
# Detecting Epinephrine with LIG/MIP Electrochemical Sensors

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## Introduction

Epinephrine, secreted by the adrenal glands, functions as a neurotransmitter that regulates physical and mental performance. It induces physiological responses such as increased heart rate, blood flow redistribution, airway dilation, and the stimulation of blood glucose release for energy provision[1], thereby facilitating a rapid systemic response and enhancing the capacity for swift reactions associated with alertness, strength, and agility[2].

The secretion of catecholamines and cortisol in animals such as cattle is profoundly affected by nutrient availability. Food scarcity or prolonged fasting is perceived as a threat, triggering the release of these hormones. Whereas catecholamines act rapidly to enhance vigilance and mobilize stored energy, cortisol acts over a more prolonged period, catabolizing muscle and adipose tissue to maintain the energy homeostasis essential for survival under nutritional challenges.

Under normal conditions, the concentration of epinephrine in blood plasma is 0.1746 nmol/L[3]. Conventional analytical methods for determining these hormone levels are invasive to the human body, financially costly, and entail long turnaround times for results[4]. The primary techniques utilized include the surgical implantation of brain electrodes, blood plasma analysis, and liquid chromatography of blood plasma[5]. An alternative for EP monitoring would be the application of low-cost electrochemical or biomimetic sensor chips capable of performing rapid analyses. However, hormone concentrations vary across different human fluids[6].

In this work, low-cost, non-enzymatic electrochemical and biomimetic sensor chips were developed to perform rapid analyses for EP detection in a controlled PBS environment. The sensors were fabricated using diode laser-induced graphene modified with molecularly imprinted polymers (MIP-Epinephrine) via drop-casting, utilizing cyclic voltammetry (CV) technique to obtain the sensor's analytical response.

**Materials and Methods**

In the sensor fabrication process, three-electrode chips were developed, comprising a reference electrode, a working electrode, and a counter electrode. The electrode geometry was designed using Inkscape vector graphics software. To fabricate the chip electrodes, a double layer of polyimide polymeric film adhered to a polyethylene (PE) plate was subjected to direct laser engraving[7] using an XTool M1 Ultra diode laser engraver. The laser carbonizes the tape at high temperatures (2500 °C) with a scanning speed of 100 mm/s and a power of 3.6 W. The tape is initially carbonized to pattern the entire sensor, as depicted in Figure 1. Subsequently, LIG modification was performed via drop-casting onto the surface of the working electrode, and finally, the modified LIG was re-pyrolyzed following the methodology described by Gonçalves (2026)[8].

**Figure 1.** Model of chip electrochemical sensor LIG.

For the preparation of the molecularly imprinted polymers, 0.02 mmol of the analyte (epinephrine hydrochloride) is dissolved in 50 mL of water. Then, 84 µL of acrylic acid, acting as the functional monomer, is added to the solution, which is left under stirring for 4 hours. Subsequently, the solution is heated to 80 °C, followed by the addition of 380.97 µL of ethylene glycol dimethacrylate (EGDMA) and 0.27 g of potassium persulfate (KPS) as the reaction initiator. The mixture is then stirred for one hour until the MIP precipitates[9,10].

Following the precipitation process, the solution is placed in an oven at 80 °C until the complete evaporation of the water, and the resulting powder is sieved to 50 mesh. Subsequently, the MIP particles undergo three washing steps to extract the analyte from the polymeric matrix. First, the powder is stirred for two hours in a solution of 30 mL of methyl alcohol and 70 mL of water. The process is then repeated using a solution of 30 mL of ethyl alcohol and 70 mL of water, and finally, a third wash is performed in 100 mL of pure water. After the washing steps, the MIP is dispersed in isopropyl alcohol at a concentration of 1 mg/mL to be utilized as the modifying solution for drop-casting.

After the carbonization process, the sensors were washed with distilled water to remove the excess solution from the working electrode and then dried at room temperature. A silver/silver chloride (Ag/AgCl) ink was used as the stabilizing ink for the reference electrode (RE). To ensure optimal electrical contact between the electrodes and the potentiostat, a highly conductive silver ink was applied to all three electrodes. Subsequently, hot melt adhesive was used to delimit the application area of the electrolytic solution for the electrochemical sensor analysis.

For the blank solution of the samples, a 0.1 M phosphate buffer solution (PBS) was employed, prepared using 0.1 M monobasic sodium phosphate monohydrate (NaH2PO4·H2O, ACS analytical grade) and 0.1 M anhydrous dibasic sodium phosphate (Na2HPO4). The reagents were dissolved in water and subsequently sonicated for 10 minutes. And, for the analyte solutions, a 1 mM epinephrine solution was prepared using epinephrine hydrochloride (C9H13NO3·HCl). The reagent was dissolved in 0.1 M PBS and then sonicated for 5 minutes.

**Results and Discuss**

Due to the oxidative process of EP, the oxidation of epinephrine generates epinephrine quinone and leucoadrenochrome, which upon reduction yields adrenochrome[11], as illustrated in Figure 2. To analyze the epinephrine detection behavior, the oxidation peak corresponding to the conversion of epinephrine to epinephrine-quinone (0.2 V) was established as the analytical detection peak during a single-cycle CV analysis, as shown in Figure 3.

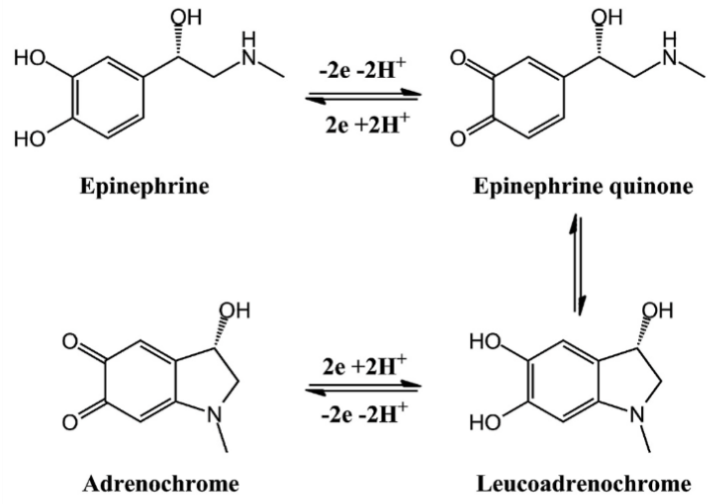


**Figure 2.** Schematic redox process from epinephrine molecule[11].

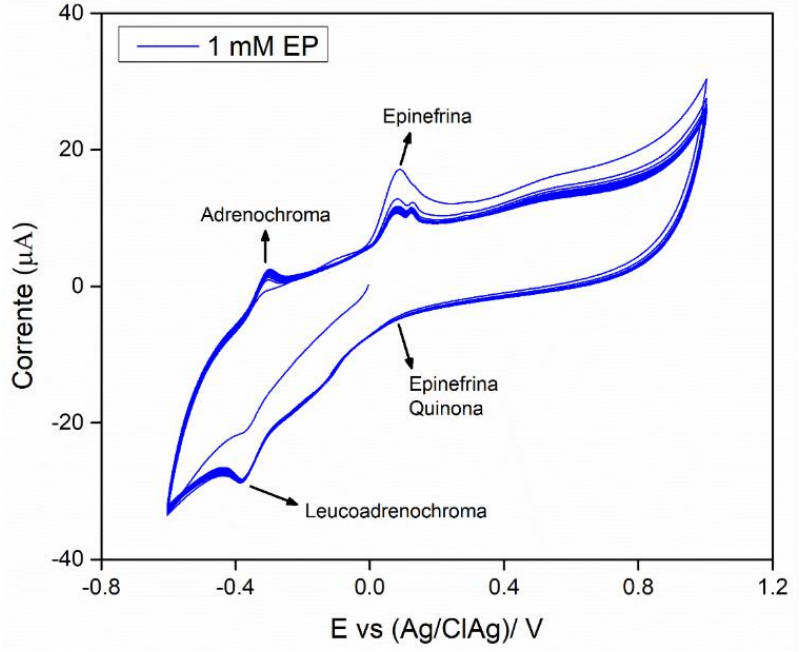


**Figure 3.** Cyclic voltammograms of different species obtained at various stages of the epinephrine redox process.

To observe the adrenochrome stabilization process, a 25-cycle CV analysis was performed on the sensor. The number of cycles required to achieve adrenochrome stabilization was three CV cycles[11], as demonstrated in Figure 4. Thus, from the third CV cycle onward, it would be possible to determine the analyzed epinephrine concentration at the oxidation peaks of both adrenochrome and epinephrine. Despite the benefit of verifying the sensor's behavior with higher precision, this multi-cycle analysis is not of primary interest for the developed device. The sensor is designed to operate over a large amplitude range for the broad detection of analyte concentrations and to perform rapid analyses; however, increasing the number of cycles would inherently result in longer analysis times.

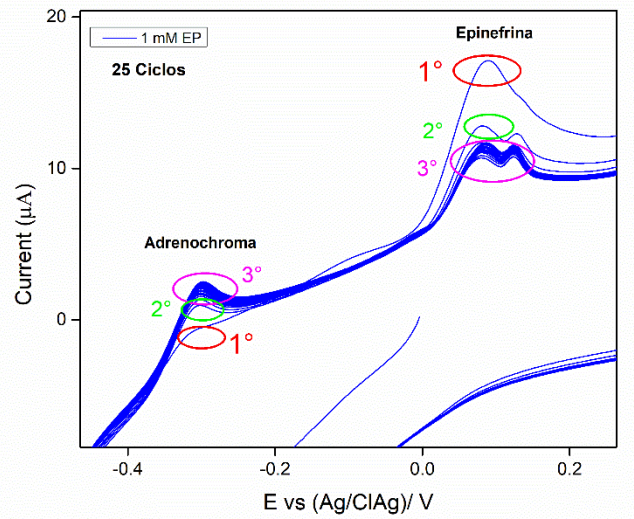


**Figure 4.** Cyclic voltammetry regarding the different peaks of adrenochrome and epinephrine during the progression of redox cycles in CV analyses.

Thus, LIG/MIP-epinephrine biomimetic sensors were tested as a means to detect the epinephrine hormone in a 0.1 M PBS solution. By observing the current difference obtained between the 1 mM epinephrine analyte solution and the blank solution (PBS), it was possible to identify a change in current at the anodic peaks, along with a current variation of 18 µA, as demonstrated in Figure 5.

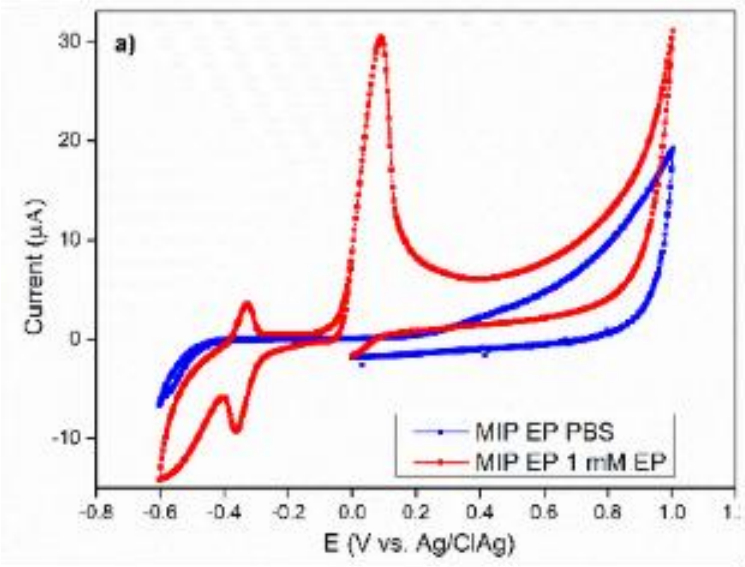


**Figure 5.** Selective study cyclic voltammetry at LIG/MIP-EP in 0,1 M PBS solution and in the presence of 1 mM epinephrine analyte in PBS solution.


## Conclusion

In this study, electrochemical sensors for epinephrine detection were investigated, utilizing a matrix derived from laser-induced graphene. This approach stands out from conventional and commercial detection techniques due to its rapid and facile preparation, as well as its low fabrication cost, enabling large-scale manufacturing and yielding rapid analytical results. The sensors were modified and evaluated using molecularly imprinted polymers to achieve enhanced efficacy and to elucidate how the epinephrine hormone interacts with the sensor structure during its redox process, up to the stabilization of the system on the sensor surface. Furthermore, it is necessary to perform an analytical curve study to determine the detection and quantification limits of the sensor, in addition to morphological and structural analyses via SEM, FTIR, and Raman spectroscopy. These characterizations are essential to elucidate how the epinephrine hormone binds to the functional sites of the LIG/MIP-epinephrine sensor and how this interaction affects the overall structure of the device.

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