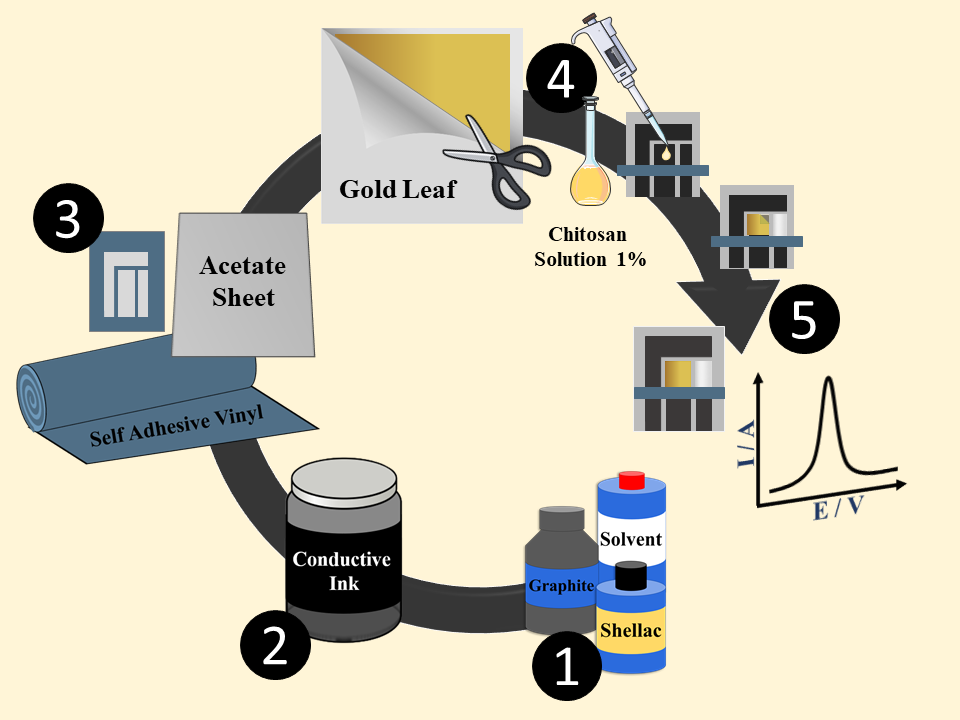
All reagents utilized in this work were of analytical grade, from Sigma- Aldrich®, USA, Synth, or Dinamica Química. Ultrapure water (Milli Q, USA) with a resistivity <18 MΩ cm was employed to prepare all aqueous solutions. The electrochemical characterization of the electrodes was conducted using a solution containing 1.0 mmol L−1 Ferrocenemethanol (≥97% w/w) and 0.1 mol L−1 KCl. Phosphate buffered saline (PBS) with a pH of 7.0 was used as the supporting electrolyte for L-tyrosine detection. Synthetic urine was prepared according to the literature with adaptations [24], which consisted of the following components: 90.0 mmol L−1 sodium chloride (99% w/w) from Synth, 170.0 mmol L−1 urea (99% w/w), 2.5 mmol L−1 calcium chloride dihydrate (≥99% w/w), 10.0 mmol L−1 anhydrous sodium sulfate (99% w/w), 0.4 mmol L−1 uric acid (99% w/w), and 7.0 mmol L−1 creatinine (≥98.5% w/w) from Sigma-Aldrich®, USA, and 2.0 mmol L−1 citric acid (99.5% w/w) and 25.0 mmol L−1 ammonium chloride (99.5% w/w) from Dinamica Química (Indaiatuba, Brazil). (Diadema, Brazil). The determination of L-tyrosine was performed in the tap water of Araras City (São Paulo State) (−22.315206, −47.380840).

*2.3 Apparatus and electrochemical measurements*

The sensor surface was characterized by scanning electron microscopy (SEM) using a Thermo Fisher Scientific Prisma. The conductive ink was blended using a SpeedMixer™ Dac 150.1 FVZ-K (FlackTec Inc., Landrum, USA), which is a double asymmetric centrifuge. All electrochemical measurements were performed with an AutoLab PGSTAT204 potentiostat/galvanostat managed by NOVA 2.1.5 software (Metrohm, Netherlands). Cyclic voltammetry (CV) measurements were performed from 0.2 to + 1.2 V, at a scan rate of 50 mV s−1 and square wave voltammograms (SWV) measurements (modulation amplitude 60 mV; Frequency 35 Hz; step potential: 5.0 mV) were used for L-tyrosine quantification. To evaluate the optimization parameters for SWV (Square Wave Voltammetry), a DoE (Design of Experiment) approach was used with Statistica 64 software from Statsoft (Dell, Tulsa, USA). The DoE aimed to identify the factors influencing the electrochemical measurement and to determine the optimal values of these factors that would give the desired result within the given parameters.

*2.4 Manufacture of the disposable electrodes modified with gold leaf*

The conductive ink was prepared according to the literature [25] by combining shellac (SH, 2.0 g), solvent for polyurethane (4.0 g), and graphite (GP, 2.0 g) as shown in step 1 (Figure 1). To ensure complete mixing, the reagents were mixed using the SpeedMixer™. The mixing procedure comprised three cycles, each running at 3500 rpm for 180 seconds (step 2). Self-adhesive vinyl was cut out with a precision printer to produce the masks. The design of these masks was created using Silhouette Studio software. Acetate sheets (AS) were used as the backing and sanded in one direction with 220 grit sandpaper (3M) to improve ink application. The sheet was then cleaned with 70% alcohol to remove all residue and the masks were glued on. A sufficient amount of ink was applied to the masks with the aid of a spatula. Immediately after the ink was applied the masks were quickly removed before the ink dried (step 3). The ink was allowed to dry for 120 minutes, after which the devices were cut out individually. Using a nail dotting pen, the silver ink (Electron Microscopy Sciences) was applied to the pseudo reference electrode, creating a system with working (0.25 cm2), auxiliary, and reference electrodes. A gold leaf (Au) was cut into a square 5 x 5 mm using scissors. A 1% chitosan solution was prepared using a 2% acetic acid solution. An amount of 10 μL of this solution was used to glue the edible gold leaves into the surface of the working electrode, named Au-SH-GP/AS. The electrodes were dried at 50°C for at least 2 hours until the chitosan solution was completely dry and the electrodes were ready for use (step 5). The entire process was carried out at room temperature (25 °C).

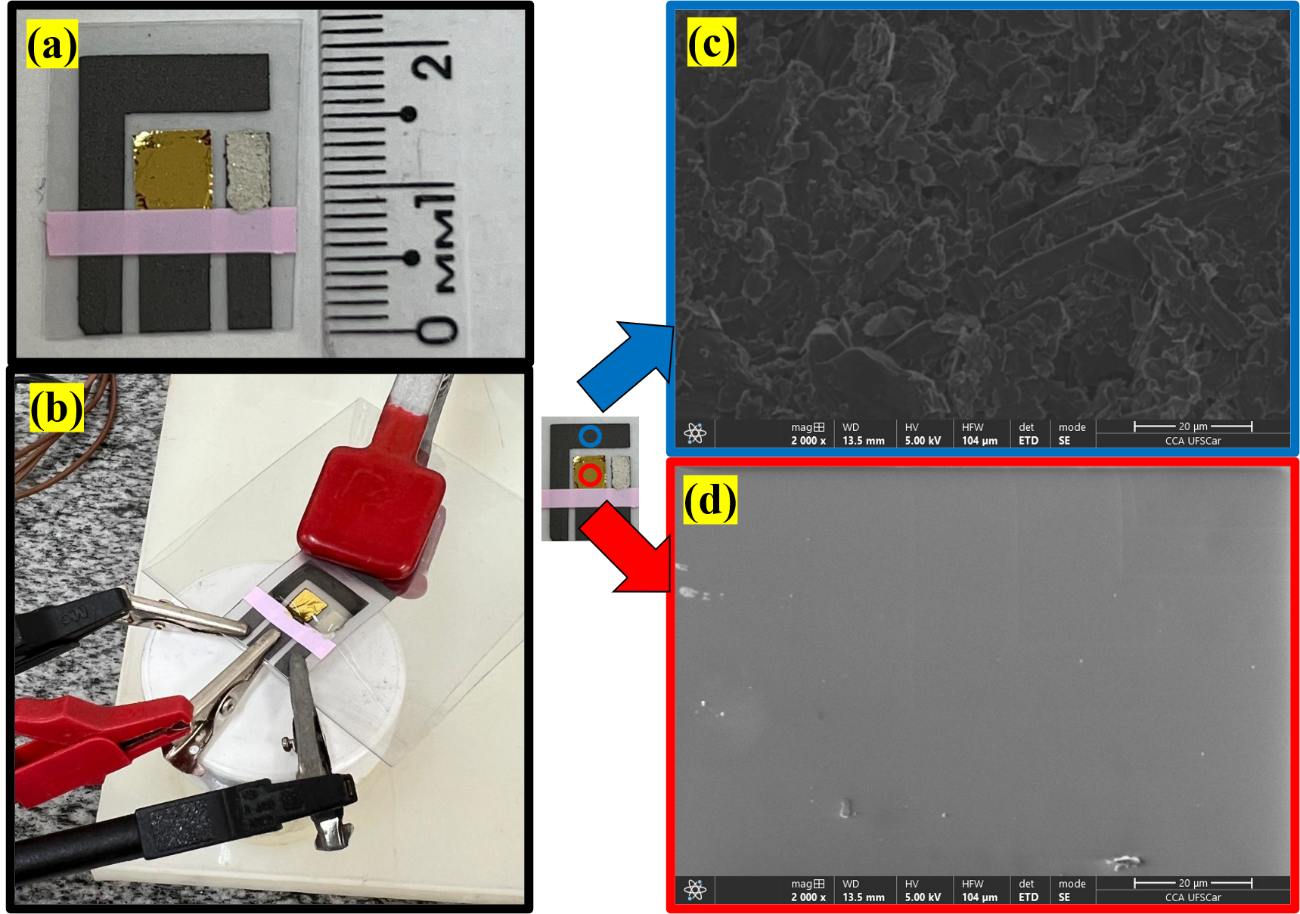


**Figure 1**. Illustration of the manufacturing process of the gold leaf SPE electrodes. The ink was prepared using a mixture of shellac, solvent for PU, and graphite (step 1). The SpeedMixer® was used to make the conductive ink (step 2). The acetate sheet was sanded and washed, and a self-adhesive vinyl cut with the Silhouette machine adhered to the surface. The ink was spread on the surface with a spatula and allowed to dry at room temperature for 2 hours (Step 3). The edible gold leaves were cut into 5 mm2 and glued to the surface of the working electrode with 1% chitosan solution (w/v) (step 4). The electrodes were dried at 50°C for at least 2 hours until the chitosan solution was completely dry and the electrodes Au-SH-GP/AS were ready for use (step 5).

**3. Results and discussion**

**3.1. Morphological characterization of the Au-SH-GP/AS**

Figure 2a shows the image of the electrode Au-SH-GP/AS with a total area of 2 cm × 1.5 cm and an area of 0.25 cm for the working electrode. Figure 2b shows the electrode connected to the potentiostat, onto the surface of which an amount of 150 L of the sample was dropped. The SEM images of Au- SH-GP /AS show the area of the working electrode modified with the gold leaf (in red) and the carbon ink (surface of the counter electrode, in blue). The image (Figure 2c) shows the graphite ink distributed over the entire surface with parallel layers [26], while Figure 2d shows the surface modified with the gold sheet, at 2000x magnification.

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**Figure 2. (a)** Photograph of the Au-SH-GP/AS electrode; **(b)** the proposed electrode connected to the potentiostat with sample dropped on the surface; SEM images obtained for Au-NP-GP/AS at 2000x magnification of **(c)** the carbon ink (counter electrode region surface) and (d) gold leaf (working electrode region surface);

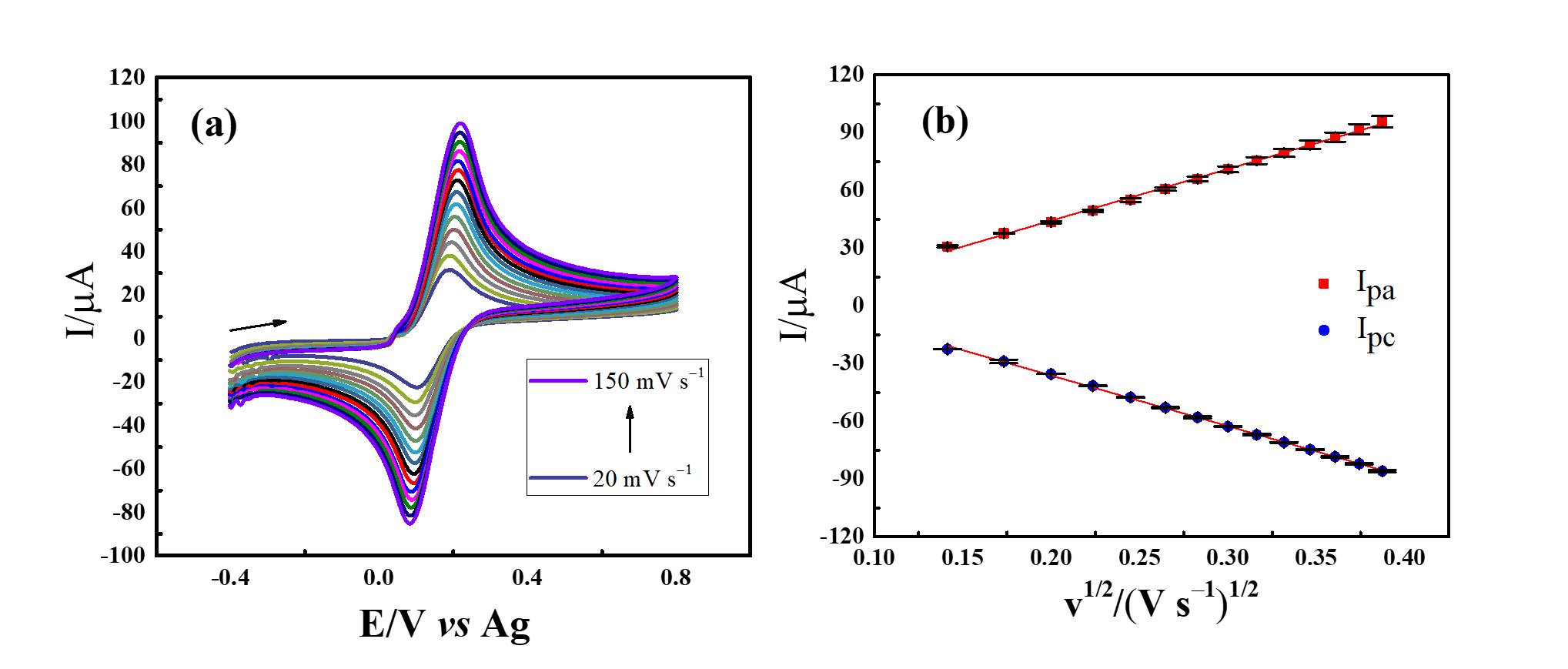
**3.2. Electrochemical characterization of the Au-SH-GP/AS**

The Au-SH-GP/AS electrode and SH-GP/AS were compared by cyclic voltammetric analysis in the presence of 1.0 mmol L ferrocenemethanol / 0.1 mol L KCl. Cyclic voltammograms (Figure 3) were obtained at a scan rate of 50 mV s and showed anodic and cathodic peaks at +20.0 and +0.09 mV for Au-SH-GP/AS and +26.0 and +19.6 mV for SH-GP/AS, respectively (*vs.* a silver ink reference electrode). The proposed sensor modified with the gold leaf showed a ΔEp of about 102 mV and anodic and cathodic peaks with current at 49.1 μA and 41.7 μA, respectively. The unmodified sensor shows anodic and cathodic peaks current at 6.7 μA and -6.0 μA, with a ΔEp of about 70 mV. This confirms that the modification of the gold increased the current by about 12 times.



**Figure 3.** Cyclic voltammograms obtained with Au-SH-GP/AS (pink line) and SH-GP/AS (blue line) electrodes in 0.1 mol LKCl in the presence of 1.0 mmol Lferrocenemethanol. Scan rate = 50 mV s

Cyclic voltammetry measurements were performed with an electrochemical probe at various scan rates from 20 to 150 mV s20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140 and 150 mV ssee Figure 4a) to evaluate the electron transfer behavior in the proposed electrode. As observed in Figure 4b, we observe an increase in both anodic peak current (Ipa) (R2 = 0.9977) and cathodic peak current (Ipc) showing a linear correlation (R2 = 0.9990) with the square root of the scan rate (*v*1/2), indicating a diffusion-controlled transport process. Furthermore, the Randles-Ševčík equation (1) [27] was used to determine the electroactive area of the Au-SH-GP/AS electrode and SH-GP/AS electrode. The result was a value of 0.36 cm2  and 0.07 cm2, in this way, the modified electrode obtained about 5 times an increase of electroactive area after modification of gold. Using the equation (%Real = (Areal/Ageo) × 100) [28], the real area of the Au-SH-GP/AS electrode was found to be %Real = 144% or 1.44 times the geometric area. The durability of the sensor was tested by CV using Au-SH-GP/AS prepared on the same day and 1 year ago in 0.1 mol L−1 KCl in the presence of 1.0 mmol L−1 ferrocenemethanol at a scan rate of 50 mV s−1. The ΔE was 133.7 mV for the sensor prepared on the same day and 158.3 mV for the sensor prepared 1 year ago, resulting in an increase of 18 % between measurements. The anodic current was 47.4 mV and the cathodic current was −39.0 mV for the sensor manufactured on the same day. An anodic current of 51.3 mV and a cathodic current of −45.3 mV were determined for the sensor manufactured 1 year ago, which represents an increase of 7.61 % for the anodic current and 13.9 % for the cathodic current after 1 year. The voltammograms of this study are shown in Figure S1. Also, 50 measurements using a unique sensor were performed in the same conditions as the aforementioned study. It has been observed that the analytical signal is still maintained without a noticeable change. This indicates the robustness of the system, allowing for multiple measurements to be performed in the same drop without compromising the signal quality is detailed in the supporting information in Figure S2.



**Figure 4.** (a) Cyclic voltammograms obtained in the presence of equimolar 1.0 mmol L Ferrocenemethanol in 0.1 mol L KCl solution, varying the scan rate from 20 to 150 mV s20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140 and 150 mV s. (b) The plot of current response *vs.* *v*1/2.

**3.3. Determination of L-tyrosine**

Cyclic voltammetric measurements in the absence and presence of 100 μmol L−1 L-tyrosine from −0.2 and 1.2 V, with a scan rate of 50 mV s−1 were obtained in 0.10 mol L−1 phosphate buffer solution (pH 7.0) (Figure 5a). The Au-SH-GP/AS electrode showed an irreversible redox process, with an oxidation peak at 0.87 V, attributed to the oxidation of L-tyrosine to oxidized L-tyrosine [29]. To compare, the SH-GP/AS electrode was used without modification for the L-tyrosine detection. We can observe that the sensor modified with gold leaf increases the performance of the system. The oxidation of L-tyrosine [30] is also reported in the literature, with a one-electron and one-proton process as shown in Figure 5b.



**Figure 5.** Cyclic voltammograms obtained with Au-NP-GP/AS and NP-GP/AS electrodes in 0.1 mol L PBS (pH 7.0) without and in the presence of 1.0 x 10  mol L L-tyrosine. Scan rate = 50 mV s(b) the electrochemical mechanism of L-tyrosine oxidation;

Square wave voltammetry (SWV) was used for L-tyrosine detection. In this sense, the optimization of SWV parameters (modulation amplitude and frequency) was performed to ensure the best electrochemical response by using the design of experiments. The pH study was conducted using PBS at different pH values of 6.0; 7.0; 8.0; 9.0 and 10.0 with 70 μmol L−1 L-tyrosine, with pH 7 performing best. This study can be found in Figure S3. For this purpose, a response curve was established to determine the optimal values of the tested factors. This study was performed with a solution containing 50 μmol L tyrosine. The experimental design used can be found in Table S1. Figures S4a and S4b show higher current values are observed in the violet region, among the evaluated values that were performed, the higher ones were obtained at 60 mV and 35 Hz modulation amplitude and frequency values, respectively. Such values were selected as the optimal conditions for further studies. The step was maintained at 5 mV in all analyses.

A calibration plot was obtained under optimized conditions to assess different concentrations of L-tyrosine (Figure 6a), ranging from 0.10 to 70 μmol L−1 (0.1, 5.0, 8.0, 20, 30, 50, and 70 μmol L−1), and a linear correlation between concentration and peak current was observed (Figure 6b). The obtained equation was: Ip (μA) = −2.163 × 10 + 0.008 CL-tyrosine (μmol L−1), with an R2 value of 0.990. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated by three and ten times the standard deviation of the intercept divided by the slope of the calibration plot, respectively [31]. The LOD value was found to be 0.013 μmol L−1 and the LOQ value was found to be 0.044 μmol L−1.



**Figure 6.** (a) SWV voltammograms recorded at different L-tyrosine concentrations 0.1, 5.0, 8.0, 20, 30, 50, and 70 μmol L−1 in 0.1 mol L−1 PBS (pH 7.0); step = 5 mV modulation amplitude: 60 mV and frequency: 35 Hz; (b) Calibration plot for L-tyrosine (Ip *vs*. CL-tyrosine).

To evaluate the accuracy of the sensor, repeatability (n=5) and reproducibility (n=5) tests were performed (Figure S5), which yielded RSD values of 0.52 % and 3.64 %, respectively, which highlights the excellent performance of the gold leaf-modified electrodes. The feasibility of using the Au-NP-GP/AS electrodes as an L-tyrosine sensor was investigated using synthetic urine and tap water samples. After enriching the samples with four different L-tyrosine concentrations (5.0, 7.0, 20, and 30 μmol L−1), recovery tests were performed, and the results are shown in Table 1. To investigate interferences, SWV measurements were performed at a concentration of 20 μmol L−1 in the presence of competing species, such as ascorbic acid, urea, and uric acid at the same concentration. As shown in Figure S6, the investigated species showed a higher signal deviation of 2.3 %, indicating that these species do not cause significant interference.

**Table 1.** Recovery values (n = 3) for L-tyrosine additions in urine and tap water samples.

|  |  |  |  |
| --- | --- | --- | --- |
| **Sample (n=3)** | **Added (mol L−1)** | **Found (mol L−1)** | **Recovery (%)** |
| Tap Water | 5.0 | 5.62 ± 0.07 | 112.4 ± 7.06 |
| 7.0 | 6.75 ± 0.22 | 96.5 ± 2.79 |
| Synthetic urine | 5.0 | 5.33 ± 0.06 | 106.7 ± 5.56 |
| 30.0 | 28.8 ± 0.04 | 95.9 ± 2.58 |
| 20.0 | 19.2 ± 0.11 | 96.2 ± 4.00 |

The analytical performance of Au- NP-GP /AS electrodes was compared with other electrochemical techniques commonly used for L- tyrosine detection (see Table 2). For example, Zribi et al [32] developed a system based on the enhanced catalytic activity of two-dimensional molybdenum disulfide nanosheets (2D-MoS2) over SPE gold electrodes. The authors obtained a wide linear range from 10 to 500 µmol L, with an extensive synthesis process that requires many reagents and at least 45 min to prepare only the 2D-MoS2. Madrakian et al. [33] and Li et al. [34] used GCE and multi-walled carbon nanotubes to develop sensors for tyrosine, and this kind of system always requires some steps such as polishing with alumina and activation in acid besides modification with carbon. This makes the sensor fabrication process more time-consuming and expensive. Rahimi-Mohseni et al. [35] develop a nano-biosensor using the co-catalytic action of tyrosinase from banana peel tissue to detect L-tyrosine. This work demonstrates a high-performance enzymatic biosensor with a linear range of 0.05 to 600 µmol L and a low LOD of 0.02 µmol L. However, more than five steps are required to construct the electrode and the SPE used is a commercial one, which makes the fabrication of the sensor more expensive. About the gold electrodes employed in the detection of L-tyrosine, the device developed by Ermiş et al. [36] is a molecularly imprınted polypyrrole-modified gold electrode for the determination of tyrosine in plasma samples. Quintana et al. [37] develop nanostructures on gold electrodes based on host–guest supramolecular interactions to detect l- tyrosine in pharmaceutical formulations. Indeed, our proposed sensor, which is fabricated in the laboratory with a simple modification that does not require chemical treatments or multiple surface modification steps, provides efficient detection of L-tyrosine being inexpensive and easy to fabricate.

**Table 2.** Analytical performance of Au-NP-GP/AS sensor for L-tyrosine determination compared with the literature

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Electrode** | **Technique** | **LDR (μmol L1)** | **LOD**  **(μmol L1)** | **Sample Application** | **Refs.** |
| 2D-MoS2-AuBSPE | CV | 10 to 500 | 0.5 | Commercial food integrator | [32] |
| AuNPs/MWCNT/GCE | DPV | 0.4 to 80.0 | 0.21 | Blood serum and pharmaceutical | [33] |
| MWCNTs-Nafion/GCE | DPV | 2 to 120 | 0.8 | Human serum | [34] |
| B.P.Tyr/M/SN-MPTS/SPE | DPV | 0.05 to 600 | 0.02 | Human plasma | [35] |
| β-CD modified gold electrode | DPV | 36 to 240 | 12 | Pharmaceutical formulations | [37] |
| MIP-polypyrrole modified gold electrode | SWV | 0.005 to 0.025 | 0.0025 | Human plasma | [36] |
| **Au-NP-GP/AS** | **SWV** | **0.1 to 70** | **0.09** | **Tap water and synthetic urine** | **This Work** |

**LDR**: Linear dynamic range; **2D-MoS2-AuBSPE:** Exfoliated 2D-MoS2 nanosheets on carbon and gold screen printed electrodes; **Pt–ZnO/CNTs/GCE:** Glassy Carbon Electrode Amplified with a nanocomposite of zinc oxide-Platinum/carbon nanotubes; **AuNPs/MWCNT/GCE:** gold nanoparticles/multiwalled carbon nanotube nanocomposite modified glassy carbon electrode; **MWCNTs-Nafion/GCE:** processed MWCNTs and Nafion on a glassy carbon electrode; **B.P.Tyr/M/SN-MPTS/SPE:** graphite screen printed electrode modified with a paper disc doped with 3-mercaptopropyl trimethoxy silane-functionalized silica nanoparticles and banana peel tissue; **β-CD modified gold electrode:** β-cyclodextrins on a gold electrode; **MIP-polypyrrole modified gold electrode**: molecularly imprinted polypyrrole modified gold electrode;

**4. Conclusion**

In summary, the demand for cleaner, more efficient, and more accurate analytical methods for analysis has driven scientific progress and led to the development of alternative electrode materials. The move toward small, inexpensive, and flexible electrodes has solved the problems of surface adsorption and poisoning and made them a viable option. Edible gold leaves, with their excellent electrical conductivity and biocompatibility, have emerged as a beneficial material that enhances electrocatalytic activity and minimizes interference with other substances. The modification of disposable sensors with gold leaf, which is cost-effective compared to sputtering, offers a promising solution. The application of square-wave voltammetry to disposable sensors modified with gold leaf has shown a linear response in the range of 0.10 to 70 μmol L with a remarkably low detection limit of 0.09 μmol L. This approach shows great potential for accurate detection of L-tyrosine and paves the way for improved diagnosis of various diseases.

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**Credit authorship contribution statement**

Jéssica R. Camargo: Investigation, Conceptualization, Validation, Data curation, Writing - review & editing. Sabrina Cleto: Investigation, Methodology, Data curation, Writing - original draft. Amanda Neumann: Investigation, Conceptualization, Validation, Data curation, Writing - review & editing. Déborah C. Azzi: Investigation, Conceptualization, Validation, Data curation, Writing - review & editing. Robert D*.* Crapnell: Conceptualization, Validation, Data curation, Resources, Writing - review & editing. Craig E. Banks: Conceptualization, Validation, Data curation, Resources, Writing - review & editing. Bruno C. Janegitz: Conceptualization, Visualization, Supervision, Project administration, Resources, Funding acquisition, Writing - review & editing.

**Competition of interest**

The authors declare that they have no competition of interest.

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