1. INTRODUCTION

A natural environment, fruits, vegetables, and waters can be contaminated by a wide variety of pollutants from agricultural, industrial, and other human activities. Pesticides as environmental pollutants are apprehensive due to their toxicity and the extensive agriculture of their use. In specific, organophosphorus compounds are widely used for agricultural and household aims [1]. Chlorpyrifos (Cps) is an organophosphate pesticide used to kill insects and worms. It acts on the nervous system of insects by inhibiting acetylcholinesterase enzyme. Cps ($C_9H_{11}Cl_3NO_3PS$) is broad spectrum of insecticide that used in agriculture to control insect and arthropod pests. Humans can be exposed to Cps molecule which is mostly hazardous by way of ingestion or inhalation. Cps interferes with signaling from the neurotransmitter acetylcholine. Cps-oxon as a Cps metabolite, binds permanently to the enzyme acetylcholinesterase, preventing this enzyme from deactivating acetylcholine in the synapse. Therefore, Cps leads to a collapsing of acetylcholine between neurons and a stronger, longer-lasting signal transfer to the next neuron [2].

Food and agricultural companies need advanced, precise, reliable, inexpensive and fast analytical techniques to ensure the quality and safety of the products. One of the most important application methods used in food analysis is sensor technology. The importance of the sensor systems for the determination of biomolecules increases with the development of technology. Sensor systems have important advantages such as sensitivity, short analysis time, remarkable sensitivity and low costs when compared to classical analytical methods including chemiluminescent enzyme immunoassay, HPLC methods, fluorescence method [3-11]. Quartz crystal microbalance (QCM) is widely used for sensor field studies. QCM as a highly sensitive method can convert a mass change into an electrical signal [12]. The used QCM sensors exhibited high sensitivity and selectivity for detection of pesticides [13,14].

The molecularly imprinted polymers have multiple features including easy preparation, cost-friendly, high stability, affinity and selectivity toward template molecule and can be employed in sensor systems [15, 16]. In this study, the molecularly imprinted poly(ethylene glycol dimethacrylate-N-methacryloyl-(L)-tryptophan (PEDMATrp) nanoparticles attached QCM sensors having high sensitivity and selectivity to Cps molecule were developed. Firstly, Cps imprinted (PEDMATrp) nanoparticles were prepared and then attached to the chip surfaces. The non-imprinted (PEDMATrp) nanoparticles attached PEDMATrp QCM chip was also prepared in the same method without the Cps molecule. Cps imprinted and non-imprinted (PEDMATrp) nanoparticles were characterized by zeta-sizer and Fourier transform infrared spectrophotometer-attenuated total reflection (FTIR-ATR) spectrophotometer measurements. Also, Cps imprinted and non-imprinted (PEDMATrp) nanoparticles attached QCM sensors were characterized using contact angle, ellipsometry, atomic force microscopy (AFM) analyses. The detection efficiency of Cps imprinted PEDMATrp QCM sensor was analyzed by applying aqueous solutions of Cps pesticides. Kinetic parameters were calculated by using Langmuir, Freundlich, Langmuir-Freundlich, Scatchard adsorption isotherm models and association kinetic analysis. In addition, intraday reproducibility of the QCM sensors was tested by applying same Cps solution four times consecutively during the same day.

Herein, a molecularly imprinted (PEDMATrp) nanoparticle based QCM sensor was developed for the determination of Cps in an aqueous solution. LOD, selectivity, sensitivity and reusability of used (PEDMATrp) nanoparticle attached QCM sensor chip system were investigated. The prepared PEDMATrp QCM sensor enables real-time monitoring of Cps determination in an aqueous solution. The molecularly imprinted (PEDMATrp) nanoparticle based QCM sensor has high selectivity and sensitivity, excellent specificity to Cps with low LOD value. Cps imprinted and non-imprinted amino acid based (PEDMATrp) nanoparticle coated QCM sensor having dual ability to form both hydrophobic matrix and functional group supplier in one mode were prepared. PEDMATrp QCM sensor was used to detect Cps molecule for the first time without needing any other spacer arm or any extra complicated processes such as ligand immobilization. The PEDMATrp QCM sensor could be a reliable alternative to current techniques to detect Cps molecules and also might play a role in protecting environmental pollution and human health.

MATERIALS and METHODS

Materials

Poly(vinyl alcohol) (PVA), sodium dodecyl sulfate (SDS), ammonium persulfate (APS), sodium bicarbonate and

sodium bisulfite were obtained from Sigma Chemical Co. (St. Louis, USA). Ethylene glycol dimethacrylate (EDMA) was purchased from Fluka A.G. (Buchs, Switzerland). The initiator α, α-azoisobutyronitrile (AIBN) used in the preparation of nanoparticle is obtained from Fluka A.G. (Buchs, Switzerland). Gold QCM chips were purchased from Maxtek Inc. (New York, USA).

Preparation of Cps imprinted and non-imprinted nanoparticles

Cps imprinted (PEDMATrp) nanoparticles were prepared by a two-phase mini-emulsion polymerization method. PVA, SDS and sodium bicarbonate were used to preparate of nanoparticles. 100 µL MATrp as functional monomer was dissolved in a crosslinker monomer, ethylene glycol dimethacrylate to form oil phase. The oil phase was slowly added to the firstly prepared aqueous phase. The mixture was homogenized at 25000 rpm by a homogenizer (T10, Ika Labortechnik, Germany). After homogenization process, the template molecule Cps (0.01 mmol) was added to the homogenized mini-emulsion solution. The obtained mixture was slowly added to the second aqueous phase while the phase has been stirred magnetically at 300 rpm (Radleys Carousel 6, Essex, UK) in a sealed-cylindrical polymerization reactor and polymerization mixture was slowly heated until reaching to the polymerizati-on temperature, 40◦C. Then, initiators, sodium bisul-fite and ammonium persulfate were added into the polymerization mixture solution. Polymerization was continued for 24 h at 600 rpm stirring rate. The non-imprinted (PEDMATrp) nanoparticles were synthesized by applying same procedure except addition of Cps.

Characterization of Cps imprinted (PEDMATrp) nanoparticles

Cps imprinted (PEDMATrp) nanoparticles were characterized by using zetasizer (NanoS, Malvern Instruments, London, UK). For data analysis, density, viscosity and refraction index of deionized water were used as 1.00 g/ml, 0.88 mPa s^{-1} and 1.33, respectively. FTIR-ATR was made for the characterization of the Cps imprinted nanoparticles (Thermo Fisher Scientific, Nicolet iS10, Waltham, MA, USA).

Preparation of Cps imprinted and non-imprinted (PEDMATrp) nanoparticles based QCM sensors

Before surface modification of the QCM chips, the gold surfaces were washed separately with 10 mL of pure ethyl alcohol, purified water and acidic piranha solution with sulfuric acid / hydrogen peroxide (3: 1 v/v) for 10 minutes. Then they are pressurized at 200 mmHg and allowed to dry under 37°C degree. Then, 10 µL of Cps imprinted (PEDMATrp) nanoparticles were dropped onto QCM chip. Figure 1 shows the schematic illustration of Cps binding/rebinding onto the PEDMATrp QCM sensor chip. The PEDMATrp QCM sensor chip was purged with alcohol and vacuum dried at 220 mmHg and 25°C. The Cps non-imprinted (PEDMATrp) nanoparticles based QCM sensor chip was also prepared by the same method.

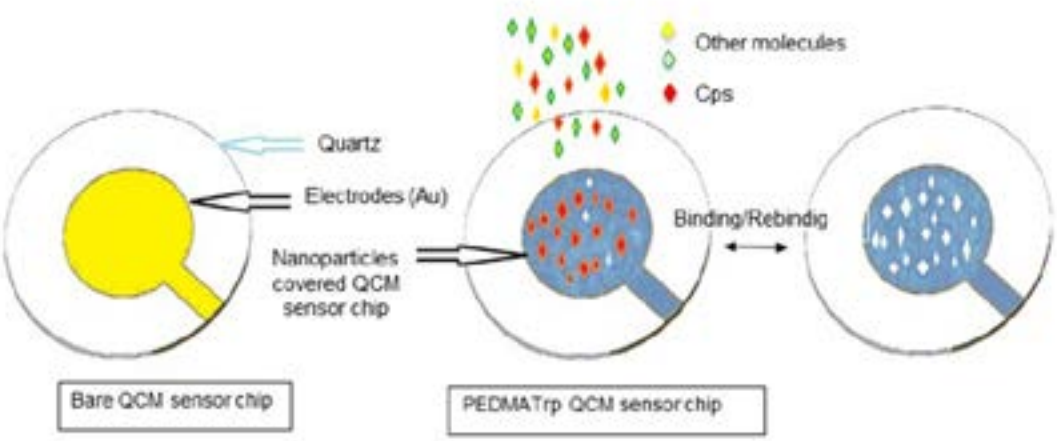

Figure 1. Schematic illustration of Cps binding/rebinding onto the PEDMATrp QCM sensor chip.

Characterization of Cps imprinted and non-imprinted (PEDMATrp) nanoparticles based QCM sensors

Atomic force microscopy (AFM), ellipsometer and contact angle measurements were made for the characterization of QCM chips. For the thickness measurements of QCM chips, measurements were taken at 62° angles and 532 nm wavelength settings, mean values were recorded. The contact angle measurements were made by the "Sessile drop" method which was used for charac-terization of solid surfaces and based on capturing the image of the dripped liquid. The data of the QCM chip surfaces were taken separately. AFM (Nanomagnetics Instruments, Oxford, UK) was used in a tapping mode to evaluate surface topography of QCM chips.

Kinetic analyses of Cps imprinted and non-imprinted (PEDMATrp) nanoparticles QCM sensors

After the preparation of Cps imprinted (PEDMATrp) nanoparticles based QCM sensors, kinetic studies are started by using an RQCM (Maxtek) software. The Cps detection was performed by Cps imprinted and non-imprinted (PEDMATrp) nanoparticles QCM chips. Firstly, PEDMATrp QCM chips were equilibrated with 0.1 M phosphate buffer (pH 5.5). Then, Cps (0.015-2.9 nM) was applied to the PEDMATrp QCM sensor systems. Then, to form cavities, 0.1 M acetic acid and 0.1 M CaCl2 solutions were applied respectively for removing Cps pesticide. Freundlich, Langmuir, and Langmuir-Freundlich adsorption isotherm models were examined to detect the equilibrium isotherm parameters.

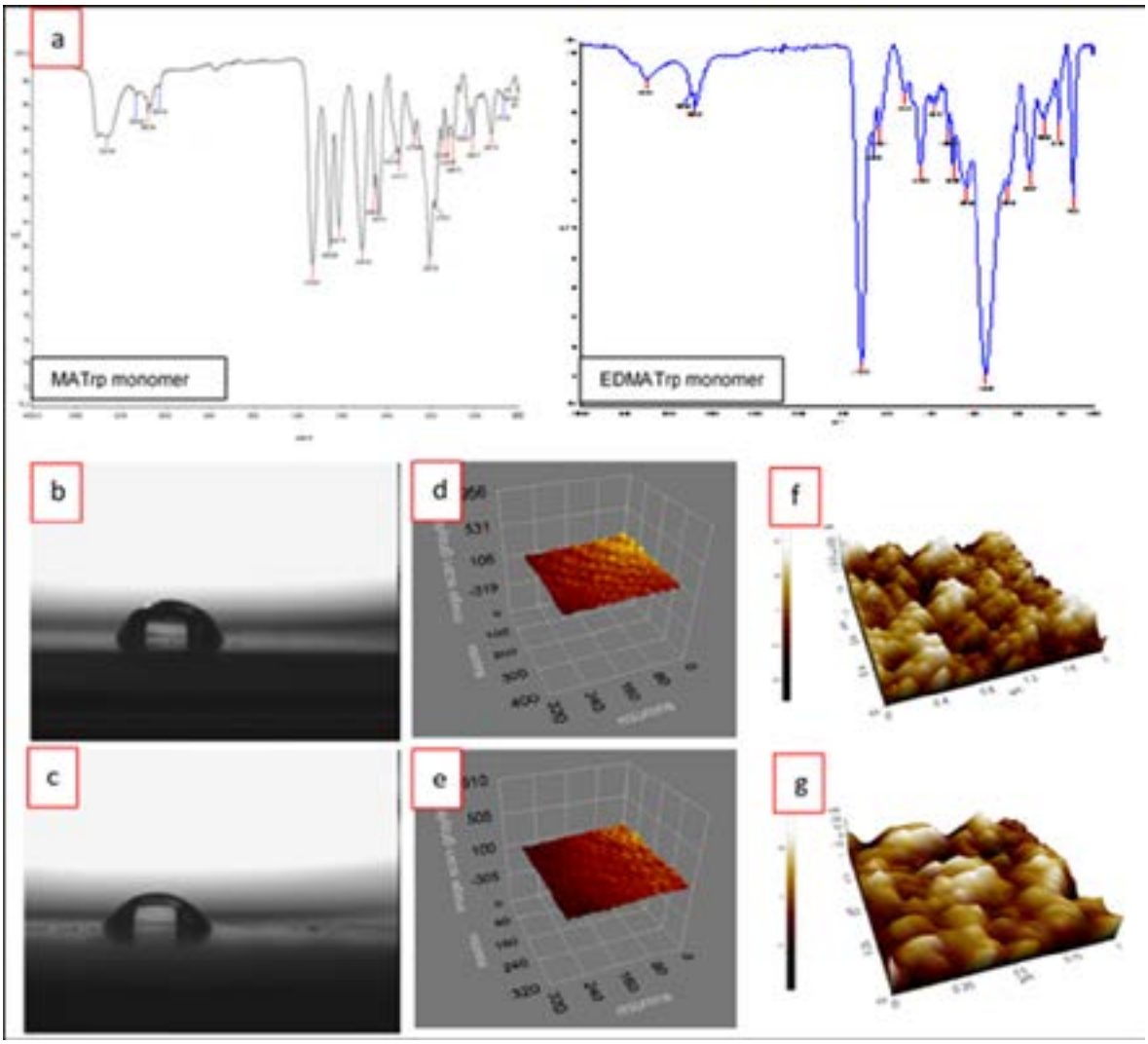

Figure 2. FTIR-ATR spectrum of MATrp and EDMATrp monomer (a). Morphological characterization of the bare gold chip and Cps imprinted PEDMATrp QCM sensor surfaces (contact angles; b. bare gold surface, c. Cps imprinted PEDMATrp QCM sensor chip). AFM studies; d. Cps imprinted PEDMATrp QCM sensor chip, e. non-imprinted PEDMATrp QCM sensor chip. Ellipsometry images; f. Cps imprinted PEDMATrp QCM sensor chip, g. non-imprinted PEDMATrp QCM sensor chip.

Selectivity and repeatability analyses of the PEDMATrp QCM sensors

Diazinon and parathion 2.9 nM were used as competitive molecules for selectivity studies of the PEDMATrp QCM sensors. Diazinon and parathion pesticides which are similar to Cps in both molecular structure and weight was used in competitive selective studies. The obtained Δm values were used for the calculation of selectivity coefficients. In the reusability studies, pH 5.5 buffer was passed for 5 minutes for equilibrium, then the sample at the same concentration was passed for 10 minutes and finally the desorption solution was passed for 5 minutes. This process was repeated 4 times and finally re-usability was obtained.

RESULTS and DISCUSSION

Characterization of Cps imprinted and non-imprinted (PEDMATrp) nanoparticles

The synthesized (PEDMATrp) nanoparticles were characterized by the zeta-sizer. Cps imprinted (PEDMATrp) nanoparticles have an average particle size of 84.62 nm with a low polydispersity index of 0.12. The non-imprinted nanoparticles also have similar physical and chemical properties with 80.72 nm average particle size and 0.14 polydispersity index value. FTIR-ATR spectrophotometer (Figure 2a) was chosen for characterization of Cps imprinted and non-imprinted nanoparticles.

Characterization of Cps imprinted and non-imprinted (PEDMATrp) nanoparticles based QCM chips

The surfaces of the bare gold chip as blank, QCM chips were characterized after modification steps by contact angle, AFM and ellipsometry measurements. As shown in Figure 2b, 2c, contact angle values were obtained to be 82.2°±1.18 and 63.4°±2.05 for both gold surface, Cps imprinted and non-imprinted PEDMATrp QCM sensors, respectively. If the contact angle is greater than 90 degrees, it can be said that surface is hydrophobic. The decrease of the contact angle values is due to the attachment of the amino acid based functional monomers to a gold surface.

The thickness of the Cps imprinted and non-imprinted PEDMATrp QCM sensor chip surfaces were measured by ellipsometry and shown in Figure 2d, 2e. Thickness measurements that are controlling the specificity, selectivity and recognition rate of the sensors were recorded as 95.3±0.34 for non-imprinted, and 84.8±0.92 nm for Based on these measurements, it is possible to say that nanoparticles were applied to the surface homogeneously.

The AFM images displayed that Cps imprinted and non-imprinted PEDMATrp QCM chip surfaces have 65.3±0.34, and 67.8±0.92 nm deepness values respectively (Figure 2f, 2g). Ellipsometer results which are consistent with AFM results emphasize that Cps imprinted and non-imprinted PEDMATrp QCM sensor chips have homogeneous and rough surfaces.

Detection of Cps by PEDMATrp QCM sensor

In these studies, Cps samples were prepared in pH 5.5 buffer solutions at 0.015-2.9 nM concentrations and their sensorgrams were taken and shown in Figure 3a with the graph. Firstly, the pH buffer solution was passed through the system for 5 minutes, then one of the Cps solutions at the specified concentrations was passed for 10 minutes, then the desorption solution was removed with 5 minutes. Cps imprinted and non-imprinted PEDMATrp QCM sensors have the linearity of 96%. Therefore, there is a direct correlation between the applied Cps aqueous solution concentrations and the sensors surfaces interacted.

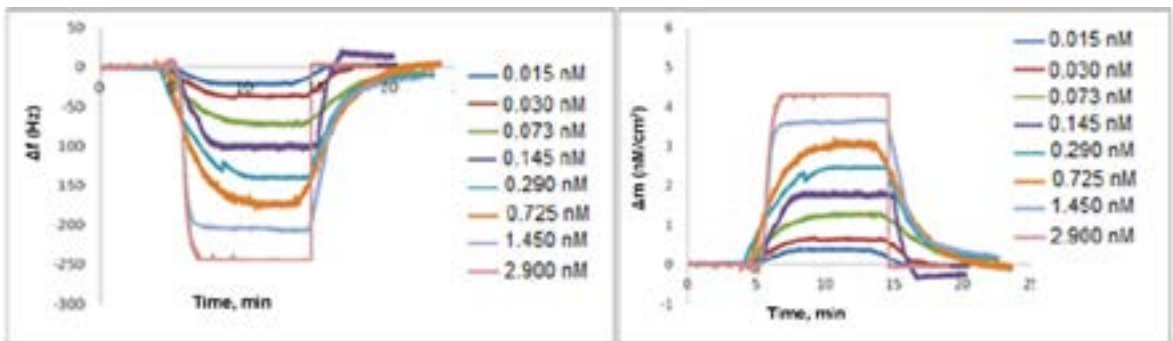

Figure 3. Effect of the applied Cps concentration to the Cps imprinted PEDMATrp QCM sensor.

Table 1. Langmuir, Freundlich and Langmuir/Freundlich adsorption isotherm models.

Langmuir		Freundlich		Langmuir- Freundlich	
Δm_{max}, nM/cm²	3.484	Δm_{max}, nM/cm²	3.401	Δm_{max}, nM/cm²	5.917
K_A, nM^{-1}	8.969	1/n	0.431	1/n	0.431
K_D, nM	0.111	R^2	0.942	K_A, nM^{-1}	0.447
R^2	0.995			K_D, nM	2.237
				R^2	0.952

The Freundlich model is an isotherm model for adsorption on heterogeneous surfaces. Langmuir isotherm is obtained by assuming that the molecules on the surface are adsorbed uniformly. Freundlich, Langmuir and Freundlich-Langmuir isotherms were taken in order to examine in QCM sensor system and given in Table 1. This result proves that the surface is homogeneous and there are no lateral interactions between adjacent adsorbed template Cps molecules. The standard deviation and slope values are determined from the calibration graph and the limit of detection was calculated. LOD = 3×s/b equation was used for the limit of detection. The s value used in these equations is the standard deviation obtained from the calibration graph, and the b value is also the slope of this graph. LOD value was found as 0.010 nM.

Selectivity and repeatability analyses of the PEDMATrp QCM sensors

Diazinon and parathion (2.9 nM) which are similar to Cps in terms of both structure and molecular weight were used as competitor molecules for selectivity studies of the PEDMATrp QCM sensors. The obtained Δm values were used for the calculation of selectivity coef-

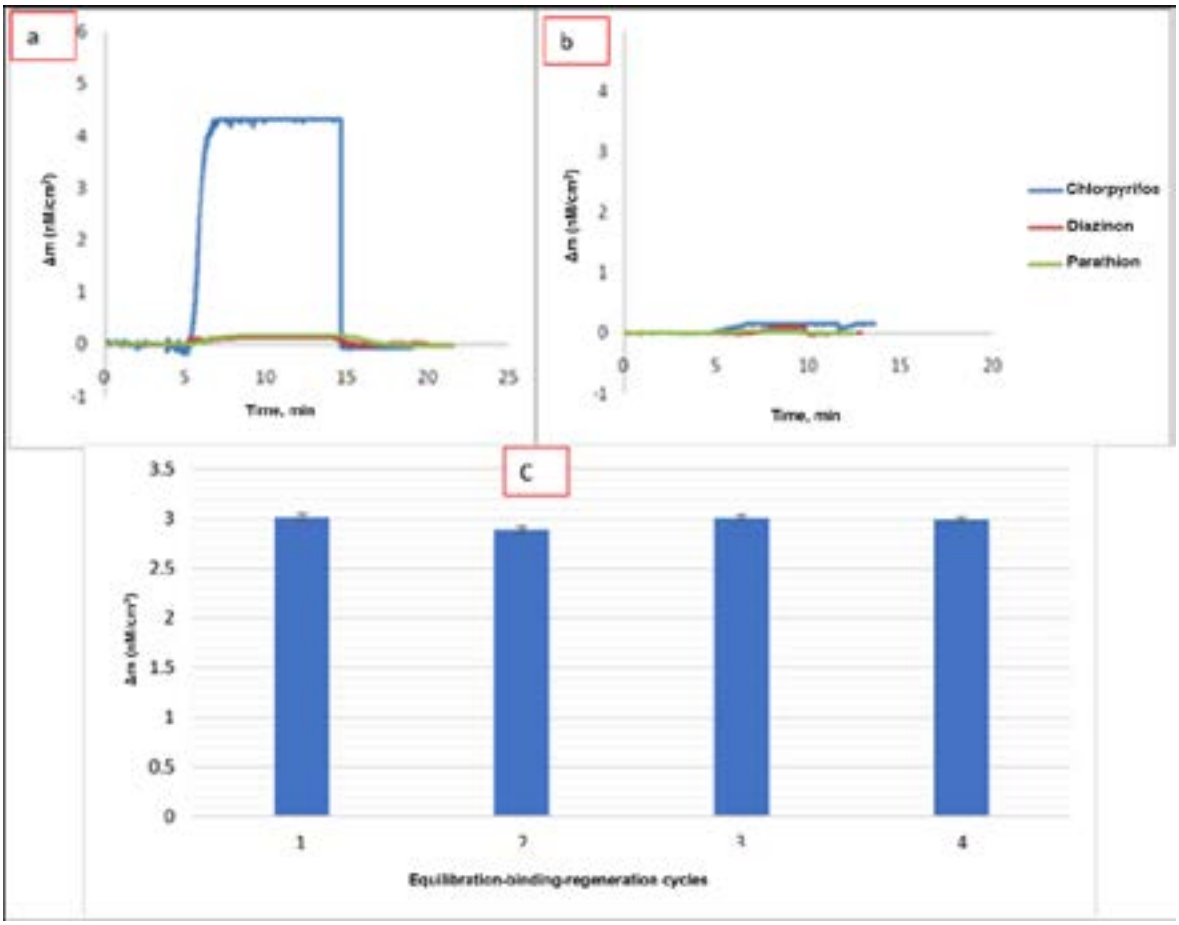

Figure 4. Comparison of selectivity efficiency of PEDMATrp QCM sensors: a. Cps imprinted PEDMATrp QCM sensor, b. Cps non-imprinted PEDMATrp QCM sensor, c. reproducibility of Cps imprinted PEDMATrp QCM sensors.

Table 2. Selectivity parameters of the PEDMATrp QCM sensors.

MIP			NIP	Langmuir	Langmuir
	Δm	k	Δm	k	k'
Cps	4.315	-	0.089	-	-
Diazinon	0.134	32.201	0.071	1.253	25.699
Parathion	0.168	25.684	0.052	1.711	15.011

ficients. The selectivities of the Cps imprinted and non-imprinted PEDMATrp QCM sensor chips against Cps molecule were visualized to emphasize the imprinting process efficiency by recording Cps molecule responses (Figure 4a,b). Selectivity coefficient (k) and relative selectivity coefficient (k´) of the prepared QCM sensor were determined by subtracting from the 1st and 2nd equations and shown in Table 2.

$$k=\Delta m_{Cps}/\Delta m_{competitor} \quad (1)$$

$$k'=k_{MIP}/k_{NIP} \quad (2)$$

These data imply that the sensor surfaces recognize the Cps molecule with a high affinity ability owing to the obtained recognition cavities by molecular imprin-ting of Cps. As a result, the imprinted PEDMATrp QCM sensor was found to show more selectivity towards the target the Cps molecule than the non-imprinted ones. In the reusability study, Cps solution in pH 5.5 buffer at a concentration of 1.45 nM was given to the system. After passing through the buffer system, the sample was given for about 5 minutes. Then 10 minutes the desorption solution was passed, the same procedure was repeated 4 times and shown in Figure 4c. As shown in the figure, almost the same Δm value was obtained for each repeated sample. Therefore, PEDMATrp QCM chips can be stable under long-term storage conditions.

Conclusion

For this aim, N-metacryloyl-(L)-tryptophan methyl ester (MATrp) monomer which was selected as a proper functional monomer was polymerized with ethylene glycol dimethacrylate (EDMA). Cps imprinted PEDMATrp nanoparticles were used to cover gold surfaces of QCM sensor chips and were characterized by several techniques including AFM, an ellipsometer, ATR-FTIR and contact angle measurements. Kinetic and affinity binding of Cps to PEDMATrp QCM sensor was investigated by binding of Cps molecule to Cps imprinted PEDMATrp QCM sensor chips. In addition, adsorption kinetics were determined by passing Cps solutions through PEDMATrp QCM sensor at different concentrations. The most proper adsorption model for the Cps molecule as an affinity system was found to be Langmuir isotherm model which is assuming that there is no lateral interaction between adjacent adsorbed molecules when a single molecule occupies a single surface site. These results proved that the prepared polymeric surfaces were highly desirable for sensitive recognition of QCM sensors for Cps detection.

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