

## Rapid Detection Technology for Pesticides Residues Based on Microelectrodes Impedance Immunosensor

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**Abstract:** Compared with conventional methods, electrochemical immunosensors have many advantages, such as low cost, high sensitivity, and rapid detection, and has certain prospects for realizing real-time-monitoring. In this paper, a design of portable pesticide residues detection instrument was presented based on an electrochemical impedance immunosensor. Firstly, we studied on an impedance immunosensor based on interdigitated array microelectrode (IDAM) coupled with magnetic nanobeads-antibody conjugates (MNAC) for the pesticide detection. Magnetic nanobeads (diameter 150 nm) coated with anti-carbofuran antibodies were used for further amplification of the binding reaction between antibody and hapten (carbofuran). Secondly, in order to develop a portable pesticide residue apparatus, we designed the impedance detection electric circuit. Main work included designing and constructing of the system circuit, designing and debugging of the system software and so on. Thirdly, the apparatus was used for the standard pesticides solutions testing combined with immunosensor to test the reliability and stability. The pesticide added standard recovery was more than 70 % and the impedance test error was less than 5 %. The results showed that the proposed instrument had a good consistence compared with the traditional analytical methods. Thus, it would be a promising rapid detection instrument for pesticide residues in agricultural products. Copyright © 2014 IFSA Publishing, S. L.

**Keywords:** Pesticide residues, Immunosensor, Interdigitated array microelectrode, Signal detection, Portable instrument.

### 1. Introduction

Pesticides are widely used in agriculture to protect seeds and crops, which lead to the most important environmental pollutants and cause severe impairment of human health. Currently, chemical analysis of pesticide residues is monitored by various analytical techniques (i.e. gas chromatography (GC), high-pressure liquid chromatography (HPLC), capillary electrophoresis (CE) and mass spectrometry

(MS)) [1-3]. These conventional methods are very sensitive and reliable, but have disadvantages such as complexity, extensive time consumption, and the need for costly, bulky instrumentation [6]. For these reasons, the development of rapid and efficient monitoring methods becomes more and more important.

In recent years the generation of antibodies against small pesticide haptens has seen significant progress leading to the introduction of several

immunoassays for environmentally sensitive small toxic molecules [7-10]. As consequence, immunochemical methods, such as enzyme-linked immunosorbent assays (ELISAs), have already gained a place in the analytical benchtop as alternative or complementary methods for routine pesticide analysis. They are fast, economic, and at least as sensitive as usual chromatographic techniques. However, the analyte detection in ELISAs need label one of the immunoreagents, which need extensive sample handling such as rather large number of washing steps. On the contrary, for immunosensors: one of the immunoreagents is immobilized on the surface of the transducer, and a direct physical signal is produced when the immunochemical interaction occurs. This label-free detection represents an essential advantage of immunosensors as compared to label-dependent immunoassays. As consequence, a great number of research papers have appeared over the last years describing the development of novel immunosensors for detecting trace amounts of pesticide residue in environmental and food samples.

Among the immunosensors, the electrochemical impedance immunosensors for pesticide residue detection have attracted extensive interest in recent years [11-12]. Electric impedance spectroscopy (EIS) is a sensitive technique, which detects the electrical response of the system studied after application of a periodic small amplitude AC signal. Impedance immunosensors detect the pesticide residue concentrations by the measurement the changes of electrical conductivity of the solution and capacity due to the Ab-Ag interaction on the electrode surface, which also can be reflected in impedimetric response. Enrique Valera's group [13-15] had reported a series of methods about impedance immunosensor for atrazine detection based on the interdigitated microelectrode array. However, the detection of pesticide residue such small molecule compound is usually performed under competitive conditions involving the competition between the free antigen (analyte) and a fixed amount of coated antigen for a limited amount (low concentration) of antibody (Ab).

As it is well known, the competitive assay is complex. In order to obtain simpler and faster immunosensing methodology, direct non-competitive electroanalytical detection of pesticide residue has also been studied [16], although the sensitivity reached is not very high due to biomolecular recognition are generally very small. It requires offer highly sensitive small molecule immunosensor technologies through careful consideration of sensor interface design and signal enhancement. The use of an array of microelectrodes can overcome the disadvantage, because one of the main benefits of using a microelectrode in a sensor application is the greater sensitivity that arises from the enhanced mass-transport at these small electrodes. Hemispherical diffusion layers are formed at such electrodes and a much faster diffusion of electroactive substances occurs due to the multi-

dimensional nature of this process, resulting in sigmoidal (or steady-state) cyclic voltammograms (CVs). The advantages are in the improved response time (faster response), greater sensitivity and increased response per unit electrode surface area (greater current density, increasing the signal-to-noise ratio) [17].

However, the use of the electrode surface as a solid phase for antibody immobilization as well as electrochemical transducer may result in a reduced electrochemical signal. Alternatively, magnetic beads (MBs) have been emerged as powerful and adaptable tool for the development of immunosensing platform for food contaminants [18-19]. It has been reported that MBs based immunoassays have many advantages over conventional ones:

- i) Magnetic nanobeads can automatically purify and concentrate target analytes by a magnet;
- ii) the matrix effect can be also minimized due to improved washing and separation steps which allows the analysis to be made without any pre-enrichment, purification, or pre-treatment steps;
- iii) MBs can provide a high surface area to immobilize the biomolecules as many as possible, leading to a lower detection limit;
- iv) MBs can quicken the velocity of assay kinetics because the beads are in suspension [20];
- v) MBs can short interaction time between biological component and the coated nanobeads because of increase in surface to volume ratio. To our knowledge, immunosensor based on MBs approach for carbofuran pesticides detection is not yet reported.

The immune responses as a signal could be quantified and handled through the instrument. By processing output, the pesticide concentration is ultimately obtained. Electrochemical analysis instrument is a universal instrument, not a specific detection instrument [21].

The development direction of biosensor technologies have been toward miniaturized multifunction biosensor array system which equipped with optical excitation and detection, current measurement and flow control systems [22]. Schöning *et al.* have fabricated a flow-injection system with dual amperometric and potentiometric organophosphorus pesticides (OP) biosensors for the simultaneous and rapid measurements of OP compounds was described [23]. Grossi *et al.* have developed an embedded portable biosensor system for bacterial concentration [24].

Here, an impedance circuit was designed to construct a portable detection instrument based on the impedance immunosensor combining the advantages of MBs and IDAM. Our work is based on the following steps:

- 1) An impedance immunosensor based on interdigitated array microelectrode (IDAM) coupled with magnetic nanobeads-antibody conjugates (MNAC) was fabricated for the pesticide detection. The linear regression equations for impedance difference and the concentration of pesticide were obtained.

2) An impedance circuit was designed to construct a portable detection instrument based on the impedance immunosensor.

3) The immunosensor and the impedance detection circuit were integrated to achieve the integration detection system, which met the requirements of detecting the pesticide residues fast and on-line test. A good precision, high stability and accuracy of the pesticide residues detection instrument in standard pesticide solutions were investigated.

## 2. Experimental and Methods

### 2.1. Chemicals and Reagents

Carbofuran was purchased from Sigma (St. Louis, USA). Affinity purified polyclonal antibodies against carbofuran were obtained from company of Germany. Phosphate buffered saline (PBS, 10 mM, pH 7.4) was purchased from Sigma-Aldrich (St. Louis, MI). All solutions were prepared with deionized water from Millipore (Milli-Q, 18.2 MΩcm, Bedford, MA). The rest of the reagents are analytically pure or above.

Magnetic nanoparticles (average diameter 150 nm, 0.5 mg Fe ml<sup>-1</sup>) conjugated with streptavidin were obtained from Molecular Probes Inc. (Eugene, OR). Magnetic nanoparticles have more than 85 % of oxide as Fe<sub>3</sub>O<sub>4</sub>, approximately 80 % wt wt<sup>-1</sup> of magnetite, and an approximately 4×10<sup>11</sup> particles mg<sup>-1</sup> Fe.

### 2.2. Apparatus and Impedance Measurement

Gold interdigitated array microelectrodes (IDAM, IME AU-1550.5) were purchased from AB-tech Scientific Inc. (Richmond, VA). Each electrode had 50 digital pairs with 15 μm digit width, 15 μm interdigit space, and a digit length of 4985 μm. The electrode area was calculated as 14.88 mm<sup>2</sup>. Gold IDAM chip was obtained from ABtech Scientific Inc. (Richmond, VA), the IDAM chip was cleaned with 0.1M sodium hydroxide (15 min), 0.1M hydrochloric acid (15 min), acetone (5 min), and deionized water, and then was dried in a stream of nitrogen.

Electrochemical Impedance measurements were performed by using an IM-6 impedance analyzer

(BAS, West Lafayette, IN) with IM-6/THALES software. All impedance measurements were conducted in the presence of deionized water was used as a redox probe. The tested frequency range was from 10 Hz to 1 MHz with amplitude of 5 mV. Bode (impedance and phase vs. frequency) and Nyquist (imaginary impedance vs. real impedance) diagrams were recorded. Pesticides residues detection system was made in our laboratory.

### 2.3. Preparation of Magnetic Nanoparticle-antibody Conjugates and Immunomagnetic Separation

1) Biotin-labeled polyclonal anti-carbofuran antibody preparation: the polyclonal anti-carbofuran antibody was labeled with biotin using the EZ-Link Sulfo-NHS-Biotinylation Kit (Pierce, Rockford, IL) according to the supplied instruction. Briefly, 100 μL antibody was mixed with 3 μL of Sulfo-NHS-Biotin solution (10 mM) into 200 μL of PBS (10 mM, pH 7.4) and incubated at room temperature for 60 min. Then, excess biotin was removed by using Slide-A-Lyzer Dialysis Cassettes. The biotin-labeled antibody was further diluted 1:2 with PBS and stored at 4°C until ready to be coupled with the streptavidin-coated magnetic nanobeads.

2) Magnetic separation:

i) 25 μL 150 nm streptavidin-coated magnetic nanobeads with 250 μL PBS. Apply magnet for 5 min then remove the waste.

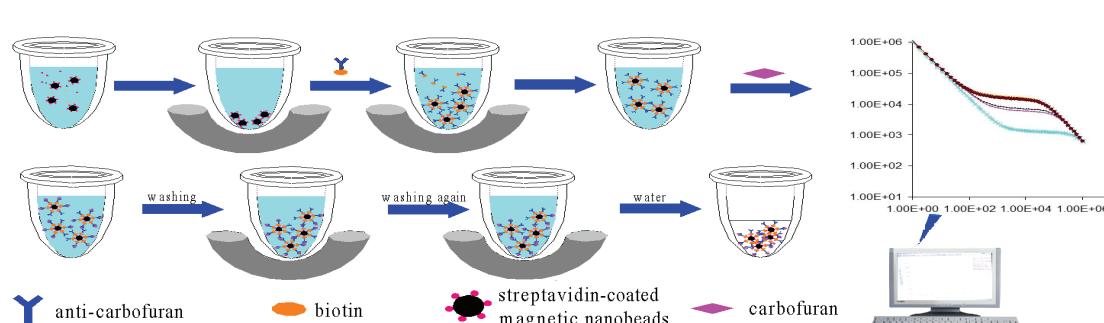
ii) Add 150 μL PBS and 75 μL biotin-antibody to 150 nm beads. Mix for 30 min (15rpm). Apply magnet for 5 min. Wash beads with 150 μL PBS. Apply magnet for 5 min, then remove the waste.

iii) Add 200 μL carbofuran samples (5 ug/mL, 500 ng/mL, 50 ng/mL and 1 ng/mL) for 30 min at 37 °C. Apply magnet for 5 min, then remove the waste. Every concentration replicates three times

iv) Wash beads with 150 μL water. Apply magnet for 5 min, then remove the waste, suspend beads in 60 μL water, then perform impedance measurements.

v) Wash beads with 150 μL water. Suspend beads in 60 μL water, and then perform impedance measurements.

The fabrication process of immunosensor was shown in Fig. 1.



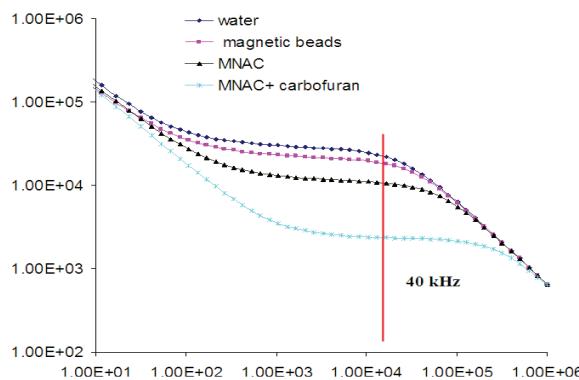
**Fig. 1.** Schematic illustration of immunosensor fabrication process.

## 2.4. Impedance Measurement and the Standard Curve Extracting

A calibration curve for normalized impedance change (NIC) and concentrations of carbofuran was drawn based on the difference of magnitude of impedance with respect to the control. The value of NIC was given by following formula:

$$NIC = (Z_{sample} - Z_{control})/Z_{control} \times 100 \%,$$

where  $Z_{control}$  is the magnitude of impedance for control sample, and  $Z_{sample}$  is the magnitude of impedance for a sample containing carbofuran. An average of three readings and their standard deviation were calculated and analyzed for each concentration of carbofuran.



**Fig. 2.** Bode diagrams of impedance spectra of IDAM based impedance biosensor for water, magnetic beads, MNAC, carbofuran (50 ng/ml) attached to MNAC. The maximum difference in NIC values was 40 kHz.

According to our previous research, a linear relationship was found in the carbofuran concentration range between 1 ng/mL and 200 ng/mL and the linear regression equations for

impedance difference and the concentration of pesticide was  $y=24.538 \lg C (\text{ng/mL}) + 34.289$  ( $R^2 = 0.9937$ ). Fig. 2 shown the maximum difference in NIC values was 40 kHz.

## 3. Experimental

### 3.1. Schematic Drawing of Detection Device Structure

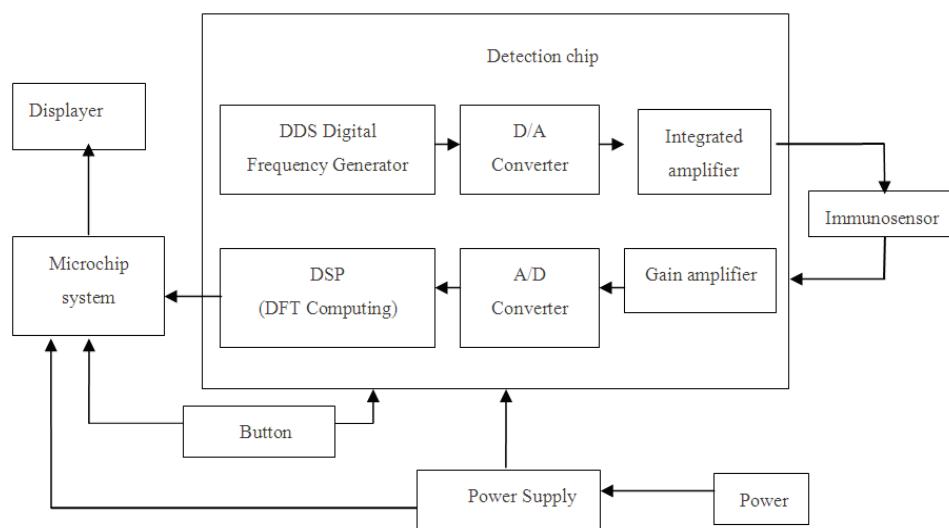
The process of impedance detection could be realized in this way: firstly, when antigen- antibody specific immune response happened, the impedance signal strength was changed. Secondly, AD5933 generated excitation signal which applied to the test impedance, and collected response signal. Thirdly, digital processing DFT module outputted the result to microcontroller. Finally, the microcontroller would compare result with the previous impedance value and get the difference. Compared the rate of change with the standard curve, the microcontroller would output the conclusions about pesticide concentration. The consequence of the detection was displayed on the LCD screen under the control of microchip. The schematic drawing of designed detection system based on the microchip was shown in Fig. 3.

### 3.2. Circuit Board Design

The wiring diagram of the circuit board as shown in Fig. 4.

Fig. 5 was PCB bare board fabricated by the plate making company.

Circuit board with components welding on was shown in Fig. 6.



**Fig. 3.** Schematic drawing of detection device structure.

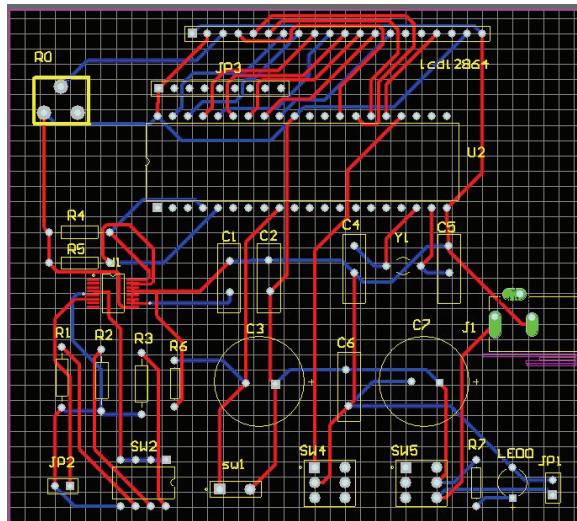


Fig. 4. Double panel wiring diagram.

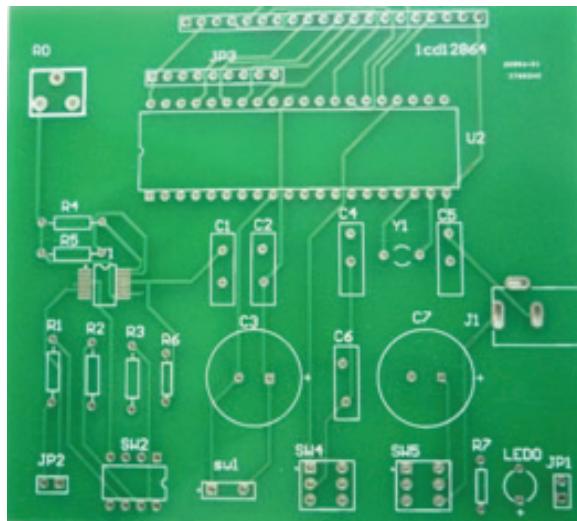


Fig. 5. PCB bare circuit board.

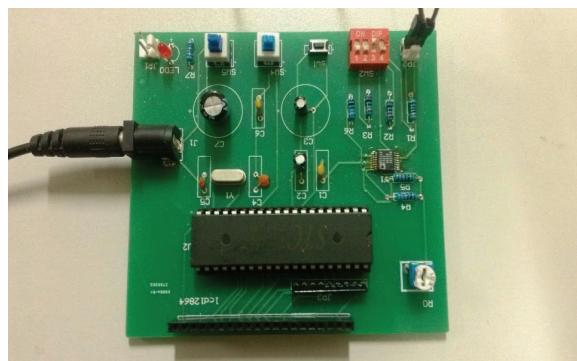


Fig. 6. Circuit board with components welding on.

## 4. Results and Discussion

### 4.1. Impedance Analyzer and Instrument Connected with the Immunosensor

Fig. 7 was the immunosensor connected with IM-6 impedance analyzer to detect the pesticide.

Fig. 8 was the immunosensor connected with rapid detection instrument to detect the pesticide.

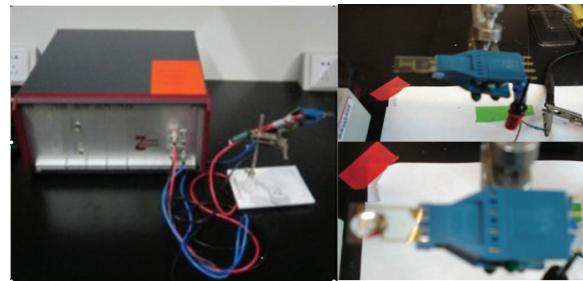


Fig. 7. Biosensor connected with IM-6 impedance analyzer.



Fig. 8. Biosensor connected with rapid detection instrument.

### 4.2. The test of Instrument's Stability

Compared the error between the two values, we got a relative error rate. Results were summarized in Table 1. The impedance test error was less than 5 % in this detection circuit. The results showed that the detection circuit was stable and the test error was acceptable.

Table 1. The measurement data of component.

| Calibration impedance (kΩ) | Measure-<br>ment point frequency (kHz) | The measure-<br>ment values of the multimeter (kΩ) | The measure-<br>ment values of design (kΩ) | Relative<br>error (%) |
|----------------------------|--|--|--|-----------------------|
| 1                          | 40                                     | 0.97   | 0.954                                      | 1.7                   |
| 5                          | 40                                     | 4.97   | 4.764                                      | 4.3                   |
| 6                          | 40                                     | 5.87   | 5.653                                      | 4.4                   |
| 10                         | 40                                     | 9.47   | 9.37                                       | 3.8                   |
| 100                        | 40                                     | 96.8   | 94.984                                     | 1.9                   |

### 4.3. The Detection of the Carbofuran Standard Pesticide

To further demonstrate the practicality of the proposed detection instrument, we tested 8 groups of 50 ng/mL concentration of standard Carbofuran solutions. Results were summarized in Table 2. It indicated that the proposed detection circuit was accurate, reliable and reproducible. It can be used for

directing analysis of practical samples. The linear regression equations for inhibition rate and the concentration of pesticide were  $y=24.538\lg C$  (ng/mL) +34.289 ( $R^2 = 0.9937$ ), where y was the value of inhibition rate and C was the concentration of pesticide.

**Table 2.** The detection result of standard pesticide solutions by immunosensor.

| Group number | Results of standard pesticide solutions |                       |              |
|--------------|---|-----------------------|--------------|
|              | Inhibition ratio (%)                    | Concentration (μg/mL) | Recovery (%) |
| 1            | 75.60                                   | 48.3                  | 96.6         |
| 2            | 74.02                                   | 41.6                  | 83.2         |
| 3            | 73.68                                   | 40.3                  | 80.6         |
| 4            | 73.54                                   | 39.8                  | 79.6         |
| 5            | 73.27                                   | 38.8                  | 77.6         |
| 6            | 74.94                                   | 45.4                  | 90.8         |
| 7            | 74.47                                   | 43.4                  | 86.8         |
| 8            | 72.30                                   | 35.4                  | 70.8         |

The overall performance of the present instrument showed the capability of the pesticide residues detection with good sensitivity and high practical value. From the Table 1, it can be seen that the impedance test error was below 5 %. We can draw the pesticide added standard recovery was more than 70 % from Table 2. The accuracy of measurement was acceptable and could meet the requirements of rapid detection of pesticide residues.

## 5. Conclusions

In this paper, we developed a miniaturization and portable pesticide residues detection instrument based on highly sensitive impedance immunosensor for carbofuran detection.

The use of MNAC provided an efficient and specific way to separate carbofuran pesticides residues from real fruit juice samples and to concentrate carbofuran attached to MNAC in the active layer of IDAM with the help of a magnetic field, thereby enhancing the sensitivity of IDAM based impedance biosensor.

The instrument was integrated by the impedance immunosensor and signal detection circuit, which had been tested on standard resistance and carbofuran concentration. The results showed that the proposed instrument was reliable, and could meet the rapid pesticide residues detection requirements. It would be a promising rapid detection instrument for pesticide residues in agricultural products.

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## References

- [1]. Santos F. J., Galceran M. T., Modern developments in gas chromatography-mass spectrometry-based environmental analysis, *Journal of Chromatography*, A, 1000, 1-2, 2003, pp. 125-151.
- [2]. S. Laschi, D. Ogończyk, I. Palchetti, M. Mascini, Evaluation of pesticide-induced acetylcholinesterase inhibition by means of disposable carbon-modified electrochemical biosensors, *Enzyme Microbial Technology*, Vol. 40, Issue 3, 2007, pp. 485-489.
- [3]. A. N. Ivanov, L. V. Lukachova, G. A. Evtugyn, E. E. Karyakina, S. G. Kiseleva, H. C. Budnikov, A. V. Orlov, G. P. Karpacheva, A. A. Karyakin, Polyaniline-modified cholinesterase sensor for pesticide determination, *Bioelectrochemistry*, Vol. 55, Issue 1-2, 2002, pp. 75-77.
- [4]. Rial-Otero R., Gaspar E. M., Moura L., et al., Chromato-graphic-based methods for pesticide determination in honey: An overview, *Talanta*, 7, 2, 2007, pp. 503-514.
- [5]. Chen G., Cao P., Liu R., A multi-residue method for fast determination of pesticides in tea by ultra performance liquid chromatography-electrospray tandem mass spectrometry combined with modified QuEChERS sample preparation procedure, *Food Chemistry*, 125, 4, 2011, pp. 1406-1411.
- [6]. Raman Suri C., Boro R., Nangia Y., Gandhi S., Sharma P., Wangoo N., Rajesh K., Shekhawat G. S., Immunoanalytical techniques for analyzing pesticides in the environment, *Trends in Analytical Chemistry*, 28, 1, 2009, pp. 29-39.
- [7]. Keay R. W., McNeil C. J., Separation-free electrochemical immunosensor for rapid determination of atrazine, *Biosensors and Bioelectronics*, 13, 9, 1998, pp. 963-970.
- [8]. Xuesong Jiang, Dongyang Li, Xia Xu, Yibin Ying, Yanbin Li, Zunzhong Ye, Jianping Wang, Immunosensors for detection of pesticide residues, *Biosensors and Bioelectronics*, 23, 11, 2008, pp. 1577-1587.
- [9]. Enrique Valera, Javier Ramón-Azcón, Ángel Rodríguez, Luis M. Castañer, F.-J. Sánchez, M.-P. Marco, Impedimetric immunosensor for atrazine detection using interdigitated  $\mu$ -electrodes (ID $\mu$ E's), *Sensors and Actuators B: Chemical*, 125, 2, 2007, pp. 526-537.
- [10]. Javier Ramón-Azcón, Enrique Valera, Ángel Rodríguez, Alejandro Barranco, Begoña Alfaro, Francisco Sanchez-Baeza, M.-Pilar Marco, An impedimetric immunosensor based on interdigitated microelectrodes (ID $\mu$ E) for the determination of atrazine residues in food samples, *Biosensors and Bioelectronics*, 23, 9, 2008, pp. 1367-1373.
- [11]. Enrique Valera, David Muñiz, Ángel Rodríguez, Fabrication of flexible interdigitated  $\mu$ -electrodes (FID $\mu$ E's) for the development of a conductimetric immunosensor for atrazine detection based on antibodies labelled with gold nanoparticles, *Microelectronic Engineering*, 87, 2, 2010, pp. 167-173.

- [16]. Hu S. Q., Xie J. W., Xu Q. H., Rong K. T., Shen G. L., Yu, R. Q., A label-free electrochemical immunosensor based on gold nanoparticles for detection of paraoxon, *Talanta*, 61, 6, 2003, pp. 769-777.
- [17]. Charlie O. Parker, Yvonne H. Lanyon, Mary Manning, Damien W. M. Arrigan, Ibtisam E. Tothill, Electrochemical Immunochip Sensor for Aflatoxin M1 Detection, *Anal. Chem.*, 81, 2009, pp. 5291-5298.
- [18]. Moreira Â. N., Conceição F. R., Conceição R. C. S., et al., Detection of *Salmonella Typhimurium* in Raw Meats using In-House Prepared Monoclonal Antibody Coated Magnetic Beads and PCR Assay of the *fimA* Gene, *Journal of Immunoassay and Immunochemistry*, 29, 1, 2007, pp. 58-69.
- [19]. Lee J. H., Choi S. J., Isolation and characteristics of sorbitol-fermenting *Escherichia coli* O157 strains from cattle, *Microbes and Infection*, 2006, 8, 8, pp. 2021-2026.
- [20]. Mattingly J. A., An enzyme immunoassay for the detection of all *Salmonella* using a combination of a myeloma protein and a hybridoma antibody, *Journal of Immunological Methods*, 1984, 73, 1, pp. 147-156.
- [21]. X. Sun, X. Y. Wang, S. Y. Du, Y. Zhu, A Weak Current Detection Circuit design of Organophosphorous Pesticides Rapid Detection Portable Instrument, *Advanced Materials Research*, Vol. 403-408, 2012, pp. 2569-2572.
- [22]. V. Scognamiglio, I. Pizzotti, G. Pezzotti, J. Cano, I. Manfredonia, K. Buonasera, F. Arduini, D. Moscone, G. Palleschi, M. T. Giardi, Towards an integrated biosensor array for simultaneous and rapid multi-analysis of endocrine disrupting chemicals, *Anal. Chim. Acta*, Vol. 751, 2012, pp. 161-170.
- [23]. M. J. Schöning, R. Krause, K. Block, M. Musahmeh, A. Mulchandani, J. Wang, A dual amperometric/potentiometric FIA-based biosensor for the distinctive detection of organophosphorus pesticides, *Sens Actuators B: Chem.*, Vol. 95, Issue 1-3, 2003, pp. 291-296.
- [24]. M. Grossi, M. Lanzoni, A. Pompei, R. Lazzarini, D. Matteuzzi, B. Riccò, An embedded portable biosensor system for bacterial concentration detection, *Biosensors and Bioelectronics*, Vol. 26, 2010, pp. 983-990.

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