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ORIGINAL ARTICLE

A Multivariate Statistical Approach to Determine the Most Effective Factors for Biological Monitoring of Pesticides Using Voltammetric Sensors

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ABSTRACT

Nowadays, there are increasing concerns about pesticides occupational and environmental adverse effects despite the wide use. Therefore, it is very important to develop reliable methods for pesticides detection, extraction, and quantification in different samples. This study was aimed to develop an electrochemical Nano-composite sensor based on molecularly imprinted polymer (MIP) for selective determination of pesticides. The MIPs and non-imprinted polymers (NIPs) for diazinon and dicloran pesticides synthesized and were used in the composition of the carbon paste electrode. The square wave voltammetry (SWV) differential technique based on a fractional 28 factorial design was used to evaluate the effects of variables on the sensor response. The results of MIP-CP electrodes showed very high recognition abilities compared to the NIP-CP. The linear ranges for diazinon and dicloran were obtained 5×10^{-10} to 1×10^{-6}, 1×10^{-9} to 1×10^{-6} mol L^{-1}, and detection limits were 2.7×10^{-10} and 4.1×10^{-10} mol L^{-1}, respectively. Modified sensors were provided the prominent selectivity and sensitivity for quantification of pesticides in urine and water real samples considering optimized conditions. Therefore, the special sample pre-treatment wasn't required prior to the analysis process. The experimental design was verified the existence of interaction between factors. Consequently, the variables including square wave frequency, square wave amplitude, and deposition potential were indicated more significant effects on the sensor response than the other factors. It can be concluded that the interaction evaluation between variables is very important to look for the optimum conditions for pesticides analysis using voltammetric sensors.

KEYWORDS: *Pesticides; Molecularly Imprinted Polymer; Voltammetric Sensor; Carbon Paste; Electrode; Biological Monitoring, Experimental Design*

INTRODUCTION

The pesticides able to enhance the agricultural production effectively and control the vector-borne diseases, but they may negatively impact on human health and unwanted side effects on the environment [1]. Some health diseases resulting from pesticides exposure included neurological abnormalities, respiratory illnesses, and reproductive, endocrinological, and dermal problems [2]. Thus, in agrarian communities there is a need for special programs to apply and monitor pesticides. Various methods should be implemented to identify different chemical classes pesticides existing in occupational and environmental samples as well as food, fruit, and vegetable matrices. The most commonly used techniques for pesticides analysis are based on gas chromatography-mass spectrometry (GC–MS), liquid chromatography-tandem mass spectrometry (LC–MS/MS), and spectrophotometry [3-5]. Different sample pre-treatment approaches may be considered besides the use of analytical methods. Solid phase extraction [6], solid phase microextraction [7-8], and other preparation techniques coupled to analytical techniques have been applied by laboratories to determine pesticides in different matrices. Therefore, it is necessary to develop and validate acceptable strategies for pesticides residues analysis [3-9]. Considering limited access and availability for universities or some countries, it is of particular to use an alternative method which able to effectively, quick, valid, and cost-effective procedures for pesticides determination [10].

On the other hand, the use of small-scale devices in laboratories to determine different compounds has being considered as a miniaturization technology. Miniaturized devices, such as electrochemical sensors may provide some characteristics requested for an analytical strategy. In addition of appropriate diagnostic abilities, these environmental friendly procedures need less extraction time and using safer and fewer hazardous chemicals. It is noteworthy that a sensor is applied as the "working electrode" in an electrochemical system. Recently, chemically modified electrodes have being widely used to increase the sensitivity and selectivity of the electrochemical sensing. These electrodes with modified surfaces exhibit unique behavior for detection of analyte [9-11-13]. The molecularly imprinted polymers (MIPs) high selectivity and sensitivity specifications for modifying the sensors composition create a high credit for this method. The selective adsorption sites formed in the polymer structure lead to increase the selectivity of sensors. The size, shape and functional groups of these sites are complementary to the analyte of interest [14-16]. Moreover, high stability and selectivity, long lifetime, and cost-effectivity offer these synthetic receptors as a worthwhile alternative to antibodies [17-18]. Nanomaterial could also be used as a modifier element in the sensor composition, resulting in the increase of its surface area and electrical conductivity. Different electrochemical methods are proposed in combination with modified sensors because of their high sensitivity, simple operation, and rapid response. The electrochemical devices can also offer on-site detection due to portability and small size [16-19-21].

The chemometrics or multivariate data analyses are appropriate methods to detect the analyte. It should be noted that factors or combination of factors may significantly affect the responses of the analytical instruments or tools. The common "one at a time" approach investigates the variables one by one; but, it is unable to identify the interaction between variables. Experimental design should be considered to detect main factors and allow controlling these factors. Multivariate fractional designs provide true conclusions with a minimum number of experiments. Factorial designs can be used for the optimization of experimental conditions of electroanalytical analysis to obtain the most satisfactory approximations of response [22-24].

In the current study, a carbon paste electrode modified with molecularly imprinted polymer and nanomaterials was developed for each pesticide to optimize factors affecting the voltammetric determination of those pesticides by a multivariate fractional design.

A multivariate statistical approach was applied to determine diclroan and diazinon in biological samples for the first time considering challenges with the biological and environmental monitoring of

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pesticides. These biomimetic sensors modified with MIPs and MWCNTs.

MATERIAL AND METHODS

2.1. Instruments and reagents:

Electrochemical analyses were performed based on the three-electrode system via AUTOLAB PGSTAT302 software. The functional group presence in the imprinted polymers was characterized by a PerkinElmer FTIR as an imaging system. Three electrode system included modified sensors, Ag/AgCl electrode, and a graphite rode as working, reference, and counter electrodes. The Methacrylic acid (MAA) and Ethylene glycol dimethacrylate (EGDMA) were obtained from Fluka (Buchs, Switzerland). The dicloran and diazinon pesticides were obtained from (Dr Ehrenstorfer, Germany), Paraffin oil (Merck) and 2,2-azobisisobutyronitrile (AIBN) from Sigma–Aldrich (Munich, Germany). Multi-wall carbon nanotubes (MWCNTs) were provided from Research Institute of the Petroleum Industry (Iran). All other chemicals such as graphite powder (1–2 μm particle size) and solvents were purchased in analytical grade from Merck (Germany).

2.2. Preparation of the molecularly imprinted polymers (MIPs):

The non-covalent bulk polymerization was employed to synthesize all polymers for pesticides of interest. Materials used for preparation MIPs included pesticide as a template molecule, MAA as a functional monomer, EGDMA as a cross-linker, chloroform (10 ml) as the porogenic solvent and AIBN as an initiator. Appropriate molar ratio of template to the functional monomer were dissolved in suitable amount of chloroform in a 75-ml glass tube, the mixture was left 20 minutes for prearrangement and then EGDMA (20 mmol) and AIBN (60 mg) were added. The molar ratios of templates to the MAA were 1:4 and 1:6 for dicloran, and diazinon, respectively. The solution was purged with nitrogen for 7 min. In order to start polymerization, a glass tube was sealed under nitrogen atmosphere and was placed in a water bath at 60 °C for 18 h. After that, synthesized polymers were dried and mechanically grounded into powders. The extensive washing was carried out using methanol via Soxhlet apparatus for removing the template molecule. Washing was continued till no template was detected in the effluent by UV-VIS spectrometry and electrochemical methods. Furthermore, the control polymer or the non-imprinted polymer (NIP) was synthesized in the same way with no added template molecule to the mixture. The explained procedure was separately repeated for each pesticide as a template molecule. The FT-IR spectroscopic analysis of unwashed and washed imprinted polymers was recorded to identify the chemical structure of the obtained particles. For two pesticides, the FTIR data displayed the similar characteristic bands related to molecular structure of these polymers. As it can be seen in Figure 1 (a, b), bands in spectrum of washed polymers shifted toward lower frequencies, illustrating the presence of the different intermolecular bonding between templates and functional molecules of MAA and EGDMA during the polymerization process. For instance, in the spectrum of diazinon pesticide, the carboxylic acid O-H stretch vibration in the washed and unwashed MIPs were 2959 and 2967cm^{-1}. The bands at about 1730 cm^{-1} may be attributed to the stretching vibrations of C=O of MAA or EGDMA. The peaks beyond 1500 cm^{-1}, the fingerprint region, were complicated to interpret due to bands overlapping. A scanning electron microscopy (SEM) was carried out to investigate the total morphology of MIPs and their size homogeneity. The results of Figure 2 (a, b) illustrates the surfaces of MIPs are irregular, rough and porous, resulting from creation of binding sites in MIP structure and successful molecular imprinting. There is spherical shape with a narrow size distribution in synthesized polymers.

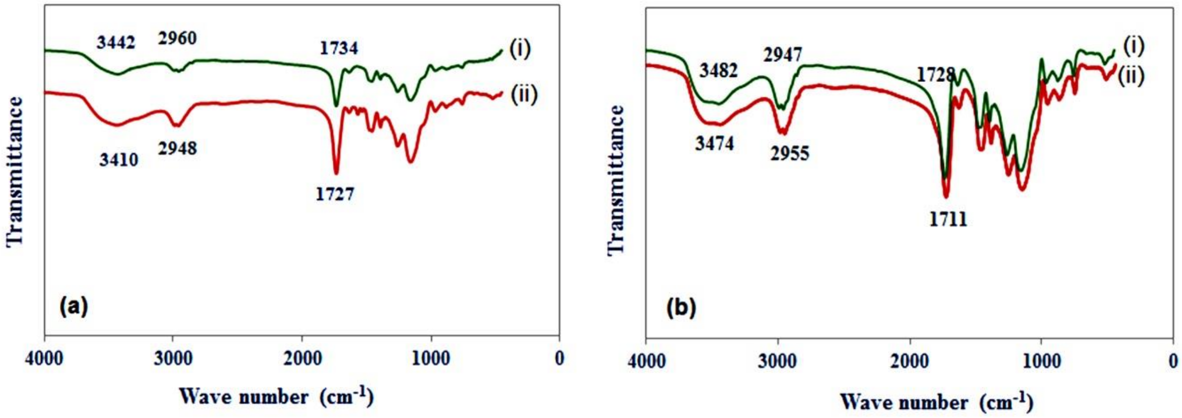

Fig 1. Infrared spectra of the washed (i) and unwashed MIP particles (ii) for diazinon (a) and Dicloran (b)

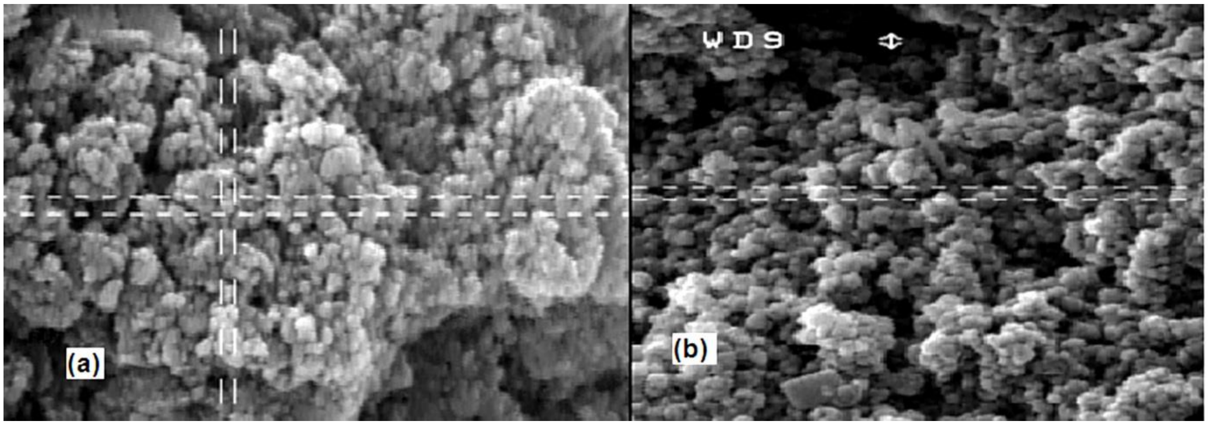

Fig 2. Scanning electron microscopy (SEM) image of prepared molecularly imprinted polymers; Dicloran (a) and Diazinon (b)

2.3. Preparation of the electrode:

The graphite powder and paraffin oil were mixed in a 75:25 (w/w%) ratio to prepare the bare carbon paste electrode (CP) as the control one. The total weight of all constituents of the paste was 0.1 g. The working electrode for each pesticide was prepared in the same procedure by mixing the appropriate amounts of graphite, paraffin oil, MIP, and MWCNTs (Table 1).

These compositions were selected based on optimization experiments. The mixture was homogenized in a mortar for at least 10 min. The final paste was packed into the end of an electrode body (id: 3 mm, paste deep: 1 cm) in which electrical contact was made with a copper rod. The electrode surface was polished using a paper to remove the excess of material. Prepared electrodes (sensors) were applied as the working electrodes in electrochemical analyses.

Table 1. The composition of carbon paste for determination of pesticides

Pesticide	**Composition of Carbon Paste (Wt. %)**			
	Graphite Powder	Binder (Paraffin oil)	MWCNTs	MIP
Dicloran	50	25	3	22
Diazinon	57	25	3	15

2.4. Experimental design for electrochemical measurements:

The experimental design method was applied to evaluate the effects of experimental conditions and instrumental parameters on the sensor response. This approach compared to the one-at-a-time approach is able to determine the main effects of parameters and estimate any interaction among parameters with a fewer measurement. In this study, the Minitab software (version 14) was used for specifying of experimental runs and analyzing of data. Eight factors affecting the sensor response including the extraction pH, extraction time, analysis pH, deposition potential and its exertion time, electrolyte concentration, square wave amplitude, and its frequency were considered as the input parameters in Minitab. A two-level full factorial design of 28 was used and a fraction of the runs (1/8) specified by this design resulting in 32 basic experiments (64 regarding two replicates) for each pesticide. The experimental runs were performed corresponding to the high (+) and low (−) points for each factor (Table 1). The result of the run in the highest current intensity for the prepared sensor was selected as the optimized one. In fact, multivariate strategy led to choose the optimized parameters in one of the predicted experimental runs for each pesticide. The electrochemical measurement of all pesticides was separately done as follow:

Extraction step: pesticide solutions with defined concentration were prepared for optimization experiments. After adjusting the level of each variable according to the runs predicted by Minitab, then the electrode was inserted into the solution stirring at a rate of 600 rpm.

Determination step: The voltammetric experiments were conducted in a cell containing auxiliary, reference, and work electrodes. After applying a certain deposition potential to the working electrode (placed in the cell), the potential was scanned in a defined range. All analytical variables in the determination step were adjusted according to predicted runs by experimental design.

2.5. Preparation of samples:

Standard stock solutions (1×10^{-2} mol L^{-1}) were prepared by weighing a suitable amount of each pesticide and diluting to 25 mL with ethanol. The working solutions for optimization experiments were made by appropriate dilution of the stock solutions. Urine, tap and river samples were considered to demonstrate the feasibility of sensor application for pesticide determination in biological and environmental real samples. These samples were analyzed directly without any special pretreatment. Only, urine samples were diluted at a ratio of 1:5 with distilled water prior to measurement. The standard addition method was used to determine the content of pesticides in real samples, taking into account the recovery of the analytical procedure. The determination of real samples was performed under optimal conditions.

RESULTS AND DISCUSSION

3.1. The composition of carbon paste electrode:

The carbon paste electrodes basically composited from mixed graphite powder in a proper binder. The modification of these electrodes with different materials such as polymers, redox mediators, and recognition elements enhances their selectivity and analytical performance. In this study, a multi-wall carbon nanotube (MWCNTs) and MIP were added to paste as modifying agents. In order to confirm the effect of modification, bare carbon paste electrode and an electrode modified with NIP were also used as blank ones. The results demonstrated significant effects of modifiers on the electrode response. The application of carbon nanotubes enhances the current intensity of the electrode due to the vast surface area, high electrical conductivity, and proper chemical stability of these particles. Furthermore, doping of the electrode with MIP improves the electrode performance because of the intrinsic properties of these polymeric materials such as long-term stability, chemical inertness, and reusability. Moreover, MIPs can accumulate target analytes on the electrode surface for increasing the sensitivity and separate them from the other compounds for improving the selectivity [25-26]. Therefore, it is remarkable that modified CPEs can be considered as a useful tool for electrochemical analysis of target molecules in different samples. It is worth mentioning that the percentage of MIP in the composition of the carbon paste has an effect on the sensor response. The results in Table 2 indicate the paste containing 15-22% MIP (for pesticides of interest) led to the highest current intensity in electrochemical experiments. It is clear that both lower and higher amounts of MIP cause the reduction of electrochemical responses due to diminishing the number of recognition sites in the sensor structure and rising the electrode resistance, respectively.

3.2. Effect of interferences:

In order to evaluate the selectivity of the MIP, the response of sensor for each pesticide was recorded under the optimal analytical conditions in presence of some commonly used pesticides (carbofuran, dichlrofention, dimethoate, and dichlorvos) and some ions. Different concentrations of these interferences were gradually added to the defined concentration of analyte and the recovery of sensor for determination of analyte was calculated. The results also showed that the presence of added agents until about 800-fold concentration could not interfere with the peak current obtained for the target pesticide. The findings proved the high selectivity of the MIP-nanocomposite electrodes toward the analytes of interest. It can be concluded that specific recognition sites in the MIP structure enhance the ability of the MIP-CP electrode to bind strongly with the desired molecule.

3.3. Optimization of the effective parameters in pesticides detection:

The experimental design methodology was applied to optimize the effect of extraction and analytical conditions for proposed sensors,. Eight inputs, presented in Table 2, including (-1) and (+1) levels were used by the Minitab to predict required experiments for each pesticide. The experimental methods (see Table 3) were performed using pesticides solutions containing defined concentration (5×10^{-7} mol L^{-1}). As it can be seen in Table 3, the highest current intensities for dicloran and diazinon sensors were obtained in indicated levels related to run numbers 29 and 9, respectively. The effects of interactions among factors to calculate sensor responses were evaluated at a 5% significance level. They were expressed by R^2 or R-Squared (adjusted) by which the degree of output variation is explained by its relationship with one or more input variables. The R^2 is always between 0 and 100%. The outcomes indicated significant interactions between factors that mean the level of one variable influences the effect caused by other factor in a positive way. According to the results of Table 4, for all pesticides, the square wave frequency (SWF) and square wave amplitude (SWA) have the significant binary interaction effects ($P<0.05$).

The degree of influence of these factors on the sensor response has been shown in Table 4. This table results review indicated that the SWF, SWA, and deposition potential (DP) factors have the greatest contribution on the sensor response which result the current intensity of the sensor significantly. So, the SWF, SWA, and DP optimization impact should be considered on electrochemical analysis better sensor response. The individually effect of main factors for dicloran and diazinon have been illustrated in the Figure 3 and 4, respectively.

The exertion of deposition potential to the working electrode results in to take place the deposition of analytes onto the electrode surface, playing a major role in the efficiency of electron transfer and analyte detection. In addition, the parameters of SWA and SWF affect the intensity of the signal and the sensitivity of the technique. The unstable current as well as large instrumental incompatibility was observed in frequencies higher than optimum value. A shift in the peak potential was found in the amplitude more than chosen level. On the other hand, in lower levels of these parameters, there was no adequate intensity of signal. So, the optimum levels of DP, SWA, and SWF caused the tangible enhancement in the current intensity [23-27-29]. The optimization of all operative variables is momentous to acquire the highest sensor response although the above-forenamed factors have more significant influence compared to the other ones. This ensures the lower detection limit leading to analyte determination in trace levels can be achieved in completely optimized conditions. In respect of each pesticide, optimum levels for all factors can be seen in selected runs 9 and 29.

Table 2. The high (+) and low (−) variable levels in the experimental design for optimization the sensor response for each pesticide

		E-pH	**E-Time (min)**	**EC (mol L^{-1})**	**SWA (V)**	**DP-Time (s)**	**DP (V)**	**SWF (Hz)**	**A-pH**
Diazinon	**High level (1)**	10	20	0.1	0.15	120	0.9	150	8
	Low level (-1)	2	5	0.03	0.02	30	-0.4	50	4
Dicloran	**High level (1)**	10	25	0.05	0.17	20	-0.1	150	8
	Low level (-1)	5	5	0.01	0.05	5	-0.6	50	2

E-pH: Extraction pH; **E-Time:** extraction time; **EC:** electrolyte concentration; **SWA:** square wave amplitude; **DP:** deposition potential; **SWF:** Square wave frequency; **DP-Time:** deposition potential exertion time; **A-pH:** Analysis pH

Table 3. The obtained results for sensors responses (μA) using the experimental design to optimize the determination of dicloran and diazinon

Run	**E-pH**	**E-time (min)**	**E-C (molL^{-1})**	**SWA (Volt)**	**DP-Time (s)**	**DP (Volt)**	**SWF (Hz)**	**A-pH**	**Current Intensity (μA) Dicloran**	**Current Intensity (μA) Diazinon**
1	1	-1	1	1	1	-1	-1	-1	19.1±2.8	40.1±2.6
2	1	1	-1	-1	1	-1	-1	1	13.2±1.4	5.3±1.5
3	1	-1	-1	1	-1	1	-1	-1	14.1±1.7	13.2±1.4
4	-1	1	-1	1	1	1	-1	-1	17.3±0.84	14.7±0.77
5	1	1	-1	1	-1	-1	1	1	21.4±0.71	5.7±0.98
6	-1	-1	-1	1	-1	-1	1	-1	13.3±1.5	30.0±2.8
7	1	-1	-1	-1	1	1	1	-1	11.9±1.9	24.9±1.2
8	1	-1	-1	1	1	1	-1	1	6.3±0.98	16.2±2.3
9	-1	-1	1	1	1	1	1	-1	9.4±1.03	63.4±2.6
10	-1	1	1	-1	-1	-1	1	1	40.0±2.1	10.9±2.4
11	1	1	1	-1	1	1	-1	-1	36.2±2.5	46.1±1.2
12	-1	-1	-1	-1	1	-1	-1	-1	4.2±0.28	8.2±1.4
13	1	1	1	-1	-1	1	-1	1	40.3±0.98	10.5±0.98
14	-1	-1	-1	-1	-1	-1	-1	1	8.0±1.2	48.0±1.4
15	-1	-1	1	-1	-1	1	-1	-1	23.1±1.4	12.2±1.6
16	1	1	1	1	1	1	1	1	34.2±1.4	9.6±0.71
17	1	-1	-1	-1	-1	1	1	1	15.0±0.85	10.1±0.98
18	1	-1	1	-1	-1	-1	1	-1	32.1±1.9	9.1±0.91
19	-1	-1	1	-1	1	1	-1	1	14.2±1.4	52.0±2.1
20	-1	1	1	1	1	-1	-1	1	32.2±1.4	15.1±0.98
21	-1	1	1	1	-1	-1	-1	-1	45.0±0.85	19.2±1.5
22	1	1	-1	-1	-1	-1	-1	-1	16.1±1.9	4.4±0.56
23	1	-1	1	-1	1	-1	1	1	18.2±1.4	17.4±1.8
24	-1	-1	1	1	-1	1	1	1	20.0±3.2	47.2±3.1
25	1	-1	1	1	-1	-1	-1	1	23.1±1.4	20.0±1.5
26	-1	-1	-1	1	1	-1	1	1	4.3±0.98	21.7±1.8
27	-1	1	-1	-1	1	1	1	1	15.3±1.7	8.2±1.5
28	-1	1	1	-1	1	-1	1	-1	32.1±2.8	18.3±1.5
29	1	1	1	1	-1	1	1	-1	77.1±1.6	10.7±0.84
30	1	1	-1	1	1	-1	1	-1	25.3±0.98	9.6±0.39
31	-1	1	-1	-1	-1	1	1	-1	27.1±2.3	7.0±1.2
32	-1	1	-1	1	-1	1	-1	1	21.0±1.4	6.1±0.84

Table 4. The most important interaction effects of factors and percentage of response variation for dicloran and diazinon

Term	Dicloran		Diazinon	
	R-Sq (adj) %	Interaction effect P-value	R-Sq (adj) %	Interaction effect P-value
A-pH × DP	7.40	0.038	13.85	0.031
A-pH × SWF	32.1	0.013	32.06	0.04
SWA × SWF	67.07	0.003	22.45	0.061
DP × SWF	37.76	0.0546	29.43	0.05
SWA× DP	37.08	0.041	19.54	0.017
SWA× DP × SWF	68.92	< 0.001	34.11	0.042
DP-time × DP × SWF	-*	-	30.53	0.001
A-pH × SWA × SWF	63.146	< 0.05	-*	-
DP × SWA × SWF	68.92	< 0.05	34.11	< 0.05

*These interactions were not investigated due to very low R^2

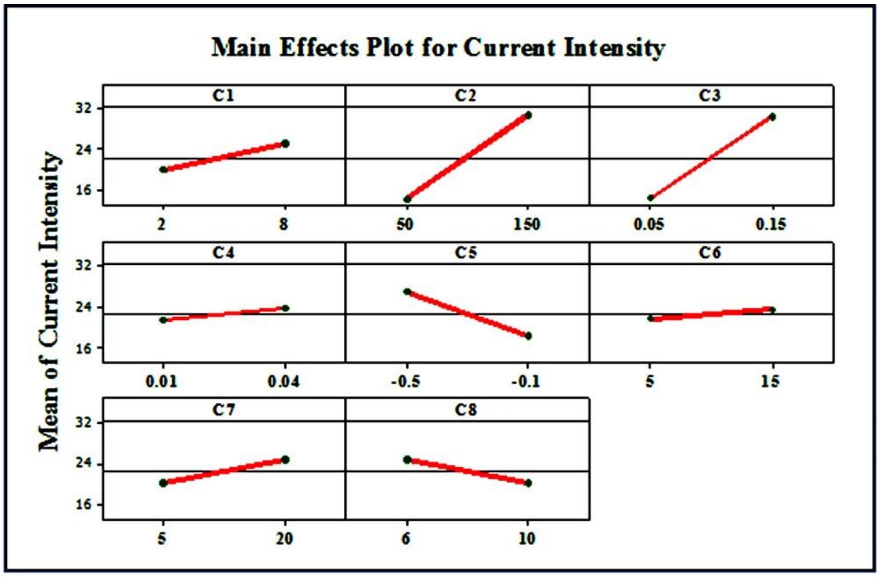

Fig. 3 Main effects plot in the factorial design for dicloran

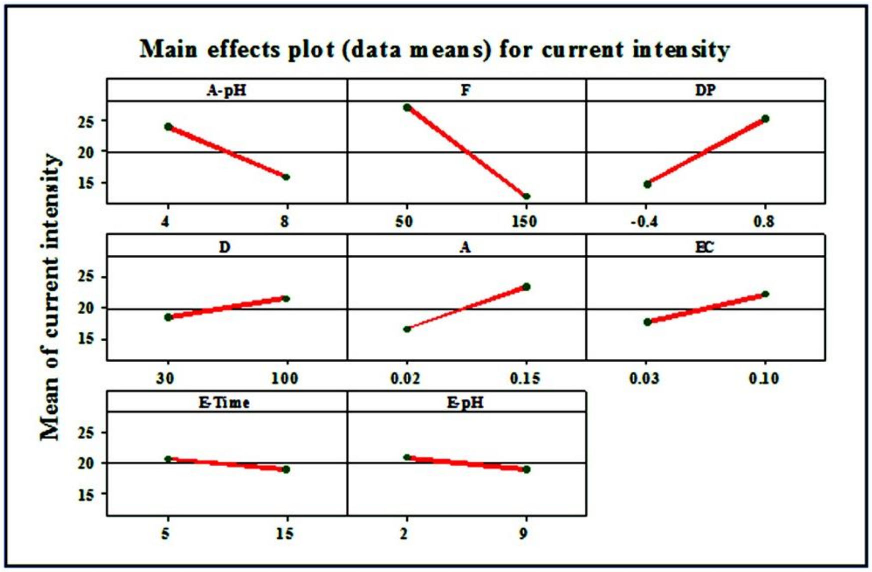

Fig 4. Main effects plot in the factorial design for diazinon

3.4. Method validation:

The optimized methodology for the pesticides determination using square wave voltammetry was used for plot the calibration curves (Figure 5). The MIP-CP sensor for each pesticide was inserted into different concentrations to perform the electrochemical analysis. The linear response ranges over the dicloran and diazinon concentrations, related R^2, limits of detection (LOD), and limit of quantification (LOQ) for dicloran and diazinon have been presented in Table 5. The following equations were applied to calculate the LOD and LOQ, where, Sb is the standard deviation of the blank and m is the slope of the calibration graph.

$$LOD = \frac{3\,S_b}{m} \qquad LOQ = \frac{10\,S_b}{m}$$

In addition, some experiments were performed to determine the reproducibility of the sensor responses. In order to do this, six measurements within one day were considered. The coefficient variations (CV) for these experiments can also be seen in Table 5. Therefore, it is statistically demonstrated that the modified electrodes have satisfactory capability to determine the pesticides of interest. In order to study the long-term stability of the proposed sensors, some experiments were performed over a period of about 2 months in the test conditions described above. Based on the results of these tests, no significant change of current intensity was observed and sensors retained about 96.5% of their initial responses, proving good stability and low errors for these devices.

Table 5. Method validation criteria for dicloran and diazinon pesticides

Method validation criteria	**Dicloran**	**Diazinon**
Calibration linear range (mol L^{-1})	1×10^{-9} to 1×10^{-6}	5×10^{-10} to 1×10^{-6}
R^2 of calibration curve	0.9964	0.9916
LOD (mol L^{-1})	4.1×10^{-10}	2.7×10^{-10}
LOQ (mol L^{-1})	1×10^{-9}	1×10^{-9}
CV (%)	3.17	3.07

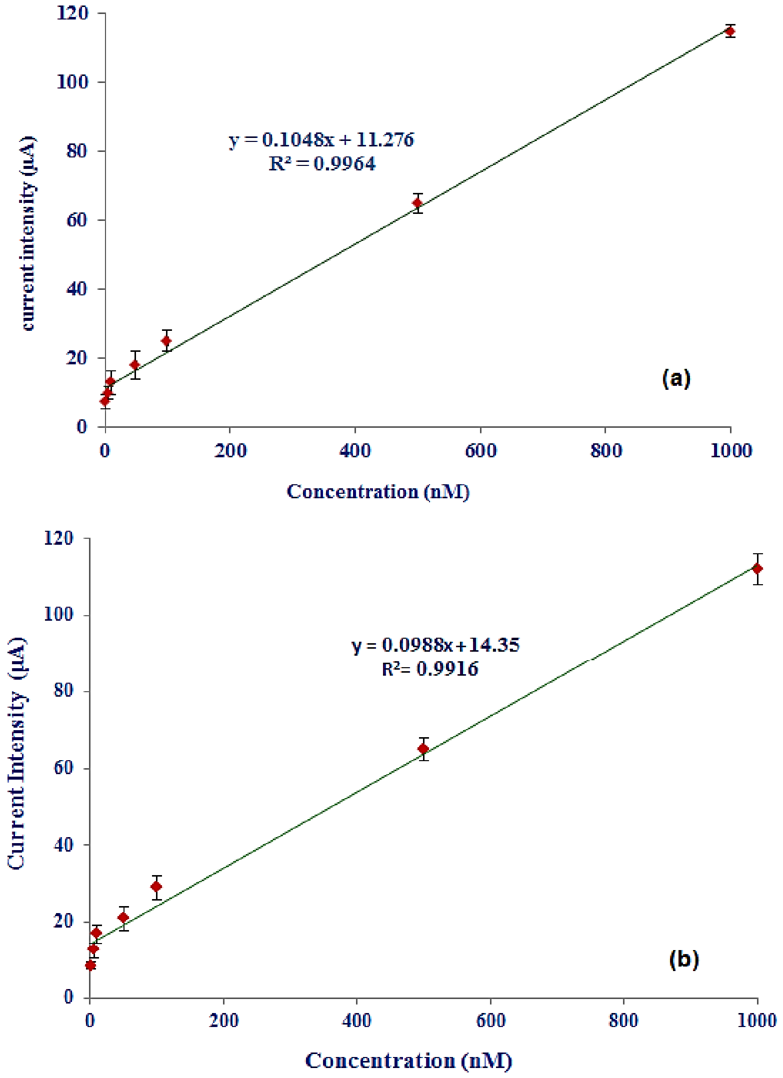

Fig 5. Calibration curve plotted for voltammetric determination of dicloran (a) and diazinon (b) with nanocomposite MIP-CP based sensor

3.5. Determination of pesticides in real samples:

The applicability of the nanocomposite MIP-based sensors was evaluated by separately determining the trace amount of each pesticide in urine and water samples. The analysis of pesticides in all real samples was done by optimized procedure using standard addition method. Samples were generally introduced for analysis directly and no digestion or other pre-treatment steps implemented prior to the voltammetric determination. The urine samples were diluted at ratio of 1:5 to 1:10 with distilled water. The performance of the nanocomposit MIP-based sensor in spiked real samples has been presented in Table 6. The obtained results confirmed that the added and measured concentrations were in good agreements and the method can be successfully applied to quantify the pesticides in biological and environmental samples. These advanced analytical procedures reduced or eliminated the prolonged sample pre-treatment procedure.

Table 6. The performance of the nanocomposit MIP-based sensor in spiked real samples

Sample	**Added (ng/ml)**	**Dicloran**			**Diazinon**		
		Found (ng/ml)	**RSD (%) (n=3)**	**Relative Recovery (%)**	**Found (ng/ml)**	**RSD (%) (n=3)**	**Relative Recovery (%)**
Tap Water	-	N.D	-	-	N.D	-	
	20	19.3	2.20	96.5	18.8	3.12	94.0
	200	190	2.11	95.0	193	2.91	96.5
	2000	1884	2.44	94.2	1909	2.06	95.45
River Water	-	< 0.2	-	-	< 0.2	-	
	20	18.9	2.22	94.5	19.5	2.44	97.5
	200	185	3.01	92.5	190	1.76	95.0
	2000	2006	2.98	100.3	1881	2.83	94.05
Urine	-	N.D	-	-	N.D	-	
	20	18.6	2.47	93.0	18.4	3.61	92.0
	200	180	2.92	90.0	186	2.77	93.0
	2000	1795	1.91	89.7	1848	3.11	92.4

CONCLUSION

In conclusion, this work used the molecularly imprinted polymers and carbon nanotubes as modifiers to fabricate the highly selective sensors for determining pesticides. The MIP could considerably improve the sensitivity and selectivity of sensors. Due to the large surface area and other special characteristics, nanomaterial could also enhance the sensor responses. Low detection limit, high sensitivity and selectivity, as well as the long-term stability can vindicate the superior sensing performances of suggested sensors towards the detection of pesticides.

The designed sensors were able to determine the pesticides in different real samples with no considerable matrix interference. It was observed that the factorial design is an effective way for evaluation interaction between the variables also to assess the optimum conditions. The electrochemical technique needs less expensive instrumentations and reagents compared to other methods like chromatographic techniques.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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