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ORIGINAL ARTICLE / ÖZGÜN MAKALE

DEVELOPMENT OF ELECTROCHEMICAL SENSOR BY MOLECULARLY IMPRINTING POLYMER FOR DETECTION OF AN INFLUENZA VIRUS NEURAMINIDASE INHIBITOR PERAMIVIR

BİR İNFLUENZA VİRÜSÜ NÖRAMİNİDAZ İNHİBİTÖRÜ OLAN PERAMİVİR'İN SAPTANMASI İÇİN MOLEKÜLER BASKI POLİMER İLE ELEKTROKİMYASAL SENSÖRÜN GELİŞTİRİLMESİ

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ABSTRACT

Objective: *Influenza viruses are the most common cause of influenza. Antiviral drugs prevent the spread of the virus through the infected cells. Peramivir is one of the antiviral drugs that is an inhibitor of influenza virus neuraminidase. In our study, we aimed to develop a MIP-based electrochemical sensor to determine Peramivir. This study is the first to create an electrochemical sensor for Peramivir. MIP(PERA)/GCE was fabricated with the electropolymerization of 4-aminophenol (4-AP) and ortophenilendiamine (o-PD) in the presence of Peramivir. The developed MIP(PERA)/GCE was applied to the commercial serum sample for analysis of Peramivir.*

Material and Method: *PERA is supplied by Tobio Novelpharma pharmaceutical company (İstanbul, Türkiye). Potassium ferricyanide ($[K_3Fe(CN)_6]$), potassium ferrocyanide ($K_4[Fe(CN)_6].3H_2O$), and potassium chloride (KCl), 4-aminophenol (4-AP) and ortophenilendiamine (o-PD), commercial human serum sample, dopamine, ascorbic acid, uric acid, paracetamol, KNO_3, Na_2SO_4 ve $MgCl_2$ were obtained from Sigma-Aldrich (St. Louis, Missouri, USA). Methanol, acetic acid, oxalic acid, hydrochloric acid, acetonitrile, sodium hydroxide, and ethanol were purchased by Merck. The redox process was investigated using cyclic voltammetry (CV) and differential pulse voltammetry (DPV) by the Dropsens µStat 400 Bipotantiostat/ Galvanostat (Metrohm, Herisau, Switzerland). Three-electrode systems consisted of a glassy carbon working electrode (GCE, 3mm^2, BASi, USA), a saturated Ag/AgCl reference electrode, and a Pt wire.*

Result and Discussion: *The sensor exhibits a linear range of 1-10 pM with a detection limit of 0.158 pM. The MIP(PERA)/GCE sensor could detect PERA from commercial serum samples with a high recovery of 101.81%.*

Keywords: *Commercial serum sample, determination, electrochemical sensor, molecularly imprinted polymer, peramivir*

ÖZ

Amaç: *Grip virüsleri, gribin en yaygın nedenidir. Antiviral ilaçlar virüsün enfekte hücreler yoluyla yayılmasını engeller. Peramivir, influenza virüsü nöraminidazının inhibitörü olan antiviral ilaçlardan biridir. Çalışmamızda Peramivir tayini için MIP tabanlı bir elektrokimyasal sensör geliştirilmesi amaçlanmıştır. Bu çalışma, Peramivir analizi için geliştirilen ilk elektrokimyasal*

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sensör çalışmasıdır. MIP(PERA)/GCE, Peramivir varlığında 4-aminofenol (4-AP) ve ortofenilendiaminin (o-PD) monomerlerin elektropolimerizasyonu ile geliştirilmiştir. Geliştirilen MIP(PERA)/GCE, Peramivir analizi için ticari serum örneğine uygulanmıştır.

Gereç ve Yöntem: *Peramivir, Tobio Novelpharma ilaç şirketi (İstanbul, Türkiye) tarafından sağlanmıştır. Potasyum ferrisiyanür ($[K_3Fe(CN)_6]$), potasyum ferrosiyanür ($K_4[Fe(CN)_6].3H_2O$) potasyum klorür(KCl), 4-aminofenol (4-AP) ve ortofenilendiamin (o-PD), sentetik insan serumu, dopamin, askorbik asit, ürik asit, parasetamol, KNO_3, Na_2SO_4 ve $MgCl_2$ Sigma-Aldrich'ten (St. Louis, Missouri, ABD) temin edilmiştir. Metanol, asetik asit, okzalik asit, hidroklorik asit, asetonitril sodyum hidroksit ve etanol Merck tarafından tedarik edilmiştir. Redoks işlemi, Dropsens μStat 400 Bipotantiostat/ Galvanostat (Metrohm, Herisau, İsviçre) tarafından döngüsel voltametri (CV) ve diferansiyel puls voltammetrisi (DPV) kullanılarak gerçekleşmiştir. Üç elektrotlu sistemler, bir camsı karbon çalışma elektrodu (GCE, 3mm^2, BASi, ABD), doymuş bir Ag/AgCl referans elektrodu ve bir Pt telinden oluşmuştur.*

Sonuç ve Tartışma: *Geliştirilen sensör, 0,158 pM en düşük tayin limiti ile 1-10 pM doğrusal bir aralık göstermiştir. Geliştirilen MIP sensörü ticari bir serum örneğine uygulanmıştır. MIP(PERA)/GCE sensörü ile %101.81'lik yüksek bir geri kazanımla ticari serum örneklerinden peramivir saptanmıştır.*

Anahtar Kelimeler: *Elektrokimyasal sensör, miktar tayini, molekül baskılanmış polimer, peramivir, ticari serum örneği*

INTRODUCTION

Influenza is a respiratory disease caused by Influenza A and Influenza B viruses. It usually occurs in people with weakened immune systems, children, and older people with a sudden onset of high fever. Influenza disease is treated with antiviral medication [1]. One of the antiviral drugs is Peramivir (Figure 1). Peramivir is a neuraminidase inhibitor. FDA approved the peramivir drug to prevent new influenza viruses from infecting cells in December 2014 [2].

Many analytical methods were performed for the determination of Peramivir. Especially, the chromatographic techniques such as RP-HPLC [3], HPLC-MS/MS [4-5], HILIC-SPE-LC-MS/MS [6], were used for the analysis of Peramivir. However, scientists have been looking for new analytical methods recently because of the expensive and long analysis time of chromatographic techniques. The electrochemical methods are strong alternatives to chromatographic techniques [7]. Electrochemical sensors have many advantages, such as high selectivity and sensitivity and being affordable and environmentally friendly.

A molecularly imprinted polymer (MIP) is produced by polymerization in the presence of a target molecule [8]. The polymerization comprises a monomer, initiator, cross-linker, and target molecule. The enzymes, biomarkers, viruses, bacteria, and pharmaceutical drugs can be used as target molecules. MIP aims to form artificial receptors for target molecules [9]. MIP has three main processes: 1-Polymerization, 2-creating a cavity specific to the target molecule 3-compatible target molecules into cavities. The polymerization is performed with different techniques, such as thermal polymerization [10], photopolymerization [11-13], and electropolymerization [14-15]. Polymerization is a critical step because of polymeric film stability and repeatability. The monomers such as acrylamide, o-phenylenediamine, methacrylic acid, 4-aminophenol, 4- aminobenzoic acid, aniline were generally used for polymerization of electrodes. Basically, the photopolymerization technique takes place under a UV lamp, while thermal polymerization is performed in the oven. Electropolymerization in the presence of a target molecule and monomer is carried out by applying potential with cyclic voltammetry [15]. The thickness of the polymeric film should be controlled by an indirect method with redox markers. The cavities specific to the target molecule in the polymeric matrix are formed for creating the MIP receptors [16]. For creating a cavity process, the best removal solution should be selected [17]. Therefore, the MIP receptor recognizes the target molecules. It is enhanced the sensitivity and selectivity of analytical methods. Moreover, the MIP-based electrochemical sensor is easy to prepare, cheap, and has high mechanical and chemical stability.

In our study, we aimed to develop a MIP-based electrochemical sensor to determine of PERA. This study is the first to create an electrochemical sensor for PERA. MIP(PERA)/GCE was fabricated

with the electropolymerization of 4-aminophenol (4-AP) and ortophenilendiamine (o-PD) in the presence of PERA. The developed MIP(PERA)/GCE was applied to the commercial serum sample for analysis of PERA.

MATERIAL AND METHOD

Reagents and Chemicals

PERA is obtained by Tobio Novelpharma pharmaceutical company (İstanbul, Türkiye). Potassium ferricyanide ($[K_3Fe(CN)_6]$), ferrocyanide ($K_4[Fe(CN)_6].3H_2O$), and 0.1 M KCl were used to use the 5 mM redox marker ($[Fe(CN)_6]^{3-/4-}$). The 4-aminophenol (4-AP) and ortophenilendiamine (o-PD) were supplied from Sigma-Aldrich (St. Louis, Missouri, USA). These monomers were used for composing the polymeric film. Moreover, methanol (MeOH), acetic acid (HAc), oxalic acid, hydrochloric acid (HCl), acetonitrile (ACN), sodium hydroxide (NaOH), and ethanol were purchased by Merck (Darmstadt, Germany) to select optimum removal solution. The commercial serum sample was supplied from Sigma-Aldrich (product no: H4522). The interference chemicals (dopamine, ascorbic acid, uric acid, paracetamol, KNO_3, Na_2SO_4, and $MgCl_2$) were acquired from Sigma-Aldrich.

Apparatus

The redox process was investigated using cyclic voltammetry (CV) and differential pulse voltammetry (DPV) by the Dropsens μStat 400 Bipotantiostat/ Galvanostat (Metrohm, Herisau, Switzerland). Three-electrode systems consisted of a glassy carbon working electrode (GCE, $3mm^2$, BASi, USA), a saturated Ag/AgCl reference electrode, and a Pt wire. All electrodes were supplied from BASi, USA.

The chemicals were weighed with precision balance (Ohaus Company, China) and dissolved with distilled water or pH 5.2 acetate buffer and sonicated with an ultrasonic bath. The solutions were kept in a refrigerator until use. The pH of the solutions was arranged with a pH meter. The commercial serum sample prepared for applying MIP(PERA)/GCE in the commercial serum sample was centrifuged at 3500 rpm for 30 min by a centrifuge from Nuve Company (NF200, Nuve Company, Türkiye). The thermal shaker (TS-100, Biosan, Riga, Latvia) was used at room temperature and 650 rpm to supply the movement of the target molecule from the polymeric matrix.

The morphology of MIP(PERA)/GCE surface and the electrochemical behavior of the films were characterized. The surface of morphology was evaluated with SEM (TESCAN GAIA 3, Czech Republic). The EIS (Metrohm Autolab, Utrecht, Netherlands) was used to examine the polymeric films regarding electrochemical behavior.

Fabrication of MIP(PERA)/GCE and NIP-based Electrochemical Sensors

The polymeric film was composed of two different monomers. To prepare the PERA stock solution, PERA was dissolved in double distilled water (ddwater). To prepare the MIP(PERA)/GCE, 2 mL 10^{-3} M 4-AP, 1 ml 10^{-3} M o-PD and 1 ml 10^{-3} M PERA, and 1 mL 5.2 acetate buffer were mixed and vortexed. The polymeric film was prepared in the same protocol without 1 ml10^{-3} M PERA to prepare the NIP-based sensor. The electrode was immersed in the polymeric film solution and electropolymerized scanning between -0.2 and 0.8 V for 5 cycles (50 mV/s scan rate). Then, the electrode was immersed in a 15 M HAc solution to create the pores in the polymeric matrix. The obtained results by MIP(PERA)/GCE was compared with NIP-based electrode in terms of analytical performances using a redox marker.

Preparation of Commercial Human Serum Sample

MIP(PERA)/GCE practicability was examined with commercial serum samples. The commercial serum sample in the presence of PERA as the target molecule was prepared. 1 mM PERA (1 ml), commercial serum (3.6 ml), 5.4 ml ACN (to precipitate the protein residues) were mixed in the centrifuge tube. PERA was not added to the commercial serum sample for the blank serum solution. Two centrifuge tubes were settled in a centrifuge as an opposite and centrifuged to separate the supernatant from the precipitate [18]. The gathered supernatant was used to evaluate the accuracy of

MIP(PERA)/GCE. Moreover, the calibration plot was obtained, and recovery studies were done in commercial serum samples.

RESULT AND DISCUSSION

Surface Characterization of the Molecularly Imprinted Polymeric Film and Non-imprinting Polymeric Film

The morphological structures of molecular imprinted polymeric film and non-imprinting polymeric film were characterized using SEM and SEM-EDX measurements (Figure 1). As expected, the non-imprinting polymeric film showed smoothness (Figure 1A), whereas the molecular imprinted polymeric film showed porosity and roughness (Figure 1B). The SEM-EDX of the MIP was also analyzed. The C and O atoms in the structure of the polymeric film were proved with The EDX spectra of the polymeric film (Figure 1C).

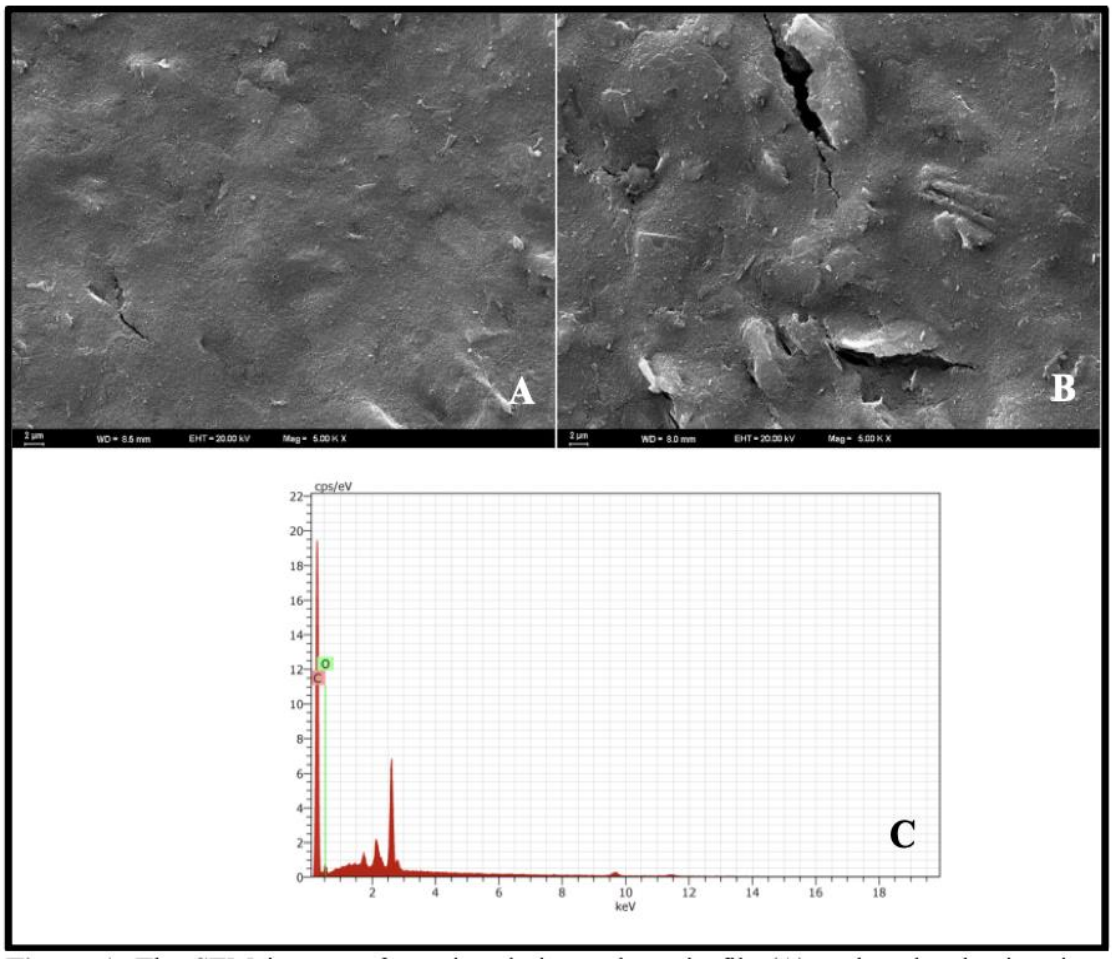


Figure 1. The SEM images of non-imprinting polymeric film(A) and molecular imprinted polymeric film (B) and EDX spectra of molecular imprinted polymeric film(C)

Electrochemical Characterization of the MIP-based Electrochemical Sensors

Electrochemical characterization of MIP-based sensors was performed with CV and EIS techniques. EIS and CV are based on charge resistance and electron transfer, respectively. After each process, the indirect measurements were performed in the solution of redox marker. The cyclic voltammograms are shown after each process in Figure 2A. Firstly, the current obtained with bare GCE was measured, and the highest current was obtained with bare GCE (black line). After

electropolymerization, the current decreased because electron transfer was slow (blue line). It was observed that the current increased (green line). Finally, the PERA was immobilized into PERA-specific cavities. The current decreased again (red line).

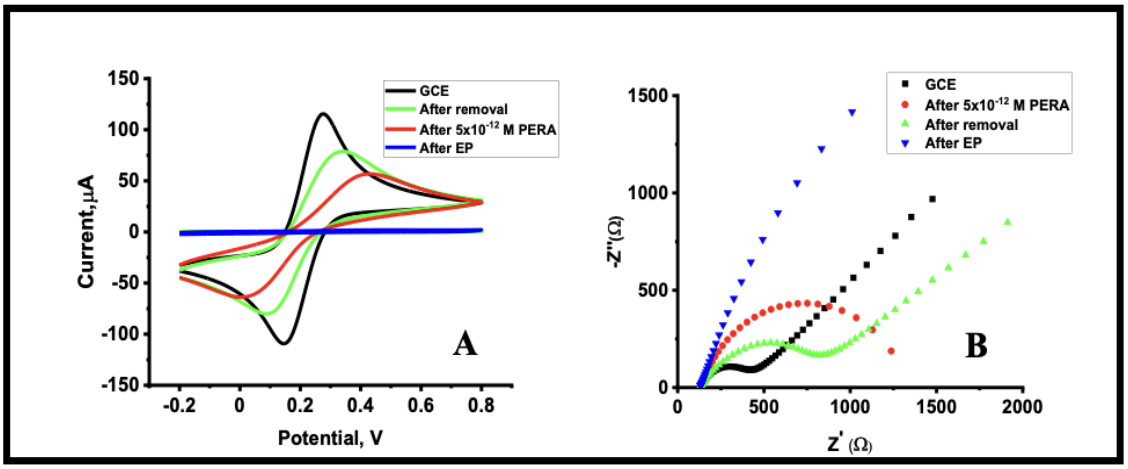


Figure 2. The cyclic voltammograms(A) and electrochemical impedance spectra(B) of MIP(PERA)/GCE in the presence of redox marker

The Nyquist plots were drawn with EIS measurement of solution of redox marker after each process in Figure 3B. The semi-circle represents the charge resistance. The lowest charge resistance was found in the bare GCE (black dots). After the EP process, it had the highest charge resistance (blue dots). After the removal process, the charge resistance decreased (green dots). Finally, the charge resistance increased again (red dots).

Optimization of Important Parameters

The crucial parameters were optimized to obtain the best MIP-based electrochemical sensor for the analysis of PERA. The o-pD:4-AP monomer ratio, monomer: template ratio, scan number, removal solution, removal time, and rebinding time were optimized. The optimization results are given in Figure 3.

The o-pD:4-AP Monomer Ratio:

Forming a polymeric matrix is an essential step for fabricating an MIP-based sensor. The o-pD and 4-AP monomers were tried separately. However, the MIP-based sensor composed of these monomers didn't respond well. For this reason, the combinations of monomers were studied. The difference (ΔI) between currents before removal and after electropolymerization was measured. Different ratios of o-pD: 4-AP (1:1; 1:2; 2:1) were tried to obtain the best polymeric film. As seen in Figure 3A., the ratio of o-pD:4-AP was found good response in 1:1.

Monomer: Template Ratio:

The monomer: template ratio was optimized after selecting the monomer and their ratio. The polymeric matrix was prepared with the combination of monomer (o-pD: 4-AP(1:1)) in the presence of PERA with an electropolymerization technique. The different ratio of the combination of monomers: template was studied between 20:1 and 2:1 while keeping the PERA constant at 1. According to Figure 3B., the best result(ΔI) was received in 10:1.

Number of Electropolymerization Scans:

The thickness of the polymeric film is related to the number of electropolymerization scans. Determining the best electropolymerization scan number is crucial for the sensitivity, repeatability, and stability of the MIIP(PERA)/GCE. The electropolymerization process on the GCE surface was carried

out by scanning the CV with a potential between -0.2 and 0.6 V. The number of EP scan were optimized from 3 to 10 cycles. The ΔI results were obtained similarly while numbers of scans 3 and 5. The results were not repeatable when scanning 3. For this reason, scan 5 was selected (Figure 3C).

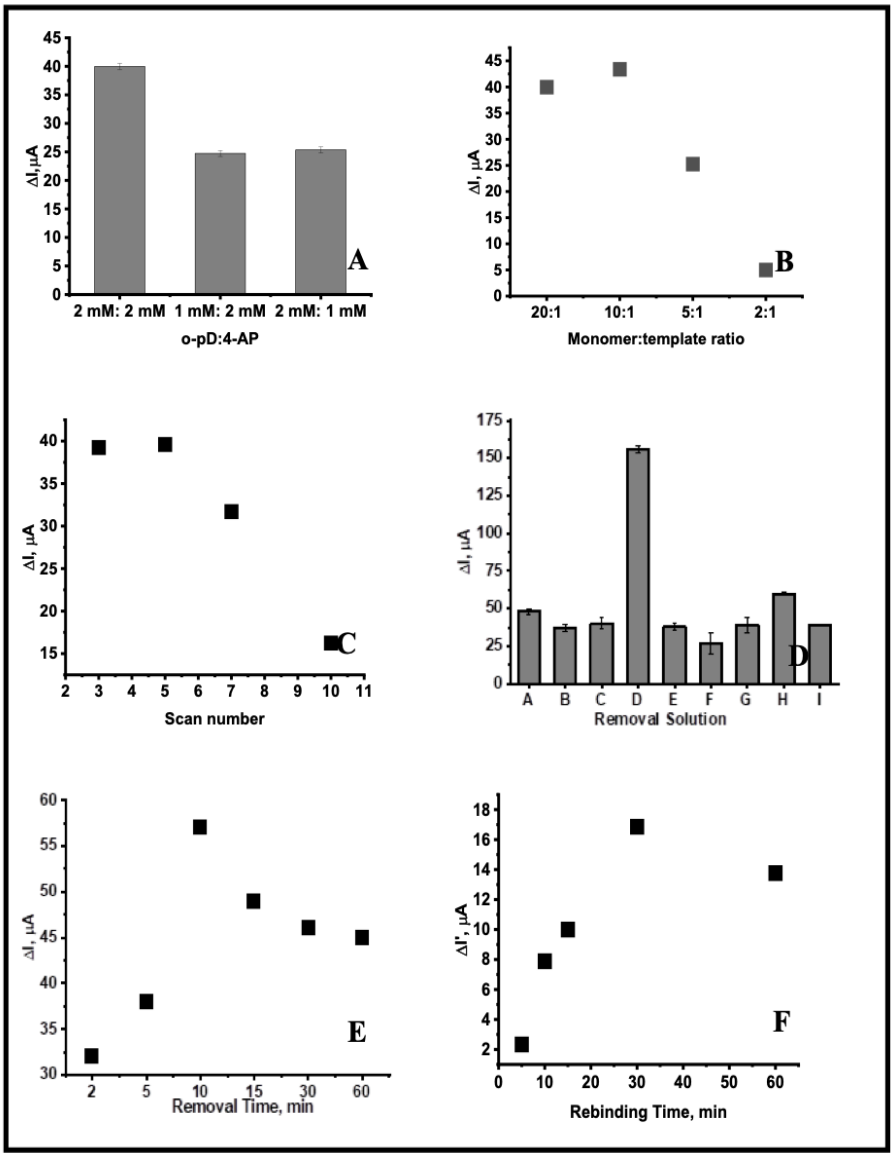


Figure 3. The optimization studies of o-pD:4-AP monomer ratio(A), monomer: template ratio(B), number of electropolymerization scan(C), removal solution(D), removal time(E) and rebinding time(F) for development of MIP(PERA)/GCE

Selection of Removal Solution and Removal Time

To remove the PERA from the cavities by broking non-covalent bonding, different removal solutions (Ethanol(A), ACN(B), NaOH. ddwater(C), 15 M HAc(D), 1M HCl(E), 1M NaOH(F), MeOH(G), 10 M HAc(H), 5 M HCl (I)) were tried. Moreover, oxalic acid and 5 M HCl were used as removal solutions; however, these solutions were not enough to remove PERA from the cavities. The obtained ΔI results were highest when 15 M HAc solution was used (Figure 3D). Therefore, the cavities were formed specific to PERA on the polymeric matrix. For determining the time of removal process

with 15 M HAc solution, the removal time from 2 to 20 min was optimized. According to Figure 3E, the removal time was chosen as 10 min.

Rebinding Time

After the removal, the PERA, the target molecule, was replaced with cavities specific to the target molecule. The 10^{-3} M PERA, the target molecule, was diluted to different concentrations. The PERA rebinding process was performed with the incubation of PERA by a thermo-shaker. The rebinding time was assessed by following the difference (ΔI') between currents after the removal process and after the rebinding process were measured. According to Figure 3F, the rebinding time was 30 min for MIP(PERA)/GCE.

Analytical Performances of MIP(PERA)/GCE and NIP-based Electrochemical Sensors

The calibration curve was drawn to examine the analytical performances of MIP(PERA)/GCE and NIP-based electrochemical sensors, and validation parameters were evaluated. Under the optimum condition, the different concentrations of PERA were incubated to MIP(PERA)/GCE. The redox solution was measured by DPV after rebinding PERA. The linear curve between 1.0 and 10 pM was obtained, plotting the concentration of PERA versus ΔI′ current. The calibration equation was found as $\Delta I' (\mu A) = 2.09x10^{12} (\mu A/M) \times C (M) + 20.21 (\mu A)$ (r = 0.998) with LOD and LOQ values of 1.58 x 10^{-13} M and 5.27 x 10^{-13} M, respectively (Table 1). LOD and LOQ values are calculated as 3×sd/m and 10×sd/m, respectively [19-20] (sd: standard deviation; m: slope of calibration curve). The calibration plots for MIP(PERA)/GCE (red dots) and NIP-based sensor (black dots) and DP voltammograms for MIP(PERA)/GCE were given in Figure 4A and Figure 4B, respectively. NIP-based sensor was prepared to control the MIP(PERA)/GCE. According to the results, the MIP(PERA)/GCE showed excellent selectivity and sensitivity for the PERA analysis.

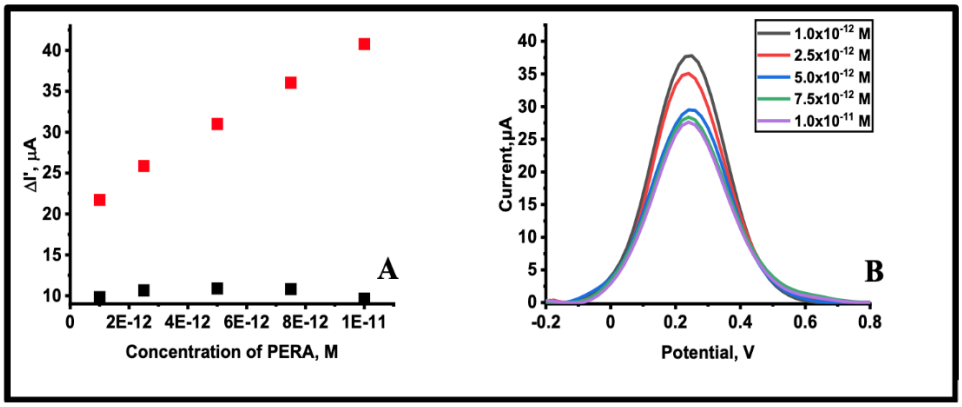


Figure 4. The calibration plots (A) for MIP(PERA)/GCE and NIP-based sensor and DP voltammograms (B) for MIP(PERA)/GCE

Analytical Application of MIP(PERA)/GCE and NIP-based Electrochemical Sensors in Commercial Human Serum Sample

MIP(PERA)/GCE practicability was investigated with commercial serum samples to demonstrate the accuracy of the sensor. The preparation of a commercial serum sample in the presence of PERA was explained in section 2.4. This sample was diluted to the required concentration of PERA and rebound to pores in the polymeric matrix. The 5 mM $[Fe(CN)_6]^{3-/4-}$ solution was measured by DPV after rebinding of PERA in the commercial serum sample. The linear curve between 1.0 and 10 pM was obtained, plotting the PERA concentration versus ΔI′ current. The calibration equation was obtained as $\Delta I' (\mu A) = 9.80x10^{11} (\mu A/M) \times C (M) + 22.85 (\mu A)$ (r = 0.998) with LOD and LOQ values of 2.79 x 10^{-13} M and 9.31 x 10^{-13} M, respectively (Table 1).

Table 1. Regression data of the calibration line for PERA on MIP(PERA)/GCE

	Standard solution	**Serum sample**
Linearity range (M)	$1x10^{-12}$-$1x10^{-11}$	$1x10^{-12}$-$1x10^{-11}$
Slope (µA/M)	$2.09x10^{12}$	$9.80\ x10^{11}$
SE of slope	$6.96x10^{10}$	$3.39x10^{10}$
Intercept (µA)	20.221	22.854
SE of intercept	0.427	0.205
Correlation coefficient (*r*)	0.998	0.998
LOD (M)	$1.58x10^{-13}$	$2.79x10^{-13}$
LOQ (M)	$5.27x10^{-13}$	$9.31x10^{-13}$
Repeatability of peak current (RSD%)*	0.507	0.498
Reproducibility of peak current (RSD%)*	0.812	0.727

**Each value is the mean of three experiments*

The calibration plots for MIP(PERA)/GCE (red dots) and NIP-based sensor (black dots) and DP voltammograms for MIP(PERA)/GCE for commercial serum samples were given in Figure 5A and Figure 5B, respectively. NIP-based sensor was prepared to control the MIP(PERA)/GCE in the commercial serum sample.

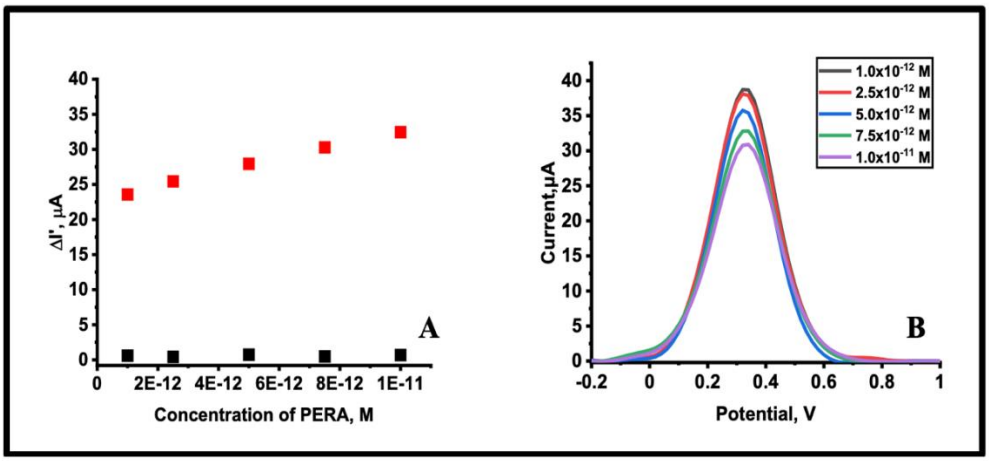


Figure 6. The calibration plots (A) for MIP(PERA)/GCE and NIP-based sensor and DP voltammograms for MIP(PERA)/GCE (B) for commercial serum samples

Furthermore, the recovery studies were performed with commercial serum samples (Table 2). Recovery and RSD % results have proven the accuracy and precision of MIP((PERA)/GCE.

Table 2. Results of the recovery experiments for commercial serum samples

	Serum Sample
Spiked amount (mg)	0.1000
Found amount (mg)*	0.1018
Average recovery (%)	101.81
RSD%	1.95
Bias%	+1.81

**Each value is the mean of three experiments*

Selectivity of MIP(PERA)/GCE and NIP-based Electrochemical Sensors

After removal, the cavities specific to PERA form in the polymeric matrix. The selectivity studies were performed by rebinding PERA and other selected compounds, such as Oseltamivir, Zanamivir, Brivudin, and Lamivudin (Figure 6). The imprinting factor (IF) was calculated by the ratio of obtained ΔI′ for MIP and NIP. The IF of PERA, Oseltamivir, Zanamivir, Brivudin, and Lamivudin were 18.69, 10, 7.5, 2.27, and 2.31, respectively. As expected, PERA shows a higher affinity to the cavities than similar compounds. The value of IF(MIP/NIP)> 1.0 confirmed that the developed sensors show the high affinity and selectivity to PERA molecule.

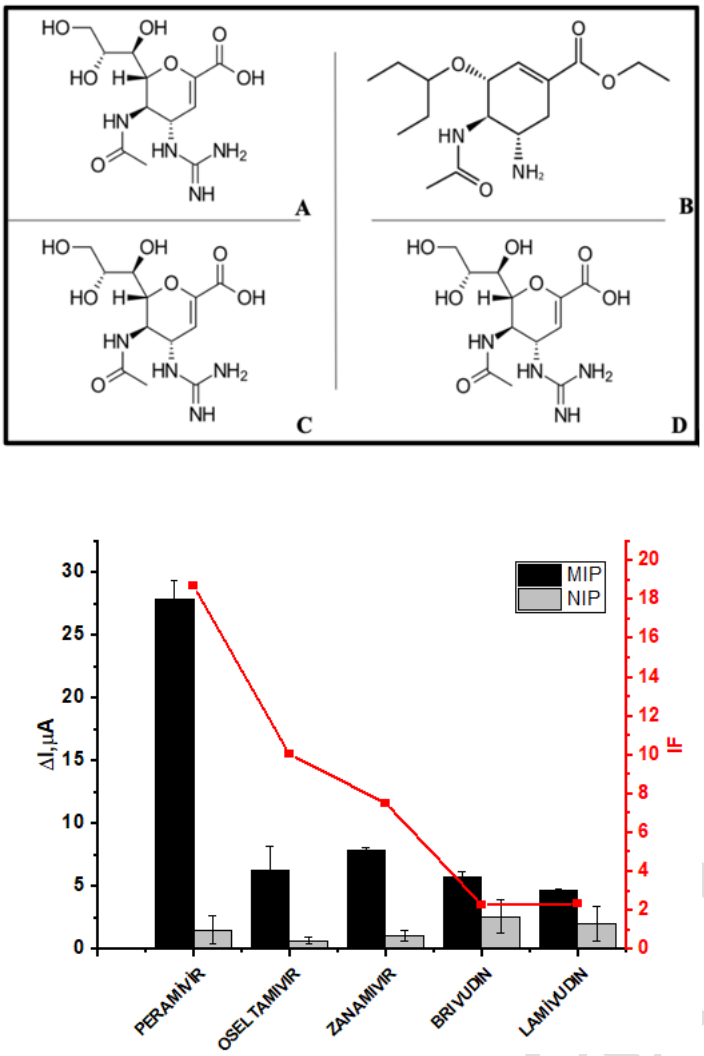


Figure 6. The structure of oseltamivir(A), zanamivir(B), brivudine(C), lamivudine(D). Selectivity studies of similar drugs and peramivir

Interference Study of MIP and NIP-based Electrochemical Sensors

The biological fluids in our body have many compounds, such as ascorbic acid, uric acid, paracetamol, KNO_3, Na_2SO_4, $MgCl_2$, and so on. In the interference study, these compounds are used as interference agents. The mixture of PERA and 10 times more interference agents were applied to the rebinding process. The 98.93-101.96% of recovery and 0.26-1.50% of RSD% showed that the interfering agents did not significantly affect the MIP(PERA)/GCE analytical performance (Table 3).

Table 3. Effect of interferents on the determination of PERA

Interferent	Recovery (%)	RSD (%)
Dopamine	100.38	1.03
Ascorbic acid	101.96	0.26
Uric acid	100.35	0.49
Paracetamol	99.41	0.82
K^+	98.93	0.89
NO_3^-	98.93	0.89
Na^+	101.07	1.50
SO_4^{2-}	101.07	1.50
Mg^{2+}	100.17	0.50
Cl^-	100.17	0.50

Stability of MIP Sensor

Stability is one of the validation parameters. The developed MIP(PERA)/GCE was tested regarding stability parameters. As a result of studies, effective results were found in daily preparation.

Conclusion

The electrochemical MIP(PERA)/GCE sensor was fabricated and used to determine PERA for the first time. The two monomers (o-pD and 4-AP) were applied to form the polymeric film consisting of the PERA. The MIP(PERA)/GCE was controlled with NIP based electrochemical sensor. The morphological and electrochemical characterization of sensors were analyzed. The required parameters were optimized to obtain the best performance of MIP(PERA)/GCE. The specificity and selectivity of the MIP(PERA)/GCE were evaluated with imprinting factors of similar structure and PERA. The MIP(PERA)/GCE analytical performance was examined, and the linear range of PERA was obtained between 1 pM and 10 pM with an LOD of 0.158 pM. The excellent recovery (101.81%) and RSD% (1.95%) results proved the feasibility and accuracy of MIP(PERA)/GCE in commercial serum samples. PERA was also analyzed in the presence of interference agents in serum samples. No interaction was observed. This study is important because it is the first MIP-based electrochemical sensor study with PERA. The developed sensor has the potential to adapt to other analytical methods.

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AUTHOR CONTRIBUTIONS

Concept: G.Ö.A., S.A.Ö.; Control: G.Ö.A., S.A.Ö.; Sources: G.Ö.A., S.A.Ö.; Data Collection and/or Processing: G.Ö.A., S.A.Ö.; Analysis and/or Interpretation: G.Ö.A., S.A.Ö.; Literature Review: G.Ö.A., S.A.Ö.; Manuscript Writing: G.Ö.A., S.A.Ö.; Critical Review: G.Ö.A., S.A.Ö.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

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