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Research Article

Electrochemical pesticide sensor based on anthraquinone substituted copper phthalocyanine

Yeliz İPEK[1,*], M. Kasım ŞENER[2], Atıf KOCA[3]

[1]*Department of Chemical Engineering, Faculty of Engineering, Munzur University, Merkez, 62000, Tunceli, Turkey*

[2]*Department of Chemistry, Faculty of Arts and Sciences, Yıldız Technical University, Esenler, 34220, Istanbul, Turkey*

[3]*Department of Chemical Engineering, Faculty of Engineering, Marmara University, Göztepe, 34722, Istanbul, Turkey*

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[*]Corresponding author's e-mail address: yelizipek@munzur.edu.tr (Y. İpek)

ABSTRACT

In this study, a biomimetic selective pesticide electrochemical sensor based on copper phthalocyanine-anthraquinone hybrid (CuPc-AQ) was studied. Electrochemical activities based on Pc ring and anthraquinone substituent of the CuPc-AQ make it valuable material for electrochemical sensor studies in detecting of pesticides. Thin film of CuPc-AQ on ITO electrode (ITO/CuPc-AQ) was exposed to water samples containing eserine and carbofuran pesticides, separately. Then in order to observe the sensor behavior of the modified electrode, square wave voltammetry (SWV), electrochemical impedance spectroscopy (EIS), and double potential step chronocoulometry (DPSCC) techniques were used. When the ITO/CuPc-AQ electrode was doped with nano-platinum (nPt) or nano-gold (nAu) particles, the sensitivity of ITO/CuPc-AQ electrode increased. The lowest detection limit (DL) (1.23x10-9 mol dm-3) was obtained with ITO/CuPc-AQ-nAu electrode using electrochemical impedance spectroscopy technique.

Keywords: Electrochemistry, Phthalocyanine, Pesticide sensor Langmuir-Blodgett

Antrakinon Substite Bakır Ftalosiyanin Temelli Elektrokimyasal Pestisit Sensörü

ÖZ

Bu çalışmada antrakinon hibrit bakır ftalosiyanin (CuPc-AQ) temelli, seçici bir elektrokimyasal pestisit sensörü üzerinde çalışılmıştır. CuPc-AQ'un, ftalosiyanin halkası ve antrakinon substituenti kaynaklı elektrokimyasal aktiviteleri, bu malzemeyi pestisitlerin saptanmasında kullanılacak olan elektrokimyasal sensor çalışmaları için değerli kılmaktadır. ITO elektrot üzerinde bulunan CuPc-AQ ince filmi (ITO/CuPc-AQ) eserin ve carbofuran pestisitleri içeren su numunelerine ayrı ayrı maruz bırakılmış ve daha sonra kare dalga voltametrisi (SWV), elektrokimyasal empedans spektroskopisi (EIS) ve çift potansiyel basamaklı kronokulometri (DPSCC) teknikleri kullanılarak modifiye elektrodun sensor davranışları incelenmiştir. ITO/CuPc-AQ elektrodu, nano-Platin (nPt) ya da nano-Altın (nAu) parçacıkları ile yüklendiğinde ITO/CuPc-AQ elektrodunun hassasiyeti artmıştır. En düşük saptama limiti değeri (DL) ($1.23x10^{-9}$ mol dm^{-3}) ITO/CuPc-AQ-nAu elektrodu ile elektrokimyasal empedans spektroskopisi tekniği kullanıldığında elde edilmiştir.

Anahtar Kelimeler: Elektrokimya, Ftalosiyanin, Pestisit sensörü, Langmuir-Blodgett,

1. INTRODUCTION

Farmers use numerous types of pesticides (herbicides, fungicides, insecticides), to protect crops and seeds and to increase the harvesting yield. However, the pesticide residues may enter into the ecosystem and food chain by means of air, water and soil.[1-4] Their increasing use in agriculture makes them the most important environmental pollutants since they are carcinogenic and cytotoxic for animals and human. Their toxicity is based on the inhibition effect on acetylcholinesterase (AChE) enzyme which has an important mission in neurologic system of animals and human.[2-4] Acetylcholine (ACh) is one of the numerous neurotransmitters in the nervous system and it has important roles in both peripheral nervous system and central nervous system. ACh carries the synaptic pulses between two neurons and when binds to the target synapse AChE which exists in the synaptic space, hydrolyzes ACh into acetate and choline rapidly. Thus, as an AChE inhibitor, pesticides cause accumulation of ACh at cholinergic synapses. As a result of this accumulation, bone marrow disorders, nerve disorders such as Alzheimer, infertility, and immunological and respiratory diseases may occure.[5-6] Detection of pesticides at the levels established by the (EPA) remains a challenge.[7] For this purpose.[8] there are some traditional detection methods such as gas chromatography (GC), high performance liquid chromatography (HPLC) combined with mass spectrometry (MS).[9-10] Although these detection methods are time-consuming and require expensive equipment and highly trained technicians, they have been used traditionally because of their sensitivity and reliability. The developing science and technology is leading new alternative detection methods for pesticides like electrochemical sensors or biosensors.[11-14] For designing pesticide biosensors, AChE enzyme has been used frequently because of the inhibitory effect of pesticides on this enzyme. However, low stability of the biological species, difficulties of immobilization on the substrates, leakage from the substrate surface, high cost, and low chemical and thermal stabilities are some disadvantages of enzyme biosensors.[15]

Metallophthalocyanines (MPcs) have been studied extensively as electrochemical catalysts and sensor recognition materials because of their properties such as excellent redox behavior, thermal and chemical stability, ability of easy modification with different metal ions and substituents, and ability of easy coating on different substrate with various techniques.[16-19] MPcs which are artificially derivate of natural protein, porphyrin, have been deeply used as mimetic enzyme receptors for the sensing of many harmful biological target species, such as insecticides and pesticides. Recently, there is a tendency towards finding new materials which can be used as recognition element of sensor instead of enzymes.[20-23] Harbeck and co-workers have modified chemically the core of phthalocyanine to increase sensor sensitivity for organophosphorus and organonitrogen pesticides such as fenthion and methiocarb and they have obtained a detection limit down to 0.03 mg dm^{-3}.[24] Pu-Hong Wang and co-workers have designed and synthesized a new phthalocyanine 2,9,16,23-tetrakis [4-(hexafluoroisopropanol)-anilinomethyl] phthalocyaninato zinc for electrochemical sarin gas sensor and obtained the detection limit as as 205.8 Hz/mg/m^3.[25] İpek and co-workers have used a terminal-alkyl substituted cobalt phthalocyanine and constructed a biomimetic electrochemical biosensor with a new electrode modification technique, “click electrochemistry” (CEC) based on a functional cobalt phthalocyanine and they detected eserine pesticide with a sensitivity of 1.15 A $dm^3\,mol^{-3}$ and DL of 1.75×10^{-7} mol dm^{-3}.[26]

The AChE enzyme is not selective for different type of pesticides, because all kind of pesticides have the same inhibitory effect on the enzyme. When there is a mixture of pesticides the enzymatic sensor detects them as the same kind. New artificial biomimetic sensors are especially advantageous for selective pesticide detection with high sensitivity. For this purpose, we prepared an electrochemical pesticide sensor based on a copper phthalocyanine–anthraquinone hybrid complex (CuPc-AQ).[27] Copper phthalocyanine–anthraquinone hybrid complex has two different redox active sides consisting of phthalocyanine ring and anthraquinone substituents for catalytic and/or sensor applications. Phthalocyanine ring can give two reduction (at -1.22 V and -1.46 V) reactions and two oxidation reactions (at 1.40 V and 0.82 V), while four anthraquinone substituents of CuPc-AQ generally give one reduction reaction (at -0.91 V).[27] These redox activities of CuPc-AQ probably provide the electrochemical applications of the complex as especially cathode active electrocatalyst and/or electrochemical sensor.

In this study, Langmuir-Blodgett monolayer coating technique was used to prepare the modified electrode with CuPc-AQ (ITO/ CuPc-AQ). The Langmuir-Blodgett film of CuPc-AQ was constructed on ITO electrode and these sensors were tested for the detection of carbofuran and eserine pesticides.

2. MATERIALS AND METHOTDS

2.1. Materials

Dichloromethane (DCM), chloroform ($CHCl_3$), $LiClO_4$, $CdCl_2.H_2O$, stearic acid (SA), KCl, H_2PtCl_6, sodium dodecyl sulfate (SDS), eserine, carbofuran, and tetrabutylammonium perchlorate (TBAP) were purchased from Sigma Aldrich. Ultra-pure water having a resistance of 18.2 MΩ was used to prepare analyte solution. The stearic acid solution was used as a supporting agent for the modification of the electrodes via Langmuir-Blodgett coating technique. KCl and H_2PtCl_6 materials were dissolved in ultra-pure water for Pt coating solution and sodium dodecyl sulfate (SDS) was added to this solution for nano Pt coating of ITO electrode. On the other hand, a gold solution (Sigma, 1000941718) was used for nano gold coating process. Herein, CuPc-AQ (Figure 1) which is the recognition material used as the functional material of the biosensor was synthesized and spectroscopic studies were performed by our research group.[27] Eserine (Sigma, E8375) and carbofuran (Aldrich, 426008) pesticides were the analytes of modified biosensor.

2.2. Electrochemical measurements

Gamry Reference 600 potentiostat/galvanostat with a three-electrode cell was used for electrode modification and sensor measurements. All the experimental studies were applied at room temperature. The working electrode was ITO electrode or modified ITO electrode (ITO/CuPc-AQ). A Pt wire electrode was used as the counter electrode, and a saturated calomel electrode (SCE) was the reference electrode. A double bridge was employed to separate the reference electrode from the solution. TBAP solution was used as the supporting electrolyte. The solution was prepared with a concentration of 0.10 mol dm^{-3} in dichloromethane (DCM) solvent. During the electrochemical studies, the absorbed O_2 from the air effects the measurements. Thus, the dissolved O_2 in the electrolytic solution was purged with high purity N_2 gas (%99.99) to obtain a nitrogen blanket and prevent O_2 absorption. Electrochemical impedance spectroscopy (EIS) measurements were performed under 10 mV AC voltage versus open circuit potential (open circuit voltage is 0.50 V) in the AC frequency range 300 kHz - 10 Hz with a 10 mV excitation AC voltage.

2.3. Electrode preparation

2.3.1. LB Coating of CuPc-AQ

Langmuir-Blodgett technique was applied for coating CuPc-AQ on ITO electrode using KSV NIMA Langmuir-Blodgett monolayer coating device. A subphase solution filled in the trough was obtained with aqueous $CdCl_2.H_2O$ solution at a concentration of 1 mg ml^{-1}. The coating material CuPc-AQ solution was prepared at concentration of 1 mg ml^{-1} with chloroform solvent and mixed with SA solution at concentration of 1 mg ml^{-1} with a volumetric ratio of 1:1. The coating mixture was dropped on the subphase solution gradually until the surface pressure reached to 0.5mN/m. To detect the ideal surface pressure for monolayer film formation on the subphase, the surface pressure (SP)-Mean molecular area (Mma) isotherm was obtained, and the ideal surface pressure was detected as 35mN/m. 20 layers of CuPc-AQ:SA were coated on ITO electrodes with 5 mm s^{-1} up and down stroke rates and the electrode was waited for 5 minutes at up position to dry the coated thin film.

2.3.2. Modification of ITO/CuPc-AQ

ITO/CuPc-AQ electrodes were modified with nano platinum and nano gold to increase the sensitivity. ITO/CuPc-AQ-nPt modified electrode was prepared with coating n-Pt on ITO/CuPc-AQ. A solution of 0.1 mol dm^{-3} KCl and 1 mmol dm^{-3} H_2PtCl_6 in 10 ml ultra-pure water which supported with 0.8 mg (1% of total weight of solution) sodium dodecyl sulfate (SDS) surfactant to prevent agglomeration of nano-Pt particles was prepared. SDS was added into this electrolyte before the addition of 1 mmol dm^{-3} H_2PtCl_6. 5 cycles of CV were applied between the potentials of 0.30 V and -0.70 V at 0.100 Vs^{-1} scan rate for the deposition of nano-Pt on the ITO/CuPc-AQ electrode.

ITO/CuPc-AQ-nAu electrode was prepared by dropping 10 µl of nano-gold solution (Sigma, 1000941718) on the 0.64 cm^2 area of ITO/CuPc-AQ electrode and drying in a vacuum oven at room temperature.

2.4. Sensor measurements

Pesticide sensor measurements of ITO/CuPc-AQ, ITO/CuPc-AQ-nPt, and ITO/CuPc-AQ-nAu electrodes as electrochemical pesticide sensors were performed with cyclic voltammetry (CV), square wave voltammetry (SWV), electrochemical impedance spectroscopy (EIS) and double potential step chrono coulometry (DPSCC) analyses with two different pesticides. The three-electrode cell and the electrolyte solution of 0.1 mol dm^{-3} lithium perchlorate ($LiClO_4$) dissolved in 0.1 mol dm^{-3} PBS were used for sensor measurements. Before sensing measurements, the stable blank measurements were obtained. Then the pesticide solution was added drop by drop, and the electrochemical measurements of CV, SWV, DPSCC, and EIS techniques were performed sequentially. The detection linear range and detection limit parameters of the electrodes were calculated.

3. RESULTS AND DISCUSSION

CuPc-AQ complex shown in Figure 1 was synthesized by our research group.

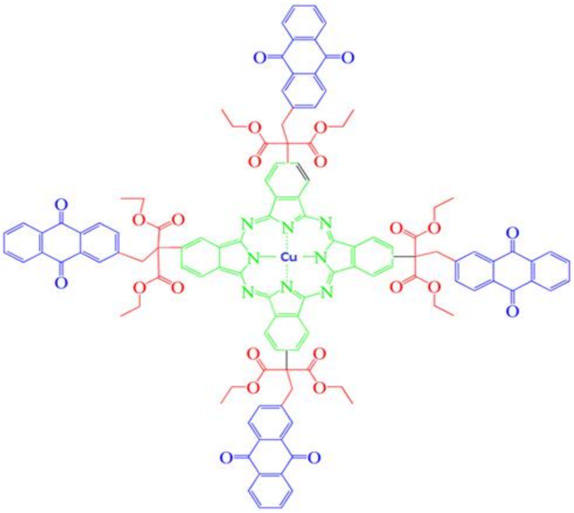

Figure 1. Molecular structure of **CuPc-AQ** phthalocyanine.

To see the usability in the electrochemical applications, its electrochemical characterizations were investigated.[27] The electro-chemical responses of CuPc-AQ in DCM solution are shown in Figure 2. When an electrical potential at cathodic region was applied to the system, three reduction reactions were recorded at -1.22 V and -1.46 V which is belong to phthalocyanine ring and at -0.91 V originated from AQ substituent of the CuPC-AQ. At anodic region two Pc ring reaction peaks were observed. CuPc-AQ has five reversible and diffusion controlled redox reactions.

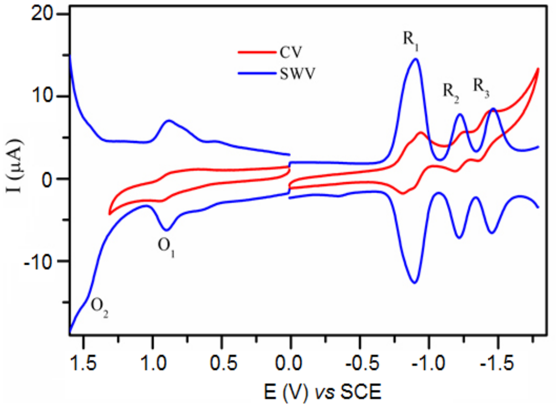

Figure 2. SWV and CV measurements of **CuPc-AQ**.

3.1. Langmuir-Blodgett film of CuPc-AQ

CuPc-AQ complex which was synthesized and electrochemically characterized by our research group was immobilized on ITO electrode with Langmuir Blodgett (LB) technique (ITO/CuPc-AQ) to determine possible practical usage of it. On the other hand, the ITO/CuPc-AQ electrode was modified with nano-platinum (ITO/CuPc-AQ-nPt) and nano gold (ITO/CuPc-AQ-nAu) as shown in Figure 3.

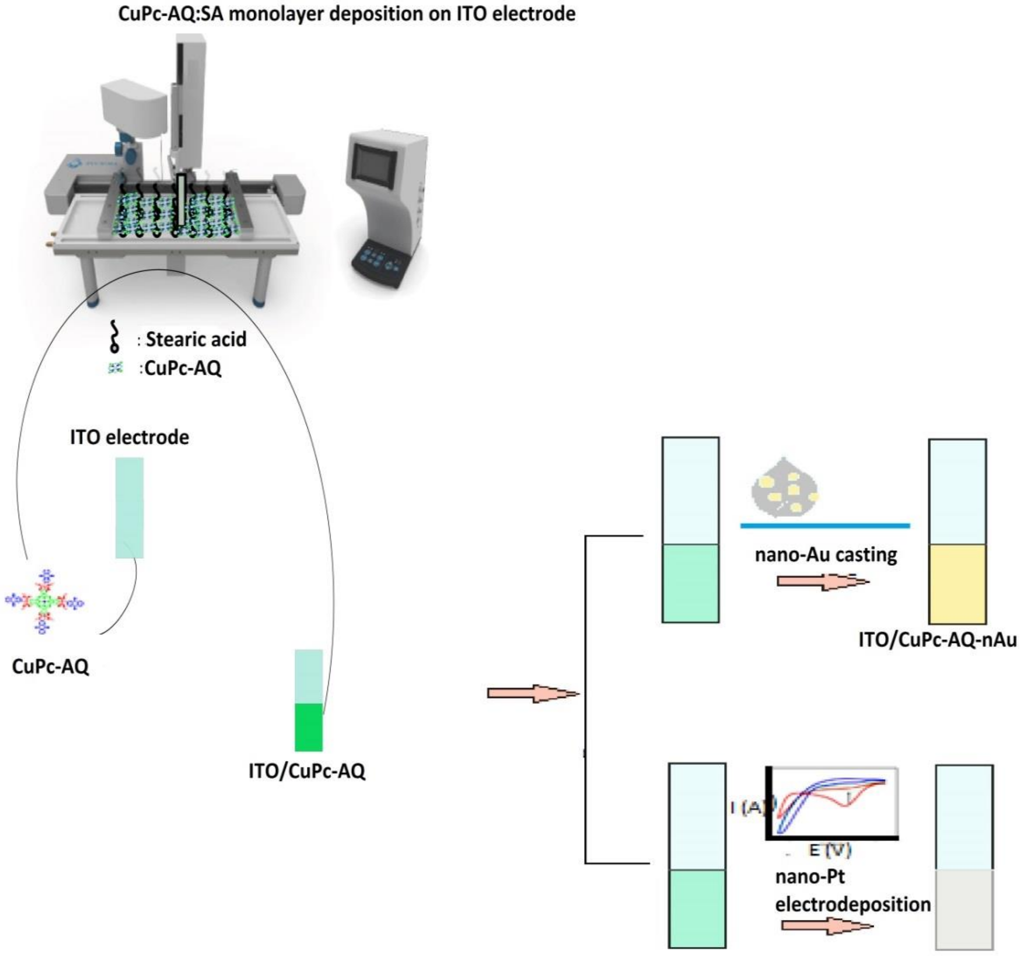

Figure 3. Preparation and modification techniques of ITO/**CuPc-AQ** pesticide biosensor.

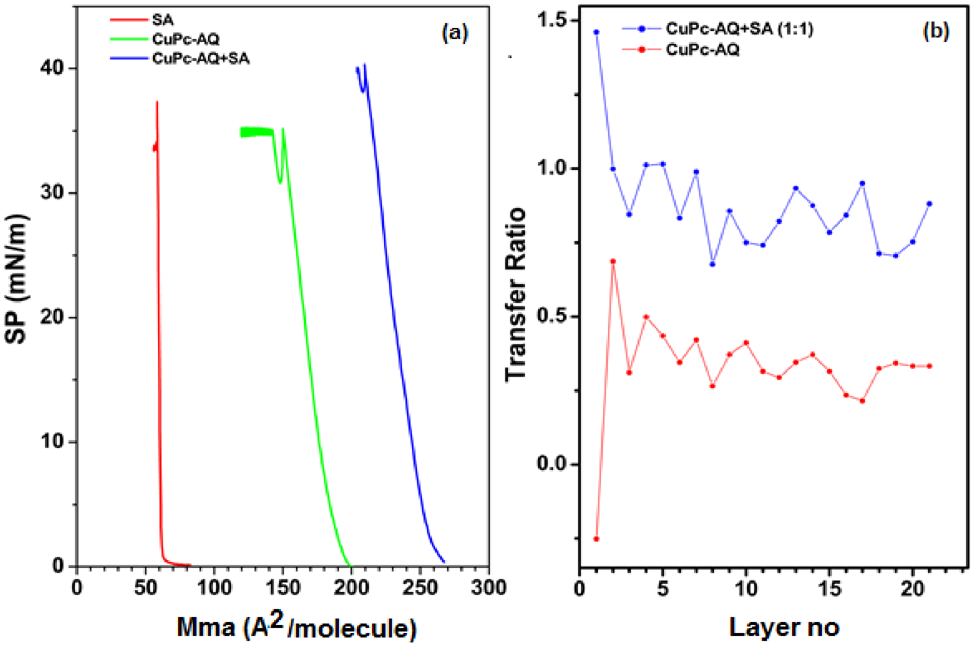

Figure 4. Langmuir-Blodgett coating results of **CuPc-AQ**-SA on ITO substrate: **a)** LB isotherms of SA, **CuPc-AQ** and **CuPc-AQ**-SA coating materials, **b)** LB transfer ratios of **CuPc-AQ** and **CuPc-AQ**-SA.

Figure 4 shows the Surface Pressure-Mean molecular area isotherm of CuPc-AQ and CuPc-AQ:SA mixture and transfer ratios. In Figure 4a, it seems that CuPc-AQ monolayer formed on the subphase of the LB trough, but it could not be coated on the ITO electrodes with a transfer ratio of 1.0. In Figure 4b, the transfer ratios of CuPc-AQ monolayer were about 0.4. To enhance the coating and obtain monolayers with 1.0 transfer ratio, stearic acid solution was used. A solution formed with a mixture of stearic acid solution and CuPc-AQ solution (1:1 by vol.) is employed to obtain 20 monolayers with a transfer ratio of 1.0±0.1. 21 µl of mixed solution was sufficient for a Y type 20 monolayers coating.

When the numbers of coated monolayers were more than 20 layers, the electrochemical measurements were not very successful because of the thick film which obstructs ion exchange during the measurements. The molecular area of CuPc-AQ was calculated from the slope of the isotherm curve as 184 Å^2 which was in agreement with those of similar phthalocyanine in the literature.[28-29] The exact molecular area of the complex indicates that it is orientated horizontally face-on on the subphase. Presence of SA in CuPc-AQ just increases the limiting area to 255 Å^2, that does not affect the electrochemical measurements.

3.2. Sensor measurements

The electrochemical reactions of ITO/CuPc-AQ, ITO/CuPc-AQ-nPt and ITO/CuPc-AQ-nAu the electrodes were studied with carbofuran and eserine pesticides. For this purpose, SWV, EIS and DPSCC techniques were applied. ITO/CuPc-AQ electrode behaved as a selective sensor for these pesticides. The SWV responses of ITO/CuPc-AQ are shown in Figure 5 for the titration of eserine and carbofuran. The electrochemical responses of the CuPc-AQ LB thin film were different than the responses of the material in the dichloromethane solution. The potentials over 1.2 V burns the ITO electrode. Thus, for sensor measurements the applied potentials were kept between -1.0V and 1.0V. In this potential range, a broad cathodic peak was observed about -0.40V which was attributed to an overlapped peak of Pc ring reduction and AQ substituent reduction peaks.

The red colored curves that show the SWV responses of ITO/CuPc-AQ electrode without pesticide addition give a reduction couple, R$_1$ at about -0.40 V. Figure 5a illustrates the SWV responses of ITO/CuPc-AQ during gradual addition of carbofuran. Because of the titration with carbofuran pesticide, the current intensity of R_1^c (represents potential scan direction from -1.2 V to 1.2 V, cathodic scan) process at -0.40 V decreased and a new peak was formed at -0.08 V while the R_1^a (represents potential scan direction from 1.2 V to -1.2V, anodic scan) at -0.39 V potential gradually decreased in current intensity. Carbofuran pesticide has benzofuran active groups which may interact with Cu central metal. Thus this interaction may be resulted with a new peak about -0.08 V. According to these changes in SWV responses during the titration process of carbofuran, this electrode has a sensor activity for carbofuran.

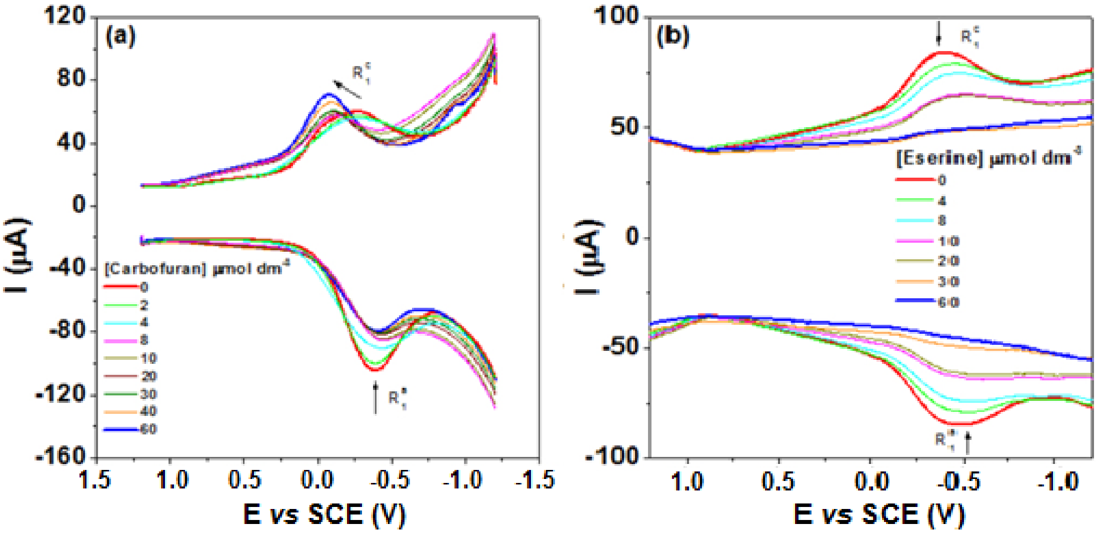

Figure 5. SWV responses of ITO/**CuPc-AQ** electrodes in PBS (pH= 7.0): **a)** During carbofuran titration, **b)** During eserine titration.

In Figure 5b, voltammetric responses of CuPc-AQ recorded during gradual addition of eserine are considerably different than carbofuran titration results. During the addition of eserine, voltammetric changes of R_1^c and R_1^a reduction current intensities continuously decreased with the increasing concentration of eserine. Eserine has functional group of indoline and has dispelled electrochemical activities of Pc ring and AQ moieties. Indoline may be interacted with both central metal and AQ substituent. These voltammetric analysis illustrates that ITO/CuPc-AQ electrode can be used as a selective sensor electrode for carbofuran and eserine while it behaves in a different manner for each pesticide. The current intensity decrease of the reduction peaks during the sensing measurements indicates that CuPc-AQ behaves as artificial sensing material instead of enzymes which were used for pesticide biosensors as organic sensing material. However, CuPc-AQ is preferable to enzymes because of its superior properties such as more stable and low cost.

The detection limits and linear ranges of the ITO/CuPc-AQ electrode were calculated from SWV measurements of carbofuran and eserine titration processes and the results are exhibited in Table 1.

The limit of detection (DL) of the ITO/CuPc-AQ electrode was calculated from Eq. (1):

$$DL = \frac{3S_B}{b} \tag{1}$$

where S_B is the standard deviation of the blank solution, and b is the slope of the analytical curve.[30] Standard deviation was calculated using four blank measurement results and the analytical curve that was obtained with pesticide concentration, and electrochemical signal data was used to calculate the slope.

To support the SWV responses, and to determine the sensor results of the other electrochemical techniques, the sensing responses of ITO/CuPc-AQ were also measured with EIS and DPSCC analysis methods. The EIS sensing measurement of ITO/CuPc-AQ with carbofuran and the calibration line derived from EIS data were illustrated in Figure 6.

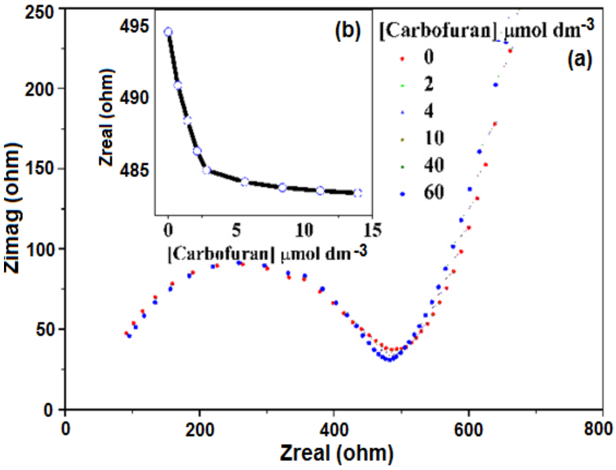

Figure 6. EIS responses and calibration line of ITO/**CuPc-AQ** electrode during the gradual addition of carbofuran.

The diameter of the Nyquist diagram semicircles gradually decreases as a function of the increasing carbofuran concentration in the electrochemical cell solution (Figure 6a). The interaction between ITO/CuPc-AQ and carbofuran increases the conductivity of the modified working electrode. The calibration line is given in Figure 6b and the detection linear range and detection limit values of this modified electrode obtained from this calibration line are tabulated in Table 1.

Table 1. Sensor parameters of modified ITO/CuPc-AQ electrodes for pesticide detection

		Pesticides				
		Eserine		**Carbofuran**		
Modified electrode	**Sensing techniques**	**Linear range (mol dm^{-3}) x 10^{6}**	**Detection Limit (mol dm^{-3})**	**Linear range mol dm^{-3}) x 10^{6}**	**DetectionLimit (mol dm^{-3})**	**Reference**
ITO/CuPc-AQ	SWV (Amperometric)	0.014 - 2.811	6.97 x 10^{-8}	2.100- 16.681	2.08 x 10^{-7}	This work
	SWV (Potentiometric)	0.014 - 1.332	3.03 x 10^{-7}	0.003 - 1.276	9.04 x 10^{-8}	This work
	EIS	0.736 – 5.395	5.93 x 10^{-8}	0.010 - 1.962	8.44 x 10^{-9}	This work
	DPSCC	0.002 - 2.822	6.58 x 10^{-8}	0.024 - 1.266	2.95 x 10^{-7}	This work
ITO/CuPc-AQ-nPt	SWV (Amperometric)	0.030 - 1.346	4.38 x 10^{-8}	0.160 - 1.963	4.66 x 10^{-8}	This work
	SWV (Potentiometric)	0.044 - 2.613	6.97 x 10^{-8}	0.257 - 2.108	7.53 x 10^{-8}	This work
	EIS	0.165 - 2.758	4.11 x 10^{-8}	0.633 - 10.86	5.83 x 10^{-9}	This work
	DPSCC	0.022 - 2.671	4.63 x 10^{-8}	0.039 -1.351	1.63 x 10^{-7}	This work
ITO/CuPc-AQ-nAu	SWV (Amperometric)	0.061 - 1.241	3.87 x 10^{-8}	0.022 - 1.880	3.71 x 10^{-8}	This work
	SWV (Potentiometric)	0.149 - 2.969	2.89 x 10^{-8}	0.033 - 2.842	1.62 x 10^{-8}	This work
	EIS	0.039 - 2.371	1.23 x 10^{-9}	0.012 - 2.855	3.99 x 10^{-9}	This work
	DPSCC	0.065 - 14.53	2.41 x 10^{-8}	0.005 - 10.85	1.82 x 10^{-8}	This work
CoO/rGO/GCE	CA (Amperometric)				1.9 x 10^{-8}	32
AChE–AuNPs–SF/Pt	CA (Amperometric)				1.0 x 10^{-9}	33
BSA/Ab/[DpAu/DMDPSE]2/Au	CA (Amperometric)				2.7 x 10^{-7}	34
AChE / TTF–TCNQ–EMIMTCB/CP	CA (Amperometric)		2.6 x 10^{-8}			35
AChE–ChO	CA (Amperometric)		1.0 x 10^{-8}			36
AChE/PAMAM dendrimers			0.03 ppb			37

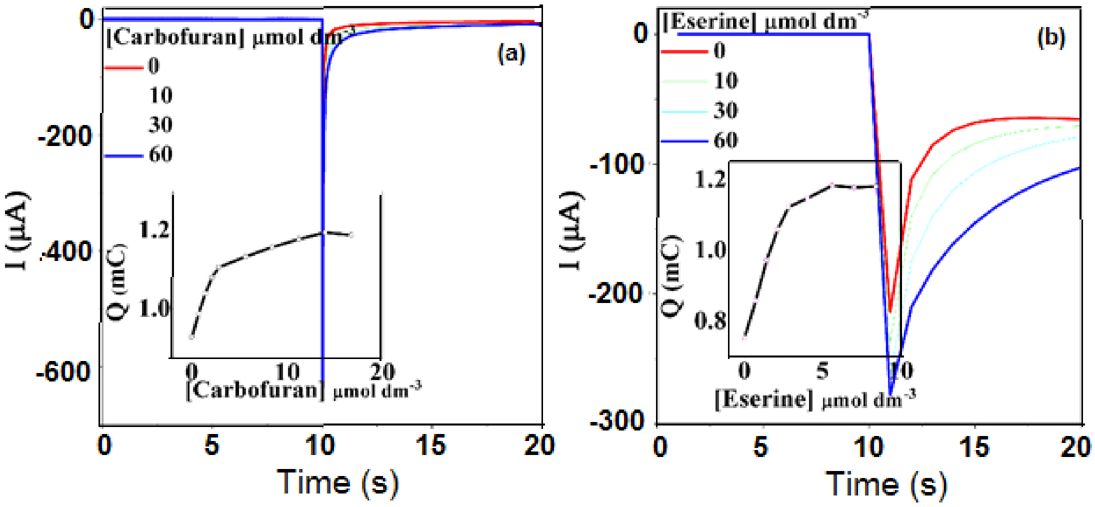

Figure 7. DPSCC responses and calibration lines of ITO/**CuPc-AQ** electrodes during the gradual addition of **a)** Carbofuran, **b)** Eserine**.**

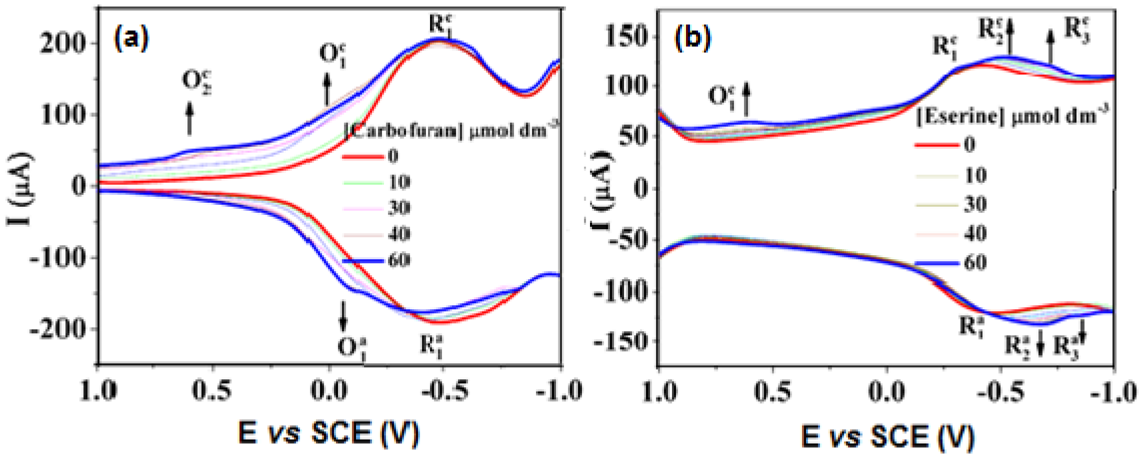

Figure 8. SWV responses of ITO/**CuPc-AQ-nPt** electrodes in PBS (pH= 7.0): **a)** During eserine titration, **b)** During carbofuran titration.

When compared with SWV analysis, the detection limit of the electrode decreases up to 5.44 x 10^{-9} mol dm^{-3}. DPSCC analyses for eserine and carbofuran were also performed between 0.0 V and -1.0 V constant potentials consecutively (Figure 7). There is an increase in the charge density during gradual addition of eserine and carbofuran during DPSCC measurements. When these three techniques are compared, the smallest DL is recorded with EIS technique.

To improve the sensing activity of CuPc-AQ, the ITO/CuPc-AQ electrodes were electrochemically coated with nano-Pt (ITO/CuPc-AQ-nPt), and sensing measurements were repeated on this electrode with the same conditions and methods. The SWV measurement results of ITO/CuPc-AQ-nPt during gradual addition of carbofuran and eserine into the phosphate buffer electrolyte are illustrated in Figure 8. Without pesticide addition, ITO/CuPc-AQ-nPt electrode gives similar voltammetric responses with ITO/CuPc-AQ. ITO/CuPc-AQ-nPt electrode gives AQ reduction peak at -0.42 V during the forward SWV scan and at the reverse SWV scan, the couples of AQ reduction peak is seen at -0.40 V. In Figure 8a, the SWV response of ITO/CuPc-AQ-nPt seems to be differing with the gradual addition of carbofuran pesticide. After gradually addition of carbofuran, a new reversible peak forms at oxidation region for ITO/CuPc-AQ-nPt, although ITO/CuPc-AQ electrode gives different response. In Figure 8b, during titration with eserine, the interaction of pesticide with ITO/CuPc-AQ-nPt affects the SWV responses and two new reduction peaks are observed at -0.67 V and -0.85 V while the reduction peaks of ITO/CuPc-AQ electrode decreases and no new peaks form. The presence of nPt particles in the recognizer film leads to formation of new electroactive structures by acting as a catalyst. This situation makes more evident the selectivity of the electrode. These voltammetric responses illustrate that CuPc-AQ is a selective sensor material against carbofuran and eserine. The sensor parameters derived from SWV analyses are exhibited in Table 1. Detection limit (DL) of ITO/CuPc-AQ-nPt is smaller than that of ITO/CuPc-AQ, which indicates that nano Pt coated on CuPc-AQ improves the sensor activity of the ITO/CuPc-AQ electrode**.** Figure 9 exhibits the EIS responses of ITO/CuPc-AQ-nPt during gradual addition of eserine into the phosphate buffer electrolyte.

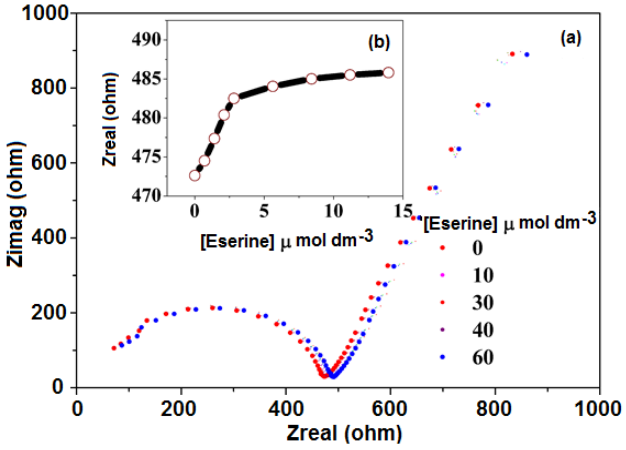

Figure 9. EIS responses of ITO/**CuPc-AQ**-nPt electrodes during the gradual addition of eserine and calibration line of the eserine sensing measurements derived from EIS responses of ITO/**CuPc-AQ**-nPt electrodes.

During gradual addition of eserine, the radius of the Nyquist diagram semicircles increases with increasing eserine concentration, which also supports the interaction of eserine with the sensor electrode. On the other hand, Figure 9 illustrates that the electrical conductivity of the ITO/CuPc-AQ-nPt electrode has improved since the semicircle radius of the electrode is shorter than those of ITO/CuPc-AQ electrode. On the other side, DL of the electrode derived from EIS analysis is also lower than that of ITO/CuPc-AQ electrode.

DPSCC sensor measurement results of ITO/CuPc-AQ-nPt electrode are shown in Figure 10. The potentials E_o= 0.0 V and E_f = -1.00 V were applied consecutively after each pesticide addition and considerable DPSCC response changes were recorded during the forward step of the technique as a function of pesticide concentration. As shown in Figure 10a, ITO/CuPc-AQ-nPt electrode gives a linear range between 3.87 x 10^{-8} and -1.460 x 10^{-6} mol dm^{-3} and DL of 1.63 x 10^{-7} mol dm^{-3} for carbofuran. However, in Figure 10b, after 8 µmol dm^{-3} eserine concentration in the phosphate buffer electrolyte solution, the electrical charge of the working electrode remained almost constant that determined the linear range of the ITO/CuPc-AQ-nPt electrode as 0.0219 x 10^{-6} and - 2.87 x 10^{-6} mol dm^{-3} and DL of 4.63 x 10^{-8} mol dm^{-3} for DPSCC technique. These DPSCC responses are in agreement with SWV responses of the electrode that two new reduction peaks are observed at -0.67 V and -0.85 V, leading the charge increase of the working electrodes between 0.0 V and -1.0 V.

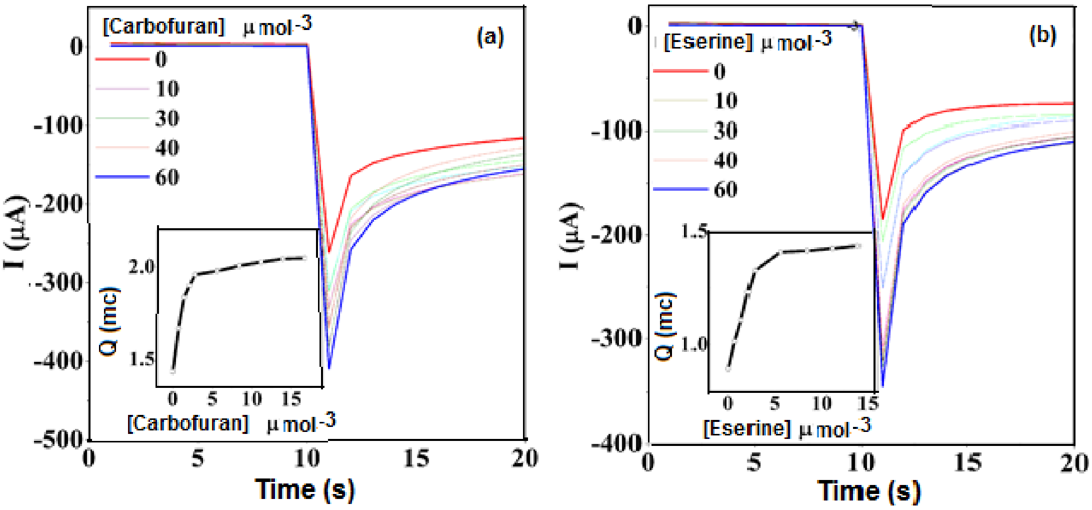

Figure 10. DPSCC responses and calibration line derived from DPSCC responses of ITO/**CuPc-AQ**-nPt electrodes: **a)** Gradual addition of carbofuran, **b)** Gradual addition of eserine.

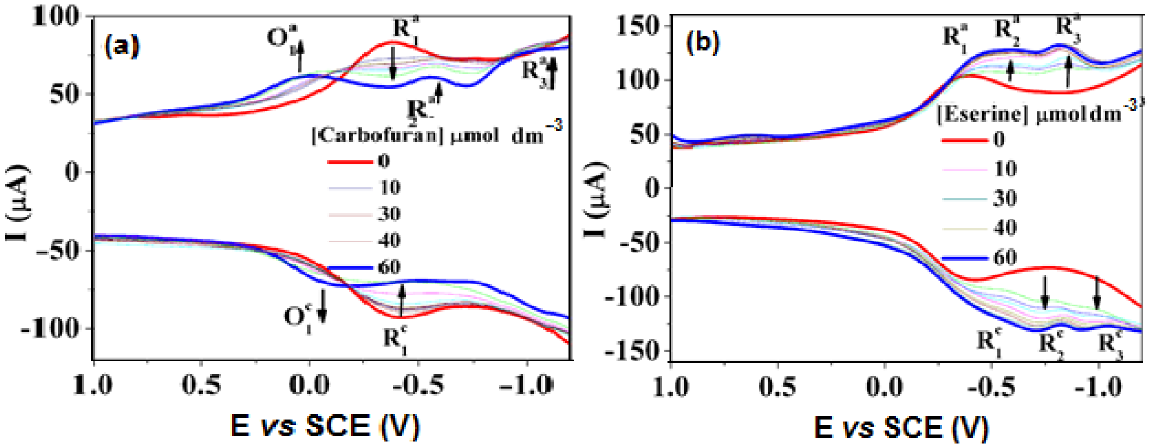

Figure 11. SWV responses of ITO/**CuPc-AQ**-nAu electrode during the gradual addition of **a)** Carbofuran, **b) Eserine.**

Similarly, the ITO/CuPc-AQ electrode was modified with Au to improve the sensor parameters and sensing measurements were carried out with SWV, EIS and DPSCC techniques. As shown in Figure 11, SWV diagrams of ITO/CuPc-AQ-nAu electrode without any pesticide addition illustrates that ITO/CuPc-AQ-nAu electrode gives similar SWV responses with those of ITO/CuPc-AQ-nPt and ITO/CuPc-AQ electrodes. A reduction couple R_1^a /R_1^c at -0.39 V and -0.43 V attributed to overlapped peaks of AQ and Pc are observed (Figure 11a). However, during gradual addition of carbofuran, the peaks of R_1^a /R_1^c couple decreases in current intensity, a cathodic peak at -0.56 V, a cathodic couple at -1.0 V, and an anodic couple at 0.04 V and -0.10 V were observed and peak current of these new waves gradually increased as a function of increasing carbofuran concentration. Two new cathodic couples are formed with the increasing concentration of eserine at about -0.60 V and -0.83 V (Figure 11b). EIS and DPSCC analyses of the electrode for carbofuran and eserine sensing were also carried out under the same conditions and basic sensor parameters were derived from the responses of these different techniques separately. The results obtained are given in Table 1. Modifying the electrode with nAu particles increased the sensitivity of the sensor against both eserine and carbofuran. The lowest detection limit was obtained with ITO/CuPc-AQ-nAu electrode as 1.23 x 10^{-9} mol dm^{-3} for eserine and 3.99 x 10^{-9} mol dm^{-3} for carbofuran.

4. CONCLUSIONS

The strong redox activity of CuPc-AQ exhibits potential usage of the complex for electrochemical technologies. In our previous study the CoPc-AQ complex was used as recognition material for eserine and carbofuran detection which was supported with nano-Au and nano-Pt in the reference 31. As a result of this study, ITO/CoPc-AQ behaved as a selective electrochemical sensor for eserine and carbofuran. Immobilization of nano-Pt and nano-Au on the ITO/CoPc-AQ electrode increased the sensitivity. The lowest detection limit of 2.30 x 10^{-9} mol dm^{-3} was obtained for eserine with EIS method using ITO/CoPc-AQ-nPt electrode while the detection limit for eserine with EIS method using ITO/CuPc-AQ-nPt was 4.11 x 10^{-8} mol dm^{-3}. Collaboration of Co as central metal of the Pc with nPt exhibited better results than that of Cu to increase the sensitivity of the sensor.

In this study, modified ITO electrode based on copper phthalocyanine-anthraquinone hybrid (CuPc-AQ) was constructed with Langmuir-Blodgett (LB) monolayer film coating technique. The modified electrode was tested as pesticide electrochemical sensor. Sensing measurements with three different electrochemical techniques denoted that ITO/CuPc-AQ electrode could be used as a selective and sensitive pesticide electrochemical sensor for eserine and carbofuran pesticides. While ITO/CuPc-AQ electrode sensed carbofuran at anodic potentials, the interaction of the electrode with eserine was detected with cathodic electrochemical signals. This situation provided the selectivity of the biomimetic sensor against carbofuran and eserine. Doping the CuPc-AQ thin film with nano-platinum and nano-gold particles increased the sensitivity of ITO/CuPc-AQ electrode.

The central metal of the anthraquinone substituted phthalocyanine did not affect the selectivity of the sensor material but affected the sensitivity. Also the presence of nPt and nAu particles as agents with catalytic effect made more evident the selectivity of the CuPc-AQ against carbofuran and eserine besides increasing the sensitivity.

New artificial biomimetic sensors instead of enzyme sensors were developed, which were more advantageous for being durable at room conditions and selective for pesticide detection with high sensitivity. The lowest detection limit was found as 1.23 x 10^{-9} mol dm^{-3} for eserine using ITO/CuPc-AQ-nAu electrode by EIS technique.

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Conflict of interest

Authors declare that there is no a conflict of interest with any person, institute, and company, etc.

REFERENCES

1. Samanta, S. *Aquat. Ecosyst. Health Manag.* **2013,** 16 (4), 454-464.

2. Hami, H.A., Azzaoui, F.Z.; Idrissi, M.; Ouammi, L.; Mokhtari, A.; Soulaymani-Bencheikh, R.; Soulaymani, A.. *Am. J. Epidemiol.* **2012,** 175 (11 Supl):S1-S145.

3. Hoai, P.M.; Sebesvari, Z.; Minh, T.B.; Viet, P.H.; Renaud, F.G. *Environ. Pollut.* **2011,** 159 (2), 3344-3350.

4. Rodriguez-Liebana, J.A.; Mingorance, M.D.; Pena, A. *Colloid Surface. A* **2013,** 435, 71-77.

5. Singh, N.K.; Chhillar, N.; Banerjee, B.D.; Bala, K.; Basu, M.; Mustafa, M. *Hum. Exp. Toxicol.* **2013,** 32 (1), 24-30.

6. Sapbamrer, R.; Nata, S. *Environ. Health Prev.* **2014,** 19 (1), 12-20.

7. Lee, K.G.; Jo, E.K. *Food Chem.* **2012,** 134 (4), 2497-2503.

8. Gaffney, S.H.; Curriero, F.C.; Strickland, P.T.; Glass, G.E.; Helzlsouer, K.J.; Breysse, P.N. *Environ. Health Perspect.* **2005,** 113 (12), 1712-1716.

9. Xu, M.L.; Liu, J.B.; Lu, J. *Appl. Spectrosc. Rev.* **2014,** 49 (2), 97-120.

10. Fatoki, O.S.; Awofolu, R.O. *J. Chromatogr A* **2003,** 983 (1-2), 225-236.

11. Luo, F.F.; Liao, S.Z.; Zhang, R.L.; Wu, Z.Y.; Yu, R.Q.; Shen, G.L. *Chinese J. Anal. Chem.* **2013,** 41 (10), 1549-1554.

12. Tran, H.V.; Reisberg, S.; Piro, B.; Nguyen, T.D.; Pham, M.C. *Electroanal.* **2013,** 25 (3), 664-670.

13. Chen, H.D.; Zuo, X.L.; Su, S.; Tang, Z.Z.; Wu, A.B.; Song, S.P.; Zhang, D.B.; Fan, C.H. *Analyst* **2008,** 133 (9), 1182-1186.

14. Svorc, L.; Rievaj, M.; Bustin, D. *Sens. Actuators B: Chem.* **2013,** 181, 294-300.

15. Eggins, B.R. *Chem. Sens. Biosens.* UK, 2002.

16. Wong, A.; Sotomayor, M.D.T. *Sens. Actuators B: Chem.* **2013,** 181, 332-339.

17. Boni, A.C.; Wong., A.; Dutra, R.A.F.; Sotomayor, M.D.P.T. *Talanta* **2011,** 85 (4), 2067-2073.

18. Shahrokhian, S.; Amini, M.K.; Mohammadpoor-Baltork, I.; Tangestaninejad, S. *Electroanal.* **2000,** 12 (11), 863-867.

19. Rahim, A.; Barros, S.B.A.; Kubota, L.T.; Gushikem, Y. *Electrochim. Acta* **2011,** 56 (27), 10116-10121.

20. Wujcik, E.K.; Londono, N.J.; Duirk, S.E.; Monty, C.N.; Masel, R.I. *Chemosphere* **2013,** 91 (8), 1176-1182.

21. Lin, T.; Lin, J.; Huang, X.; Liu, J. *Comm. Comp. Interface Sci.* **2011,** 214, 257-262.

22. Vial, L.; Dumy, P. *New J. Chem.* **2009**, .33 (5), 939-946.

23. Bhattachayay, D.; Pal, P.; Banerjee, S.; Sanyal, S.K.; Turner, A.P.F.; Sarkar, P. *Anal. Lett.* **2008,** 41 (8), 1387-1397.

24. Erbahar, D.D.; Gurol, I.; Gumus, G.; Musluoglu, E.; Ozturk, Z.Z.; Ahsen, V.; Harbeck, M. *Sens. Actuators B: Chem.* **2012,** 173, 562-568.

25. Wang, P.H.; Yu, J.H.; Li, Z.J.; Ding, Z.J.; Guo, L.; Du, B. *Sens. Actuators B: Chem.* **2013,** 188, 1306-1311.

26. Ipek, Y.; Dincer, H.; Koca, A. *Sens. Actuators B: Chem.* **2014,** 193, 830-837.

27. Sezer, B.; Sener, M.K.; Koca, A.; Erdogmus, A.; Avciata, U. *Synth. Met.* **2010,** 160 (19-20), 2155-2166.

28. Wiederkehr, N.A. *Phys. Chem. Glasses: B* **2008,** 49 (6), 326-333.

29. Valli, L. *Adv. Colloid Interface*. **2005,** 116 (1-3), 13-44.

30. Mahmoudian, M.R.; Alias, Y.; Basirun, W.J.; MengWoi, P.; Jamali-Sheini, F.; Sookhakian, M.; Silakhori, M. *J. Electroanal. Chem.* **2015,** 751, 30-36.

31. İpek, Y.; Şener, M.K.; Koca, A.. *J. Porphyrins Phthalocyanines* **2015,** 19, 708-718.

32. Wang, M.; Huang, J.; Wang, M.; Zhang, D. Chen, J. *Food Chem.* **2014,** 151, 191-197.

33. Yin, H.; Ai., S.; Xu, J.; Shi, W.; Zhu, L. *J. Electroanal. Chem.* **2009,** 637(1-2), 21-27.

34. Sun, X.; Zhu, Y.; Wang, X. *Food Control* **2012,** 28, 184-191.

35. Zamfir, L.G.; Rotariu, L.; Bala, C. *Biosens. Bioelectron.* **2013,** 46, 61-67.

36. Snejdarkova, M.; Svobodova, L.; Evtugyn, G.; Budnikov, H.; Karyakin, A.; Nikolelis, D.P.; Hianik, T. *Anal. Chim. Acta* **2004,** 514, 79-88.

37. Snejdarkova, M.; Svobodova, L.; Nikolelis, D.P.; Wang, J.; Hianik, T. *Electroanalysis* **2003,** 15(14), 1185-1191.

ORCID

ID 0000-0002-9390-9875 (Y. İpek)

ID 0000-0003-3560-2995 (M. Kasım Şener)

ID 0000-0003-0141-5817 (A. Koca)