

Voltammetric Determination of Carbofuran Pesticide in Biological and Environmental Samples Using a Molecularly Imprinted Polymer Sensor, a Multivariate Optimization

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Abstract—Increasing application of pesticides and their toxicity made it essential to develop reliable and accurate methods of their trace quantification. This research was aimed to develop an electrochemical nano-composite sensor based on molecularly imprinted polymer (MIP) for selective and sensitive determination of carbofuran. The proposed sensor was made based on incorporation of carbofuran MIP in the carbon paste. The square wave voltammetric (SWV) method was applied for the electrochemical measurements. The experimental design was then used to optimize parameters affecting the sensor response according to selected experimental domain. The obtained linear range after optimization of the parameters was from 1×10^{-9} to 1×10^{-6} M. The calculated detection limit was 3×10^{-10} M. Under optimized extraction and determination conditions, the proposed modified sensor indicated the considerable sensitivity and selectivity to determine carbofuran in some real samples (human urine, tap and river water) without any special sample pre-treatment before the analysis.

Keywords: carbofuran, molecularly imprinted polymer, voltammetric sensor, biological monitoring, experimental design

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During the last decades, increasing agricultural production has led to remarkable pesticides consumption. Farmers apply pesticides to control different pests or weeds and improve agricultural productivity. However, the use of fertilizers and pesticides has caused serious problems especially in developing countries. Human exposure to these compounds can lead to different adverse effects. Also, pesticides may enter the soil, surface, and groundwater from various sources resulting in environmental contamination [1, 2]. Carbofuran (Scheme 1), as a carbamate pesticide, is used to control insects and nematodes. Carbofuran can cause various symptoms depending on the amount and route of the exposure. Some of reported symptoms include eye and skin irritation, headache, dizziness, central nervous system depression, muscle twitching, breathing difficulty, and wheezing [3, 4].

According to the above mentioned, there may be a need for fast and reliable methods for pesticides detection in occupational and environmental samples as

well as in food products. Most of traditional techniques for pesticides determination include gas or liquid chromatography [5], spectrophotometry [6, 7], and immunoassay [6]. Solid phase extraction [8], solid phase microextraction [9, 10], and other preparation techniques in combination with mass spectrometry have been reported in the literature to determine pesticides in different matrices too. Although these methods have acceptable sensitivity, sometimes desired sensitivity for pesticides detection, especially organophosphorus group using routine detectors in chromatographic methods, is low. On the other hand, pre-treatments or extraction steps which are compulsory before determining the analytes at ppb levels by advanced techniques are sophisticated and time-consuming. In spite of many studies supporting classical methods for pesticides detection, experts are faced with some limitations during employing them. These techniques are expensive and need a well-equipped laboratory along with well-trained operators. In addi-

tion, there is no easy access to enough and qualified instruments in many developing countries for the quantification of trace pesticide residues. For these reasons, development of novel, simple, and inexpensive methodologies is welcomed to determine these compounds in a single run and on-site [11].

Furthermore, rapid growing of miniaturization technology has affected the analytical systems and provided the use of small-scale devices in laboratories to determine different analytes. In this regard, electrochemical sensors, as miniaturized devices, have appropriate diagnostic abilities and can be used to monitor trace and even ultra-trace compounds like pesticides. In recent years, the modification of sensors by various agents has led to introduction of new and very appealing analytical tools for selective determination of many chemicals [12]. In a study by Maltzman et al. [13], a two-electrode electrochemical cell based on a mitochondrial modified bioanode was successfully used to determine such pesticides as atrazine, paraquat, parathion, and permethrin. Motaharian et al. [14] applied an electrochemical sensor based on molecularly imprinted polymer nanoparticles to detect diazinon in well water and fruit samples resulting in high adsorption capacity and excellent sensitivity. Bow et al. [15] proposed a highly selective analytical sensor based on MIP for selective and sensitive determination of endosulfan in contaminated water. Molecular imprinted polymers have a tremendous potential in development of sensors structure as recognition materials. These synthetic polymers increase the selectivity of sensors because of containing the selective adsorption sites. The size, shape, and functional groups of these sites are complementary to the analyte of interest [16, 17]. Notably, some parameters affect the properties of the final polymer including functional monomers, initiator, porogenic solvent, and the monomers to cross-linker ratio. Indeed, to synthesize an optimal and selective polymer, the selection of effective parameters must be carefully considered [18–20]. Molecularly imprinted polymer, as a sensing material, makes sensitive sensors for determining the analytes in complex matrices. High stability and selectivity, long lifetime, and cost-effectiveness make these synthetic receptors very attractive materials and offer them as a promising alternative to antibodies [21]. It is especially noticeable that modification of the electrode by MIPs as well as various nanostructures can propose a robust and interesting methodology for trace quantification of compounds [22–25]. Different electrochemical techniques in combination with modified sensors have been successfully used to detect the electro-active analytes in numerous samples.

Besides the preparation of a sensor, the application of chemometrics or multivariate data analysis is suggested to optimize and interpret obtained data rapidly. Optimization studies have largely focused on the use of the chemometric approach and experimental design to determine the critical factors affecting

responses of the analytical instruments or tools. These methods allow simultaneous proceeding one or multiple parameters. They can be used to optimize experimental conditions of electroanalytical analysis to obtain the most satisfactory results [16, 26, 27].

This study was aimed to develop a nanocomposite carbon sensor based on molecularly imprinted polymer as well as the optimization of main factors affecting the voltammetric determination of carbofuran in environmental and biological samples by a chemometric approach.

EXPERIMENTAL

Instruments and reagents. Electrochemical analyses were performed by a three-electrode system using an AUTOLAB PGSTAT302. The Fourier-transform infrared spectroscope (FTIR, Perkin Elmer) was used as an imaging system. The modified sensors were applied as working electrodes. In addition, an Ag/AgCl electrode and a graphite rod were used as the reference and counter electrodes, respectively. Methacrylic acid (MAA) and ethylene glycol dimethacrylate (EGDMA) were obtained from Fluka (Buchs, Switzerland). Carbofuran (Dr Ehrenstorfer, Germany), paraffin oil (Merck Co.), and 2,2-azobisisobutyronitrile (AIBN) from Sigma–Aldrich (Munich, Germany) were used. 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 of analytical grade were purchased from Merck Co. (Germany).

Preparation of the molecularly imprinted polymer. To synthesize the molecularly imprinted polymer, non-covalent bulk polymerization was applied. Following the complexation of the template molecule carbofuran (1 mmol) with functional monomers MAA (4 mmol), the polymerization of MAA around the template was employed by cross-linker EGDMA (20 mmol) in the presence of initiator AIBN (60 mg) in a porogen solvent. Indeed, all polymer compounds were added to a 75-mL glass tube containing 10 mL of chloroform (porogen) and the mixture was purged with nitrogen for 7 min. The glass tube was sealed under nitrogen atmosphere and placed in a water bath at 60°C for 18 h to start the polymerization. After this time, the synthesized polymer was dried and ground to obtain a homogenous powder. To remove the template molecule, extensive washing was done via Soxhlet extraction with methanol for 48 h. Finally, extra washing was performed by methanol to remove carbofuran completely, so that no template molecules were detected in the effluent by UV-Vis spectrometry and square wave voltammetry methods. Furthermore, the non-imprinted polymer (NIP) was prepared with the same procedure without the addition of the template molecule to the mixture. The FTIR spectroscopic analysis of unwashed and washed carbofuran

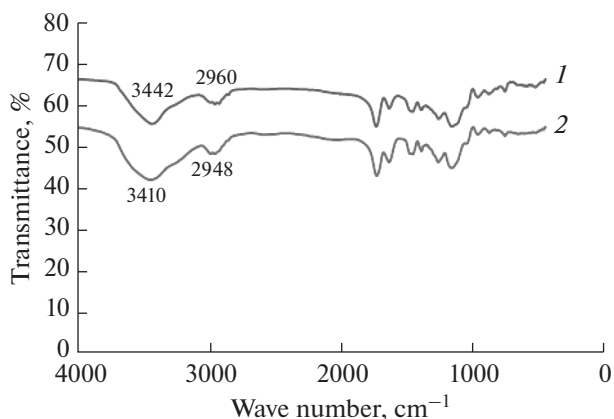


Fig. 1. Infrared spectra of the washed (1) and unwashed molecularly imprinted polymer particles (2).

imprinted polymer materials (Fig. 1) indicated the similarity in molecular structures of these polymers. As it can be seen, the carboxylic acid O–H stretch vibration in the washed and unwashed MIPs was observed at 2948 and 2960 cm^{-1} , respectively. This displacement toward lower frequencies can be attributed to the interaction of hydrogen with carbofuran in the unwashed MIP. The carbonyl group C=O stretching peak was observed at 1734 and 1727 cm^{-1} for washed and unwashed polymers, respectively, and they might be related to MAA and EGDMA. In order to maximize the imprinting effect, the functionalities of the template and functional monomer were matched in an appropriate mode, for instance H-bond

donor with H-bond acceptor. The peaks appeared at 3410 and 3442 cm^{-1} could reflect the possible presence of the N–H bond. The displacement of these peaks and the other ones in both polymers confirmed the interaction between the functional monomer and template molecule. The peaks beyond 1500 cm^{-1} , the fingerprint region, were difficult to interpret because many bands overlapped. Figure 2 shows the scanning electron microscope image of prepared MIP for carbofuran. The scanning electron microscopy was done for total investigation of the MIP morphology and homogeneity of the size. According to the observations, the formation of carbofuran imprinted particles was successfully performed and there was a spherical shape with a narrow size distribution.

Preparation of the electrode. Graphite powder and paraffin oil were mixed in a 75 : 25 (w/w, %) ratio to prepare the bare carbon paste electrode (CPE). The total weight of the paste considered was 0.1 g and its composition percentages were calculated based on this weight. The modified electrodes were prepared in the same procedure by mixing 52% of graphite, 25% of paraffin oil, 20% of MIP, and 3% of MWCNTs (electrode No. 5 in Table 1). As it can be seen in Table 1, different percentages of graphite, MWCNTs, paraffin oil, and MIP or NIP were mixed for preparing all electrodes. The mixture was homogenized in a mortar for at least 10 min. The final paste was packed into the end of an electrode body (i.d.—3 mm, paste depth—1 cm) in which an electrical contact was made with a copper rod. To remove the excess of material, the electrode surface was polished using an office paper. To deter-

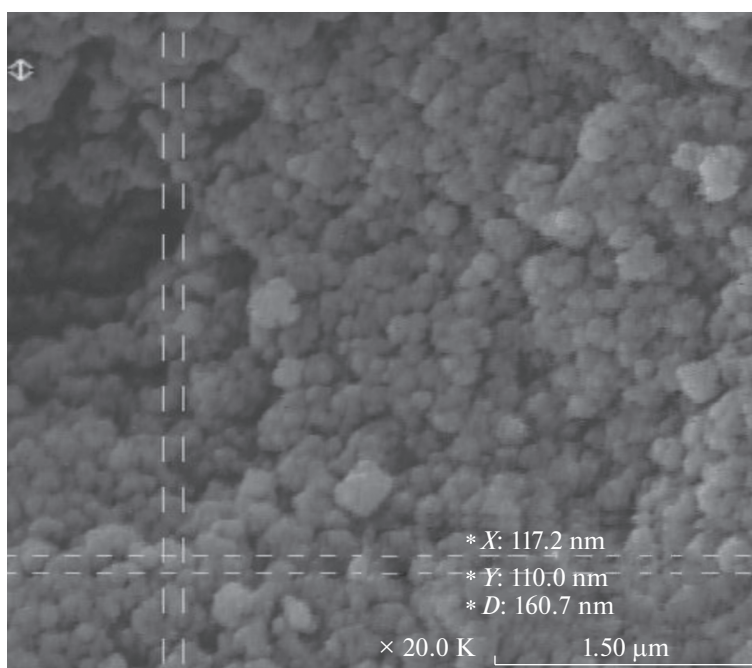


Fig. 2. Scanning electron microscopy image of prepared molecularly imprinted polymers.

Table 1. The high (+) and low (–) factor levels in the experimental design for the sensor response optimization

Level	E–pH	E–time, min	EC, M	SWA, V	DP–time, s	DP, V	SWF, Hz	A–pH
High (+)	10	20	0.05	0.1	25	–0.1	150	9
Low (–)	2	5	0.01	0.05	5	–1	50	4

Notations: 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.

mine the role of each component in the electrode response improvement, several electrodes with different compositions were prepared.

Experimental design approach for electrochemical measurements. Experimental design can be applied to optimize experimental conditions and instrumental parameters to obtain the most satisfactory results. This approach needs less measurements compared to one-at-a-time experiment and it can estimate any interaction between the factors. A list of the main and interaction effects is evaluated using analysis of variance (ANOVA) table to determine their statistical significances. In this work, the Minitab software (version 14) was used for data analysis. At the first step of the experimental design, eight factors affecting the sensor response were selected, including the extraction pH, extraction time, analysis pH, deposition potential and its exertion time, electrolyte concentration, square wave amplitude and its frequency. A two-level full factorial design of 2^8 was used and a fraction of the runs (1/8) specified by this design was picked involving 32 basic experiments (64 with two replicates). Table 1 indicates the tested values corresponding to the high (+) and low (–) points for each factor. In fact, multivariate strategy was used to optimize the experimental variables for square wave voltammetry for carbofuran determination. The electrochemical measurement of carbofuran was done according to the following steps.

Extraction. Each prepared electrode was inserted into the carbofuran solution with defined concentration and the extraction pH and extraction time variables were adjusted according to the runs determined by Minitab. All solutions were stirred at the stirring rate of 600 rpm.

Determination. The electrochemical cell containing KCl solution was used for voltammetry experiments. At first, a negative deposition potential was applied to the electrode (placed in the cell) and then the potential was scanned in the defined range. The analytical variables in each run were adjusted to predicted ones.

Preparation of samples. The stock solution of carbofuran (1×10^{-2} M) was prepared in ethanol. The working solutions for experiments optimization were made by diluting a certain amount of the stock solution with deionized water. Unexposed urine, tap and river samples were considered as biological and environmental real samples to evaluate the possibility of carbofuran determination by the proposed sensor. There was no need for special pre-treatment for these

samples. Only urine samples were diluted 1 : 5 with deionized water. The specified amount of carbofuran was spiked in all solutions to do the optimization process. The working electrode (MIP-CPE) was dipped into the solutions to extract carbofuran. Then, the measurement was completed via inserting three electrodes into an electrochemical cell with selected optimal characteristics. After applying deposition potential of 1 V, the square wave voltammograms were recorded in the scan range from –0.05 to 0.5 V.

RESULTS AND DISCUSSION

Optimization of the carbon paste electrode composition. Basically, carbon paste is made of graphite powder and binder. However, this paste can be modified by other components as modifying agents. In this study, the MWCNTs and MIP were added to the paste. To have a better comparison, a bare CPE and the electrode modified by NIP were also made. Eventually, electrochemical experiments were carried out by various prepared CPEs to select the most efficient electrode composition for the determination of carbofuran. Table 2 indicates the responses of different prepared electrodes in the same electrochemical conditions. Obtained results confirm the significant effect of the modifier and its amount on the electrode response. The improving effect of nanoparticles on the properties of CPE can be attributed to the vast surface area, high electrical conductivity, and proper chemical stability of these particles resulting in desired new sensors with appropriate characteristics.

Furthermore, MIPs, as synthetic antibody mimics, can be an excellent sensing material to be used in carbon paste electrodes for their performance improvement. It seems clear that MIPs are desirable recognition elements because of their intrinsic properties such as high selectivity to the analyte of interest, physiochemical stability, reusability, and applicability in rough chemical solutions. According to Table 2, the carbon paste modified with both modifiers (MIP and MWCNTs) indicated the best electrochemical response and it can be considered as a special kind of sensor for a selective determination of the target molecule. On the other hand, the percentage of MIP in the composition of the carbon paste affects the sensor response. Results showed that the paste containing 20% of MIP (electrode No. 5) led to the highest current intensity, thus, it was selected for later experiments. The increase in MIP percentage enhances the

Table 2. Optimization of carbon paste composition for carbofuran quantification (5×10^{-7} M)

Electrode No.	Composition of carbon paste, % (w/w)					Anodic current, μA
	graphite powder	binder (paraffin oil)	MWCNTs	MIP	NIP	
1	75	25	0	0	0	3 ± 2
2	72	25	3	0	0	5 ± 2
3	55	25	0	20	0	9 ± 3
4	57	25	3	15	0	12 ± 3
5	52	25	3	20	0	18 ± 2
6	47	25	3	25	0	12 ± 3
7	57	25	3	0	15	5 ± 2
8	52	25	3	0	20	7 ± 2
9	47	25	3	0	25	5 ± 1

Table 3. The response (anodic current, μA) of the prepared sensor for carbofuran (1×10^{-7} M) in the simultaneous presence of some interferences ($n = 3$)

Added concentration	Simultaneous presence of some pesticides	Simultaneous presence of ions	Without the interferences	<i>P</i> -value
100-fold	30 ± 2	29 ± 3	30 ± 3	>0.05
500-fold	29 ± 1	30 ± 2		>0.05
800-fold	28 ± 3	31 ± 2		>0.05
1000-fold	20 ± 4	19 ± 3		<0.05

electrode resistance resulting in the reduction of its electrochemical responses. It is worth mentioning that the optimization of instrumental and analysis conditions in the next steps of the study has led to the further response enhancement of the selected electrode.

Effect of interferences. In order to assess the selectivity of the prepared sensor, it was inserted into the solutions of carbofuran as well as some commonly used pesticides and some ions using the optimal analytical conditions. The response of the sensor was obtained for carbofuran in the simultaneous presence of different concentrations of these interferences that were gradually added to the defined concentration of carbofuran. Table 3 shows the responses of the electrode. The simultaneous presence of dicloran, diazinon, dichlorfention, and dimethoate as well as different ions could not interfere carbofuran peak current. According to the results, the mentioned compounds had no interfering effect on the analyte signal until about 1000-fold concentration. Obtained findings confirmed high selectivity of the MIP-nanocomposite electrode toward carbofuran in the presence of other compounds. Therefore, it was demonstrated that the MIP-CPE was able to interact strongly with the target molecule due to specific cavities in the MIP structure.

Optimization of the important parameters in carbofuran detection. The effect of different parameters on carbofuran detection was investigated using the designed sensor to optimize the extraction and elec-

trochemical determination conditions. For applying the experimental design methodology, eight mentioned factors in Table 1 were selected as inputs. All experiments were performed using carbofuran solutions containing defined concentration (5×10^{-7} M). Table 4 exhibits chosen runs for the optimization of carbofuran electrochemical determination and obtained results for the sensor response in μA . As it can be seen in Table 4, the highest current intensity was obtained in levels indicated in the run number 20. These levels (-1 and $+1$) can be seen in Table 1. On the other hand, the interactions between factors were investigated and data were evaluated by *t*-test and ANOVA at the 5% significance level. In statistical analysis of data provided by Minitab, the interaction effect of variables on the response is expressed by R^2 -(adjusted). This parameter (R^2) is the percentage of response variation that is explained by its relationship with one or more effective factors. R^2 is always between 0 and 100%. According to obtained data, in some cases significant interactions have taken place between factors. This means that the influence of one factor on the sensor response optimization may depend on the values of other factors. Based on Table 5, the binary interaction effect of square wave frequency (SWF) and square wave amplitude (SWA) is significant ($P < 0.05$) and the contribution of these factors to sensor response is 42%. Also, data in Table 5 indicate the contribution of SWF, SWA, and deposi-

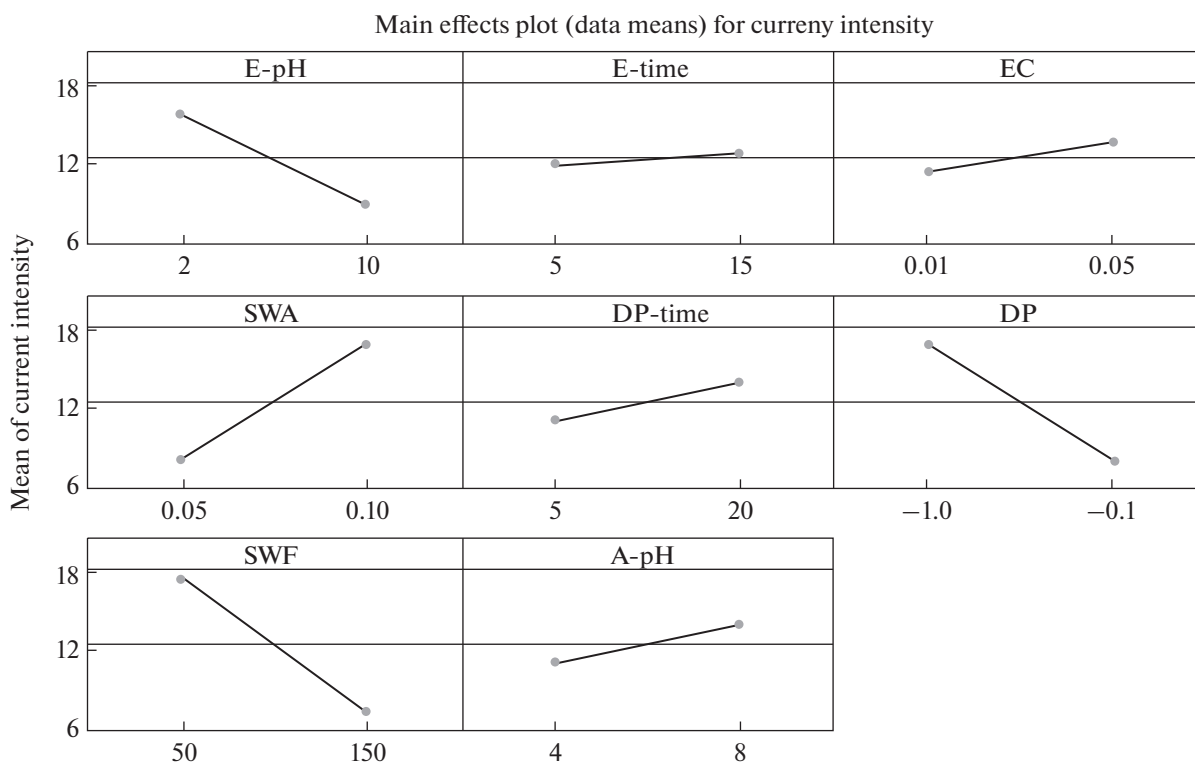


Fig. 3. Main effects plot in the factorial design.

tion potential (**DP**) to the sensor response is significant (50%, $P < 0.001$) and these three factors have the highest effect on the sensor current intensity increasing. In this respect, the effect of each factor on the response as well as their interaction effects are depicted in Figs. 3 and 4, respectively. It seems clear that the optimization of SWF, SWA, and DP has a more remarkable effect on the sensor response than the other factors and they should be considered for the electrochemical determination of analyte. As it can be seen in Table 4, in other runs in which these three factors had the same levels as in run 20 (runs 21 and 25) a higher increase in current intensity was observed. The exertion of deposition potential to the working electrode causes the molecule of interest to be deposited onto the surface of the electrode leading to more efficient detection. In addition, both SWA and SWF are of up-most importance in SWV because they affect the signal intensity and technique sensitivity. The optimum levels for amplitude and frequency were selected as 0.1 V and 50 Hz, respectively. In frequencies higher than 50 Hz, unstable as well as large current fluctuations were observed. The higher amplitude caused the wider peak current. On the other hand, in lower levels of these parameters no adequate signal intensity was observed [28, 29]. Therefore, the optimum levels of DP, SWA, and SWF made a considerable enhance in the current intensity. It is worth mentioning that although 50% of the sensor response depends on

above-mentioned factors, all effective factors should be optimized to obtain the highest sensor response. Each factor may have an interaction effect on other factors, and it can contribute to the response enhancement. In completely optimized conditions, the lower detection limit can be achieved resulting in the analyte determination at trace levels. Accordingly, the optimum levels for other factors were selected as follow: extraction pH – 2, extraction time – 20 min, analysis pH – 9, deposition potential exertion time – 25 s, electrolyte concentration – 0.05 M.

Comparison of the prepared molecularly imprinted and non-imprinted polymers. To completely compare the ability of the prepared sensors to adsorb carbofuran, the difference between their responses was studied after the optimization of all effective parameters influencing its electrochemical determination. The square wave voltammograms related to the determination of carbofuran by MIP-CPE, NIP-CPE, and bare CPE can be seen in Fig. 5. There was a significant difference ($P > 0.05$) between sensors in analyte adsorption proving the ability of MIP to selectively adsorb molecules due to its special structure.

Method validation. After the optimization of parameters affecting the electrochemical determination, the calibration curve was plotted using the prepared MIP-CPE and different carbofuran concentrations. The sensor was inserted into carbofuran solutions and the electrochemical analysis was performed

Table 4. The experimental design for the optimization of carbofuran electrochemical determination and obtained results for the sensor response

Run	E-pH	E-time, min	E-C, M	SWA, V	DP-time, s	DP, V	SWF, Hz	A- pH	Current intensity, μA
1	1	-1	1	1	1	-1	-1	-1	17 \pm 2
2	1	1	-1	-1	1	-1	-1	1	12 \pm 1
3	1	-1	-1	1	-1	1	-1	-1	11 \pm 2
4	-1	1	-1	1	1	1	-1	-1	17 \pm 1
5	1	1	-1	1	-1	-1	1	1	8.7 \pm 0.4
6	-1	-1	-1	1	-1	-1	1	-1	12 \pm 1
7	1	-1	-1	-1	1	1	1	-1	2.9 \pm 0.4
8	1	-1	-1	1	1	1	-1	1	11 \pm 2
9	-1	-1	1	1	1	1	1	-1	5.7 \pm 0.8
10	-1	1	1	-1	-1	-1	1	1	6 \pm 1
11	1	1	1	-1	1	1	-1	-1	4.9 \pm 0.3
12	-1	-1	-1	-1	1	-1	-1	-1	29 \pm 3
13	1	1	1	-1	-1	1	-1	1	5.5 \pm 0.6
14	-1	-1	-1	-1	-1	-1	-1	1	5.0 \pm 0.4
15	-1	-1	1	-1	-1	1	-1	-1	5.3 \pm 0.6
16	1	1	1	1	1	1	1	1	4.7 \pm 0.3
17	1	-1	-1	-1	-1	1	1	1	3.0 \pm 0.9
18	1	-1	1	-1	-1	-1	1	-1	6.1 \pm 0.8
19	-1	-1	1	-1	1	1	-1	1	13 \pm 2
20	-1	1	1	1	1	-1	-1	1	50 \pm 2
21	-1	1	1	1	-1	-1	-1	-1	38 \pm 2
22	1	1	-1	-1	-1	-1	-1	-1	5 \pm 1
23	1	-1	1	-1	1	-1	1	1	9.1 \pm 0.6
24	-1	-1	1	1	-1	1	1	1	5 \pm 1
25	1	-1	1	1	-1	-1	-1	1	34 \pm 3
26	-1	-1	-1	1	1	-1	1	1	22 \pm 2
27	-1	1	-1	-1	1	1	1	1	6.4 \pm 0.9
28	-1	1	1	-1	1	-1	1	-1	10 \pm 1
29	1	1	1	1	-1	1	1	-1	3 \pm 1
30	1	1	-1	1	1	-1	1	-1	8 \pm 2
31	-1	1	-1	-1	-1	1	1	-1	4 \pm 1
32	-1	1	-1	1	-1	1	-1	1	25 \pm 2

in optimized conditions. The linear relationship between the anodic current intensity and analyte concentration was obtained over the range from 1×10^{-9} to 1×10^{-6} M. Since it is more important to accurately quantify analyte trace amounts, the calibration curve was drawn in two concentration regions causing the higher linearity in lower concentrations. The equation of calibration curve and R^2 were obtained $Y = 0.1012X + 10.519$ and 0.9946, respectively. The limits of detection (LOD) and quantification (LOQ) were 3×10^{-10} and 1×10^{-9} M, respectively. The following equations were applied to calculate the LOD and LOQ:

$$\text{LOD} = 3s_b/m, \quad \text{LOQ} = 10s_b/m,$$

where s_b is the standard deviation of the blank and m is the slope of calibration graph.

In addition, day to day and within a day measurements were used to determine the reproducibility of the sensor responses. To do this, 6 measurements on 6 consecutive days as well as 6 measurements within one day were considered. The coefficients of variation for day to day and within a day experiments were 4.7 and 3.9%, respectively. Therefore, satisfactory capability of the modified electrode to quantify carbofuran was statistically demonstrated. To evaluate the long-

Table 5. The most important interaction effects of factors and percentage of response variation

Term	Interaction effect <i>P</i> -value	<i>R</i> ² (adj), %
E-pH × E-time	0.021	12.69
E-pH × SWA	0.247	27.03
E-pH × SWF	0.152	28.78
SWA × DP	0.065	32.86
SWA × SWF	0.048	42.30
DP × SWF	0.156	35.97
DP-time × DP	0.359	14.70
SWA × DP × SWF	<0.001	51.00
A-pH × DP × SWF	<0.001	35.37
A × DP × DP-time	<0.001	30.52

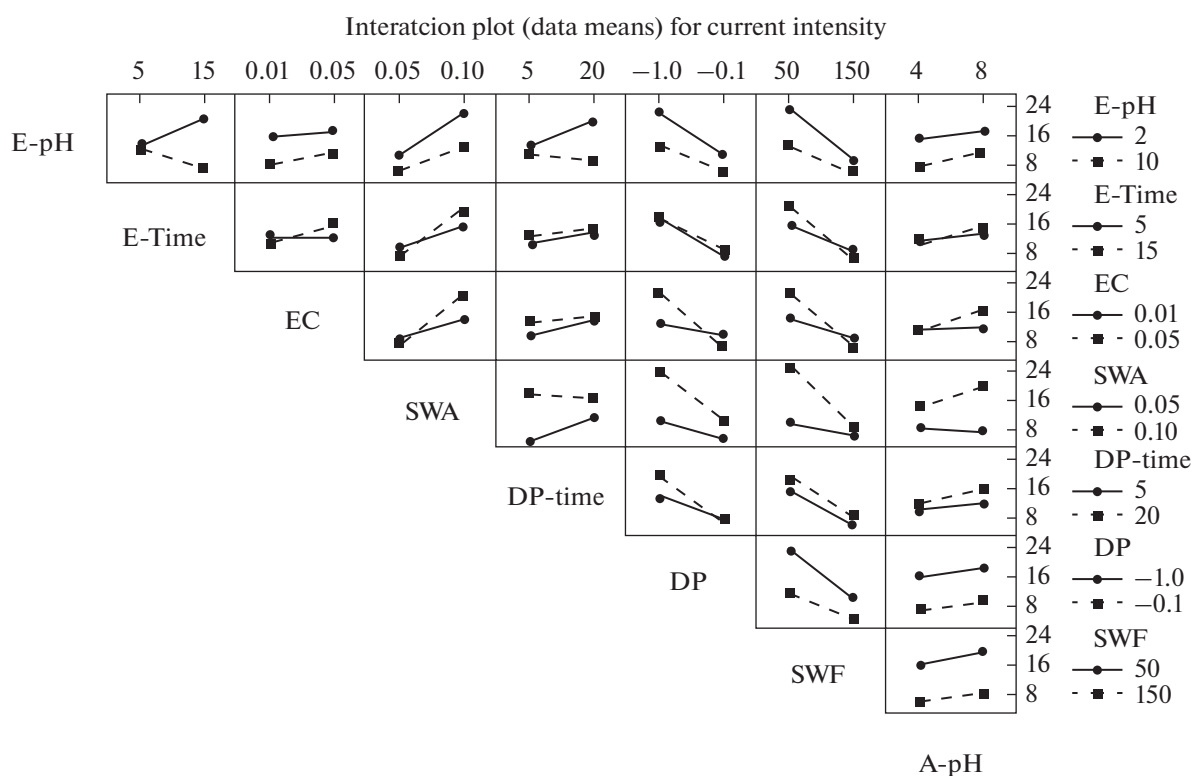
term stability of the proposed sensor, some experiments were carried out in a period of one month. There was no significant change in the current intensity for recorded responses and the sensor retained 96% of its initial response.

Determination of carbofuran in spiked urine and water samples. The nanocomposite MIP-based sensor was applied for the determination of carbofuran trace amounts in urine and water samples. Since real urine samples containing carbofuran were not available,

spiked urine samples were used. The detection of carbofuran in all real samples was done by optimized procedure (see above) using the standard addition method. There was no need for digestion or other special pre-treatment steps prior to the voltammetric analysis, because the extraction and electrochemical determination steps were separately performed. Following the addition of different carbofuran amounts into the samples, the extraction and determination steps were done, and the recoveries were calculated for the analyte (Table 6). The obtained results confirm that the proposed electrode in combination with optimized voltammetry procedure can be sufficiently acceptable for monitoring carbofuran in biological and environmental samples. From a technological point of view, such modern analytical tools result in eliminating or reducing the time-consuming and tedious steps of sample pre-treatment. The novel sensor proposed in this study introduces a fast, simple, sensitive, and inexpensive tool to determine the analyte of interest in complex matrices.

CONCLUSIONS

In this work, the molecularly imprinted polymer and carbon nanotubes were used as modifiers in fabrication of a highly selective square wave voltammetric sensor for carbofuran determination. It was shown that the MIP had a considerable effect on the sensor selec-

**Fig. 4.** Interaction effects plot in the factorial design.

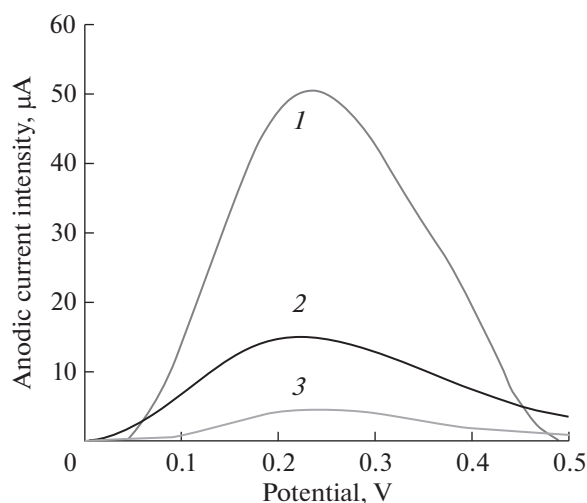


Fig. 5. The voltammograms of MIP-CPE (1), NIP-CPE (2), and bare CPE (3) for the determination of the defined concentration of carbofuran (5×10^{-7} M).

tivity. In addition, by applying MWCNTs better responses for the detection of the analyte were obtained. Low detection limit, high sensitivity, ease of preparation, and long-term stability can be mentioned as the advantages of the introduced sensor comparing to other existing tools. The designed sensor was successfully used for the determination of carbofuran in different real samples with no considerable matrix interference. The estimation of possible interactions of

effective parameters on the current intensity was allowed using the experimental design.

FUNDING

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Table 6. The performance of the nanocomposite molecularly imprinted polymer based sensor in spiked real samples

Sample	Added, ng/mL	Found, ng/mL	RSD, % ($n = 3$)	Relative recovery, %
Tap water	–	N.D. ^a	–	–
	20	19	2.5	94
	200	192	2.3	96
	2000	1904	1.9	95
River water	–	<0.2	–	–
	20	19	3.3	97
	200	189	2.4	95
	2000	1889	3.0	94
Urine	–	N.D. ^a	–	–
	20	19	2.0	91
	200	188	2.2	94
	2000	1821	3.1	91

^a N.D. – not detected.

CONFLICT OF INTEREST

It is confirmed that there is no conflict of interest.

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