

Electrochemical Detection of Fenthion Insecticide in Olive Oils by a Sensitive Non-Enzymatic Biomimetic Sensor Enhanced with Metal Nanoparticles [†]

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Abstract: Fenthion, an organophosphate insecticide, is a cholinesterase inhibitor and is highly toxic. An electrochemical sensor based on molecularly imprinted polymer is developed here for its detection. For this purpose, 2-aminothiophenol mixed with gold nanoparticles was immobilized on screen-printed gold electrodes. The FEN pattern was then fixed before being covered with 2-aminothiophenol. Cyclic voltammetry, differential pulse voltammetry and electrochemical impedance spectroscopy methods were used for the electrochemical characterization. The low detection limit was 0.05 mg/Kg over a range of 0.01–17.3 µg/mL. The sensor was successfully applied for the determination of FEN in olive oil samples with high recovery values.

Keywords: fenthion; molecularly imprinted polymer (MIP); screen-printed gold electrode (Au-SPE); electrochemical sensor; olive oils



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1. Introduction

Olive oil production is located in the countries of the Mediterranean basin, specifically in Spain, Portugal, Italy, Greece, Turkey, Tunisia and Morocco [1]. However, production in other countries, such as Australia and the United States, is increasing.

Organophosphorus insecticides are the pesticides used in the largest quantities in olive groves to control pests. The most commonly used are those belonging to the class of organophosphate insecticides, because of their relatively low persistence under natural conditions, their ease of synthesis, their low cost and their high effectiveness in eradicating insects. Fat-soluble pesticides tend to concentrate in oils and toxic residues in lipids have been reported [2,3]. In addition, however, residues of POs in the environment present significant risks to the ecosystem and agricultural products; due to their lack of specificity, they affect the nervous system of non-target species, such as mammals, birds and aquatic fauna. Fenthion (FEN) is among the most commonly used pesticides [4]. The Codex Alimentarius Commission of the Food and Agriculture Organization of the United Nations (FAO) have set maximum residue limits (MRLs) for pesticides in olives and olive oil (e.g., 1 and 2 mg/kg for FEN) [5]. Many different detection methods have been used for the determination of residues of organophosphorus pesticides in olive oil. The most commonly used techniques are gas chromatography (GC) methods, which require the extraction of pesticides from samples. Reversed phase liquid chromatography–gas chromatography was also applied to olive oil [6]. These techniques are generally expensive, and require large quantities of samples and organic solvents as well as cleaning and preconcentration steps. Complementary analytical methods, such as enzymatic biosensors, which are based on

the inhibition of acetylcholinesterase, were also developed for the detection of FEN [6]. However, enzymes have drawbacks such as difficulty in purification due to their instability, sensitivity and selectivity, which often depend on the nature of the materials. Immunosensing applications are also used for the FEN detection of organophosphorus insecticides, such as indirect enzyme-linked immunosorbent assay (ELISA) [7] and electrochemical immunosensor [8] applications. Their main limitations lie in the availability of antibodies and the fact that they cannot be used for the determination of low-molecular-weight hapten analytes [9,10].

Alternative methods based on molecularly imprinted polymers (MIPs) have also been adopted. They can mimic the functions of biological receptors but with fewer stability constraints. There are few applications of MIPs for the detection of FEN [11].

Due to the growing concern about the presence of pesticide residues in olive oil, it is necessary to implement procedures that can be applied in the field, with sensitive and selective detection.

The objective of this work was to develop a new low-cost, selective and highly sensitive electrochemical sensor, based on MIP, for the determination of FEN in olive oil samples.

This electrochemical sensor was fabricated by immobilizing a 2-aminothiophenol (2-ATP) complex mixed with gold nanoparticles (AuNPs) onto a screen-printed gold electrode (Au-SPE) via Au-S bonds. Then, the FEN template was bound onto Au-SPE/ATP-AuNPs before being coated with 2-ATP. The synthesis process of the electrochemical MIP sensor was straightforward. More generally, we believe that the results obtained open up new opportunities to detect other organophosphate insecticides in various food products and in the environment.

2. Materials and Methods

2.1. Reagents and Solutions

Fenthion (FEN), malathion (MAL), dimethoate (DMT), ethanol (99.8%), methanol, hydrochloric acid (HCL), acetonitrile, gold nanoparticles (AuNPs), potassium chloride, 2-aminothiophenol (2-ATP), phosphate-buffered saline (PBS) and ferri-ferrocyanide ($K_4[Fe(CN)_6]$, $K_3[Fe(CN)_6]$), were all purchased from Sigma-Aldrich, Saint-Quenti-Fallavier, France. Ultra-pure water was used throughout the experiments.

2.2. Synthesis of MIP and NIP Materials on the Gold Electrode

As shown in Figure 1, the overall process for the preparation of the MIP sensor can be summarized by the following steps: First, the functionalization process was performed by immobilizing 0.1 M of 2-aminothiophenol (2-ATP), mixed with 1 mL of AuNPs, on the surface of the gold electrodes followed by incubation for 12 h at room temperature. In the second step, the FEN pattern was deposited on the modified surface and incubated for 2 h at room temperature. In the third step, the second layer containing 5 mM 2-ATP was electropolymerized with 0.1 M KCl and 0.05 M PBS (pH = 6.8) over the potential range (−0.2 V to 0.6 V) at a scan rate of 100 mV/s for 10 cycles. Finally, the printed pattern was extracted in HCl solution (0.5 M) for 20 min. Electrochemical detection of FEN by the MIP sensor was performed by placing 10 μ L of each concentration of FEN on the working electrode for 30 min. The electrochemical characteristics of the stepwise MIP sensor fabrication were studied in a $[Fe(CN)_6]^{3-/4-}$ 5 mM solution containing PBS (pH = 7.2). The redox probe $[Fe(CN)_6]^{3-/4-}$ was chosen as a marker to study the changes at the electrode surface after each step of the sensor preparation. Similarly, a non-printed film (Au-SPE/NIP) was prepared using the same procedure, but without adding the template into the polymer solution. This was carried out in order to ensure that the observed effects during the MIP detection steps were only related to the printing characteristics.

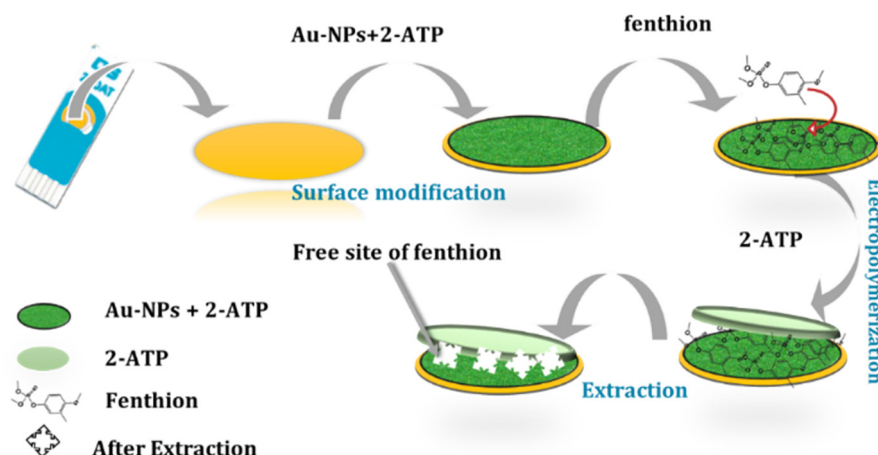


Figure 1. Representation of the experimental procedure of stepwise MIP sensor fabrication.

2.3. Electrochemical Measurements

A portable instrument (PalmSens³, Houten, The Netherlands) was used for performing the electrochemical measurements. The screen-printed gold electrodes (Au-SPE) consisted of a three-electrode system (purchased from Dropsens, Asturias, Spain), with a gold working electrode. (0.19 cm²), a silver reference electrode and a gold counter electrode (0.54 cm²). The following three electrochemical techniques, cyclic voltammetry (CV), differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS), were applied. They were used during the development and detection phases to evaluate the sensor response by measuring changes in current and resistance. The DPV measurements were carried out by scanning the potential from -0.1 V to 0.2 V with a scan rate of 50 mV/s. The EIS was measured at a bias potential of 10 mV over a frequency range of 0.1 Hz to 50 kHz. The impedance data were appropriately adjusted using the Randles equivalent circuit.

2.4. Analysis of Olive Oil Samples

The prepared MIP sensor was applied to olive oil samples to detect the presence of FEN. For this purpose, the extract of a Moroccan extra virgin olive oil collected from fields in the province of Taounate, Morocco, supposed to be free of residues of the pesticide FEN, a contaminated oil collected from an olive field in the province of Ouarzazate in Morocco, and a commercial oil called Al Hora, were pretreated. The two olive oil samples were pre-treated as follows: 1 mL of the oil samples was macerated and mixed with 5 mL of methanol/water ($4:1$, v/v), for 1 h at room temperature. This allowed the remaining pesticide residues in the samples to be extracted. This solution was centrifuged for 15 min at 6000 rpm, which accelerated the transfer of the pesticide residues into the solution. Next, the solvent methanol was evaporated from the supernatant at 65 °C (boiling point of methanol), for 5 min and the extract was collected in an Eppendorf tube. Finally, we deposited a volume of 10 μ L of this mixture on the surface of the MIP sensor.

3. Results and Discussion

3.1. Electropolymerization of FEN Imprinted Film

After the functionalization step of the Au-SPE electrode, by 2-ATP with Au-NPs, the prepared pre-electropolymerization complex, composed of a mixture of FEN with acetonitrile, was electropolymerized via reactions between FEN and the second layer containing 2-ATP.

In order to study the surface changes of Au-SPE (bare gold, after electropolymerization and extraction), the CV was used for further characterization in a $[\text{Fe}(\text{CN})_6]^{3-/4-}$ 5 mM solution, over the potential range of -0.4 V to 0.6 V at a scan rate of 20 mV/s. As shown in Figure 2, the peak anode current (I_a) and potential (E_a) were recorded. As can be seen, the CV signal of the bare gold electrode was lower than the MIP current, indicating that FEN

had been successfully trapped on the Au-SPE electrodes, preventing $[\text{Fe}(\text{CN})_6]^{3-/4-}$ from diffusing onto the Au-SPE surface. In addition, the NIP sensor had a lower peak current due to the absence of FEN molecules in the polymer.

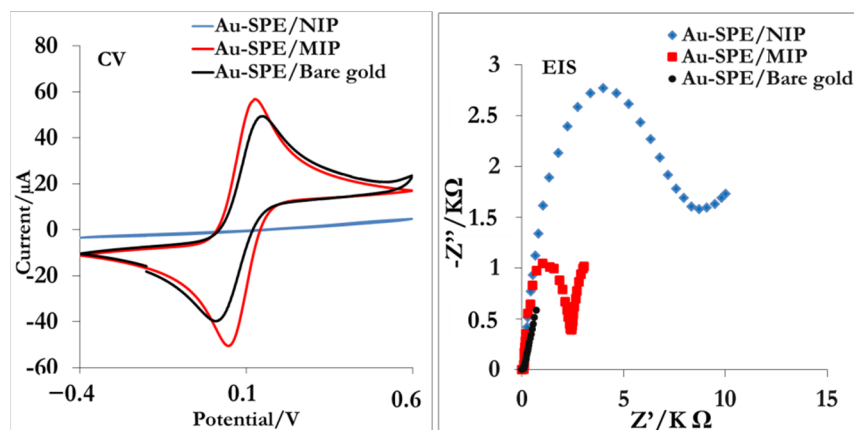


Figure 2. Cyclic voltammograms, Nyquist plots of 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ solution at bare Au-SPE, Au-SPE/MIP and Au-SPE/NIP.

Impedance spectroscopy was also used. As can be seen, the results obtained from the EIS and the CV are consistent with each other.

3.2. Molecular Recognition by MIP and NIP Sensors

To verify the retention capacity of the sensor for different concentrations of FEN, DPV and EIS techniques were used. Figure 3a shows the DPV and EIS responses of the modified electrode for the detection of FEN in the range of 0.01 $\mu\text{g/mL}$ to 17.3 $\mu\text{g/mL}$. The $[\text{Fe}(\text{CN})_6]^{3-/4-}$ was used as a mediator between the printed electrodes and the standard solutions.

In Figure 3a, it can be seen that the redox current peaks increased with increasing FEN concentrations. This increase may have been due to the conductivity of the film covering the surface of the Au-SPE. This confirms the binding of the FEN molecules that hindered the electron transport of the redox probe $[\text{Fe}(\text{CN})_6]^{3-/4-}$.

The same results were observed for the EIS technique. The semicircles of the Nyquist diagrams decreased as the FEN concentrations increased. Thus, the increase in FEN concentration produced a decrease in charge transfer resistance. The deposition of FEN concentrations resulted in an increase in the overall conductivity of the electrode surface. This demonstrates that the FEN molecules were well captured by the MIP sites.

When analyzing the NIP data obtained under the same conditions, it was found that the current peaks varies slightly. This was probably due to the absence of FEN during electropolymerization that did not involve the creation of specific cavities (Figure 3b). The results of the NIP Nyquist plots show semi-circular patterns and negligible changes in resistance values (R_{tc}). This means that the NIP sensor was not specific to FEN molecules, confirming that the responses obtained by the MIP sensor were only due to the presence of FEN-specific cavities.

3.3. Calibration Curve and Detection Limit

After optimizing the manufacturing process parameters, the MIP sensor was used for synthetic FEN detection.

Figure 4 shows the calibration curve referring to the sensor responses to FEN exposure.

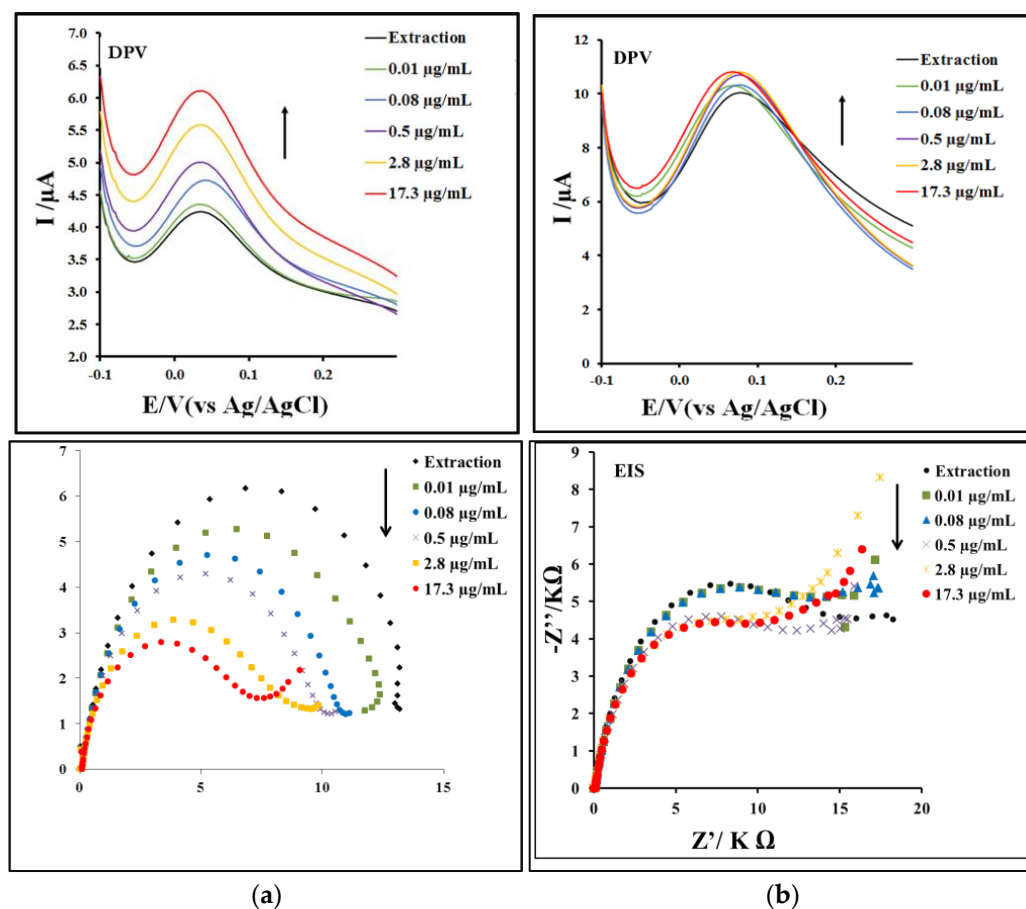


Figure 3. Voltammograms, Nyquist plots of different FEN concentrations for (a) MIP and (b) NIP sensors.

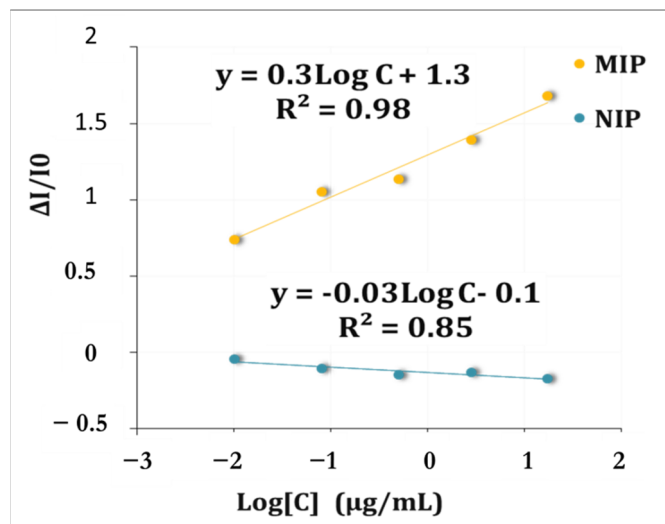


Figure 4. Calibration curves obtained by DPV for MIP and NIP sensors towards FEN.

The DPV technique was performed in a potential window of -0.1 to 0.2 V. The working range for synthetic detection was 0.01 to 17.3 $\mu\text{g/mL}$.

As a result, a satisfactory logarithmic relationship between the MIP sensor responses and FEN concentrations was achieved ($Y = 0.3\text{Log } C + 1.3$; $R^2 = 0.98$).

The limits of detection (LOD) and quantification (LOQ) were calculated using:

$$\text{LOD/LOQ} = k_{i\text{LOD/LOQ}} \times s/m, \quad (1)$$

where k_i corresponds to the signal/noise ratio, $k = 3.3$ for the LOD and $k = 10$ for the quantification limit (LOQ), s is the standard deviation of the intercept and m is the slope [12]. The LOD value was found to be 0.05 mg/Kg for the DPV measurements.

3.4. Selectivity of the MIP Sensor

To examine the selectivity of the MIP electrochemical sensor towards FEN, the interference of some similar molecular structures, including dimethoate (DMT) and malathion (MAL), was examined [11,13,14]. The interference test was carried out with satisfactory results. Figure 5 clearly shows that FEN's MIP sensor has much higher current responses compared to both analogues. It is, therefore, better suited for the selective detection of FEN.

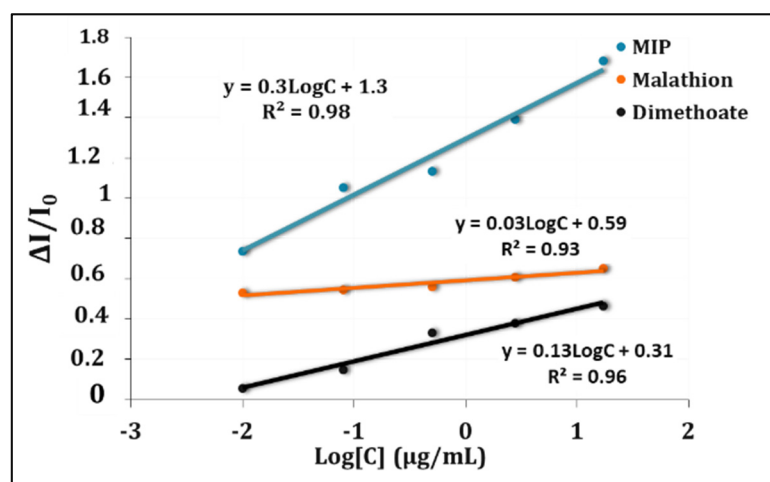


Figure 5. Calibration curves obtained by DPV for MIP towards FEN and interferences.

3.5. Analysis of Olive Oil Samples

The developed MIP sensor was tested for the determination of FEN in contaminated olive oil samples. Using the DPV technique, the responses of the MIP sensor were exploited. In fact, the difference between the maximum current values of the blank and the sample can be calculated using Equation (2) to obtain the FEN concentration in the real samples.

$$y = 0.3 \log C + 1.3 \quad (2)$$

The results obtained are summarized in Table 1. These results show a satisfactory measurement accuracy of the MIP sensor with an acceptable RSD of 0.14% for olive oils. The measured FEN content of the olive oil samples was 0.25 pg/mL for the Al Horra commercial oil and 0.74 pg/mL for the Ouarzazate field oil.

Table 1. Detection of fen in olive oil samples.

Samples	Concentrations (mg/kg)	RSD (%) (n = 2)
Commercial oil Al Horra	0.3125	0.14
Ouarzazate field oil	0.925	0.20

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