

Interdigitated μ -electrodes for development of an impedimetric immunosensor for atrazine detection

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Abstract—This contribution describes the development of an impedimetric immunosensor for atrazine detection. This immunosensor is based on the use of interdigitated metallic μ -electrodes (ID μ Es). The method described in this work does not use any redox mediator and relies on the direct detection of the immunochemical competitive reaction between the pesticide and a haptenized-protein immobilized on interdigitated μ -electrodes for the specific antibody. The immunoreagents used were specifically developed to detect atrazine. The immunochemical detection of this pesticide is achieved without using any label. The immunosensor shows a limit of detection of $8.34 \pm 1.37 \mu\text{g L}^{-1}$, which is lower than the Maximum Residue Level (MRL) ($50 \mu\text{g L}^{-1}$) established by EU (European Union) for residues of atrazine as herbicide in the wine grapes and other foodstuff products.

I. INTRODUCTION

The combination of the recent advances obtained in electronic technology and biotechnology have resulted in the refinement and extension of the limits of detection of the diagnostic devices.

In particular, the electrochemical biosensors have revolutionized the modern chemical analysis because their simplicity and fast response [1], [2]. Nowadays, they are between the potential applications of new materials and devices.

The biomolecular recognition event conducted on the developed transducers produces changes on electric properties such as the relative permittivity that can be detected, for example by using impedance spectroscopy.

Impedance spectroscopy is a sensitive technique based on monitoring the electrical response of the device under test after the application of a periodic AC signal of small amplitude in a wide range of frequencies [3], [4], which provides significant information about the electric properties of the sensor-sample interface and the underlying reactions [5]-[7].

Between the biosensors, immunosensors (biosensors that

use antibodies as biorecognition elements) have demonstrated a large selectivity to detect a particular target. Antibodies can be produced, in principle against all kind of substances and their features can be tailored according to the requirements of each application.

In this communication we will present a label-free immunosensor to detect atrazine, a widely used pesticide. In this device the biomolecular recognition reaction is recorded measuring the changes in the impedimetric spectrum of interdigitated μ -electrodes (ID μ E) in which specific antibodies have been immobilized.

II. EXPERIMENTAL PROCEDURE AND DETECTION METHOD

A. Immunosensor description

The immunosensor developed combines non-isolated interdigitated μ -electrodes (ID μ Es) with immunoreagents specifically developed to the atrazine detection.

For the fabrication of these devices, thin Au/Cr ($\sim 150 \text{ nm}$) interdigitated μ -electrodes with bar shape and $10 \mu\text{m}$ pitch were patterned on a Pyrex glass substrate (0.7 mm (± 0.05) thickness), and finally separated in small arrays (1 cm^2) of six devices. In Figure 1 can be seen the pattern of the interdigitated μ -electrodes fabricated (1a), an enlarged SEM picture of a detail of the fingers and gaps (1b), as well as a size comparison (1c).

On the other hand, the chemical changes on the immunosensor surface follow basically two steps, Step 1: antigen immobilization on the ID μ E and Step 2: specific antibody capture in the competition step. This procedure is schematically represented in Figure 2.

B. Detection Method

The detection method used in this device consists on the impedance measurements of the system in a broad range of

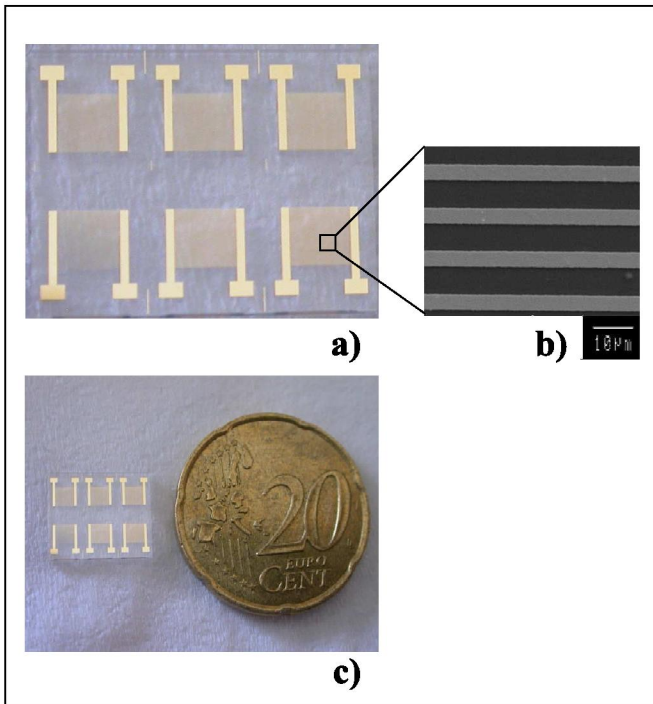


Fig. 1. Interdigitated μ -electrode array (six devices) fabricated: a) optical image; b) SEM image; c) size comparison.

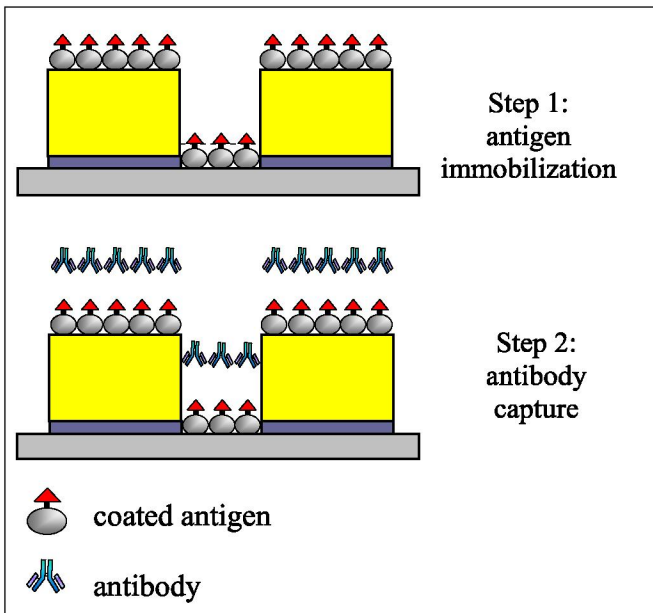


Fig. 2. Schematic diagram of the complete assay system performed on the ID μ E.

frequencies and the fitting of the measurements to the parameters of an equivalent circuit used to model impedance data. Impedimetric measurements must be taken after each functionalization step, when the antigen is immobilized on the ID μ E and after the competition step is developed. Thus, the variation of the selected parameter is plotted against concentration of atrazine to obtain the response curve.

Including the atrazine during the competition step, a fraction of the immobilized antibodies will not be available on the electrodes. Then, the amount of captured antibody will be lower than in the reference, depending on the pesticide

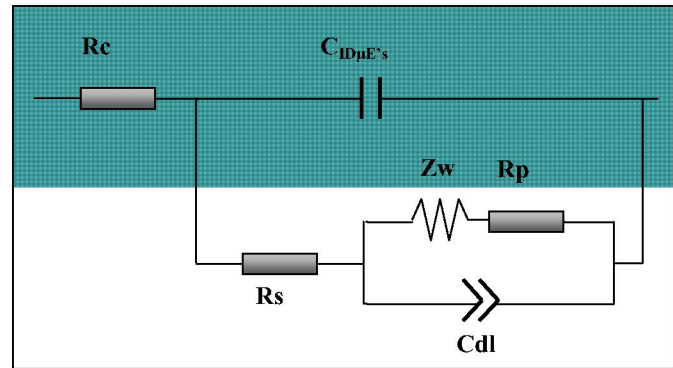


Fig. 3. Equivalent circuit of the implemented system.

concentration. This change in the antibody concentration is equivalent to the concentration of the atrazine used.

The equivalent circuit used (Figure 3) was found adequate to fit correctly the data over the entire frequency range (40 Hz-1 MHz). The circuit includes the following 6 elements:

- (i) resistance of the contacts, R_c ;
- (ii) capacitance of the ID μ E, $C_{ID\mu E}$;
- (iii) ohmic resistance of the solution (diluted PBS solution), R_s ;
- (iv) polarization resistance, R_p ;
- (v) Warburg impedance from the diffusion, W ;
- (vi) Double-layer capacitance, C_{dl} .

As it was corroborated by the measurements, the changes of the ID μ E surface show evident modifications in the impedance. Thus, the measuring system reflects mainly the chemical changes on the immunosensor surface. Then, the signal follows the changes in the concentration of the immobilized antigen, the amount of the captured antibody and the competitive equilibrium between analyte, specific antibody and the competitor antigen.

III. ATRAZINE RESIDUES DETECTION

In order to detect atrazine residues, the detection method explained above was applied. Thus, in order to detect residual amounts of atrazine, different concentrations of this pesticide were added during the competition step. Therefore, the resultant amount of specific antibody on the ID μ Es is different for each sample.

From all the elements of the equivalent circuit, the one which provided more confident and meaningful results was the ohmic resistance of the solution R_s . Therefore, the change of this parameter was used for quantify the atrazine detection.

All measurements were performed using PBS (Phosphate Buffered Saline) as medium. Since the ID μ Es fabricated are metallic and not protected by an insulator, the PBS conductivity was adjusted ($1.6 \mu S cm^{-1}$) by dilution to provide accurate and sensitive impedance measurements.

As it can be seen in Figure 4, the atrazine response of the sensor follows an inverse law and hence the response is larger at low concentrations of atrazine. This is a result of the competitive method of detection used in this work.

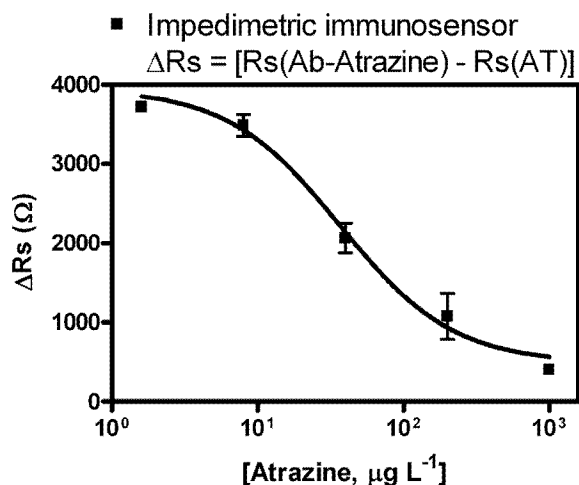


Fig. 4. Response curve of the impedimetric immunosensor for the atrazine detection in relation with the R_s variation. Measures were taken in diluted PBS solution. Reprinted from Sensors and Actuators B: Chemical, 125 / 2, E. Valera et al., Impedimetric immunosensor for atrazine detection using interdigitated μ -electrodes (ID μ E's), 526-537 (2007), with permission from Elsevier.

As it was commented before, impedance measurements were taken in Step 1 and in Step 2. Thus, the change in the value of R_s (denoted ΔR_s in Figure 4) is plotted against the atrazine concentrations.

The limit of detection (LOD) obtained for the atrazine residues detection using the immunosensor presented in this work is $8.34 \pm 1.37 \mu\text{g L}^{-1}$. The Maximum Residue Level required by EC for the atrazine in the wine grapes is $50 \mu\text{g L}^{-1}$. Therefore, the LOD obtained using the impedimetric device presented in this work is well below this MRL.

IV. CONCLUSION

An impedimetric immunosensor for the quantification of atrazine residues has been developed. This immunosensor has already demonstrated high sensibility to atrazine, detecting atrazine concentrations below the Maximum Residue Level required by EC for the atrazine in the wine grapes.

Although in this work, the sensor characterization has been performed for atrazine, this immunosensor concept has been thought of as a more general sensing methodology for the detection of any chemical if the appropriate antibody and competitor are available.

The fabrication of the sensor described above is fast, simple and inexpensive in mass production, the results are sensitive and the operation is user-friendly. The assay relies on the immunochemical competitive reaction between the pesticide and the immobilized antigen on ID μ Es for a small amount of the specific antibody. The use of a non-passivated interdigitated μ -electrode increases the device sensitivity to the changes on the chemical sensitive layer at the cost of use a low conductivity buffer instead the conventional isotonic buffers.

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