Direct competitive immunosensor for Imidacloprid pesticide detection on gold nanoparticle-modified electrodes

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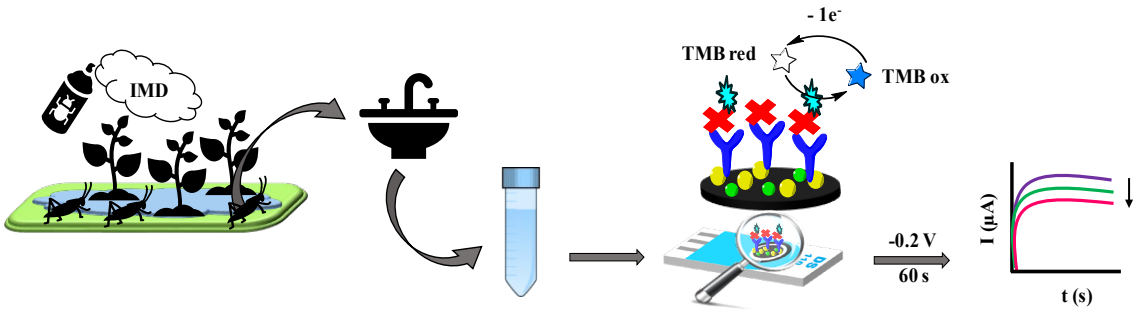
ABSTRACT

A direct competitive immunosensor for the electrochemical determination of Imidacloprid (IMD) pesticide on gold nanoparticle-modified screen-printed carbon electrodes (AuNP-SPCE) is here reported for the first time. Self-obtained specific monoclonal antibodies are immobilized on the AuNP-SPCE taking advantage of the AuNPs biofunctionalization abilities. In our biosensor design, free IMD in the sample competes with IMD conjugated with horseradish peroxidase (IMD-HRP) for the recognition by the antibodies. After that, 3,3′,5,5′-Tetramethylbenzidine (TMB) is enzymatically oxidized by HRP, followed by the oxidized TMB reduction back at the surface of the SPCE. This process gives an associated catalytic current (analytical signal) that is inversely proportional to the IMD amount. The main parameters affecting the analytical signal have been optimized, reaching a good precision (repeatability with a RSD of 6%), accuracy (relative error of 6%), stability (up to one month), selectivity and an excellent limit of detection (LOD of 22 pmol L^{-1}), below the maximum levels allowed by the legislation, with a wide response range (50 – 10000 pmol L^{-1}). The detection through antibodies also allows to have an excellent selectivity against other pesticides potentially present in real samples. Low matrix effects were found when analysisng IMD in tap water and watermelon samples. The electrochemical immunosensor was also validated with HPLC-MS/MS, the reference method used in official laboratories for IMD analysis, through statistical tests. Our findings make the electrochemical immunosensor as an outstanding method for the rapid and sensitive determination of IMD at the point-of-use.

GRAPHICAL ABSTRACT



KEYWORDS

Electrochemical immunosensor, Screen-printed Carbon Electrodes, Imidacloprid, Point-of-use, Pesticides, Nanoparticles

1. INTRODUCTION

According to the World Health Organization (WHO), more than 200 diseases are produced by the contamination of food, either by microbiological or chemical agents [1]. Therefore, it is necessary to keep a strict food control to ensure food safety. Pesticides are among the most toxic chemical agents for any living being that can cause cancer, problems in the reproductive and central nervous systems and finally death due to the continuous exposure. Most exposed people are the agricultural workers and also the consumers of fruits and vegetables, so exhaustive controls of pesticides must be carried in such samples, as well as in wastewater.

The official methods for the determination of pesticides are usually based on chromatographic techniques such as high-performance liquid chromatography-mass spectrometry-mass spectrometry (HPLC-MS/MS) and gas chromatography–mass spectrometry-mass spectrometry (CG-MS/MS) [2–5]. To perform these analyses, specialized personnel is needed, requiring long procedures, as well as a large volume of reagents with a high cost. In this context, point-of-care (POC) biosensors have emerged to overcome such limitations. In particular, electrochemistry is being increasingly used to determine pesticides (imidacloprid, acetamiprid, thiamethoxan, parathion, etc.) [6–9] and heavy metals (mercury, lead, cadmium, chromium, etc.) [10–15]. These compounds can be directly measured through red-ox processes [9,16–18] but biosensors using enzymes or monoclonal antibodies have emerged as advantageous alternatives with improved sensitivity and selectivity [19–21]. Competitive biosensors [22] are of special relevance here, since the small size of the pesticide molecules usually makes difficult to perform typical sandwich assays.

The main advantages of such electrochemical biosensors are related to the low cost of instruments and analysis, low volume of reagents and samples, portability, simplicity and high sensitivity [23,24].

Currently, the most used electrochemical platforms are screen-printed carbon electrodes (SPCE) [25]. These are small devices that contain an electrochemical cell made by a reference electrode, a working electrode and a counter electrode. The SPCEs have an easily modifiable carbon working electrode in which metallic nanoparticles such as Ni [7,26], Cu [27–29], Pt [30,31], Au [32–34], bi-metallic nanoparticles [35–38], nanowires [39], nanotubes [40] or graphene oxide [19,41] can be deposited, increasing the electroactive surface and catalysing redox reactions. In particular, gold nanoparticles (AuNP) are the most widely used ones, since they increase the effectiveness of the immobilization of recognition elements such as antibodies and enzymes, as well as facilitate the electronic transfer between the biomaterial and the electrode.

In this scenario, we have developed a direct competitive immunosensor for Imidacloprid (IMD) pesticide on AuNP-modified SPCEs. IMD is a pesticide belonging the neonicotinoid family widely used as a neuroactive insecticide for the control of pests, seed treatment and as a systemic insecticide, having a long residual effect on the soil [42]. Such pesticide is a relevant target, since it can cause the syndrome called Colonial Collapse Disorder (CCD) [43,44] in colonies of bees. The disappearance of bees leads to an important environmental problem reducing pollination and thereby decreasing the diversity of flora and plant species. Moreover, it is also classified as a probable carcinogen by the Environmental Protection Agency (EPA) [45,46]. Maximum Residue Limits (MRLs) are established for food, for human and animal consumption, that are exposed to pesticides, which in the European Union are 0.5 μg L^{-1} for total pesticides and 0.1 μg L^{-1} for each individual pesticide [47,48]. This legislation is a requirement for the

commercialization of the raw material and its products. Therefore, very sensitive methods for IMD detection are strongly required.

In our particular approach, a competitive direct assay between free IMD and IMD labelled with horseradish peroxidase (HRP) enzyme is performed. Such direct format presents clear advantages compared with indirect assays [49], avoiding the use of secondary antibodies and leading to a faster and cheaper methodology suitable for *in field* analysis.

2. EXPERIMENTAL SECTION

2.1. Materials

Imidacloprid PESTANAL® (IMD), Glyphosate PESTANAL® (GLY), Parathion PESTANAL® (PAR), Thiamethoxam PESTANAL® (THIA), Carbendazim PESTANAL® (CARB), Phosphate buffered saline (PBS) 10 mmol L^{-1} pH 7.4, Bovine Serum Albumin fraction V (BSA), anti-mouse IgG (produced in goat), 3,3',5,5' tetramethylbenzidine (TMB) and potassium hexacyanoferrate (III) were purchased from Sigma-Aldrich (Spain). Permethrin (PERM) was provided by LGC Standars (Spain). Monoclonal antibody to IMD (mAb-IMD) and horseradish peroxidase conjugated to IMD (IMD-HRP) were kindly provided by the University of Valencia and IATA-CSIC (Valencia, Spain). Gold-standard solution ($HAuCl_4$. $3H_2O$ in 12.7 % HCl) and Hydrochloric Acid 25 % (HCl) were obtained from Merck (Spain). WAT020515 - Sep-Pak C18 Plus Short Cartridge was provided by Waters (Spain). Imidacloprid ELISA detection kit was purchased from Abraxis (USA). Ultrapure water obtained with a EMD Millipore™ Direct-Q5™ purification system from Millipore Ibérica SA. (Madrid, Spain) was used throughout this work. All chemicals employed are of analytical reagent grade.

Working solutions of mAb-IMD and IMD-HRP were daily prepared in 10 mmol L^{-1} pH 7.4 PBS buffer.

HPLC-MS/MS experiments for the determination of IMD were carried out using a liquid chromatograph model 1260 Infinity from Agilent (Germany) coupled to a triple quadrupole mass spectrometer model 6460 also from Agilent (Germany). The mobile phases were (A) water/methanol (80 : 20) with 5 mmol L^{-1} ammonium acetate and (B) acetonitrile with 5 mmol L^{-1} ammonium acetate.

Screen-printed carbon electrodes (SPCEs) (carbon working and counter electrodes and a silver/silver chloride quasi-reference electrode) (ref. DRP-110) were purchased from Dropsens (Spain). Electrochemical measurements were performed at room temperature using a potentiostat/galvanostat μAutolab Type II from Ecochemie (The Netherlands).

A JEOL 6610LV scanning electron microscope was used to characterize the sensor surface.

2.2. Methods

2.2.1. Direct competitive immunoassay

AuNPs were generated following a previously reported protocol [11][32], consisting in applying a constant current (-100 μA) for 180 s in a solution of 1 mmol L^{-1} $HAuCl_4 \cdot 3H_2O$ in HCl 0.1 mol L^{-1}. In this way, a homogeneous distribution of AuNPs of 16 ± 3 nm electrodeposited on the working area of the SPCE electrode is obtained, as expected (**Figure 1**). Then, the electrodes were washed with ultrapure water and stored at 4°C before further use.

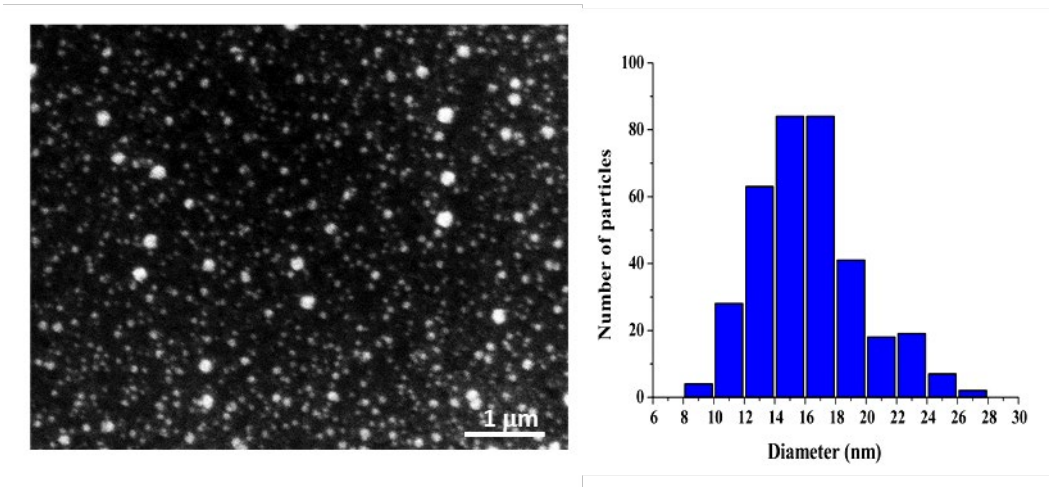


Figure 1. SEM images and histogram plot of the size distribution of AuNPs electrodeposited on the working area of the SPCE electrode.

The effect of the AuNPs on the electroactive area of the electrode was also studied (see S1 at the supporting information). As expected, we found an increase in the electroactive area from 6.63 ± 0.36 mm2 (unmodified electrodes) to 7.70 ± 0.22 mm2 (AuNP-modified electrodes) that may lead to a slight improve in the sensor response

The scheme of the direct competitive immunoassay is presented in **Figure 2**. 10 µL of 6 µg mL^{-1} mAb-IMD solution in 10 mmol L^{-1} PBS (pH 7.4) were dropped on the surface of the AuNP-modified working electrode and incubated at 4 ºC overnight. After washing with water, 40 µL of 1% BSA were dropped on the electrode and incubated for 40 min to block the surface and avoid unspecific absorptions. 10 µL of sample or free IMD at different concentrations in 10 mmol L^{-1} PBS (pH 7.4) were dropped and left there for 1 h, before washing. Then, 10 µL of 3 µg mL^{-1} IMD-HRP solution were added and incubated for 1 h.

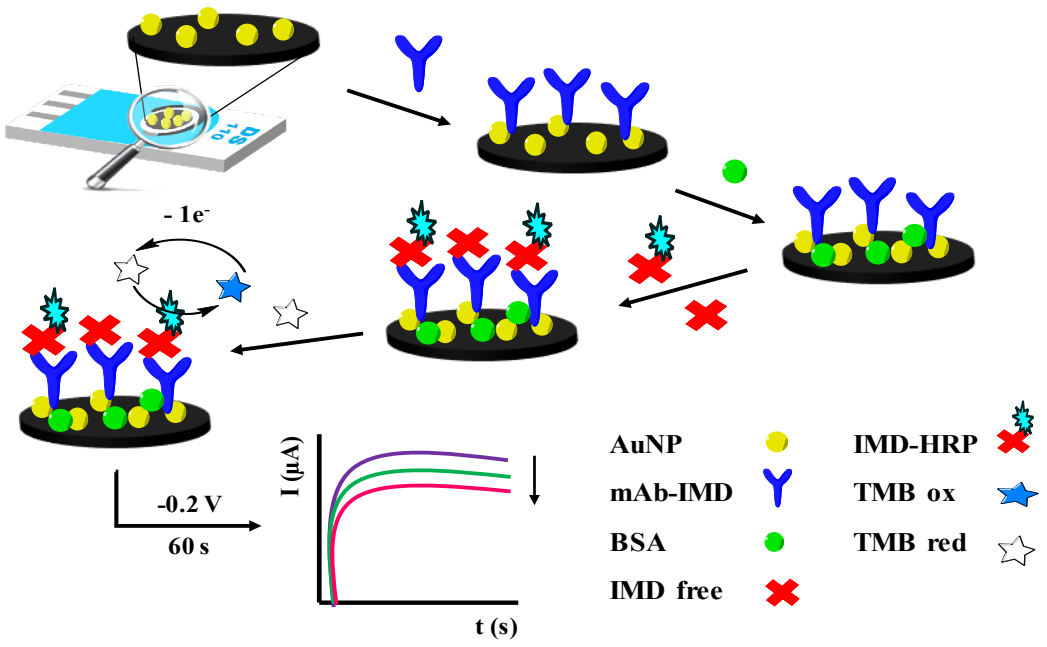


Figure 2. Scheme of the direct competitive immunosensor for the detection of IMD on AuNP-SPCE using monoclonal antibodies.

2.2.2. Electrochemical detection

The enzymatic reaction was carried out by placing 40 µL of TMB solution and incubating for 1 min. Chronoamperometric detection was performed applying a constant potential of -0.2 V during 60 s, recording the current associated to the oxidized TMB reduction process [50,51]. The analytical signal was the absolute value of the current recorded at 60 s. All measurements were done by triplicate. For the optimization studies shown at **section 3.1**, the variation of the signal to noise ratio (S/N) under different conditions was evaluated. A new electrode/immunosensor was used for each measurement, which avoids fouling, unspecific adsorptions and leaching of reagents.

2.2.3. Real samples preparation

Watermelons and tomatoes were bought from a local supermarket. Samples from such vegetables were ground and spiked with IMD standards. After that, the samples were filtered with a HyperSep C18 cartridge (Thermo Scientific, Spain) before the immunoassay. The water sample was obtained from the laboratory tap and directly spiked with IMD.

3. RESULTS AND DISCUSSION

3.1. Optimization of the main parameters affecting the analytical signal

The main paramaters affecting the analytical signal, such as the enzymatic reaction time, the monoclonal antibody (mAb-IMD) concentration, the IMD-HRP conjugate concentration and the blocking of the electrode for avoiding unspecific adsoprtions were optimized.

The effect of the enzymatic reaction time (in the range 0-15 min) on the signal/noise ratio (S/N) was evaluated fixing 10 μg mL^{-1} of mAb-IMD (immobilized on the electrode overnight at 4 °C) and 1 μg mL^{-1} of IMD-HRP (1 h of reaction). The results shown in **Figure 3A** suggest that the highest S/N ratio is obtained for 1 min of reaction, with an acceptable standard deviation. Thus, that time was selected as optimum for the sensor development.

Regarding the monoclonal antibody (mAb-IMD) concentration, antibody from different solutions (from 0 to 9 μg mL^{-1}) was immobilized on the electrode (overnight at 4 °C) so as to evaluate the optimum mAb-IMD concentration. IMD-HRP concentration was fixed at 1 μg mL^{-1} (1 h of reaction). At **Figure 3B**, it can be observed that S/N ratio increases

with the mAb-IMD concentration, reaching saturation of the electrode for 6 μg mL^{-1}, which is selected as the optimum for the immunosensor development.

The concentration of the enzyme-conjugated analyte (IMD-HRP) is also a key parameter in the development of a competitive immunoassay. Different concentrations of IMD-HRP in the range 0 to 15 μg mL^{-1} were evaluated, fixing the rest of parameters at the optimized levels. As shown in **Figure 3C** the minimum concentration of conjugate giving the maximum S/N ratio corresponds to 3 μg mL^{-1}, so it was chosen as optimum for the competitive assay.

Finally, the need and conditions of the blocking of the electrode surface with BSA for avoiding unspecific absorptions was also evaluated. The responses of electrodes modified with the specific antibody to IMD (mAb-IMD) were compared with those of electrodes modified with an unspecific antibody (anti mouse IgG) for different amounts of BSA under the optimized IMD biosensing conditions. In **Figure 3D**, it is observed that significant unspecific absorptions are obtained in the absence of BSA while 1% of BSA is enough for reducing those negative effects without affecting the specific signal. In view of these results, 1% BSA was selected as optimum for the sensor development.

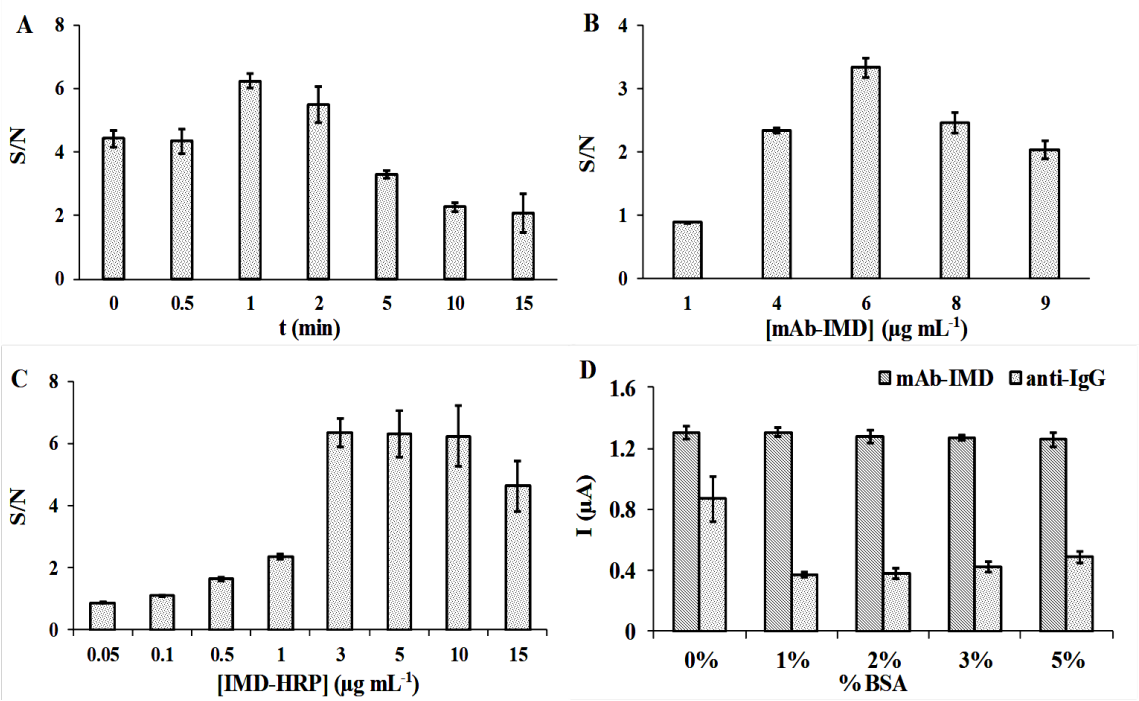


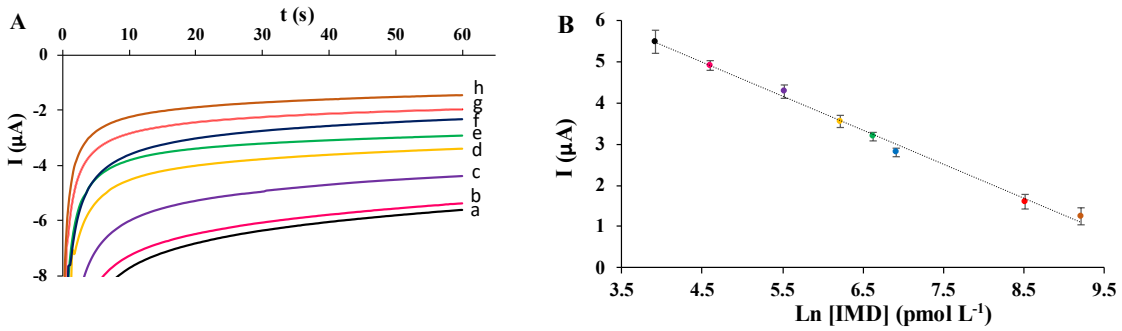
Figure 3. Results of the optimization of the main parameters affecting the analytical signal: (A) enzymatic reaction time, (B) mAb-IMD concentration, (C) IMD-HRP concentration and (D) BSA concentration.

3.2. Imidacloprid detection in standard solutions: comparison with standard methods

The optimized competitive immunoassay was applied for the detection of IMD from standard solutions in the range 50-20000 pmol L^{-1}. Increasing concentrations of IMD lead to a decrease in the absolute value of the cathodic current corresponding to the TMB reduction process, as displayed in **Figure 4A**. Plotting the analytical signal (absolute value of the current generated at 60 s) vs the IMD concentration, a logarithmic relationship in the range 50-10000 pmol L^{-1} is found (**Figure 4B**), and it can be adjusted to the following equation:

$$I(\mu A) = -0.822\, Ln\,[IMD](pmol\, L^{-1}) + 8.673 \qquad r = 0.997$$

The limit of detection, calculated as three times the deviation of the blank divided by the slope of the calibration plot, was 22 pmol L^{-1} of IMD, which is below the maximum levels allowed by the legislation (391 pmol L^{-1} for the individual pesticides). The good precision of the method was demonstrated comparing the calibration plots of three assays performed in different days (repeatability), which gives a relative standard deviation (RSD) of 6%.



The accuracy of the method was estimated in terms of % of relative error for 750 pmol L^{-1}, considering that:

$$\%\ Relative\ error = \frac{real\ value - experimental\ value}{real\ value}\ x\ 100$$

According to the equation of the calibration plot, an experimental IMD concentration of 795 pmol L^{-1} is estimated when a concentration of 750 pmol L^{-1} is analysed. From these data, a 6 % of relative error is calculated, which evidences the good accuracy of our method.

The performance of the biosensor was also compared with that of HPLC-MS/MS and ELISA analysis. As summarized in **Table 1**, our system shows a lower detection limit and a wider linear range compared with such well-stablished methods, being our methodology faster, simpler and cheaper.

Table 1. Comparative summary of the analytical performance of standard methods for IMD detection and the electrochemical immunosensor developed in this work.

Technique	Linear Range (pmol L^{-1})	LOD (pmol L^{-1})
HPLC-MS/MS	391-15646	117
ELISA kit	293-4694	235
AuNP-SPCE immunosensor (this work)	50-10000	22

Moreover, such performance is better than that reported for alternative electrochemical methods for pesticide analysis based on direct detection and enzymatic inhibition (**Table 2**), having our approach also clear advantages in terms of selectivity and time/cost of analysis respectively. Comparing with our previous work based on an indirect immunosensor [49], the direct format presents clear advantages in terms of time and cost of analysis, since we avoid the use of secondary antibodies. Moreover, the increase in the electroactive area of the working electrode by the AuNPs modification may also contribute to the improvement in the limit of detection calculated. The similar dynamic range of response obtained is probably due to the use of the same enzymatic system and the same electrode in both approaches.

Table 2. Analytical characteristics of different electrochemical methods for pesticide detection.

Pesticide	Approach	Linear Range	LOD	Ref.
Imidacloprid	Direct Immunosensor	50-10000 pmol L^{-1}	22 pmol L^{-1}	This work
Imidacloprid	Indirect immunosensor	50-10000 pmol L^{-1}	24 pmol L^{-1}	[49]
Parathion	Immunosensor	0.1-1000 ng L^{-1}	52 pg L^{-1}	[52]
Chlorpyrifos-methyl	Immunosensor	0.4-20 ng mL^{-1}	22.6 ng L^{-1}	[53]
Paraoxon	Enzimatic sensor	0.1-1 µmol L^{-1}	0.1 µmol L^{-1}	[54]
Malaoxon	Enzimatic sensor	2-50 µg mL^{-1}	2 µg mL^{-1}	[55]
Parathion	Enzimatic sensor	Up to 36.4 µmol L^{-1}	10 nmol L^{-1}	[56]
Chlortoluron	Inhibition sensor	0.001-0.1 µmol L^{-1}	0.47 nmol L^{-1}	[57]
Chlorpyrifos	Inhibition sensor	1-50000 µmol L^{-1}	5 µmol L^{-1}	[58]
Paraoxon	Inhibition sensor	Up to 40 µg mL^{-1}	2 µg mL^{-1}	[59]
Imidacloprid	Direct detection	30-200 µmol L^{-1}	440 nmol L^{-1}	[16]
Imidacloprid	Direct detection	0.5-15 µmol L^{-1}	0.10 µmol L^{-1}	[17]
Imidacloprid	Direct detection	1-5 µmol L^{-1}	0.93 µmol L^{-1}	[18]
Hydrazine	Direct detection	Up to 936 µmol L^{-1}	0.57 nmol L^{-1}	[60]
Carbofuran	Direct detection	1-250 µmol L^{-1}	0.22 µmol L^{-1}	[61]

3.3. Sensor selectivity and stability

Permethrin (PERM), glyphosate (GLY) and parathion (PAR) were selected as control pesticides potentially present in real samples. Pesticides with chemical structure similar to that of IMD, such as thiamethoxam (THIA) and carbendazim (CAR) were also evaluated. 10000 pmol L^{-1} solutions of IMD were mixed with the same amount of each of the control pesticides, finding no significant variations in the analytical signal of the pure IMD (**Figure 5A**) which demonstrates the selectivity of our immunosensor.

Different electrodes were prepared with immobilized mAb-IMD and stored at 4 ºC to study the stability of the immunosensor. A concentration of 1000 pmol L^{-1} of IMD was assayed in different days. As shown in **Figure 5B**, the devices are stable at least for one month. Longer-term stability study was out of the scope of this work.

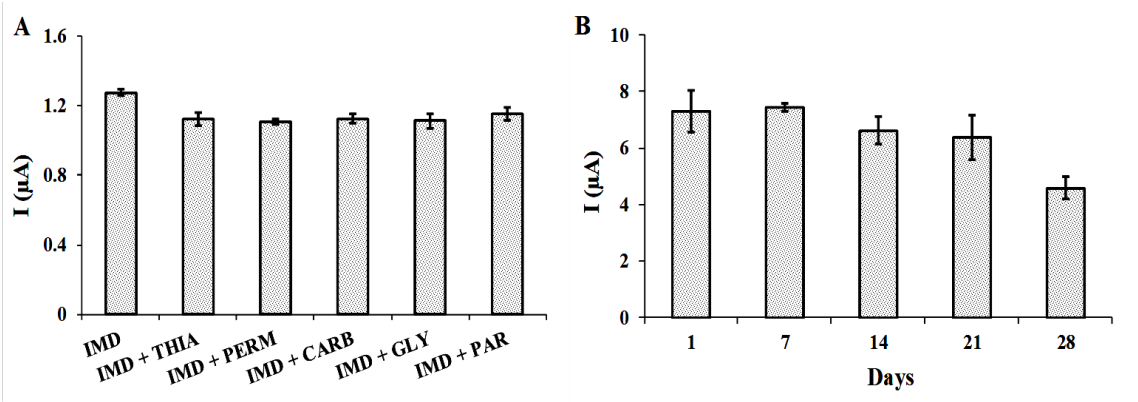


Figure 5. A) Analytical signals obtained for direct competitive immunoassays carried out for mixtures of 10000 pmol L^{-1} of IMD and 10000 pmol L^{-1} of different potential interfering pesticides. B) Long-term stability of the immunosensor. IMD concentration: 1000 pmol L^{-1}.

3.4. IMD analysis in real samples: study of matrix effects

Spiking of IMD concentrations in the range 50-10000 pmol L^{-1} was done in tap water, watermelon and tomato samples to determine whether pesticide detection is affected by such real sample matrixes. As shown in **Figure 6**, the calibration plots obtained in tap water and watermelon were similar to the standard one in buffer medium, suggesting low matrix effects. However, tomato matrix seems to exert an important negative effect in the immunosensor performance, as reflected by the high decrease in the slope of the curve.

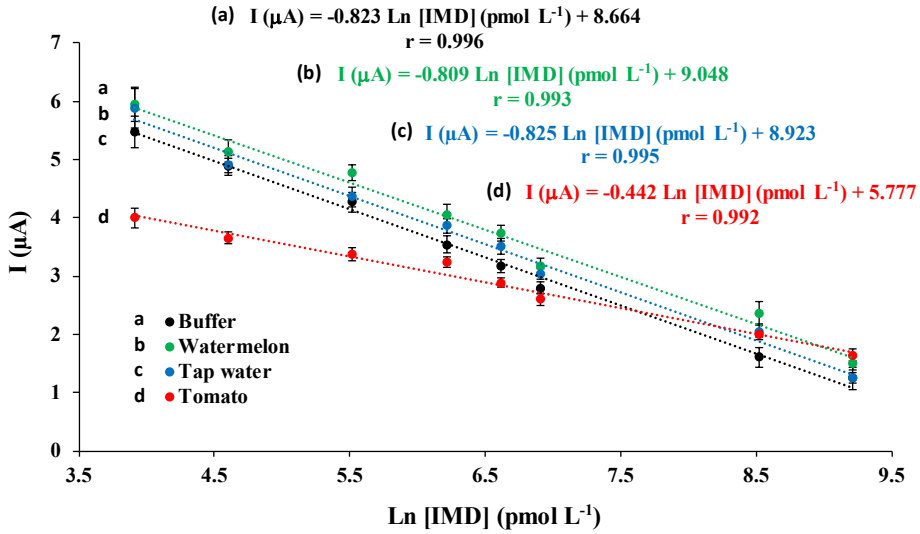


Figure 6. Evaluation of matrix effects. Relationship between the analytical signal (current recorded at 60 s) and the logarithm of the IMD concentration spiked in buffer (10mM PBS pH 7.4), watermelon, tap water and tomato.

These findings demonstrate the potential of our technology for the IMD detection in tap water and watermelon samples. Analysis of more complex matrixes such as tomato would require standard additions experiments for fixing the matrix effect.

3.5. Immunosensor validation: comparison with HPLC-MS/MS

Our immunosensor was validated by comparison with HPCL-MS/MS, the reference method used in official laboratories for IMD analysis. The study consisted in the analysis with both methods of a tap water sample doped with an unknown amount of IMD, and the further comparison of the methods by the student's t test, according to the following equation:

$$t = \frac{\bar{X}_A - \bar{X}_B}{S_C} \sqrt{\frac{N_A \times N_B}{N_A + N_B}}$$

where $\overline{X}_A$ and $\overline{X}_B$ are the average of the IMD concentration estimated by the electrochemical immunosensor (675 pmol L^{-1}) and the HPLC-MS/MS method (701 pmol L^{-1}), N_A and N_B are the number of replicates (10 for both methods) and S_c is the joint standard deviation, calculated as follows:

$$S_C = \sqrt{\frac{(N_A - 1) \times S_A^2 + (N_B - 1) \times S_B^2}{N_A + N_B - 2}}$$

where S_A (35) and S_B (30) are the individual standard deviation of each method.

In this way, a experimental *t* of 1.750 is obtained. Since the tabulated value for a 95 confidence interval and 18 degrees of freedom (N_A+N_B-2) is 2.101, we assume the absence of systematic errors in our immunosensor and a good agreement/validation with the reference method.

4. CONCLUSION

A direct competitive immunosensor on AuNPs-modified screen-printed electrodes using specific monoclonal antibodies has been successfully developed for IMD electrochemical detection. The system exhibits an excellent analytical performance in terms of range of response, limit of detection, repeatibility, precision, accuracy, selectivity and long-term stability. Low matrix effects are found in samples such as tap water and watermelon. Such performance is better than that reported for alternative biosensing methods for IMD pesticide analysis, with also advantages in terms of selectivity and time/cost of analysis.

Our device has also been validated with HPLC-MS/MS, the reference method used in official laboratories. Moreover, our findings represent a methodological improvement compared with indirect competitive immunoassays previously reported by our group [49] in terms of simplicity, time and cost of analysis. All this makes our immunosensor a reliable and advantageous alternative for the *in field* determination of IMD pesticide.

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REFERENCES

[1] WHO | World Health Organization, (n.d.). https://www.who.int/ (accessed June 18, 2019).

[2] C. Anagnostopoulos, G.E. Miliadis, Development and validation of an easy multiresidue method for the determination of multiclass pesticide residues using GC-MS/MS and LC-MS/MS in olive oil and olives, Talanta. 112 (2013) 1–10. doi:10.1016/j.talanta.2013.03.051.

[3] Z. Xiao, Y. Yang, Y. Li, X. Fan, S. Ding, Determination of neonicotinoid insecticides residues in eels using subcritical water extraction and ultra-performance liquid chromatography-tandem mass spectrometry, Anal. Chim. Acta. 777 (2013) 32–40. doi:10.1016/j.aca.2013.03.026.

[4] J.L. Vilchez, R. ElKhattabi, J. Fernandez, A. GonzalezCasado, A. Navalon, Determination of imidacloprid in water and soil samples by gas chromatography mass spectrometry, J. Chromatogr. A. 746 (1996) 289–294. doi:10.1016/0021-

9673(96)00402-5.

[5] Q. Fang, L. Wang, Q. Cheng, J. Cai, Y. Wang, M. Yang, X. Hua, F. Liu, A bare-eye based one-step signal amplified semiquantitative immunochromatographic assay for the detection of imidacloprid in Chinese cabbage samples, Anal. Chim. Acta. 881 (2015) 82–89. doi:10.1016/j.aca.2015.04.047.

[6] A. Kumaravel, M. Chandrasekaran, Sensors and Actuators B : Chemical Nanosilver / surfactant modified glassy carbon electrode for the sensing of thiamethoxam, Sensors Actuators B. Chem. 174 (2012) 380–388. doi:10.1016/j.snb.2012.08.054.

[7] M. Khairy, H.A. Ayoub, C.E. Banks, Non-enzymatic electrochemical platform for parathion pesticide sensing based on nanometer-sized nickel oxide modified screen-printed electrodes, Food Chem. 255 (2018) 104–111. doi:10.1016/j.foodchem.2018.02.004.

[8] A. Rhouati, M. Majdinasab, A. Hayat, A perspective on non-enzymatic electrochemical nanosensors for direct detection of pesticides, Curr. Opin. Electrochem. 11 (2018) 12–18. doi:10.1016/j.coelec.2018.06.013.

[9] W. Huixiang, H. Danqun, Z. Yanan, M. Na, H. Jingzhou, L. Miao, S. Caihong, H. Changjun, A non-enzymatic electro-chemical sensor for organophosphorus nerve agents mimics and pesticides detection, Sensors Actuators, B Chem. 252 (2017) 1118–1124. doi:10.1016/j.snb.2017.07.004.

[10] X. Liu, Y. Yao, Y. Ying, J. Ping, Recent advances in nanomaterial-enabled screen-printed electrochemical sensors for heavy metal detection, TrAC Trends Anal. Chem. 115 (2019) 187–202. doi:10.1016/j.trac.2019.03.021.

[11] D. Martín-Yerga, M.B. González-García, A. Costa-García, Use of nanohybrid materials as electrochemical transducers for mercury sensors, Sensors Actuators, B Chem. 165 (2012) 143–150. doi:10.1016/j.snb.2012.02.031.

[12] D. Martín-Yerga, M.B. González-García, A. Costa-García, Electrochemical determination of mercury: A review, Talanta. 116 (2013) 1091–1104. doi:10.1016/j.talanta.2013.07.056.

[13] Y. Liu, T. Li, C. Ling, Z. Chen, Y. Deng, N. He, Electrochemical sensor for Cd2+ and Pb2+ detection based on nano-porous pseudo carbon paste electrode, Chinese Chem. Lett. (2019) 2–7. doi:10.1016/j.cclet.2019.05.020.

[14] Z. Guo, M.L. Seol, C. Gao, M.S. Kim, J.H. Ahn, Y.K. Choi, X.J. Huang, Functionalized porous Si nanowires for selective and simultaneous electrochemical detection of Cd(II) and Pb(II) ions, Electrochim. Acta. 211 (2016) 998–1005. doi:10.1016/j.electacta.2016.06.141.

[15] R. Ouyang, W. Zhang, S. Zhou, Z.L. Xue, L. Xu, Y. Gu, Y. Miao, Improved Bi film wrapped single walled carbon nanotubes for ultrasensitive electrochemical detection of trace Cr(VI), Electrochim. Acta. 113 (2013) 686–693. doi:10.1016/j.electacta.2013.09.110.

[16] W. Lei, Q. Wu, W. Si, Z. Gu, Y. Zhang, J. Deng, Q. Hao, Electrochemical determination of imidacloprid using poly(carbazole)/ chemically reduced graphene oxide modified glassy carbon electrode, Sensors Actuators, B Chem. 183 (2013) 102–109. doi:10.1016/j.snb.2013.03.118.

[17] M. Zhang, H.T. Zhao, T.J. Xie, X. Yang, A.J. Dong, H. Zhang, J. Wang, Z.Y. Wang, Molecularly imprinted polymer on graphene surface for selective and sensitive electrochemical sensing imidacloprid, Sensors Actuators, B Chem. 252

(2017) 991–1002. doi:10.1016/j.snb.2017.04.159.

[18] A. Kumaravel, M. Chandrasekaran, Electrochemical determination of imidacloprid using nanosilver Nafion®/nanoTiO2Nafion® composite modified glassy carbon electrode, Sensors Actuators, B Chem. 158 (2011) 319–326. doi:10.1016/j.snb.2011.06.028.

[19] M.K.L. da Silva, H.C. Vanzela, L.M. Defavari, I. Cesarino, Determination of carbamate pesticide in food using a biosensor based on reduced graphene oxide and acetylcholinesterase enzyme, Sensors Actuators, B Chem. 277 (2018) 555–561. doi:10.1016/j.snb.2018.09.051.

[20] F.A. Esteve-Turrillas, A. Abad-Somovilla, G. Quiñones-Reyes, C. Agulló, J. V. Mercader, A. Abad-Fuentes, Monoclonal antibody-based immunoassays for cyprodinil residue analysis in QuEChERS-based fruit extracts, Food Chem. 187 (2015) 530–536. doi:10.1016/j.foodchem.2015.04.119.

[21] Z. Bin, C. Yanhong, X. Jiaojiao, Y. Jing, Acetylcholinesterase biosensor based on functionalized surfae of carbon nanotubes for monocrotophos detection, Anal. Biochem. 560 (2018) 12–18. doi:10.1016/j.ab.2018.08.024.

[22] M. Zouari, S. Campuzano, J.M. Pingarrón, N. Raouafi, Competitive RNA-RNA hybridization-based integrated nanostructured- disposable electrode for highly sensitive determination of miRNAs in cancer cells Biosensors and Bioelectronics Competitive RNA-RNA hybridization-based integrated nanostructured-disposable electrode for highly sensitive determination of miRNAs in cancer cells, Biosens. Bioelectron. 91 (2016) 40–45. doi:10.1016/j.bios.2016.12.033.

[23] H. Lebik-elhadi, Z. Frontistis, H. Ait-amar, S. Amrani, Electrochemical oxidation of pesticide thiamethoxam on boron doped diamond anode : Role of operating

parameters and matrix effect, Process Saf. Environ. Prot. 116 (2018) 535–541. doi:10.1016/j.psep.2018.03.021.

[24] P. Chorti, J. Fischer, V. Vyskocil, A. Economou, J. Barek, Electrochimica Acta Voltammetric Determination of Insecticide Thiamethoxam on Silver Solid Amalgam Electrode, Electrochim. Acta. 140 (2014) 5–10. doi:10.1016/j.electacta.2014.01.081.

[25] Z. Chu, J. Peng, W. Jin, Advanced nanomaterial inks for screen-printed chemical sensors, Sensors Actuators, B Chem. 243 (2017) 919–926. doi:10.1016/j.snb.2016.12.022.

[26] B. Pérez-Fernández, D. Martín-Yerga, A. Costa-García, Electrodeposition of nickel nanoflowers on screen-printed electrodes and its application to non-enzymatic determination of sugars, RSC Adv. 6 (2016) 83748–83757. doi:10.1039/C6RA15578B.

[27] B. Pérez-Fernández, D. Martín-Yerga, A. Costa-García, Galvanostatic electrodeposition of copper nanoparticles on screen-printed carbon electrodes and their application for reducing sugars determination, Talanta. 175 (2017) 108–113. doi:10.1016/j.talanta.2017.07.026.

[28] F. Kardaş, M. Beytur, O. Akyıldırım, H. Yüksek, M.L. Yola, N. Atar, Electrochemical detection of atrazine in wastewater samples by copper oxide (CuO) nanoparticles ionic liquid modified electrode, J. Mol. Liq. 248 (2017) 360–363. doi:10.1016/j.molliq.2017.10.085.

[29] F. Pino, C.C. Mayorga-Martinez, A. Merkoçi, High-performance sensor based on copper oxide nanoparticles for dual detection of phenolic compounds and a pesticide, Electrochem. Commun. 71 (2016) 33–37.

doi:10.1016/j.elecom.2016.08.001.

[30] H. Zhao, C. Zhou, Y. Teng, C. Chen, M. Lan, Applied Surface Science Novel Pt nanowires modified screen-printed gold electrode by electrodeposited method, Appl. Surf. Sci. 257 (2011) 3793–3797. doi:10.1016/j.apsusc.2010.11.151.

[31] A. Youse, A. Babaei, M. Delavar, Application of modified screen-printed carbon electrode with MWCNTs-Pt- doped CdS nanocomposite as a sensitive sensor for determination of natamycin in yoghurt drink and cheese, 822 (2018) 1–9. doi:10.1016/j.jelechem.2018.05.008.

[32] G. Martínez-Paredes, M.B. González-García, A. Costa-García, In situ electrochemical generation of gold nanostructured screen-printed carbon electrodes. Application to the detection of lead underpotential deposition, Electrochim. Acta. 54 (2009) 4801–4808. doi:10.1016/j.electacta.2009.03.085.

[33] Y. Song, J. Chen, M. Sun, C. Gong, Y. Shen, Y. Song, L. Wang, A simple electrochemical biosensor based on AuNPs / MPS / Au electrode sensing layer for monitoring carbamate pesticides in real samples, 304 (2016) 103–109.

[34] C. Malarkodi, S. Rajeshkumar, G. Annadurai, Detection of environmentally hazardous pesticide in fruit and vegetable samples using gold nanoparticles, Food Control. 80 (2017) 11–18. doi:10.1016/j.foodcont.2017.04.023.

[35] X. Tian, L. Liu, Y. Li, C. Yang, Z. Zhou, Y. Nie, Y. Wang, Nonenzymatic electrochemical sensor based on CuO-TiO2 for sensitive and selective detection of methyl parathion pesticide in ground water, Sensors Actuators, B Chem. 256 (2018) 135–142. doi:10.1016/j.snb.2017.10.066.

[36] X. Lu, L. Tao, Y. Li, H. Huang, F. Gao, A highly sensitive electrochemical

platform based on the bimetallic Pd@Au nanowires network for organophosphorus pesticides detection, Sensors Actuators, B Chem. 284 (2019) 103–109. doi:10.1016/j.snb.2018.12.125.

[37] M. Chen, B. Lou, Z. Ni, B. Xu, PtCo nanoparticles supported on expanded graphite as electrocatalyst for direct methanol fuel cell, Electrochim. Acta. 165 (2015) 105–109. doi:10.1016/j.electacta.2015.03.007.

[38] X. Quan, Y. Mei, H. Xu, B. Sun, X. Zhang, Optimization of Pt-Pd alloy catalyst and supporting materials for oxygen reduction in air-cathode microbial fuel cells, Electrochim. Acta. 165 (2015) 72–77. doi:10.1016/j.electacta.2015.02.235.

[39] D. Huo, Q. Li, Y. Zhang, C. Hou, Y. Lei, A highly efficient organophosphorus pesticides sensor based on CuO nanowires–SWCNTs hybrid nanocomposite, Sensors Actuators B Chem. 199 (2014) 410–417. doi:10.1016/j.snb.2014.04.016.

[40] Y. Zeng, D. Yu, Y. Yu, T. Zhou, G. Shi, Differential pulse voltammetric determination of methyl parathion based on multiwalled carbon nanotubes-poly(acrylamide) nanocomposite film modified electrode, J. Hazard. Mater. 217–218 (2012) 315–322. doi:10.1016/j.jhazmat.2012.03.033.

[41] A.S. Calvo, C. Botas, D. Martín-Yerga, P. Álvarez, R. Menéndez, A. Costa-García, Comparative Study of Screen-Printed Electrodes Modified with Graphene Oxides Reduced by a Constant Current, J. Electrochem. Soc. 162 (2015) B282–B290. doi:10.1149/2.1021510jes.

[42] E. Taillebois, A. Cartereau, A.K. Jones, S.H. Thany, Neonicotinoid insecticides mode of action on insect nicotinic acetylcholine receptors using binding studies, Pestic. Biochem. Physiol. (2018) 0–1. doi:10.1016/j.pestbp.2018.04.007.

[43] T. Farooqui, A potential link among biogenic amines-based pesticides, learning and memory, and colony collapse disorder: A unique hypothesis, Neurochem. Int. 62 (2013) 122–136. doi:10.1016/j.neuint.2012.09.020.

[44] R.D. Booton, Y. Iwasa, J.A.R. Marshall, D.Z. Childs, Stress-mediated Allee effects can cause the sudden collapse of honey bee colonies, J. Theor. Biol. 420 (2017) 213–219. doi:10.1016/j.jtbi.2017.03.009.

[45] United States Environmental Protection Agency | US EPA, (n.d.). https://www.epa.gov/ (accessed June 18, 2019).

[46] U.S. EPA, Preliminary Pollinator Assessment to Support the Registration Review of Imidacloprid, Off. Chem. Saf. Pollition Prev. (2016) 305.

[47] E.L. Caso, C.D.E.L.O.S. Plaguicidas, Análisis de la directiva europea 98/83/CE: paradigma de la justificación y establecimiento de los valores paramétricos. El caso concreto de los plaguicidas, (2012) 21–35.

[48] D. Oficial, D.E.L.A. Comisi, P. Europeo, P. Europeo, C. Alimentarius, Scientific support for preparing an EU position for the 45th Session of the Codex Committee on Pesticide Residues (CCPR), EFSA J. 11 (2013) 1–5. doi:10.2903/j.efsa.2013.3312.

[49] B. Pérez-Fernández, J.V. Mercader, B.I. Checa-Orrego, A. de la Escosura-Muñiz, A. Costa-García, A monoclonal antibody-based immunosensor for the electrochemical detection of imidacloprid pesticide, Analyst. 144 (2019). doi:10.1039/c9an00176j.

[50] J. Biscay, M.B.G. García, A.C. García, Electrochemical biotin determination based on a screen printed carbon electrode array and magnetic beads, Sensors

Actuators, B Chem. 205 (2014) 426–432. doi:10.1016/j.snb.2014.08.042.

[51] G. Vásquez, A. Rey, C. Rivera, C. Iregui, J. Orozco, Amperometric biosensor based on a single antibody of dual function for rapid detection of Streptococcus agalactiae, Biosens. Bioelectron. 87 (2017) 453–458. doi:10.1016/j.bios.2016.08.082.

[52] J. Mehta, P. Vinayak, S.K. Tuteja, V.A. Chhabra, N. Bhardwaj, A.K. Paul, K. Kim, A. Deep, Graphene modified screen printed immunosensor for highly sensitive detection of parathion, Biosens. Bioelectron. 83 (2016) 339–346. doi:10.1016/j.bios.2016.04.058.

[53] W. Wei, X. Zong, X. Wang, L. Yin, Y. Pu, S. Liu, A disposable amperometric immunosensor for chlorpyrifos-methyl based on immunogen / platinum doped silica sol – gel film modified screen-printed carbon electrode, Food Chem. 135 (2012) 888–892. doi:10.1016/j.foodchem.2012.06.037.

[54] S. Sajjadi, H. Ghourchian, H. Tavakoli, Choline oxidase as a selective recognition element for determination of paraoxon, 24 (2009) 2509–2514. doi:10.1016/j.bios.2009.01.008.

[55] A.N. Ivanov, R.R. Younusov, G.A. Evtugyn, F. Arduini, D. Moscone, G. Palleschi, Acetylcholinesterase biosensor based on single-walled carbon nanotubes — Co phtalocyanine for organophosphorus pesticides detection, Talanta. 85 (2011) 216–221. doi:10.1016/j.talanta.2011.03.045.

[56] J. Bao, C. Hou, Q. Dong, X. Ma, J. Chen, D. Huo, M. Yang, K. Hussein, A. El, W. Chen, Y. Lei, ELP-OPH / BSA / TiO 2 nano fi bers / c-MWCNTs based biosensor for sensitive and selective determination of p -nitrophenyl substituted organophosphate pesticides in aqueous system, Biosens. Bioelectron. 85 (2016)

935–942. doi:10.1016/j.bios.2016.05.094.

[57] M. Haddaoui, N. Raouafi, Chemical Chlortoluron-induced enzymatic activity inhibition in tyrosinase / ZnO NPs / SPCE biosensor for the detection of ppb levels of herbicide, Sensors Actuators B. Chem. 219 (2015) 171–178. doi:10.1016/j.snb.2015.05.023.

[58] D. Catalina, S. Carvajal, G. Peñuela, Effect of chlorpyrifos on the inhibition of the enzyme acetylcholinesterase by cross-linking in water-supply samples and milk from dairy cattle, Talanta. 111 (2013) 1–7. doi:10.1016/j.talanta.2013.03.036.

[59] F. Arduini, S. Guidone, A. Amine, G. Palleschi, D. Moscone, Acetylcholinesterase biosensor based on self-assembled monolayer-modified gold-screen printed electrodes for organophosphorus insecticide detection, Sensors Actuators B. Chem. 179 (2013) 201–208. doi:10.1016/j.snb.2012.10.016.

[60] C. Karuppiah, S. Palanisamy, S. Chen, S. Kannan, P. Periakaruppan, A novel and sensitive amperometric hydrazine sensor based on gold nanoparticles decorated graphite nanosheets modified screen printed carbon electrode, Electrochim. Acta. 139 (2014) 157–164. doi:10.1016/j.electacta.2014.06.158.

[61] A. Jirasirichote, E. Punrat, A. Suea-ngam, O. Chailapakul, Voltammetric detection of carbofuran determination using screen-printed carbon electrodes modi fi ed with gold nanoparticles and graphene oxide, Talanta. 175 (2017) 331–337. doi:10.1016/j.talanta.2017.07.050.