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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 immobilised 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 characterisation. The low detection limit is 0.04 µg/mL 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. Among the most commonly used pesticides is mainly fenthion (FEN) [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 has also been applied to olive oil [6]. These techniques are generally expensive, 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, have also been 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 Organophosphorus insecticides FEN detection, such as indirect enzyme-linked immunosorbent assay (ELISA) [7] and electrochemical immunosensor [8]. Their main limitations lie in the availability of antibodies and 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 is 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 immobilising 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, Hydro-chloric acid (HCL), acetonitrile, Gold nanoparticles (AuNPs), Potassium chloride, 2-aminothiophenol (2-ATP), phosphate buffered saline (PBS), ferri-ferrocyanide ($K4[Fe(CN)_6]$, $K3[Fe(CN)_6]$), were all purchased from Sigma-Aldrich, 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 summarised 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 and incubated for 12 hours at room temperature. In the second step, the FEN pattern was deposited on the modified surface and incubated for 2 hours at room temperature. In the third step, the second layer containing 5 mM 2-ATP was electropolymerised 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 minutes. Electrochemical detection of FEN by the MIP sensor was performed by placing 10 µL 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.

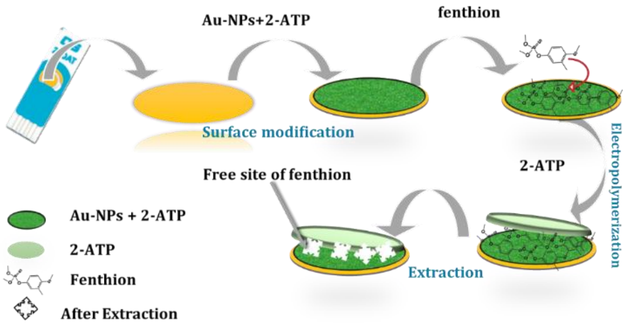


Figure 1. Representation of the experimental procedure of stepwise MIP sensor fabrication.

2.3. Electrochemical Measurements

A portable instrument (PalmSens3, Spain) was used for performing the electrochemical measurements. Screen-printed gold electrodes (Au-SPE) consisting of a three-electrode system (purchased from Dropsens), with a gold working electrode. (0.19 cm^2), a silver reference electrode and a gold counter electrode (0.54 cm^2). 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 was 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, and 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 hour at room temperature. This allowed the remaining pesticide residues in the samples to be extracted. This solution was centrifuged for 15 minutes 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. Finally, we deposited a volume of 10 µL 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 by reactions between FEN and the second layer containing 2-ATP.

In order to study the surface changes of Au-SPE (bare gold, after Electropolymerisation and extraction), the CV was used for further characterisation in a $[Fe(CN)_6]^{3-/4-}$ 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 (Ia) and potential (Ea) were recorded. As can be seen, the CV signal of the bare gold electrode is lower than the MIP current, indicating that FEN has been successfully trapped on the Au-SPE electrodes, preventing $[Fe(CN)_6]^{3-/4-}$ from diffusing onto the Au-SPE surface. In addition, the NIP sensor has a lower peak current due to the absence of FEN molecules in the polymer.

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

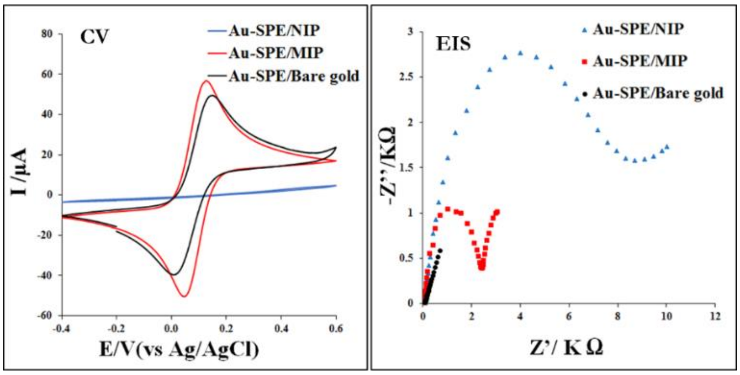


Figure 2. Cyclic voltammograms, Nyquist plots of 5 mM $[Fe(CN)_6]^{3-/4-}$ solution at bare Au-SPE, Au-SPE/MIP and Au-SPE/NIP.

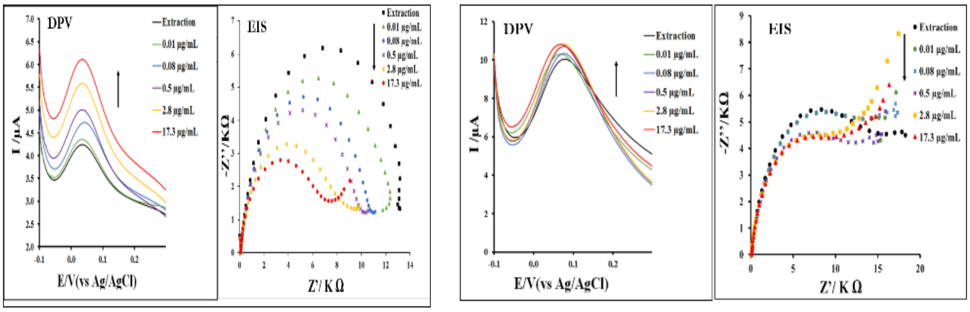
3.2. Molecular Recognition by MIP and NIP Sensors

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 µg/mL to 17.3 µg/mL. The $[Fe(CN)_6]^{3-/4-}$ was used as a mediator between the printed electrodes and the standard solutions.

In Figure 3a, the redox current peaks increase with increasing FEN concentrations. This increase may be 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 $[Fe(CN)_6]^{3-/4-}$.

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

When analysing the NIP data obtained under the same conditions, the current peaks vary slightly. This is probably due to the absence of FEN during electropolymerisation not involving the creation of specific cavities (Figure 3b). The results of the NIP Nyquist plots show semi-circular patterns and negligible change in resistance values (Rtc). 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.



(**a**) (**b**)

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

3.3. Calibration Curve and Detection Limit.

After optimising 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.

The DPV technique was performed between a potential window of -0.1 to 0.2 V. The working range for synthetic detection was 0.01 to 17.3 µg/mL.

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

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

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

Where k_i corresponds to the signal/noise ratio, (k = 3.3) for LOD and (k = 10) for quantification limit (LOQ), s is the standard deviation of the intercept and m is the slope [12]. LOD value were found to be 0.04 µg/mL for DPV measurements.

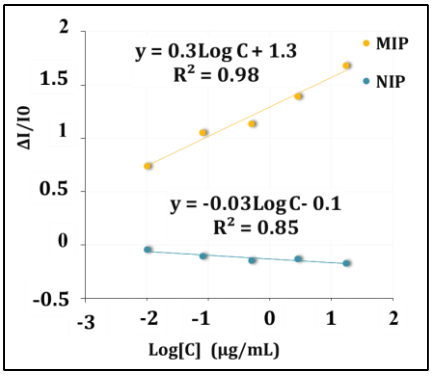


Figure 4. Calibration curves obtained by DPV for MIP and NIP sensors towards FEN.

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.

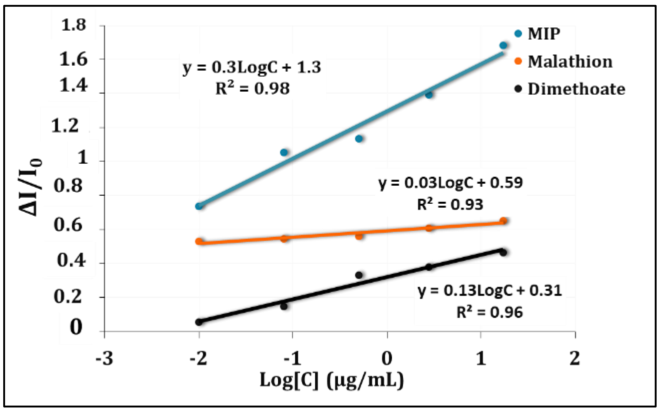


Figure 5. Calibration curves obtained by DPV for MIP towards FEN and interferences.

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 is reported in equation (2) to obtain the FEN concentration in the real samples.

$$y = 0.3\text{Log } C + 1.3, \qquad (2)$$

The results obtained are summarised 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 (pg/mL)	RSD (%) (n=2)
Commercial oil Al Horra	0.25	0.14
Commercial oil Al Horra	0.74	0.20

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Conflicts of Interest: The authors declare no conflict of interest.

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