

# Sleepy but Active: Screen-printed Gold Nanoparticle Electrodes Modified with Sleepy Plant (*Mimosa pudica*) Peroxidase for Hydrogen Peroxide Detection

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## Abstract

The sleepy plant (*Mimosa pudica*) is renowned for its rapid leaf-folding response to touch, embodying an intriguing blend of dormancy and activity. Inspired by this unique behavior, we have developed a novel electrochemical sensor using screen-printed gold nanoparticle electrodes (SPGNPE) treated with *Mimosa pudica* peroxidase (MPP) to quantify hydrogen peroxide ( $H_2O_2$ ). The MPP exhibits a specific activity of 122.9 U/mg and operates optimally at pH 4.0 and a temperature of 55°C, indicating its robust performance under mildly acidic and moderately high-temperature conditions. The enzyme's inactivation rate constant ( $k_{inact}$ ) was  $-0.018 \text{ min}^{-1}$ , suggesting a stable enzymatic activity over time. Cyclic voltammetry (CV) experiments using potassium ferrocyanide as a redox probe revealed a significant increase in current signal in the presence of MPP, indicating effective interaction of electrons with the redox compounds and the electrode interface. The linear relationship between the square root of the scan rate and the anodic and cathodic peaks suggested a surface-controlled, semi-reversible process involving the migration of the electrochemically active species to the electrode interface. A low limit of detection (LOD) of  $0.4 \mu\text{M}$  was achieved, accompanied by a sensitivity of  $0.039 \mu\text{A}/\mu\text{M}$ , demonstrating the electrode's capability for precise and sensitive  $H_2O_2$  quantification. This study highlights the unique application of the sleepy plant peroxidase, revealing its potential as a robust and sensitive novel bioelement in electrochemical sensing platforms. The synergy between nanomaterials and biological catalysts opens new avenues for environmentally friendly and efficient detection systems.

**Keywords:** Sleepy plant; Peroxidase; Hydrogen peroxide; Electrochemical detection; Biosensing; Screen-printed electrodes.

## 1. Introduction

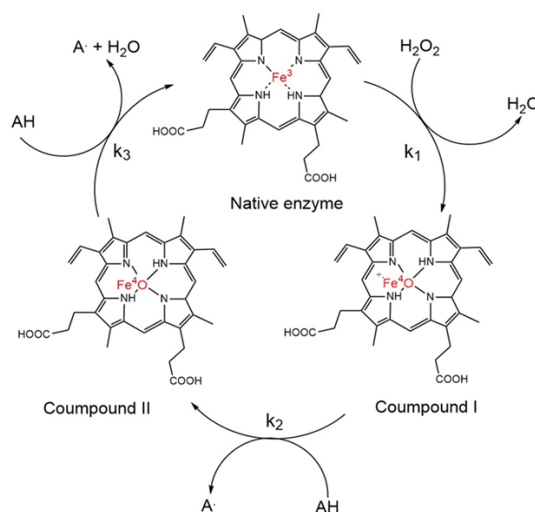
Peroxidases (PODs) are enzymes with a wide range of functions that catalyze the oxidation of diverse substrates using  $H_2O_2$ . This enzyme family plays a crucial role in numerous biological processes, including defense mechanisms in plants and animals, hormone biosynthesis, and the degradation of toxic substances [1–3]. PODs are grouped into various types based on their origin, structure, and function, with plant, fungal, and bacterial PODs being the most studied. Among these, plant PODs are particularly significant due to their involvement in the oxidative stress response, lignin biosynthesis, and pathogen defense [3].

The catalytic activity of PODs revolves around the reduction of  $H_2O_2$  to water while oxidizing organic or inorganic substrates [4]. As shown in **Fig. 1**, POD binds to its substrate, typically a peroxide compound such as  $H_2O_2$ . Upon binding, the enzyme interacts with the peroxide,

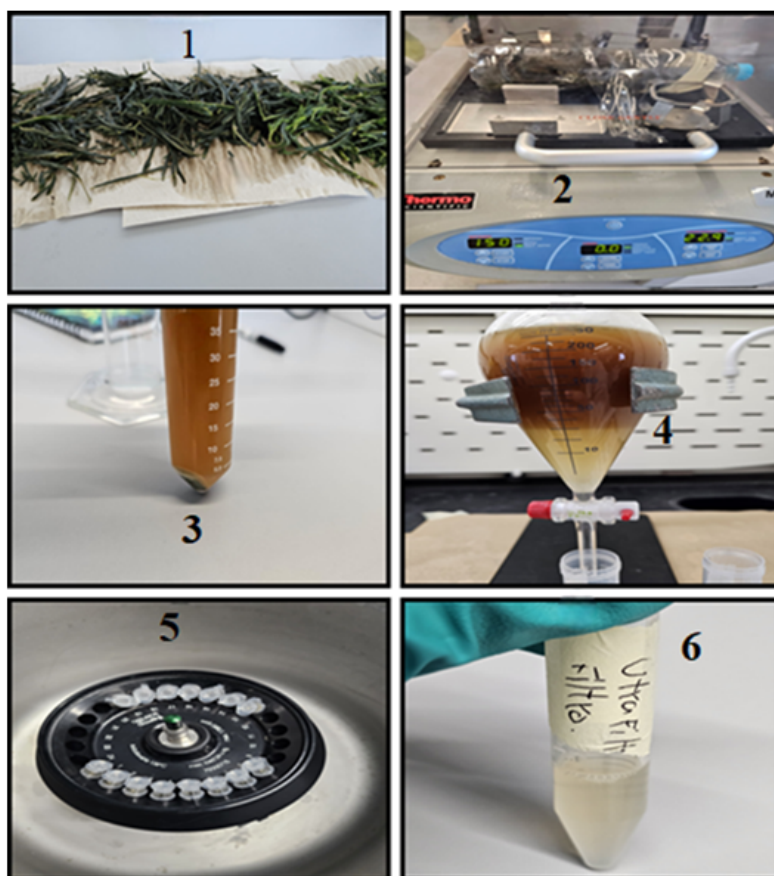
generating an intermediate known as Compound I. During this step, the iron atom located in the heme group at the enzyme's active site oxidizes. Compound I then reacts with the substrate, oxidizing it. This oxidation step is essential for the enzyme's role in breaking down or modifying specific substrates. Following oxidation of the substrate (AH), Compound I is reduced to Compound II, involving electron transfer within the enzyme. The enzyme reverts to its original native state through the action of an external reducing agent, which reduces Compound II back to the enzyme's native form, thus completing the catalytic cycle.

This reaction not only highlights the importance of PODs in biological systems but also makes them valuable tools in various industrial and analytical applications. For instance, PODs are used in biosensors to quantify  $H_2O_2$ , a common byproduct in many biological and chemical processes, including oxidative stress, enzyme reactions, and industrial effluents [5–9]. Detecting  $H_2O_2$  with high sensitivity and specificity is essential for advancements in medical diagnostics, environmental monitoring, and food safety [10–12].

The species *Mimosa pudica*, known as sleepy plant, is a member of the Fabaceae family and exhibits a rapid leaf-folding response to mechanical stimuli (thigmonasty), which has triggered extensive studies on the plant's physiological and biochemical mechanisms. Beyond this characteristic behavior, the plant is of biochemical interest due to the presence of a unique peroxidase (MPP), which has shown promising catalytic properties compared to conventional plant PODs [13–16]. MPP exhibits high stability and efficient activity toward  $H_2O_2$ , making it suitable for analytical applications requiring robust catalytic performance [17–19]. **Fig. 2** illustrates *Mimosa pudica*'s peroxidase (MPP) extraction process. The extraction begins with harvested leaves and continues through homogenization, clarification, centrifugation, and ultrafiltration, before immobilization on the screen-printed gold nanoparticle electrodes (SPGNE).



**Figure 1.** Mechanism of the catalytic cycle of PODs.



**Figure 2.** Sleepy plant (*Mimosa pudica*) peroxidase (MPP) extraction and partial purification: (1) fresh leaves, (2) homogenization in extraction buffer, (3) crude extract collection, (4) clarification/pigment removal, (5) centrifugation, and (6) concentration by ultrafiltration.

MPP is particularly noteworthy because it embodies an intriguing blend of dormancy and activity, much like the plant itself. While the "sleepy" nature of the plant suggests a state of rest, its POD is highly active in catalyzing reactions involving  $H_2O_2$ . This duality makes MPP an attractive candidate for incorporation into biosensors, with required stability and catalytic efficiency.

Analytical applications are a key feature of electrochemical biosensors, which are tools that integrate a biomolecule component, such as an enzyme, with an electrochemical transducer [20–22]. The transducer converts the biological event into a measurable electrical signal. Because of their exceptional sensitivity, selectivity, rapid response, and potential for miniaturization, these biosensors are suitable for applications such as clinical diagnostics, environmental monitoring, and food safety testing [22].

Electrochemical biosensors with integrated nanomaterials have revealed enhanced performance. Nanomaterials, such as gold nanoparticles, graphene, and carbon nanotubes, provide enlarged surface areas with superior conductivity and marked biocompatibility, facilitating

enzyme immobilization and electron transfer [23–25]. Gold nanoparticles stand out for their facile production, chemical stability, and strong affinity for biomolecules, enabling effective enzyme immobilization and enhanced catalytic activity. Screen-printed electrodes (SPEs) are widely used in electrochemical sensor development and can be mass-produced with reproducible characteristics, making them ideal for commercial sensor applications [26,27].

The incorporation of nanomaterials into SPEs further improves their performance, enabling the development of highly sensitive and selective biosensors [28,29]. In recent years, the modification of SPEs with gold nanoparticles (SPGNEs) has attracted significant attention in biosensing. The SPGNE technology represents an ideal enzyme immobilization substrate and a stable surface for enzyme-electrode interactions [23,30,31]. Moreover, the SPGNE and MPP combination yields a robust, precise sensing platform for detecting analytes such as  $H_2O_2$ .

The present study examines the fabrication of a new electrochemical biosensor designed for  $H_2O_2$  determination, utilizing screen-printed gold nanoparticle electrodes modified with MPP. We chose MPP as the biosensor, given its unique enzymatic properties, which reflect *Mimosa pudica*'s characteristic balance of dormancy and activity. This biosensor leverages the synergistic effects of MPP and AuNPs, enhancing sensitivity, stability, and specificity in  $H_2O_2$  detection.  $H_2O_2$  is a critical analyte in diverse areas, including clinical diagnostics, environmental monitoring, and food safety. Its detection is essential for monitoring oxidative stress, assessing the effectiveness of antioxidant therapies, and detecting contamination in industrial effluents. The construction of reliable and productive  $H_2O_2$  sensors is therefore of great significance. Traditional methods for  $H_2O_2$  detection, such as spectrophotometry and fluorescence, often require complex procedures and expensive reagents. In contrast, electrochemical sensors constitute a straightforward, cost-effective, and highly sensitive alternative.

To our knowledge, MPP has not been used in SPGNEs for electrochemical sensing. This platform stands apart from conventional HRP-based biosensors by proposing MPP as an unconventional, low-cost, and catalytically competent alternative for  $H_2O_2$  detection. In this study, MPP was immobilized onto AuNP-modified SPEs through physical adsorption, leveraging the enzyme's high affinity for the gold surface. The resulting MPP-modified electrodes were characterized for their electrochemical properties, using cyclic voltammetry (CV). The biosensor's performance was assessed in terms of its sensitivity, limit of detection (LOD), linearity, and stability. These findings underscore the potential of MPP as a biorecognition element in electrochemical sensing platforms, offering a novel approach to  $H_2O_2$  detection that combines biological uniqueness with technological innovation.

## 2. Material and Methods

### 2.1. Extraction and Purification of *Mimosa pudica* Peroxidase (MPP)

Sleepy plant (*Mimosa pudica*) leaves were harvested in the Santander region of Colombia. The collected material was finely ground, thoroughly washed, and continuously stirred in 100 mM phosphate buffer (pH 8.0) at room temperature for 24 hours. The resulting homogenate was filtered and centrifuged at 7 000 rpm for 10 minutes. The pellet was discarded. To eliminate pigmented compounds, a biphasic system consisting of 14% (w/v) polyethylene glycol (PEG) and 10% (w/v) ammonium sulfate  $((NH_4)_2SO_4)$  was used. After 3 hours, phase separation occurred, namely pigments and polymeric compounds concentrated in the upper PEG phase, while peroxidase

activity remained in the lower aqueous phase. The enzyme-rich phase was then concentrated by ultrafiltration at 5 000 rpm for 30 minutes at 4°C using Amicon centrifuge filters with a 10 kDa molecular weight cutoff. The partially purified MPP was stored at 5°C until further use.

## 2.2. Peroxidase Activity Assay

The enzymatic activity of MPP was evaluated spectrophotometrically as described previously. For this, 10  $\mu\text{L}$  of the enzyme preparation was mixed with 2.5 mL of 30 mM phosphate-buffered saline (PBS, pH 8.0) containing 10 mM guaiacol and 4.4 mM  $\text{H}_2\text{O}_2$ . The reaction was performed at 25°C, and the increase in absorbance at 470 nm ( $\epsilon = 26\,000\text{ M}^{-1}\text{ cm}^{-1}$ ) was recorded. One unit of enzymatic activity (U) was defined as the amount of enzyme catalyzing the oxidation of 1  $\mu\text{mol}$  of substrate per minute. Specific activity was expressed in units per milligram of protein, with protein concentration determined using the Pierce™ BCA Protein Assay Kit.

## 2.3. pH and Thermal Stability of MPP

To assess pH stability, MPP was incubated in a universal buffer system (10 mM, composed of  $\text{CH}_3\text{COOH}$ ,  $\text{H}_3\text{PO}_4$ , and  $\text{H}_3\text{BO}_3$  adjusted with NaOH). Thermal stability was evaluated using a preheated 990  $\mu\text{L}$  solution of 10 mM Tris–HCl buffer (pH 8.0) maintained at 65 °C. Then, 10  $\mu\text{L}$  of MPP was added, and the mixture was kept at the same temperature. At specific time intervals, 10  $\mu\text{L}$  aliquots were withdrawn, immediately mixed with 90  $\mu\text{L}$  of buffer, and allowed to reactivate at 25°C for 30 minutes to recover any reversibly inactivated enzyme. Residual activity was then measured.

## 2.4. Immobilization of MPP onto Screen-Printed Gold Nanoparticle Electrodes

Before immobilization, the electrodes were cleaned with an ethanol–water mixture and dried under nitrogen. A 5  $\mu\text{L}$  aliquot of the MPP solution was deposited onto the screen-printed electrode surface and left to dry at room temperature for approximately 10 hours. Subsequently, 2  $\mu\text{L}$  of 0.5 % Nafion solution was applied to stabilize the enzyme film and minimize interference. Subsequently, electrodes were gently rinsed with 10 mM phosphate buffer (pH 7.0) to remove any loosely bound enzyme. After modification, the electrodes were stored at 4 °C in a closed container to prevent dehydration. Under these storage conditions, the modified electrodes retained more than 90 % of their initial response for at least 7 days, indicating suitable short-term shelf stability.

## 2.5. Electrochemical Characterization of MPP-Modified Electrodes

Cyclic voltammetry (CV) was performed using an Autolab PGSTAT101 potentiostat (Echo Chemie, Utrecht, The Netherlands) operated via NOVA 1.10.1.9 software (Metrohm, Filderstadt, Germany) using a screen-printed three-electrode configuration provided by DropSens (Oviedo, Spain). The electrodes consisted of a 4 mm-diameter graphene working electrode, a silver pseudo-reference electrode, and a carbon counter electrode. All experiments were conducted under ambient conditions. Prior to each measurement, the solution was stirred magnetically and purged with nitrogen for 30 seconds. CV was performed in 10 mM PBS (pH 7.8) on both unmodified and MPP-modified electrodes to assess redox behavior. The bioelectrocatalytic reduction of  $\text{H}_2\text{O}_2$  by MPP was evaluated using a scan rate of 50 mV/s at 25°C.



### 3. Results and Discussion

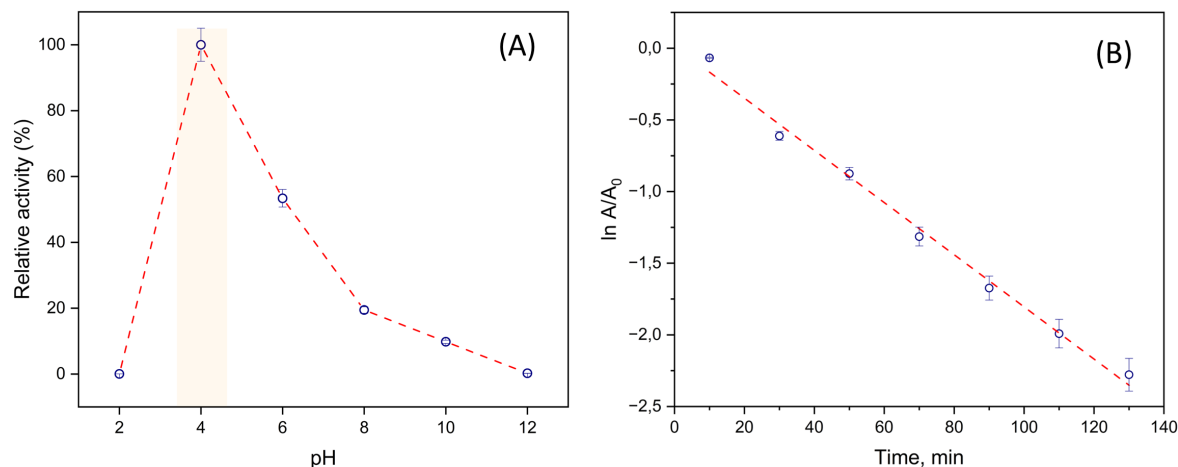
#### 3.1. Partial purification and stability evaluation of sleepy plant peroxidase

In a preceding study [19], researchers found that the raw extract from sleepy plant exhibited significant POD activity. Before proceeding with partial purification, the extraction conditions for MPP were determined. The most effective extraction was achieved using a 100 mM phosphate buffer at pH 8.0, with a maximum specific activity observed after 24 hours of extraction. The crude extract showed a specific activity of 6.20 U/mg, indicating the presence of various impurities and potential inhibitors. As noted by Sakharov *et al.*, [7, 32–34], many plant tissue extracts contain high concentrations of pigments. To generate a clarified extract, the pigments were eliminated using an aqueous biphasic system, specifically PEG–ammonium sulfate. Following the removal of pigments, a substantial increase in specific activity to 78.30 U/mg was observed, suggesting that the pigments may have interfered with enzyme activity, and their elimination was crucial for enhancing purity. Further purification was conducted using ultracentrifugation at 5 000 rpm for 30 minutes at 4°C employing Amicon membranes with a defined molecular weight exclusion limit of 10 000 kDa. This step increased specific enzyme activity to 122.9 U/mg. This final step not only removed low-molecular-weight contaminants but also concentrated the POD, leading to a highly purified enzyme preparation.

#### 3.2. Stability of sleepy plant peroxidase

Enzyme stability is remarkably influenced by pH, since it may alter the ionization states of side chains, thereby altering activity [25, 35]. It is widely reported that POD activity varies with pH [36]. Consequently, the stability of MPP was evaluated at different pH levels at room temperature. **Fig. 3A** shows the pH stability profile of sleepy plant POD reveals a clear dependence of enzyme activity on pH, with the highest relative activity detected at pH 4. At this particular pH, the enzyme retains 100% of its activity, indicating that acidic conditions are optimal for its functionality. As the pH increases, the enzyme's activity decreases markedly, with relative activities of 53.4% at pH 6 and only 19% at pH 8. This decline in activity suggests a progressive loss of enzyme stability as the environment becomes more alkaline. At extreme pH levels, such as pH 2 and pH 12, the POD activity drops almost completely, to 0.014% and 0.125%, respectively, indicating that the MPP is nearly inactive under these conditions. These results underscore the importance of maintaining a slightly acidic environment to preserve the catalytic efficiency of sleepy plant POD, which could have implications for its application in various biotechnological processes that require stable enzyme performance under specific pH conditions.

Additionally, the thermostability study of sleepy plant POD revealed a robust performance under mildly acidic and moderately high-temperature conditions, specifically at 55 °C. **Fig. 3B** reveals a linear relationship, reflecting a first-order kinetic behavior in the enzyme's thermal inactivation. The calculated inactivation rate constant ( $k_{\text{inact}}$ ) of  $-0.018 \text{ min}^{-1}$  suggests that the enzyme retains its activity over time with relatively slow inactivation at this temperature. These stability features indicate that MPP can maintain its catalytic function under mildly acidic and moderately elevated temperature conditions, which is advantageous for practical applications. In operational terms, this suggests that the biosensor can function reliably in environments where temperature or pH are not strictly controlled, supporting its potential for routine or field-based measurements [33, 37].

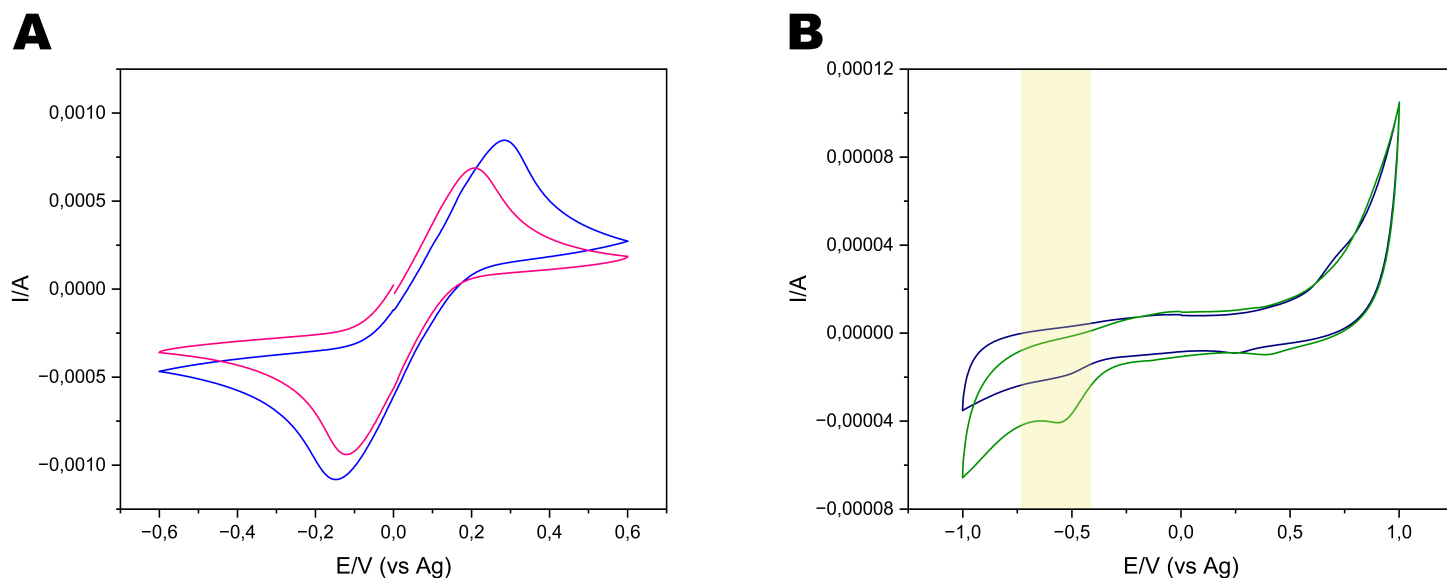


**Figure 3.** Effect of pH (A) and temperature (B) on MPP activity.

### 3.3. Electrochemical studies

The electrochemical studies conducted with the SPGNEs modified MPP demonstrated promising performance for the detection of  $H_2O_2$ . The successful immobilization was preliminarily confirmed by electrochemical impedance spectroscopy (EIS), which showed an increased charge-transfer resistance after enzyme modification (Figure S1, Supporting Information). The MPP was immobilized onto the surface of the SPGNE, and the assessed CVs of the ferri/ferrocyanide redox pair using both the modified and bare electrodes are presented in **Fig. 4A**. The increase in signal current observed for the electrode modified with MPP in contrast to the bare electrode, could be explained by the greater electron transfer facilitated by the enzyme and the conductive properties of the gold nanoparticles on the SPE. Gold nanoparticles provide an extensive surface area and excellent conductivity, improving the electron transfer kinetics between the redox species ( $K_3Fe(CN)_6$ ) and the electrode surface [38]. Once immobilized, MPP enhances electron transfer and catalytic performance due to its redox-active nature. This modification reduces the resistance to electron flow, allowing for a more efficient interaction between the ferri/ferrocyanide redox couple and the electrode surface [6, 39, 40]. In contrast, the bare electrode lacks this catalytic layer, resulting in a higher barrier for electron transfer and lower current response.

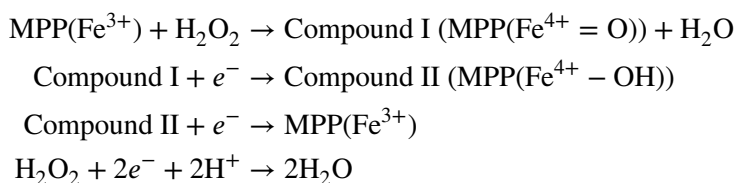
As illustrated in **Fig. 4B**, the CVs obtained from the MPP-SPGNE with and without 1 mM  $H_2O_2$  offer relevant insights into the electrocatalytic capabilities of the modified electrode. In absence of  $H_2O_2$ , the CV exhibits minimal current response across the scanned potential range, revealing no significant redox processes at the electrode surface. This low signal response is expected, as the MPP-SPGNE alone, without the presence of a substrate such as  $H_2O_2$ , does not promote notable electron transfer. Upon the introduction of 1 mM  $H_2O_2$  into the electrochemical cell, the voltammogram shows a reduction potential current increase at -0.5 V, which can be attributed to the bioelectrocatalytic reduction of  $H_2O_2$  by the MPP immobilized on the electrode [41–43]. The significant increase in current response compared to the blue curve is a clear indication of the efficient interaction between MPP and  $H_2O_2$ , wherein the POD facilitates electron transfer by catalysing the reduction of  $H_2O_2$ .



**Figure 4.** (A) CVs of the bare SPGNE (pink) and MPP-SPGNE (blue) recorded in the presence of 10 mM  $\text{K}_3\text{Fe}(\text{CN})_6$  with 0.1 M KCl, at a scan rate of  $50 \text{ mV s}^{-1}$ . (B) CVs of the MPP-SPGNE without (blue) and with (green) 1 mM  $\text{H}_2\text{O}_2$ , recorded at a rate of scanning of  $50 \text{ mV s}^{-1}$ .

This bioelectrocatalytic process occurs when the enzyme interacts with the peroxide, transferring electrons from the electrode to the  $\text{H}_2\text{O}_2$  molecules, reducing them to water [44]. The peak at approximately -0.5 V suggests a well-defined electrochemical process, agreeing closely with previously reported potentials for  $\text{H}_2\text{O}_2$  reduction using peroxidase-based systems [8, 34, 45–47].

The electrochemical reduction of  $\text{H}_2\text{O}_2$  on the MPP-modified electrode can be summarized as follows:

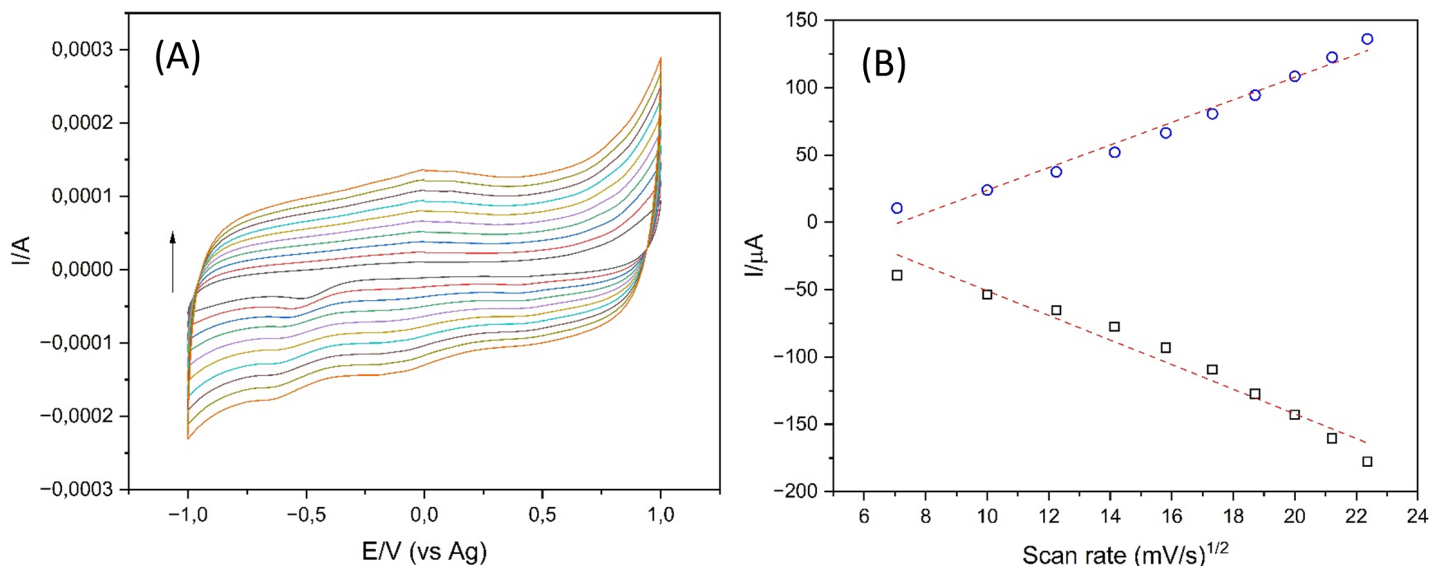


In this sequence, MPP acts as a bioelectrocatalyst, accelerating the reduction of  $\text{H}_2\text{O}_2$  while direct electron transfer occurs between the enzyme's heme center and the gold nanoparticle-modified electrode (SPGNE). The cathodic peak at -0.5 V indicates the electron flow linked to  $\text{H}_2\text{O}_2$  reduction in this catalytic cycle.

The influence of scan velocity on the electrochemical signal of MPP-immobilized SPGNEs was evaluated to understand the electron transfer kinetics and the interaction between the enzyme and the electrode surface [48].

The scan-rate experiment shown in **Fig. 5** was performed under specific time constraints and therefore recorded with a single electrode. Consequently, these CVs are presented as representative data and should be interpreted with caution. As the scan rate increases, the corresponding CV shows a simultaneous enhancement of anodic and cathodic peak currents, indicating that the electrochemical process is diffusion-controlled. The peak current exhibits a linear correlation

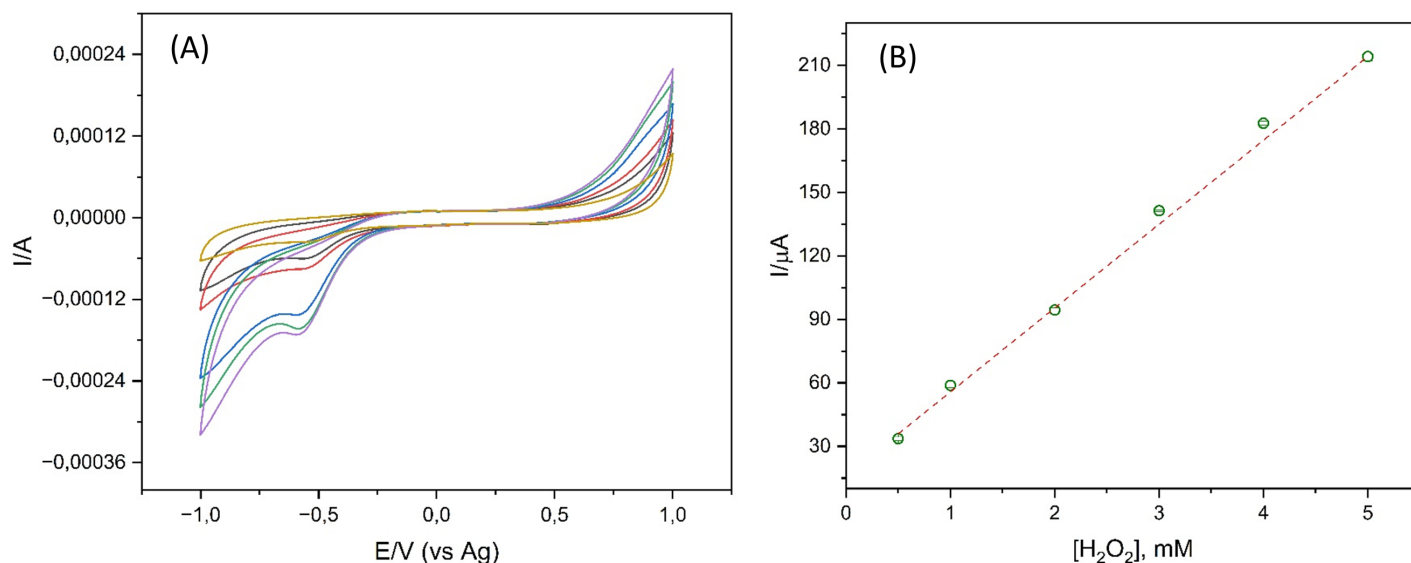




**Figure 5.** Cyclic voltammograms (A) and relationship between the square root of the scan rate and cathodic and anodic currents (B) for MPP-SPGNE in a 100 mM phosphate buffer at pH 8.0, evaluated at varying scan rates (These are representative data from a single electrode).

with the square root of the scan rate, further supporting the diffusion-controlled mechanism and suggesting that the reaction rate is influenced by the diffusion of the electrochemical species towards the electrode [49–51]. Additionally, the shift in peak potential with increasing scan rates may imply a slight resistive effect in the electron transfer process, which is typical in systems where biomolecules are immobilized on electrode surfaces [36]. The consistent increase in peak current with increasing scan rate also confirms the strong interaction between the immobilized MPP and the electrode, reinforcing its suitability for fast and efficient electrocatalytic reactions, such as  $\text{H}_2\text{O}_2$  detection.

CVs for MPP-SPGNE were recorded at increasing  $\text{H}_2\text{O}_2$  concentrations (0.5 mM to 5 mM), as shown in **Fig. 6A**. As the concentration of  $\text{H}_2\text{O}_2$  increased, the reduction peak current exhibited a significant and proportional increase, confirming the electrochemical activity of the POD in facilitating the bioelectroreduction of  $\text{H}_2\text{O}_2$ . The CVs exhibited well-defined reduction peaks, with increasing peak intensities as the  $\text{H}_2\text{O}_2$  concentration increased, a characteristic of an efficient enzyme-electrode interaction and direct electron transfer during the catalytic reduction of  $\text{H}_2\text{O}_2$ . The calibration curve (Fig. 6B), based on the reduction peak currents at varying  $\text{H}_2\text{O}_2$  concentrations, follows a linear trend as described by the equation  $y = 39.62x + 16.10$ , where  $y$ , the reduction current ( $\mu\text{A}$ ), is a function of the  $\text{H}_2\text{O}_2$  concentration. The obtained correlation coefficient ( $R^2$ : 0.998) indicates excellent linearity between the current signal and  $\text{H}_2\text{O}_2$  concentration over the tested range. This linear relationship suggests that the MPP-SPGNE can quantitatively detect  $\text{H}_2\text{O}_2$  within this concentration range, with the slope of the calibration curve ( $0.039 \mu\text{A}/\mu\text{M}$ ) representing the electrode's sensitivity to  $\text{H}_2\text{O}_2$ . The sensitivity of the MPP-SPGNE system can therefore be determined as  $39.62 \mu\text{A}/\mu\text{M}$ , which reflects the efficient catalytic activity of MPP and its efficient binding to the electrode surface. The linear range for  $\text{H}_2\text{O}_2$  detection extends from 0.5 mM to 5 mM, as supported by the consistent linearity of the calibration plot within this range. This is advantageous for practical applications, as it covers a broad concentration spectrum relevant to various industrial and environmental monitoring processes. The limit of detection (LOD) is calculated by multiplying the standard deviation of



**Figure 6.** MPP-SPGNE cyclic voltammograms (A) and calibration curve (B) at varying  $H_2O_2$  concentrations (Scan rate 50 mV/s).

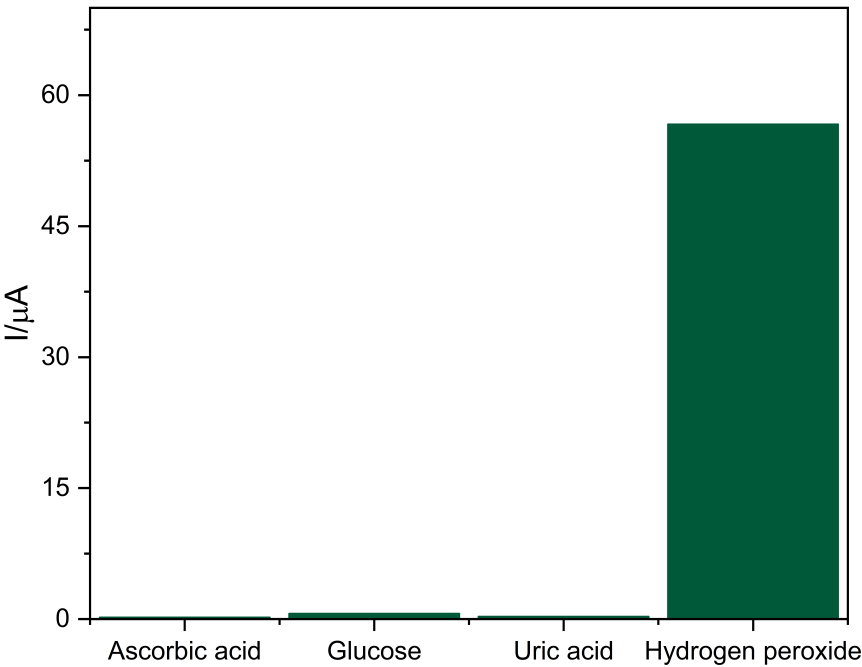
the blank response by three and then dividing by the slope of the linear equation, resulting in a value of  $0.4 \mu M$  [22]. Assuming minimal noise in the baseline current, the LOD for  $H_2O_2$  detection using the MPP-SPGNE is expected to be in the low micromolar range, reflecting the high sensitivity of the system.

The interference study was conducted to evaluate the selectivity of the MPP-SPGNE biosensor toward  $H_2O_2$  in the presence of potential interferents commonly found in biological and food matrices, such as ascorbic acid, glucose, and uric acid. As shown in **Fig. 7**, the current responses for these compounds were negligible compared to the pronounced signal observed for  $H_2O_2$ . This demonstrates that the electrochemical response is specifically associated with the catalytic activity of MPP toward  $H_2O_2$  rather than non-specific redox processes. The high selectivity may be attributed to the intrinsic substrate preference of plant peroxidases and the favorable electron transfer environment provided by the AuNP-modified electrode surface. Overall, the MPP-SPGNE biosensor shows strong anti-interference capability, reinforcing its practical applicability in real sample analysis.

Finally, to evaluate the performance of MPP-SPGNE for  $H_2O_2$  detection, its electrochemical parameters, namely redox potential, linear range, limit of detection (LOD), and sensitivity, were compared among modified electrodes previously reported in the literature [53–55]. The results from this study, alongside similar works, are summarized in **Table 1**. The MPP/SPGNE biosensor exhibits a competitive detection limit and a linear range, comparable to those of sensors based on commercial horseradish peroxidase (HRP) and other plant peroxidases. Although its analytical sensitivity is lower than that of HRP/AuNP electrodes, the main advantages of the MPP-SPGNE platform include (i) the use of a low-cost, locally available, and renewable natural enzyme source, reducing reliance on commercial HRP, (ii) straightforward and reagent-free enzyme immobilization, involving simple physical adsorption without cross-linkers, and (iii) good operational and thermal stability, making it suitable for routine analyses.

**Table 1.** Electrochemical parameter comparison of modified electrodes for hydrogen peroxide detection.

Modified Electrode	Redox Potential (V)	Linear Range (mM)	LOD ( $\mu$ M)	Sensitivity ( $\mu$ A/mM)	Reference
HRP/AuNP/Au electrode	-0.3	0.5–1.2	0.16	1808	[25]
SPP/ITO	-0.07	0.02–0.1	0.19	0.027	[52]
HRP/Graphene/GE	-0.08	0.025–3.5	0.05	92.82	[53]
GGP/SPGE	-0.65	0.1–4	150	–	[54]
GGP/GO	-0.64	0.5–6	45.21	–	[55]
MMP/SPGNE	-0.5	0.5–5	0.4	0.039	This work



**Figure 7.** Interference assessment of the MPP-SPGNE towards common electro-active species. Current responses were recorded in the presence of 1 mM ascorbic acid, glucose, uric acid, and 1 mM H<sub>2</sub>O<sub>2</sub> at 50 mV under the same experimental conditions.

We also acknowledge the limitations of the proposed biosensor, particularly its lower current sensitivity compared to some HRP-based nano-structured electrodes. This trade-off relates to the intrinsic catalytic differences between MPP and HRP, and we indicate that future optimization may involve controlled orientation or covalent immobilization strategies to enhance electron transfer efficiency. This additional comparative discussion strengthens the justification of the proposed approach while clearly situating it within the context of existing technologies.

## 4. Conclusions

*Mimosa pudica*, commonly known for its unique touch-sensitive behavior, has inspired the design of a cutting-edge electrochemical sensor founded on SPGNPE modified with MPP for H<sub>2</sub>O<sub>2</sub> detection. The POD showed a notable specific activity of 122.9 U/mg and demonstrated optimal catalytic performance at pH 4.0 and a temperature of 55 °C, showing stability under mildly acidic and moderately high-temperature conditions. With an inactivation rate constant of  $-0.018 \text{ min}^{-1}$ , MPP exhibited prolonged enzymatic activity, further confirming its robustness. Results from CV experiments indicated effective electron transfer between the redox species and the electrode surface, with a process that is quasi-reversible and controlled by surface interactions. The sensor achieved a LOD of 0.4  $\mu\text{M}$  and a sensitivity of 0.039  $\mu\text{A}/\mu\text{M}$ , demonstrating its precise and sensitive H<sub>2</sub>O<sub>2</sub> quantification capability. This work underscores the potential of MPP as a bioelement in electrochemical sensors, with the combination of biological catalysts and nanomaterials offering a promising approach for creating environmentally sustainable and highly efficient detection systems. A key novelty of this work is that it represents the first report of MPP immobilized on a SPGNE surface for direct electrochemical H<sub>2</sub>O<sub>2</sub> detection, offering a new biocatalytic alternative to traditional HRP-based systems. This demonstrates that MPP has significant potential as a low-cost, renewable, and efficient catalytic element in future biosensing technologies. Long-term operational stability and multi-cycle reproducibility were not evaluated in this study, representing important directions for future sensor optimization.

## 5. Acknowledgments

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## 6. Conflict of interest

The authors have no conflicts of interest to declare.

## References

- [1] Freitas CDT, Costa JH, Germano TA, de O. Rocha R, Ramos MV, Bezerra LP. Class III plant peroxidases: From classification to physiological functions. *International Journal of Biological Macromolecules*, 263: 130306, 2024.  
<https://doi.org/10.1016/j.ijbiomac.2024.130306>
- [2] Hiner ANP, Ruiz JH, Rodri JN, López A, Cánovas FG, Brisset NC, Smith AT, Arnao MB, Acosta M. Reactions of the class II peroxidases, lignin peroxidase and *Arthromyces ramosus* peroxidase, with hydrogen peroxide: Catalase-like activity, compound III formation, and enzyme inactivation. *Journal of Biological Chemistry*, 277(30): 26879–26885, 2002.  
<https://doi.org/10.1074/jbc.M200002200>
- [3] Mathé C, Barre A, Jourda C, Dunand C. Evolution and expression of class III peroxidases. *Archives of Biochemistry and Biophysics*, 500(1): 58–65, 2010.  
<https://doi.org/10.1016/j.abb.2010.04.007>

- [4] De Oliveira F. K, Santos L. O, Buffon J. G. Mechanism of action, sources, and application of peroxidases. *Food Research International*, 143: 110266, 2021.  
<https://doi.org/10.1016/j.foodres.2021.110266>
- [5] Pandey V. P, Awasthi M, Singh S, Tiwari S, Dwivedi U. N. A Comprehensive Review on Function and Application of Plant Peroxidases. *Biochemistry & Analytical Biochemistry*, 06(01): 308, 2017.  
<https://doi.org/10.4172/2161-1009.1000308>
- [6] Ayala M., Roman R., Vazquez-Duhalt R. A catalytic approach to estimate the redox potential of heme-peroxidases, *Biochemical and Biophysical Research Communications (BBRC)*, 357:804–808, 2007.  
<https://doi.org/10.1016/j.bbrc.2007.04.020>
- [7] Brusova Z., Ferapontova E.E., Sakharov I.Y., Magner E., Gorton L. Bioelectrocatalysis of plant peroxidases immobilized on graphite in aqueous and mixed solvent media, *Electroanalysis*, 17:460–468, 2005.  
<https://doi.org/10.1002/elan.200403182>
- [8] Škulj S., Kožić M., Barišić A., Vega A. Biarnés X., Piantanida I., Barisic I., Bertoša B. Comparison of two peroxidases with high potential for biotechnology applications – HRP vs. APEX2. *Computational and Structural Biotechnology Journal*, 23:742–751, 2024.  
<https://doi.org/10.1016/j.csbj.2024.01.001>
- [9] Alpeeva, I.S., Sakharov, I.Y. Luminol oxidation catalyzed by royal palm leaf peroxidase. *Applied Biochemistry and Microbiology*. 43, 25–28 (2007).  
<https://doi.org/10.1134/S0003683807010048>
- [10] Bhapkar S., Choudhari U., Jadhav U., Jagtap S. Evaluation of soybean peroxidase–copper phosphate mediated organic–inorganic hybrid for hydrogen peroxide biosensor application. *Sensors International*, 4, 2023.  
<https://doi.org/10.1016/j.sintl.2023.100242>
- [11] Sellami K., Couvert A., Nasrallah N., Maachi R., Abouseoud M., Amrane A. Peroxidase enzymes as green catalysts for bioremediation and biotechnological applications: A review. *Science of the Total Environment*, 806:150500, 2022.  
<https://doi.org/10.1016/j.scitotenv.2021.150500>
- [12] Bilal M., Barceló D., Iqbal H.M.N. Nanostructured materials for harnessing the power of horseradish peroxidase for tailored environmental applications, 749: 142360, 2020.  
<https://doi.org/10.1016/j.scitotenv.2020.142360>
- [13] Noor M., Muhammad G., Hanif H., Hussain M.A., Iqbal M.M., Mehmood U., Taslimi P., Shafiq Z. Structure, chemical modification, and functional applications of mucilage from *Mimosa pudica* seeds – A review, *International Journal of Biological Macromolecules*, 2024.

- <https://doi.org/10.1016/j.ijbiomac.2024.132390>
- [14] Hagihara T., Mano H., Miura T., Hasebe M., Toyota M. Calcium-mediated rapid movements defend against herbivorous insects in *Mimosa pudica*, *Nature Communications*, 13, 2022.  
<https://doi.org/10.1038/s41467-022-34106-x>
- [15] Dhanush C., Aravindh S., Jesreena J.S., Nagadharshini R., Jano N., Almeer R., Velu S.K.P. Biomimetic synthesis of carbon dots from *Mimosa pudica* leaves for enhanced bioimaging, *Waste and Biomass Valorization*, 2024.  
<https://doi.org/10.1007/s12649-024-02675-1>
- [16] Adurosakin O.E., Iweala E.J., Otiike J.O., Dike E.D., Uche M.E., Owanta J.I., Ugbogu O.C., Chinedu S.N., Ugbogu E.A. Ethnomedicinal uses, phytochemistry, pharmacological activities and toxicological effects of *Mimosa pudica* – A review, *Pharmacological Research modern Chinese Medicine*, 2023.  
<https://doi.org/10.1016/j.prmcm.2023.100241>
- [17] Adegoke H.I., Gbenga A.A. Bio-assisted synthesis of zinc oxide nanoparticles from *Mimosa pudica* aqueous leaf extract: Structure and antibacterial activity. *Chemistry Africa*, 6:1283–1296, 2023.  
<https://doi.org/10.1007/s42250-022-00581-4>
- [18] Aishan Y., Funano S., Sato A., Ito Y., Ota N., Yalikun Y., Tanaka Y. Bio-actuated microvalve in microfluidics using sensing and actuating function of *Mimosa pudica*, *Scientific Reports*, 12:7653, 2022.  
<https://doi.org/10.1038/s41598-022-11637-3>
- [19] Yu I, Gerardo S, Ardilal B, Sakharova IV. Peroxidasa de plantas tropicales, *Revista Colombiana de Química*, 28:97–106, 2010.
- [20] Cerdeira Ferreira LM, Lima D, Marcolino-Junior LH, Bergamini MF, Kuss S, Campanhã Vicentini F. Cutting-edge biorecognition strategies to boost the detection performance of COVID-19 electrochemical biosensors: A review, *Biochemistry*, 157: 108632, 2024.  
<https://doi.org/10.1016/j.bioelechem.2023.108632>
- [21] Rauf S, Nawaz H, Akhtar K, Ghauri MA, Khalid AM. Studies on sildenafil citrate (Viagra) interaction with DNA using electrochemical DNA biosensor. *Biosensors and Bioelectronics*. 22: 2471–2477, 2007.  
<https://doi.org/10.1016/j.bios.2006.09.023>
- [22] Forzato C, Vida V, Berti F. Biosensors and Sensing Systems for Rapid Analysis of Phenolic Compounds from Plants: A Comprehensive Review, *Biosensors*, 10(9): 105, 2020.  
<https://doi.org/10.3390/bios10090105>
- [23] Boruah JS, Kalita P, Chowdhury D, Barthakur M. Conjugation of citrate capped gold nanoparticles with gabapentin to use as biosensor, *Materials Today Proceedings*. 46(14):6404–6408, 2019.



- <https://doi.org/10.1016/j.matpr.2020.06.422>
- [24] Orduz AE, Gutiérrez JA, Blanco SI, Castillo JJ. Amperometric detection of triclosan with screen-printed carbon nanotube electrodes modified with Guinea Grass (*Panicum maximum*) peroxidase, *Universitas Scientiarum*. 24(2):363–379, 2019.
- <https://doi.org/10.11144/javeriana.sc24-2.adot>
- [25] Alvarez-Paguay J, Fernández L, Bolaños-Méndez D, González G, Espinoza-Montero PJ. Evaluation of an electrochemical biosensor based on carbon nanotubes, hydroxyapatite and horseradish peroxidase for the detection of hydrogen peroxide, *Sensing and Bio-sensing Research*. 37, 2022.
- <https://doi.org/10.1016/j.sbsr.2022.100514>
- [26] Zakiyyah, S.N., Irkham, Einaga, Y., Gultom, N.S., Fauzia, R.P., Kadja, G.T.M., Gaffar, S., Ozsoz, M., Hartati, Y.W.: Green Synthesis of Ceria Nanoparticles from Cassava Tubers for Electrochemical Aptasensor Detection of SARS-CoV-2 on a Screen-Printed Carbon Electrode, *ACS Applied Bio Materials*, 7(4):2488-2498, 2024.
- <https://doi.org/10.1021/acsabm.4c00088>
- [27] Valsalakumar VC, Joseph AS, Piyus J, Vasudevan S. Polyaniline-Graphene Oxide Composites Decorated with ZrO<sub>2</sub> Nanoparticles for Use in Screen-Printed Electrodes for Real-Time l-Tyrosine Sensing. *ACS Applied Nano Materials*. 6, 8382–8395, 2023.
- <https://doi.org/10.1021/acsanm.3c00659>
- [28] Castillo, J., Guarín-Guio, P.A., Ortiz, L.: Bio-Electrocatalytic Reduction of Hydrogen Peroxide by Peroxidase from Guinea Grass (*Panicum Maximum*) Immobilized on Graphene and Graphene Oxide Screen-Printed Electrodes, *Ingeniería y Universidad*, 26, 2022.
- <https://doi.org/10.11144/javeriana.iued26.brhp>
- [29] González-Hernández J, Moya-Alvarado G, Alvarado-Gámez AL, Urcuyo R, Barquero-Quirós M, Arcos-Martínez MJ. Electrochemical biosensor for quantitative determination of fentanyl based on immobilized cytochrome c on multi-walled carbon nanotubes modified screen-printed carbon electrodes, *Microchimica Acta*, 189, 2022.
- <https://doi.org/10.1007/s00604-022-05578-x>
- [30] Org, W.E., Farghali, R.A., Ahmed, R.A. Gold Nanoparticles-Modified Screen-Printed Carbon Electrode for Voltammetric Determination of Sildenafil Citrate (Viagra) in Pure Form, Biological and Pharmaceutical Formulations, *International Journal of Electrochemical Science*, 2015.
- [https://doi.org/10.1016/S1452-3981\(23\)05088-5](https://doi.org/10.1016/S1452-3981(23)05088-5)
- [31] Parkhe VS, Tiwari AP. Gold nanoparticles-based biosensors: pioneering solutions for bacterial and viral pathogen detection a comprehensive review, *World Journal of Microbiology and Biotechnology*, 40:269, 2024.
- <https://doi.org/10.1007/s11274-024-04072-1>
- [32] Sakharov, I.Y.: Palm tree peroxidases, *Biochemistry (Moscow)*, 69, 823–829, 2004.

- <https://doi.org/10.1023/B:BIRY.0000040213.91951.bc>
- [33] Sakharov IY, Vorobiev AC, Leon JJC. Synthesis of polyelectrolyte complexes of polyaniline and sulfonated polystyrene by palm tree peroxidase, *Enzyme Microbial Technology*, 33, 661–667, 2003.
- [https://doi.org/10.1016/S0141-0229\(03\)00188-1](https://doi.org/10.1016/S0141-0229(03)00188-1)
- [34] Alpeeva IS, Niculescu-Nistor M, Leon JC, Csöregi E, Sakharov IY. Palm tree peroxidase-based biosensor with unique characteristics for hydrogen peroxide monitoring, *Biosens Bioelectron.* 21, 742–748, 2005.
- <https://doi.org/10.1016/j.bios.2005.01.008>
- [35] Thulasiprevinnah S, Bashir S, Ramesh K, Ramesh S. Recent advances in electrochemical biosensors for the determination of biomolecules on modified and unmodified electrodes, 2024.
- <https://doi.org/10.1007/s13738-024-03050-6>
- [36] Mathebe NGR, Morrin A, Iwuoha EI. Electrochemistry and scanning electron microscopy of polyaniline/ peroxidase-based biosensor. In: *Talanta*. pp. 115–120. Elsevier 2004.
- [37] Caramyshev AV, Evtushenko EG, Ivanov VF, Barceló AR, Roig MG, Shnyrov VL, van Huystee RB, Kurochkin IN, Vorobiev AK, Sakharov IY. Synthesis of conducting polyelectrolyte complexes of polyaniline and poly (2-acrylamido-3-methyl-1-propanesulfonic acid) catalyzed by pH-stable palm tree peroxidase, *Biomacromolecules*, 6: 1360–1366, 2005.
- <https://doi.org/10.1021/bm049370w>
- [38] Li Y, Schluesener HJ, Xu S. Gold nanoparticle-based biosensors, *Gold Bull*, 43: 29–41, 2010.
- <https://doi.org/10.1007/BF03214964>
- [39] *Biocatalysis Based on Heme Peroxidases*. Springer, 2010.
- <https://link.springer.com/book/10.1007/978-3-642-12627-7>
- [40] Castillo J, Ferapontova E, Hushpulia D, Tasca F, Tishkov V, Chubar T, Gazaryan I, Gorton L. Direct electrochemistry and bioelectrocatalysis of  $H_2O_2$  reduction of recombinant tobacco peroxidase on graphite. Effect of peroxidase single-point mutation on  $Ca^{2+}$ -modulated catalytic activity, *Journal of Electroanalytical Chemistry*, 588: 112–121, 2006.
- <https://doi.org/10.1016/j.jelechem.2005.12.010>
- [41] Roth JP, Cramer CJ. Direct examination of  $H_2O_2$  activation by a heme peroxidase, *Journal of the American Chemical Society*, 130: 7802–7803, 2008.
- <https://doi.org/10.1021/ja802098c>
- [42] Caglar B, İçer F, Özdokur KV, Caglar S, Özdemir AO, Guner EK, Beşer BM, Altay A, Çırak Ç, Doğan B, Tabak A. A novel amperometric  $H_2O_2$  biosensor constructed by cross peroxidase entrapped on  $BiFeO_3$  nanoparticles, *Materials Chemistry and Physics*, 262, 2021.

- <https://doi.org/10.1016/j.matchemphys.2021.124287>
- [43] Uribe PA, Ortiz CC, Centeno DA, Castillo JJ, Blanco SI, Gutierrez JA. Self-assembled Pt screen printed electrodes with a novel peroxidase *Panicum maximum* and zinc oxide nanoparticles for H<sub>2</sub>O<sub>2</sub> detection. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 561:18–24, 2019.
- <https://doi.org/10.1016/j.colsurfa.2018.10.051>
- [44] Roth JP, Cramer CJ. Direct examination of H<sub>2</sub>O<sub>2</sub> activation by a heme peroxidase. *Journal of the American Chemical Society*, 130:7802–7803, 2008.
- <https://doi.org/10.1021/ja802098c>
- [45] Centeno DA, Solano XH, Castillo JJ. A new peroxidase from leaves of guinea grass (*Panicum maximum*): A potential biocatalyst to build amperometric biosensors. *Bioelectrochemistry*, 116:33–38, 2017.
- <https://doi.org/10.1016/j.bioelechem.2017.03.005>
- [46] Mathebe NGR, Morrin A, Iwuoha EI. Electrochemistry and scanning electron microscopy of polyaniline/peroxidase-based biosensor. *Talanta*, 64:115–120, 2004.
- <https://doi.org/10.1016/j.talanta.2003.11.050>
- [47] Gaspar S, Catalin Popescu I, Gazaryan IG, Bautista AG, Sakharov IY, Mattiasson B, Csöregi E. Biosensors based on novel plant peroxidases: a comparative study, *Electrochimica Acta*, 2000.
- [https://doi.org/10.1016/S0013-4686\(00\)00580-6](https://doi.org/10.1016/S0013-4686(00)00580-6)
- [48] Adachi T, Kitazumi Y, Shirai O, Kano K. Direct electron transfer-type bioelectrocatalysis of redox enzymes at nanostructured electrodes, 10(2):236, 2020.
- <https://doi.org/10.3390/catal10020236>
- [49] Ferapontova EE, Castillo J, Hushpulia D, Tishkov V, Chubar T, Gazaryan I, Gorton L. Direct electrochemistry of recombinant tobacco peroxidase on gold. *Electrochemistry Communications*, 7:1291–1297, 2005.
- <https://doi.org/10.1016/j.elecom.2005.09.004>
- [50] Dequaire M, Limoges B, Moiroux J, Savéant JM. Mediated electrochemistry of horseradish peroxidase. Catalysis and inhibition. *Journal of the American Chemical Society*, 124:240–253, 2002.
- <https://doi.org/10.1021/ja0170706>
- [51] Léger C, Bertrand P. Direct electrochemistry of redox enzymes as a tool for mechanistic studies, *Chemical Reviews*, 108(7):2379–2438, 2008.
- <https://doi.org/10.1021/cr0680742>
- [52] Bhapkar S, Choudhari U, Jadhav U, Jagtap S. Evaluation of soybean peroxidase–copper phosphate mediated organic-inorganic hybrid for hydrogen peroxide biosensor application. *Sensors International*, 4, 2023.

<https://doi.org/10.1016/j.sintl.2023.100242>

- [53] Nandini S, Nalini S, Manjunatha R, Shanmugam S, Melo JS, Suresh GS. Electrochemical biosensor for the selective determination of hydrogen peroxide based on the co-deposition of palladium, horseradish peroxidase on functionalized-graphene modified graphite electrode as composite. *Journal of Electroanalytical Chemistry*, 689:233-242, 2013.

<https://doi.org/10.1016/j.jelechem.2012.11.004>

- [54] Centeno DA, Solano XH, Castillo JJ. A new peroxidase from leaves of guinea grass (*Panicum maximum*): A potential biocatalyst to build amperometric biosensors. *Bioelectrochemistry*, 116:33–38, 2017.

<https://doi.org/10.1016/j.bioelechem.2017.03.005>

- [55] Castillo J, Guarín-Guio PA, Ortiz L. Bio-electrocatalytic reduction of hydrogen peroxide by peroxidase from guinea grass (*Panicum maximum*) immobilized on graphene and graphene oxide screen-printed electrodes. *Ingeniería y Universidad*, 26, 2022.

<https://doi.org/10.11144/javeriana.iued26.brhp>

### Somnolienta pero Activa: Electrodos Serigrafiados de Nanopartículas de Oro Modificados con Peroxidasa de la Planta Dormilona (*Mimosa pudica*) para la Detección de Peróxido de Hidrógeno

**Resumen:** La planta dormilona (*Mimosa pudica*) es reconocida por su rápida respuesta de plegamiento de hojas al tacto, lo que representa una combinación intrigante de somnolencia y actividad. Inspirados en este comportamiento único, desarrollamos un novedoso sensor electroquímico empleando electrodos serigrafiados de nanopartículas de oro (SPGNPE) tratados con peroxidasa de *Mimosa pudica* (MPP) para cuantificar peróxido de hidrógeno ( $H_2O_2$ ). La MPP presenta una actividad específica de 122.9 U/mg y opera de manera óptima a un pH de 4.0 y una temperatura de 55 °C, lo cual indica un desempeño robusto en condiciones ligeramente ácidas y de temperatura moderadamente alta. La constante de inactivación de la enzima ( $k_{inact}$ ) fue de  $-0.018 \text{ min}^{-1}$ , lo que sugiere una actividad enzimática estable a lo largo del tiempo. Los experimentos de voltametría cíclica (CV) usando ferrocianuro de potasio como sonda redox mostraron un aumento significativo en la señal de corriente en presencia de MPP, lo que indica una interacción efectiva de los electrones con los compuestos redox y la interfaz del electrodo. La relación lineal entre la raíz cuadrada de la velocidad de barrido y los picos anódicos y catódicos sugirió un proceso semirreversible controlado por la superficie, involucrando la migración de especies electroquímicamente activas hacia la interfaz del electrodo. Se obtuvo un límite bajo de detección (LOD) de  $0.4 \mu\text{M}$ , así como una sensibilidad de  $0.039 \mu\text{A}/\mu\text{M}$ , lo que demuestra la capacidad del electrodo para una cuantificación precisa y sensible de  $H_2O_2$ . Este estudio resalta la aplicación única de la peroxidasa de la planta dormilona, revelando su potencial como un bioelemento novedoso, robusto y sensible en plataformas de sensores electroquímicos. La sinergia entre nanomateriales y catalizadores biológicos abre nuevas posibilidades para el desarrollo de sistemas de detección eficientes y ambientalmente amigables.

**Palabras Clave:** Biosensado; Detección electroquímica; Electrodos serigrafiados; Peróxido de hidrógeno; Peroxidasa; Planta dormilona

### **Sonolenta, porém Ativa: Eletrodos Serigrafados de Nanopartículas de Ouro Modificados com Peroxidase da Planta Dormideira (*Mimosa pudica*) para Detecção de Peróxido de Hidrogênio**

**Resumo:** A planta dormideira (*Mimosa pudica*) é conhecida por sua rápida resposta de dobramento das folhas ao toque, representando uma combinação intrigante de sonolência e atividade. Inspirados nesse comportamento único, desenvolvemos um novo sensor eletroquímico utilizando eletrodos serigrafados de nanopartículas de ouro (SPGNPE) tratados com peroxidase de *Mimosa pudica* (MPP) para quantificar peróxido de hidrogênio ( $H_2O_2$ ). A MPP apresentou atividade específica de 122,9 U/mg e operou de forma ideal num pH 4,0 e temperatura de 55 °C, indicando um desempenho robusto em condições levemente ácidas e de temperatura moderadamente elevada. A constante de inativação da enzima ( $k_{inact}$ ) foi de  $-0,018 \text{ min}^{-1}$ , sugerindo uma atividade enzimática estável ao longo do tempo. Experimentos de voltametria cíclica (CV) utilizando ferrocianeto de potássio como sonda redox mostraram um aumento significativo no sinal de corrente na presença de MPP, indicando uma interação eficaz dos elétrons com os compostos redox e a interface do eletrodo. A relação linear entre a raiz quadrada da velocidade de varredura e os picos anódico e catódico sugeriu um processo semirreversível controlado pela superfície, envolvendo a migração de espécies eletroquimicamente ativas para a interface do eletrodo. Foi obtido um baixo limite de detecção (LOD) de  $0,4 \mu\text{M}$  e uma sensibilidade de  $0,039 \mu\text{A}/\mu\text{M}$ , o que demonstrou a capacidade do eletrodo para quantificação precisa e sensível de  $H_2O_2$ . Este estudo destaca a aplicação singular da peroxidase da planta dormideira, revelando seu potencial como um bioelemento novo, robusto e sensível em plataformas de sensores eletroquímicos. A sinergia entre nanomateriais e catalisadores biológicos abre novas possibilidades para o desenvolvimento de sistemas de detecção eficientes e ambientalmente amigáveis.

**Palavras-chave:** Biossensoriamento; Detecção eletroquímica; Eletrodos serigrafados; Peróxido de hidrogênio; Peroxidase; Planta dormideira.



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