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Novel Electrochemical Sensor Based on Molecularly Imprinted Polymer for Detection of Gefitinib

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In this research, for the first time, a novel electrochemical sensor was proposed for the detection of Gefitinib (Gefi) as an anticancer drug based on a molecularly imprinted polymer (MIP) on the glassy carbon electrode (GCE) surface. The present research aimed to introduce a selective electrochemical sensor through electropolymerization of o-aminophenol (o-AP) as a monomer on the GCE surface for the determination of Gefi as a template molecule. Afterward, the proposed sensor was assayed by scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), atomic force microscopy (AFM), and electrochemical methods including electrochemical impedance spectroscopy (EIS), differential pulse voltammetry (DPV), and cyclic voltammetry (CV). The introduced sensor illustrated a good linear range of 5 to 1000 µM and a limit of detection of 1.6 µM for Gefi detection. Moreover, the modified electrode to determine Gefi in the blood serum sample was successfully employed. The recoveries were from 98.8 to 100.5% and the RSD was less than 2.7% which illustrated the applicability of the proposed sensor in the real sample.

Keywords: Gefitinib, Molecularly imprinted polymer, Electropolymerization, Sensor

INTRODUCTION

Gefitinib (Gefi) under the brand name IRESA is a medicine used to treat some types of breast cancer, lung cancer, and other types of cancer [1,2]. The chemical structure of Gefi is shown in Fig. 1. This drug is an epidermal growth factor receptor inhibitor that disrupts the signaling through these receptors in the target cells [3]. Gefi belongs to a group of anticancer drugs called tyrosine kinase inhibitors. These drugs inhibit messenger molecules (tyrosine kinase) that deliver growth messages to cells, this prevents the growth and proliferation of cancer cells [4]. In 2003, the US Food and Drug Administration approved this drug as the third line of treatment for non-small cell lung cancer, in those who are treated with platinum chemotherapy drugs and regimens containing docetaxel. Then in 2005, this approval was withdrawn due to the lack of sufficient scientific evidence on its effectiveness in increasing the survival and longevity of patients. Finally, in 2015, the US Food and Drug Administration re-approved this drug as the first line of treatment for this type of cancer [5]. Therefore, selective determination of Gefi is necessary. On the other hand, the Gefi analysis can promote new drug research and development related to Gefi. Considering the complexity of biological and pharmaceutical samples and the low amount of analyte in them, techniques with excellent selectivity, low-

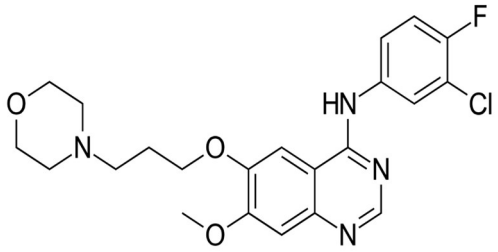

Fig. 1. Chemical structure of Gefitinib.

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cost, simple, and easy operational features are needed [6].

It is worth mentioning that electrochemical methods have taken much attention to detecting pharmaceuticals due to a series of advantages including simple operation, rapid response, high sensitivity, selectivity, and in situ determination [7]. Furthermore, it is important to make an effort to provide selective and stable electrodes that can improve the electrochemical performance for Gefi detection.

The term molecularly imprinted polymers (MIPs) refers to polymers that, during the synthesis, certain sites are created for a specific target in the polymer [8]. The chemical and physical interactions between the functional parts of the polymer matrix and the functional groups of the template are remembered during polymerization, and after washing and removing the template, imprinted cavities with specific sizes and shapes on the MIP film are formed. Therefore, the MIPs act selectively to detect the target molecule. Recently, the conjugation of MIP technology with an electrochemical sensor as an important molecular recognition tool has been applied for incrementing the electrochemical sensor selectivity [9].

MIPs are used in various applications such as catalyst, drug delivery, membrane, cell culture, and crystallization due to their special characteristics [10]. There are a number of methods for preparing MIPs, including electropolymerization, sol-gels, bulk polymerization, self-assembled monolayers, and precipitation polymerization [11]. The electropolymerization strategy is important compared to other techniques because it has different advantages including high reproducibility, low cost and easy preparation, and a wide range of molecules and macromolecules that can be imprinted [12-14].

In this study, a novel and easy technique was explored for the selective determination of Gefi; this technique is based on creating an MIP sensor through electropolymerization. Molecularly imprinted membranes were immobilized on the glassy carbon electrode (GCE) surface with an electropolymerization technique employing Gefi as the template and o-AP as monomer. Moreover, the properties of the sensor were characterized by various methods including EIS, CV, DPV, AFM, SEM, and FT-IR. To the best of our knowledge, no study has been introduced on modified electrodes based on MIP for the detection of Gefi.

EXPERIMENTAL

Materials and Reagents

All chemical reagents including potassium chloride (KCl), ferro/ferricyanide ($[Fe(CN)_6]^{3-/4-}$), o-AP (C_6H_7NO), acetic acid (CH_3COOH), sodium acetate (CH_3COONa), and Gefi were purchased from Sigma-Aldrich. A solution of 5 mM $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl was used in all experiments. The buffer of 0.1 M acetate was prepared using 0.1 M acetic acid and 0.1 M sodium acetate. The doubly distilled water is applied for the experiments.

Instrument

To carry out the electrochemical data a voltalab potentiostat/galvanostat/EIS electrochemical (Eco-Chemie, Switzerland) is utilized as an analyzer which has been coupled with a common cell. The three-electrode system was employed with a reference electrode (Ag/AgCl/saturated KCl), the auxiliary electrode (Platinum wire), and the working electrode (GCE). SEM (Hitachi S4160) and AFM (AFM, Bruker) were employed to characterize the surface morphology of electrodes. Confirming the accuracy of the suggested sensor, the FTIR spectra were performed using a Bruker Fourier transform infrared (VERTEX 70).

Preparation Steps of the Modified Electrodes

The first step is to prepare the GCE surface so that the electrode is completely clean and free of contamination. For this purpose, the GCE surface was polished with alumina slurry and, then, sonicated in a solution of ethanol and doubly distilled water. In the second step, the clean GCE is placed in the polymerization solution including 0.1 M acetate buffer, 10 mM o-AP, and 5 mM Gefi. Then on the surface of the GCE by applying 8 cycles of CV in the range of -0.2 to 1 V and a scan rate of 50 mV s^{-1} electropolymerization was performed. In the next step, the polymerized electrode is placed in a washing solution consisting of hydrochloric acid (HCl) and doubly distilled water with a ratio of 1:3 for 2 min under a stirrer. In this step, after eluting Gefi with the washing solution, creating sites in the MIP layer becomes supplementary to Gefi in shape and size. Therefore, formed cavities can tightly rebind with Gefi. It is special for the template molecule compared to compounds with similar structures, which can form a bond again with the template

species in a very selective manner. Furthermore, as a control sensor, the non-imprinted polymer (NIP) was supplied, employing the same method in the absence of the Gefi.

Preparation of Real Samples

In order to examine the introduced sensor's applicability, a blood serum sample was obtained from the local clinical laboratory. Then, 2 ml of methanol was added to 1.5 ml of the serum sample for protein separation. After stirring for 2 min, the precipitated proteins were separated by centrifugation. The obtained clear solution was passed through the filter and the final volume of the sample was diluted to 10 ml using a buffer solution.

RESULTS AND DISCUSSION

Investigating the Structural Characteristics and Morphology of the Electrodes

SEM is one of the most important tools in identifying the morphology of particles. Figure 2 shows the SEM spectrum of unleached MIP (A) and leached MIP (B). As seen in Fig. 2A, MIP film has been uniformly polymerized on the GCE surface and an uneven polymer layer consisting of spherical particles has been fabricated on it. In fact, the topography of the particles is saturated before removing the analyte. In Fig. 2B, after washing and removing the analyte, a porous surface with many holes related to the exit of the chelating molecule on the polymer film can be seen. Therefore, by comparing the images of the leached and unleached polymer, we can see that the exit of the analyte molecule creates a surface with many pores, which is related to the exit of the analyte molecule from the polymer film, and this porosity leads to the reconnection of the analyte on the modified electrode surface.

In order to check the morphology of polymers, the AFM technique was used (Fig. 3). In the MIP topography image before washing (Fig. 3A), there are areas with bright spherical morphology, which have a relatively smooth surface with uniform distribution, and a certain accumulation of particles can be seen. The 3D topography image of MIP after washing has a different morphology than before washing. As can be seen in the image (Fig. 3B), the surface becomes more uneven and the smoothness is less visible, which indicates the production of holes from the polymer

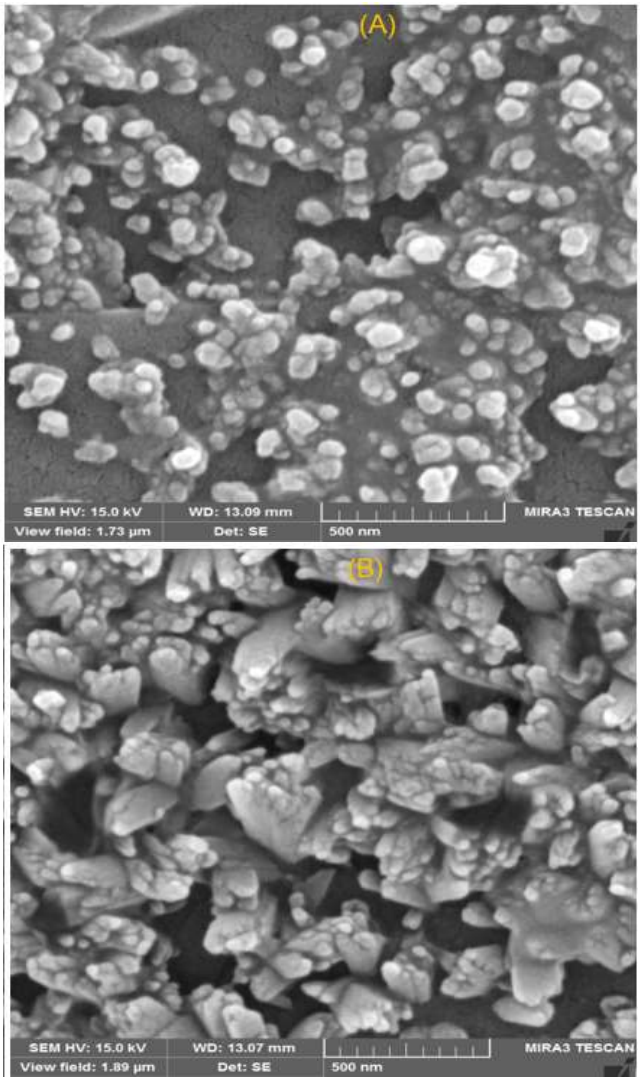

Fig. 2. SEM of unleached MIP (A) and leached MIP (B).

matrix and the increase of porosity on the sensor surface.

FT-IR analysis was used to assess the accuracy of the sensor. The FT-IR spectra of Gefi, unleached MIP, and leached MIP are shown in Fig. 4. From the comparison of the spectra, it can be seen that the spectrum of unleached MIP is similar to the spectrum of MIP due to the presence of Gefi in the structure, and they have similar functional groups. A strong peak is related to the OH group at 3335 cm^{-1} corresponding to Gefi, which is observed by a shift in the unleached MIP spectrum. While the intensity of this peak in the leached MIP spectrum can be seen with a change in intensity, which is proof of the exit of the analyte from the

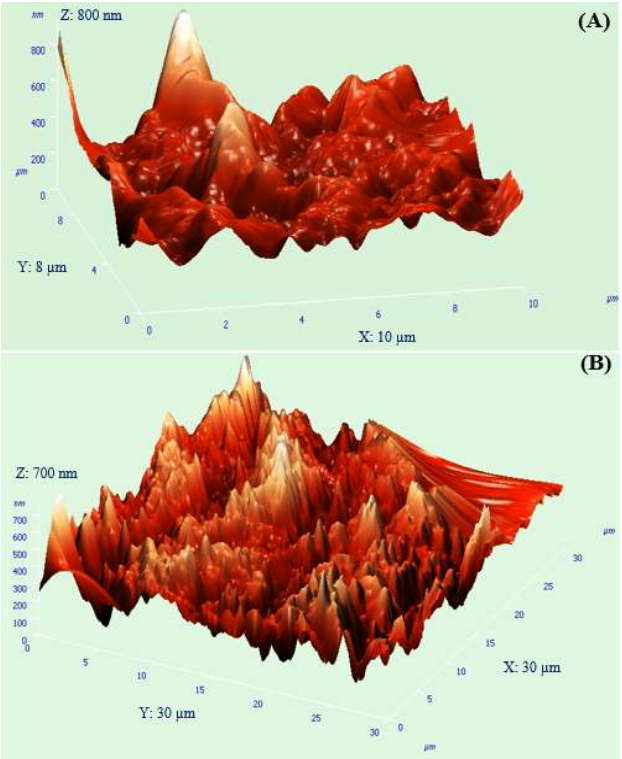

Fig. 3. AFM images of MIP before washing (A) and after washing (B).

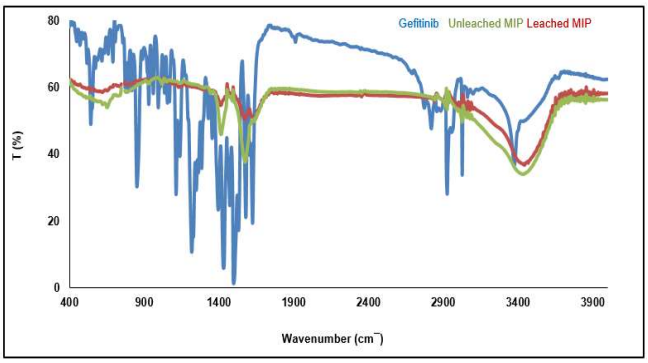

Fig. 4. FT-IR spectra related to Gefitinib, MIP before washing, and MIP after washing.

leached MIP and shows that the target molecule has been separated from the binding site. FT-IR spectrum of the unleached polymer has a strong observed peak in the range of 1579 cm^{-1} corresponding to the N-H group, in the range of 1406 cm^{-1} corresponding to the C-F group, and the range of 651 cm^{-1} corresponding to the group C-Cl. As can be seen in the unleached MIP spectrum, these peaks are greatly reduced or completely removed after the washing step, which indicates the exit of the target molecule from the polymer surface. Therefore, the decrease in intensity and removal of peaks after washing and of course the observed difference in signal intensity, wavelength, and peak shape confirm the exit of the analyte from the polymer film and also the successful polymerization process.

Electrochemical Properties of the Modified Electrodes

To obtain information about the characteristics of electrode reactions, CV has been used more than any other method. The value of this method is due to the considerable information that is quickly related to the manner and nature of the electrode reactions (either cathodic or anodic). Figure 5A shows the voltammograms of different stages of electrode modification in a solution of 5 mM ferro/ ferricyanide and 0.1 M potassium chloride. A pair of reversible peaks are observed in the voltammogram of the unmodified electrode (curve a), which indicates a reversible electrochemical process. The electropolymerization of the polymer layer on the electrode surface and the formation of a non-conductive film were confirmed by the absence of the oxidation/reduction peak, which is caused by the lack of access of the redox probe to the conductive surface of the electrode. This shows that the surface is blocked for electron transfer (curve b). In addition, after washing the electrode surface with the washing solution and removing the drug from the polymer film, the oxidation-reduction current of the probe increases again (curve c) because by removing the analyte from the polymer film, a number of holes are created in the polymer. The probe can be brought to the surface of the electrode and as a result oxidation/reduction occurs. In order to demonstrate the ability of the modified electrode to absorb the analyte, a 5 µl drop of the drug solution was placed on the surface of the modified electrode and after 10 min (optimized time) its voltammogram was recorded (curve d). As can be seen, the current of this curve has decreased compared to curve c, which can be attributed to the binding of the drug to the formed holes, which causes some holes to be closed by the analyte. It can also be seen that almost no electrochemical response is observed in the NIP electrode (curve e) due to the blocking of electron transfer by the non-

patterned molecular polymer matrix that covers the entire surface of the electrode. These results show the most effective modifiers for the design of an electrochemical sensor suitable for measuring the selectivity of Gefi. The voltammograms related to the DPV technique also confirm the above results (Fig. 5B).

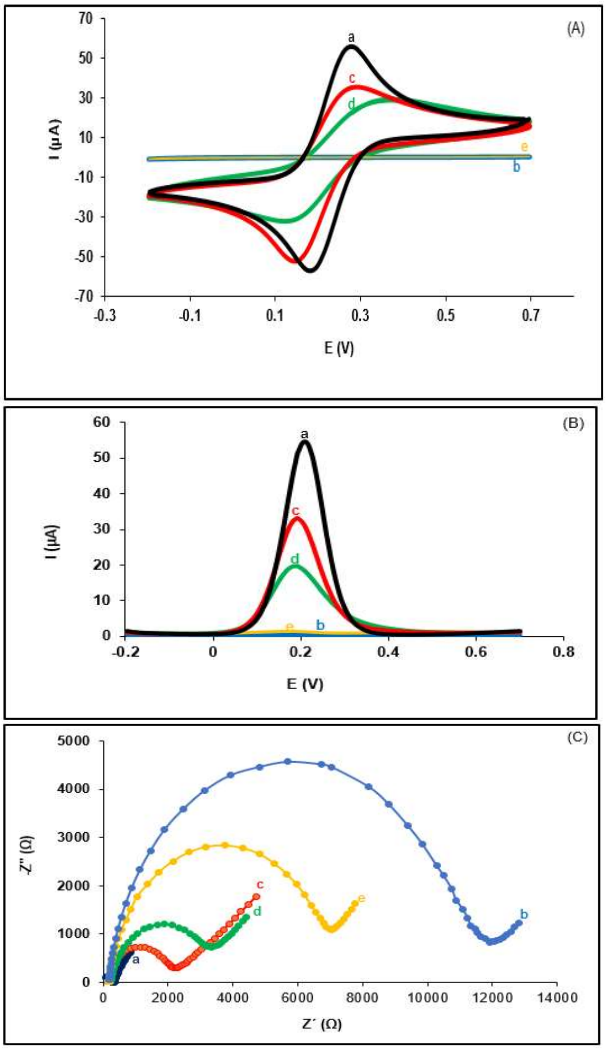

Fig. 5. (A) CV curves recorded related to different stages of electrode surface modification, (B) DPV curves recorded related to different stages of electrode surface modification and (C) EIS related to different stages of electrode surface modification, unmodified electrode (a), GCE modified with MIP before (b) and after washing (c), Gefi incubation on the electrode surface (d) and modified GCE with NIP (e) in a solution of 5 mM ferro/ferricyanide and 0.1 M potassium chloride.

Also, using the EIS method, the steps of making the electrode were investigated in a solution of 5 mM ferro/ferricyanide and 0.1 M potassium chloride (Fig. 5C). This method has the most application in obtaining qualitative information on electrochemical reactions on the surface of the electrode. With the electropolymerization of the polymer layer on the surface of the GCE (curve a) and the formation of a non-conductive film on the surface of the electrode, the charge transfer resistance is greatly increased because the polymer film prevents the transfer of electrons (curve b). By washing the electrode surface and removing the analyte molecule from the polymer film, the charge transfer resistance decreases (curve c). In the next step, after removing the drug and placing a drop of the drug solution on the surface of the electrode, the charge transfer resistance increases again, which indicates the interaction of the analyte with the holes in the polymer tissue (curve d). Curve e corresponds to the electrode modified with NIP, which has a higher charge transfer resistance than curve d.

Optimization of Experimental Conditions

In order to improve the response and increase the efficiency and selectivity of the electrode modified with MIP, parameters such as the number of cycles and the time of analyte incubation to the surface of the modified electrode were optimized using the DPV method.

DPV responses were recorded in a solution of 5 mM ferro/ferricyanide and 0.1 M potassium chloride for different cycles. For this purpose, different modified electrodes with various cycles were prepared in polymerization solution and then washed and tested in 5 mM ferro/ferricyanide and 0.1 M potassium chloride. As can be seen in Fig. 6, in the number of cycles less than 8, less current difference is seen, which is due to the thinness and brittleness of the polymer film formed on the surface of the modified electrode. In the number of cycles more than 8, fewer current changes are observed, which is due to the thickness of the polymer film formed on the surface of the modified electrode and preventing optimal washing of the template molecule in the washing step. Therefore, the number of 8 cycles of voltammogram was chosen as the optimal number of cycles for the formation of MIP film.

In order to check the connection performance by MIP, after removing the analyte molecule using washing solution

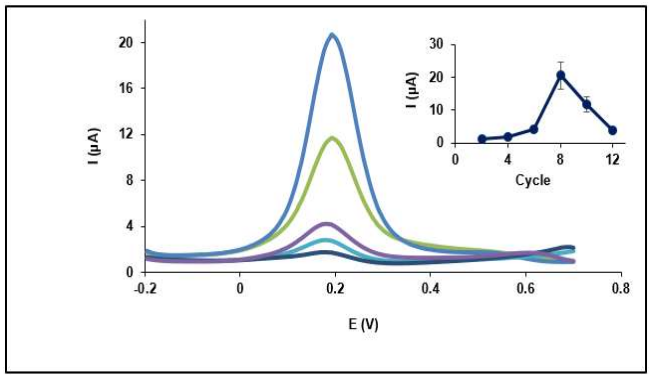

Fig. 6. Optimizing the number of cycles using a modified GCE in a solution of 5 mM ferro/ferricyanide and 0.1 M potassium chloride.

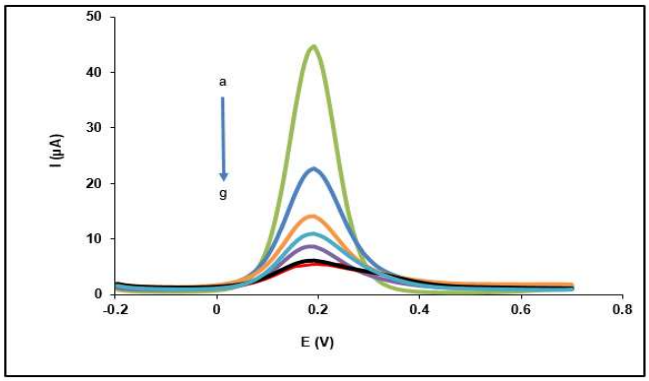

Fig. 7. Examining the optimal time for the incubation of analyte molecules by the polymer film on the surface of the modified GCE in the time range (a to g) from 2 to 14 min in a solution of 5 mM ferro/ferricyanide and 0.1 M Potassium chloride.

(2 min), the modified electrode with MIP was examined in the time interval of 2 to 14 min. A 5 µl drop of the drug solution was placed on the surface of the modified electrode and the voltammogram was recorded every 2 min with a solution of 5 mM ferro/ferricyanide and 0.1 M potassium chloride. As can be seen in Fig. 7, by increasing the time from 2 to 14 min, at the same time as the binding sites on the polymer surface are occupied by the template, the penetration of the probe into the electrode surface and the oxidation/reduction process are prevented and lead to the current decreases and after 10 min the current changes remain constant. Therefore, it can be concluded that the maximum drug absorption by the modified electrode occurred in 10 min. Therefore, the time of 10 min was chosen as the optimum time for analyte reabsorption in electrochemical sensor fabrication.

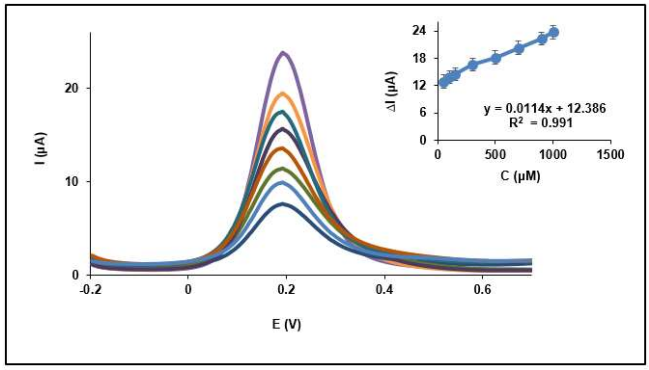

Fig. 8. DPV curves of various concentrations of Gefi in a solution of 5 mM ferro/ferricyanide and 0.1 M potassium chloride. In the margin of the diagram, the current changes according to the concentrations of the analyte.

Investigating the Analytical Performance of the Modified GCE

In order to check the analytical efficiency of the sensor, different concentrations of the drug were placed on the electrode surface under optimal conditions and 10 min as the optimal reabsorption time, and DPV responses in a solution of 5 mM ferro/ferricyanide and 0.1 M of potassium chloride were recorded. As can be seen in Fig. 8, with the increase of analyte concentration, the amount of current decreases gradually. By increasing the concentration of the analyte, the binding of more analyte molecules and the blocking of the detection sites from the penetration of the probe are prevented. The linear regression equation for the modified electrode was calculated as Ip = 0.0114 [Gefi] (µM) + 12.386 (R^2 = 0.991) in the linear range of 5 to 1000 µM and with a detection limit (LOD) of 1.6 µM based on S/N = 3.

Selectivity of the Proposed Sensor

Selectivity is important as an effective parameter in the practical application of the modified electrode. Analytes with similar structure and properties to Gefi including Uric acid, Osimertinib, and Riboflavin were evaluated (Fig. 9). For this purpose, possible interfering analytes were measured with a

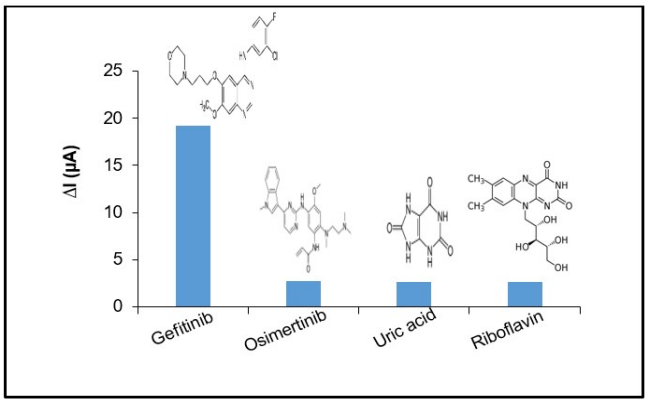

Fig. 9. Investigating the selectivity of the modified electrode for the measurement of Gefi and comparing it with possible interfering analytes.

concentration 100 times the concentration of the analyte and under the same laboratory conditions. DPV response shows that the changes in the electrochemical response caused by the non-specific binding of possible interfering analytes are very small and negligible compared to the electrochemical response of Gefi. The selectivity of the modified electrode is related to the strong and selective interactions that occur between the MIP and the substrate on the surface of the modifier.

Repeatability, Reproducibility, and Stability of the Proposed Sensor

To check the repeatability of the modified electrode, the current for a specified concentration of the drug solution with an optimal duration of 10 min was measured using a modified electrode 9 times in a solution of 5 mM ferro/ferricyanide and 0.1 M potassium chloride. The calculated relative standard deviation (RSD) value was equal to 2.03%, which indicates the good reproducibility of the electrode response (Fig. 10). Also, in order to check the reproducibility of the peak current response of Gefi, it was investigated using 5 modified electrodes prepared under the same conditions. The value of the RSD was 3.24%, which indicates the good reproducibility of the modified electrode (Fig. 11). Also, to check the stability of the designed sensor, the modified electrode was tested under optimal conditions for 10 days. For this purpose, the modified electrode was kept in proper conditions and evaluated every 5 days. The results show that the current

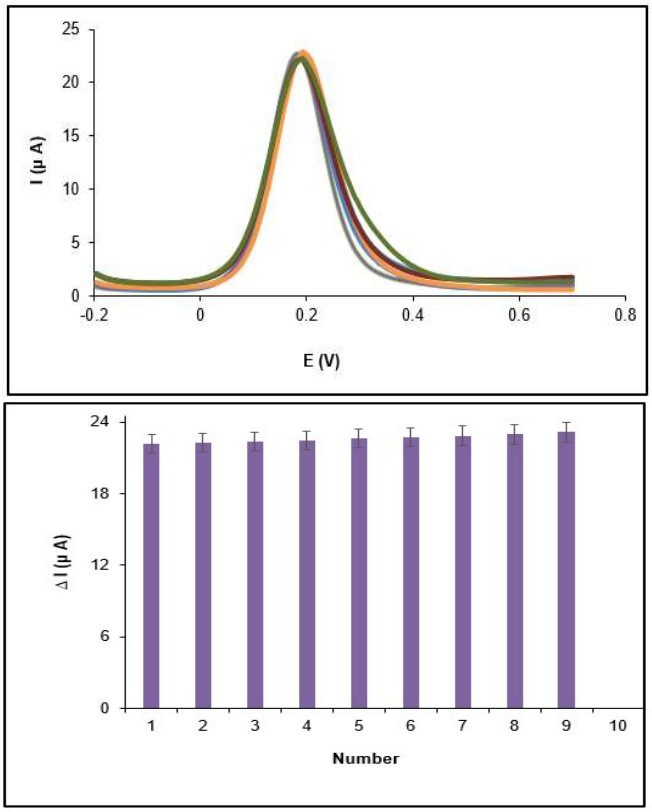

Fig. 10. Checking the repeatability of the modified electrode in a solution of 5 mM ferro/ferricyanide and 0.1 M potassium chloride.

value for the proposed sensor maintained its efficiency after five days at 99.2% and after ten days at 90.8%, which indicates good stability.

Real Sample Analysis

To check the ability of the designed sensor in the analysis of real samples, the amount of Gefi in the blood serum sample was evaluated. Examining the results of the DPV method to analyze the real sample was done according to the optimal laboratory conditions and using the standard addition technique. As listed in Table 1, the results obtained from this method are in good agreement with the actual values of Gefi in the real sample.

CONCLUSIONS

The preparation and production of selective MIPs for the

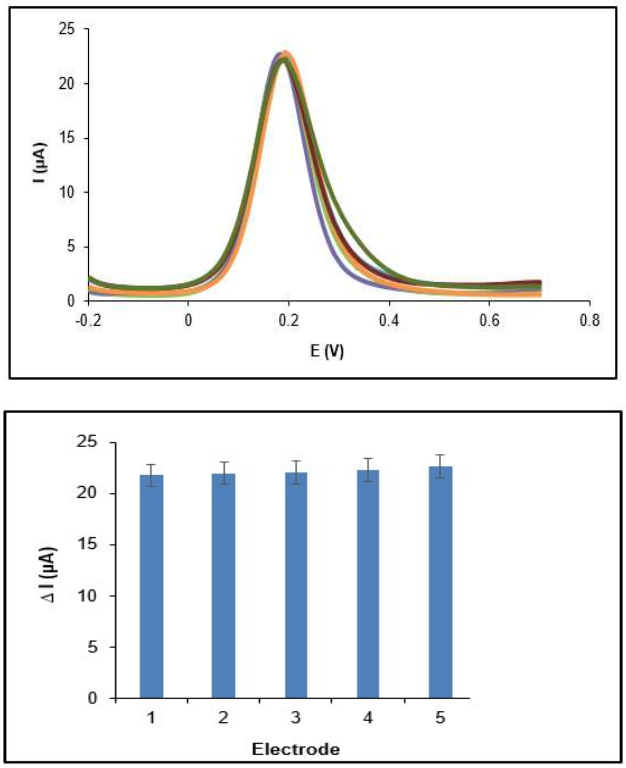

Fig. 11. Checking the reproducibility of 5 modified electrodes in a solution of 5 mM ferro/ferricyanide and 0.1 M potassium chloride.

Table 1. Gefi Detection in the Real Sample Using the Designed Sensor (n = 3)

Sample	Added (μM)	Found (μM)	RSD (%)	Recovery (%)
	-	-	-	-
Serum	5	5.83	2.7	98.8
	10	10.95	2.3	100.5

specific identification of target molecules requires a study of the components and methods used to produce, identify, and use these synthesized materials. In this study, a cost-effective, simple, and fast electrochemical sensor based on MIP was designed to detect the sensitivity and selectivity of Gefi. The electrode has several special analytical characteristics such as quick and easy preparation, cheapness, stability in the chemical environment, wide linear range, fast response, good reproducibility and reproducibility, high sensitivity, and selectivity for measuring Gefi. Under the optimal conditions, a linear relationship between the peak current and the concentration of Gefi was obtained in the range of 5 to 1000 μM with a detection limit of 1.6 μM.

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