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ORIGINAL ARTICLE / ÖZGÜN MAKALE

THE FABRICATION OF MIP BASED ELECTROCHEMICAL SENSOR FOR THE DETERMINATION OF DOBUTAMINE

DOBUTAMİN TAYİNİ İÇİN MIP ESASLI ELEKTROKİMYASAL SENSÖRÜN GELİŞTİRİLMESİ

Göksu ÖZÇELİKAY-AKYILDIZ[1]*, Sariye İrem KAYA[2]

[1]Ankara University, Faculty of Pharmacy, Department of Analytical Chemistry, 06560, Ankara, Türkiye
[2]University of Health Science, Gülhane Faculty of Pharmacy, Department of Analytical Chemistry, 06018, Ankara, Türkiye

ABSTRACT

Objective: *Dobutamine (DBT), one of the most common synthetic catecholamines, is important in the renal, cardiovascular, hormonal, and central nervous systems. In our study, it is proposed to produce the first MIP-based electrochemical sensor for DBT analysis. MIP(DBT)/GCE was developed by electropolymerization of 4-aminobenzoic acid (4-ABA) in the presence of DBT. Analytical performance and validation evaluation were performed on both standard solution and commercial serum samples.*
Material and Method: *DBT was supplied by the Vem pharmaceutical company. For the preparation of 5 mM $[Fe(CN)_6]^{3-/4-}$ solution, known amounts of potassium ferricyanide ($[K_3Fe(CN)_6]$) and potassium ferrocyanide ($K_4[Fe(CN)_6].3H_2O$ mixed in 0.1 M KCl solution. The 4-ABA was used to create the polymeric film in the presence of DBT. Electrochemical measurements were actualized in IVIUM compactStat.h potentiostat (Eindhoven, The Netherlands) using a three-electrode system consisting of Pt wire, Ag/AgCl reference electrode, and glassy carbon electrode (GCE).*
Result and Discussion: *The MIP(DBT)/GCE sensor was applied to standard solution and commercial serum samples. When DBT concentrations were plotted against ΔI values, a linear response between 1×10^{-13} and 1×10^{-12} M was obtained in both environments. LOD results were found to be 0.087×10^{-13} M and 0.033×10^{-13} M in standard solution and commercial human serum, respectively. Recovery% and RSD% were found to be 99.68-100.32% and 1.38-1.66%, respectively.*
Keywords: *Commercial samples of human serum, determination, dobutamine, electrochemical MIP sensor, validation*

ÖZ

Amaç: *En yaygın sentetik katekolaminlerden biri olan dobutamin (DBT), renal, kardiyovasküler, hormonal ve merkezi sinir sistemlerinde önemlidir. Çalışmamızda DBT analizi için ilk MIP tabanlı elektrokimyasal sensörün üretilmesi önerilmiştir. MIP(DBT)/GCE, 4-aminobenzoik asidin (4-ABA) DBT varlığında elektropolimerleştirilmesiyle geliştirildi. Analitik performans ve doğrulama değerlendirmesi hem standart çözelti hem de ticari serum örneklerinde gerçekleştirildi.*
Gereç ve Yöntem: *DBT, Vem ilaç firmasından temin edilmiştir. 5 mM $[Fe(CN)_6]^{3-/4-}$ çözeltisinin hazırlanması için, potasyum ferrisiyanür ($[K_3Fe(CN)_6]$), potasyum ferrosiyanür ($K_4[Fe(CN))_6].3H_2O$, 0.1 M KCl'de karıştırıldı. DBT varlığında polimerik filmi oluşturmak için 4-ABA kullanıldı.*

* **Corresponding Author / Sorumlu Yazar:** Göksu Özçelikay-Akyıldız
e-mail / e-posta: goksu.ozcelikay91@gmail.com, **Phone / Tel.:** +905349160728

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Elektrokimyasal ölçümler, IVIUM kompaktStat.h potansiyostatta (Eindhoven, Hollanda) Pt teli, Ag/AgCl referans elektrotu ve camsı karbon elektrottan (GCE) oluşan üç elektrotlu bir sistem kullanılarak gerçekleştirildi.

Sonuç ve Tartışma: *MIP(DBT)/GCE sensörü, DBT'yi belirlemek için standart çözeltide ve insan serumunun ticari numunelerinde test edildi. DBT konsantrasyonları ΔI değerlerine göre çizildiğinde her iki ortamda da 1×10^{-13} ile 1×10^{-12} M arasında doğrusal bir yanıt elde edildi. TS sonuçları ise sırasıyla standart çözelti ve ticari insan serumda 0.087×10^{-13} ve 0.033×10^{-13} olarak bulundu. %Geri kazanım ve %BSS sırasıyla %99.68-100.32 ve %1.38-1.66 olarak belirlendi.*

Anahtar Kelimeler: *Dobutamin, elektrokimyasal MIP sensör, insan serumunun ticari örnekleri, tayin, validasyon*

INTRODUCTION

Organic compounds that play a physiological regulatory role and are found in the structure of disease markers and pharmaceutical substances are called catecholamines. Dobutamin (DBT) (Figure 1), one of the most common synthetic catecholamines, is widely used in clinical practice as a cardiostimulator, antimutagen, antioxidant, and anticarcinogen. DBT's catechol, secondary amine, and phenol functional groups have a high potential to participate in redox reactions, and therefore, it is an electroactive compound.

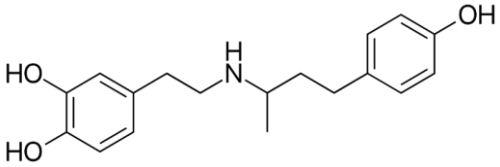

Figure 1. Chemical structure of DBT

Many analytical methods were performed to determine DBT. Especially the chromatographic techniques such as ultra-performance liquid chromatography-mass spectrometry (UHPLC-MS/MS) [1], two-dimensional liquid chromatography-mass spectrometry (2D-LC-MS) [2], high-performance liquid chromatography-mass spectrometry (HPLC–MS/MS) [3], reverse phase-high performance liquid chromatography (RP-HPLC) [4], high-performance liquid chromatography (HPLC) [5,6], were used to analyze DBT. However, scientists have been looking for new analytical methods recently because of the high cost of chromatographic techniques and the long analysis time. The electrochemical methods are strong alternatives to chromatographic techniques [7]. The voltammetric sensors record the current as a function of potential. In the measurement, the potential is variously applied either step by step or continuously to obtain a voltammogram. Several electrochemical sensors based on the voltammetry technique include cyclic, differential pulse, square wave, linear sweep, stripping, and hydrodynamic [8]. Electrochemical sensor has several advantages such as high sensitivity and selectivity to analyze the drugs, rapid response time, small sampling volume, and cost-effective protocols. There is no need for expensive equipment for signal transduction since the electron-transfer process directly generates an electronic signal [9].

Due to all these advantages of electrochemical methods, it would be attractive to develop electrochemical methods to detect DBT. Shvedene et al. (2002) performed electrochemical DBT determination using a screen-printed electrode modified with 3-(4-tolylazo)phenyl-boronic acid and ionic liquid graphite powder compositions [10]. Zhang (2004) determined DBT by the adsorptive stripping voltammetry method using electrodes modified with poly(acridine orange) film [11]. Chernyshov et al. (2008) reported that carbon paste electrodes were prepared and applied for the electrochemical determination of DBT. This electrode consists of graphite powder and ionic liquids [12]. Rastogi et al. (2012) used electrodes modified with sulfonic acid and acrylamide-derived copolymer to determine neurotransmitter substances, including DBT, by ion exchange voltammetry [13]. Ling et al. (2013) developed a nanosensor for the electrochemical oxidation of DBT [14]. The microflower-structured magnesium oxide-nafion composite layer-modified glassy carbon electrode

(GCE) was used. Asadian et al. (2014) produced graphene nanoribbon/polyaniline modified GCE (GNS/PAN/GCE) by in situ electropolymerization process. It showed that this modification agent enhanced the performances of electrodes at 10-fold [15]. Ekram et al. (2018) developed a Sr_2PdO_3 nano perovskite mixed with carbon nanotubes (CNT) modified new electrochemical sensor for the DBT analysis. With the modification, sensitivity, repeatability, and stability performance of nanosensors were improved [16]. Atta et al. (2019) determined the DBT by nanosensor. The electrode surface was modified by layer-by-layer technique. The multi-walled carbon nanotubes (MWCNT), ionic liquid (ILC), graphene (RGO), and 18-crown-6 (CW) were dropped to the electrode surface, respectively. This observed increase in current is associated with the ILC filling the gaps between the fMWCNT and G layers [17]. Ibrahim and Temerk (2020) fabricated the new nanosensor modified with nanocomposite (In_2O_3/ fMWCNT) for the determination of DBT. This modification agent provided the synergistic effect of In_2O_3 and fMWCNTs. It was enhanced the selectivity and sensitivity of the electrochemical sensor [18].

With the introduction of the key-lock theory by German chemist Hermann Emil Fischer, he opened a new page in the studies on the concept of molecular interaction from the past to the present [19]. Among molecular interaction applications, the molecular imprinting technique stands out. The molecular imprinting technique is a technique used to develop polymers with selective molecule recognition sites [20]. Polymer preparation components in the molecular imprinting technique; It consist of a functional monomer, target molecule, cross-linker, and initiator. The first step of polymer synthesis is the interaction of the target molecule (imprint molecule) and the functional monomer to form a target molecule complex. While this complex is being formed, covalent, non-covalent, semi-covalent, or metal-coordinated interactions occur between the target molecule and the functional monomer. This process is called the polymerization process [21].

With this study, the first MIP-based electrochemical sensor was fabricated for DBT analysis. For the formation of polymerization of 4-ABA, the electropolymerization technique was used in the presence of DBT. The developed MIP(DBT)/GCE was applied to a commercial serum sample for DBT analysis.

MATERIAL AND METHOD

Reagents and Chemicals

DBT was supplied from the Vem pharmaceutical drug company (İstanbul, Türkiye). The commercial serum sample (product no: H4522), uric acid, ascorbic acid, paracetamol, Na_2SO_4, $MgCl_2$, $[K_3Fe(CN)_6]$, $K_4[Fe(CN)_6].3H_2O$ and KCl were supplied from Sigma-Aldrich (St. Louis, Missouri, USA). For the preparation of 5 mM $[Fe(CN)_6]^{3-/4-}$ solution, known amounts of potassium ferricyanide ($[K_3Fe(CN)_6]$) and potassium ferrocyanide ($K_4[Fe(CN)_6].3H_2O$ mixed in 0.1 M KCl solution. The 4-ABA was used to create the polymeric film. Moreover, the preparation of buffer, phosphate buffer $NaH_2PO_4.2H_2O$, H_3PO_4, acetic acid sodium salt trihydrate, acetic acid, and Na_2HPO_4 were purchased from Merck (Darmstadt, Germany) to realize the removal process and create the cavities in a polymeric matrix.

Apparatus

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed for electrochemical measurements. Electrochemical measurements were actualized in IVIUM compactStat.h potentiostat (Eindhoven, The Netherlands) using a three-electrode system consisting of Pt wire as counter electrode, Ag/AgCl as reference electrode, and glassy carbon (GC) as working electrode. The three electrodes were supplied to BASi company (USA). All chemical compounds (drugs, monomers, and so on) were weighted with balance (Ohaus company, China). The prepared solutions were kept in the ultrasonic bath for the sonication of the solution. The pH of the buffer was adjusted with a pH meter. A centrifuge (3500 rpm, 30 min) (NF200, Nuve Company, Türkiye) was used for the separation of precipitation from the supernatant. The supernatant taken was used to apply commercial serum samples. The thermo-shaker (650rpm, 25◦C) (TS-100, Biosan, Riga, Latvia) was used for the rebinding process. The surface characterization of MIP(DBT)/GCE was performed by a scanning

electron microscope (SEM) with an energy-dispersive X-ray spectrometer (EDX)(SEM_EDX, TESCAN GAIA 3, Czech Republic) and electrochemical impedance spectroscopy (EIS) (Metrohm, Autolab, Utrecht, Netherland).

Fabrication of MIP(DBT)/GCE and NIP-Based Electrochemical Sensors

Alumina was applied to the GCE surface on the polishing pad to clean the electrode surface thoroughly. Then, the GCE surface was washed with distilled water (dwater) and methanol, respectively. To prepare the polymeric film solution, 0.2 mM DBT, and 0.6 mM 4-ABA were mixed as a 1:3 ratio (v/v) in the pH 7.0 phosphate buffer. The GCE was electropolymerized in a polymeric film solution by scanning the potential with CV between −0.2 and 1.5 V. To obtain the cavity in the polymeric matrix, the GCE was immersed in the removal solution (pH 10.5 phosphate buffer), and the CV was performed by scanning the potential between −0.2 and 1.5 V. To control the MIP sensor, the NIP sensor was formed using the same fabrication protocol without the analyte.

Preparation of Commercial Human Serum Sample

MIP(DBT)/GCE practicability was examined with commercial serum samples. The commercial serum sample in the presence of DBT as the target molecule was prepared. 1 mM DBT (1 ml), commercial serum (3.6 ml), and 5.4 ml ACN (to precipitate the protein residues) were mixed in the centrifuge tube. DBT 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. The gathered supernatant was used to evaluate the accuracy of MIP(DBT)/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

Characterization of the polymeric film formed on the GCE surface is carried out in terms of the surface's morphological properties and electrochemical properties. When examining surface morphology, the main goals are to contrast the surface characteristics of MIP with those of NIP and to pinpoint the distinct characteristics of MIP. Images obtained as a result of SEM measurements for MIP and NIP are shown in Figure 2. As can be clearly observed in the MIP image (Figure 2A), a very porous structure and, therefore, a rough appearance have been formed. The layered view of the polymeric structure is also visible in the figure. Observation of the features of the polymeric structure in this image supports the formation of MIP on the GCE surface. On the other hand, uniformity and smoothness are present in the NIP's SEM image (Figure 2B). This reveals that NIP is not suitable for binding to the target molecule. EDX spectra showing the presence of C, N, and O atoms expected to be in the MIP structure are shown in Figure 2C.

Electrochemical Characterization of the MIP-Based Electrochemical Sensors

The main methods preferred for evaluating electrochemical properties are CV and EIS. In both methods, the characteristic behavior of the redox probe is examined. As mentioned before, the redox probe in this study is a $5x10^{-3}$ M $[Fe(CN)_6]^{3-/4-}$ solution. Changes in the redox probe's anodic/cathodic peak currents were examined using the CV method (Figure 3A). When the GCE surface is unmodified before polymerization, the reversible peaks of the redox probe are observed as quite clear and high as a result of oxidoreduction, which occurs easily. As a result of coating the GCE surface with polymer after electropolymerization, a non-conductive surface is formed that prevents electron transfer. This manifests in the absence of redox probe peaks observed in the CV voltammogram. Thanks to the removal step, the template molecule DBT is removed from the polymer network of MIP, and the resulting cavities create spaces that re-enable electron transfer. This step is displayed as a re-increase of the reversible peaks of the redox probe in the CV voltammogram. Binding of DBT molecules to imprinted cavities through the rebinding step results in blocking electron transfer and decreasing oxidoreduction peaks.

Nyquist plots obtained with EIS measurements of the redox probe are displayed in Figure 3B. Here, the results regarding the charge transfer resistance (R_{ct}) value at different stages of MIP can be observed. Low R_{ct} values express fast electron transfer and easy oxidoreduction. The values recorded at different stages of MIP are included in Table 1 and confirm this.

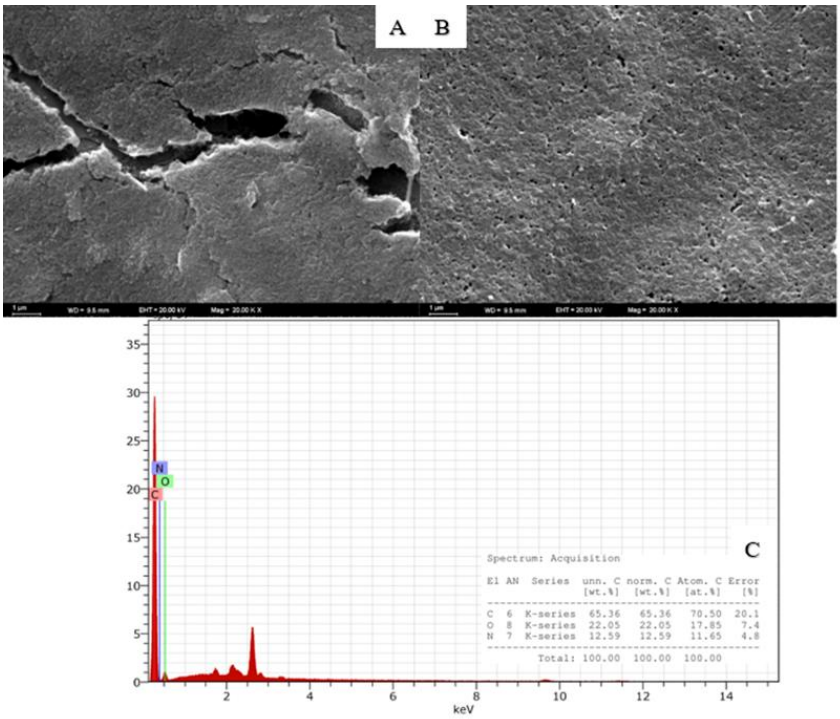

Figure 2. SEM images of A) MIP(DBT)/GCE and B) NIP(DBT)/GCE, EDX spectra of C) MIP(DBT)/GCE

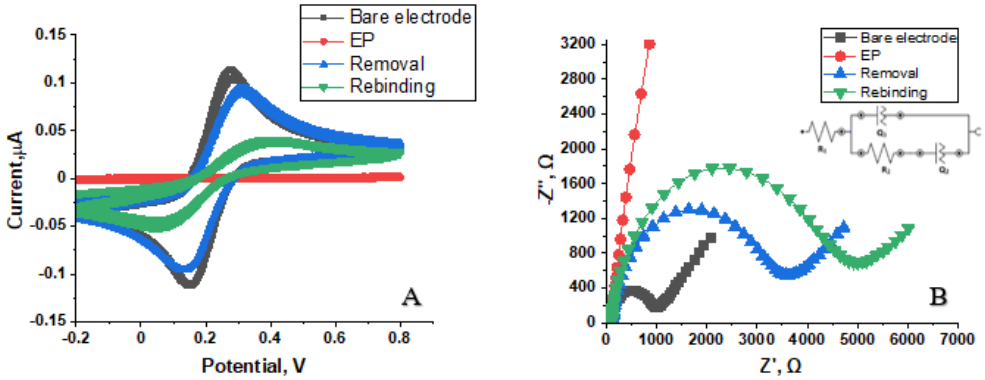

Figure 3. A) CV voltammograms B) Nyquist plots measured at different stages of MIP(DBT)/ GCE (Inset: R(Q[RQ]) equivalent circuit)

Table 1. R_{ct} values measured at different stages of MIP(DBT)/GCE

	R_{ct} (Ω)
Bare electrode	804.5
EP	24019
Removal	2813
Rebinding	3616.6

Optimization of Important Parameters

Critical parameters of the MIP process (number of cycles for EP, removal process, rebinding time, etc.) were optimized carefully and step by step for the determination of DBT. The DBT:4-ABA monomer ratio, number of scans, removal solution, removal cycles, and rebinding time were optimized. The optimization results are given in Figure 4.

The DBT:4-ABA ratio

The polymeric film formation is the most important parameter in the molecular imprinting process. According to the target molecule structure, the 4-ABA was selected. While this complex is being formed, non-covalent interactions occur between the 4-ABA and the DBT. While the results are evaluated, the difference of peak currents (Δ*Ip)* which are obtained after the polymerization process and removal process were calculated. Different proportions of 4-ABA and DBT were prepared and polymerized onto the electrode surface by an electrochemical process. When the 4-ABA ratio was 1 and 2 while keeping the DBT constant, it was not sufficient for the formation of the polymeric film. For this reason, the DBT:4-ABA ratio was selected as 1:3 (Figure 4A).

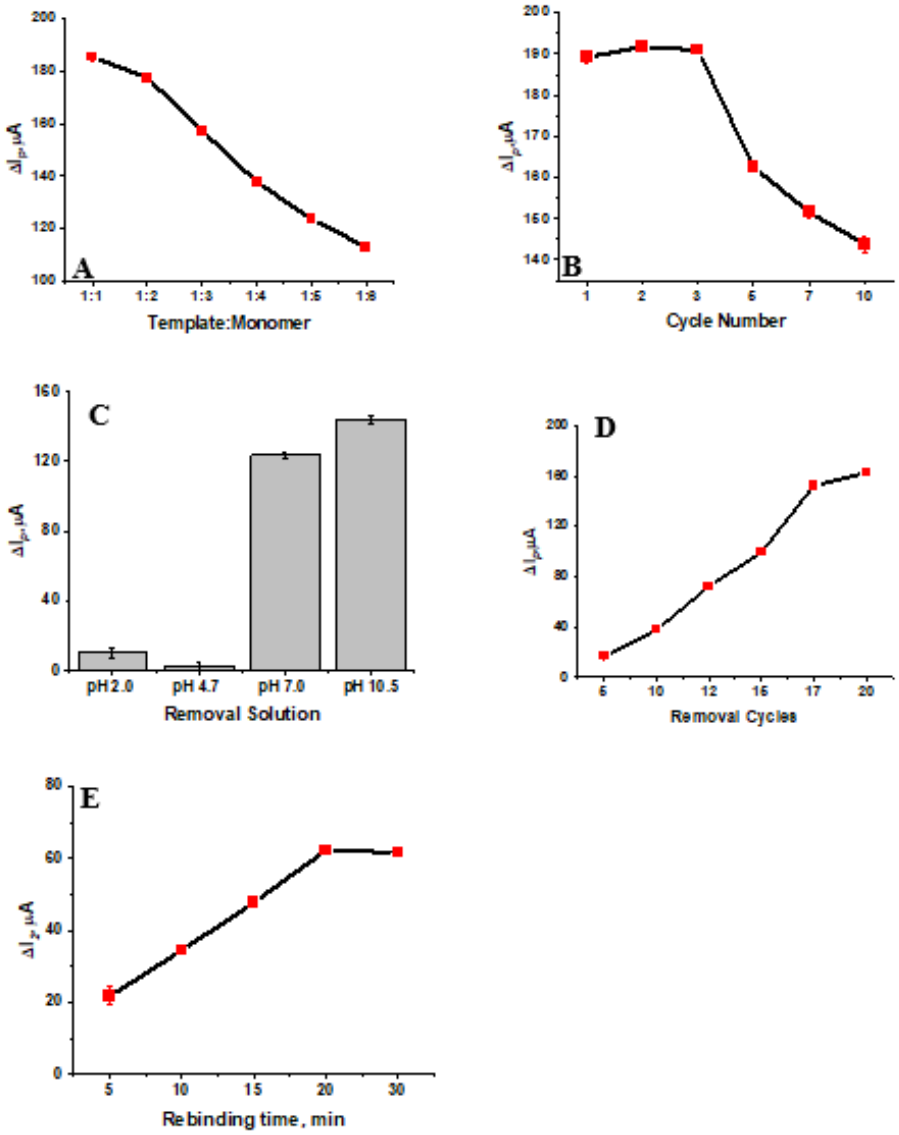

Figure 4. The optimization parameters of DBT:4-ABA monomer ratio(A), cycle number(B), removal solution(C), removal cycles(D), rebinding time(E) for development of MIP(DBT)/GCE

Cycle Number for the Electropolymerization

The electropolymerzation process arranges the polymer thickness. Another important parameter is to be determined by the cycle number. This step provides the sensitivity, repeatability, and stability of polymeric structures. For the electropolymerization process on the GCE surface, the repeated CVs were taken between −0.2 and 1.5 V. The number of cycles for electropolymerization was optimized from 1 to 10 cycles. The ΔI_p values were obtained similarly while numbers of 1, 2, 3. The cycle numbers were not sufficient for the formation of the polymeric film up to 5 cycles. For this reason, 5 cycles were selected for electropolymerization in the next stages (Figure 4B).

Selection of Removal Solution and Removal Cycles

The formation of a polymeric matrix in the presence of DBT provides the cavities that are suitable to shape, structure, and functional groups of DBT when the removal of the DBT from the polymeric matrix. Non-covalent bonds between DBT and 4-ABA are broken by exposure to various solutions and buffers of different pH environments. Some removal solutions (15 M acetic acid, 5 M NaOH, acetone, acetonitrile, ethanol) were applied by treatment of shaking in the thermal-shaker (650 rpm, 25◦C). This removal process didn't affect the cavities in the polymeric matrix. For this reason, buffers in different pH environments (pH 2.0, 7.0, and 10.5 phosphate buffer, pH 4.7 acetate buffer) were tried for electrochemical removal. The basic medium was given a good response for the removal process (Figure 4C). Therefore, pH 10.5 phosphate buffer was selected as the best removal solution. For the removal process, the CV was performed by scanning the potential between −0.2 and 1.5 V. To obtain the suitable removal cycles, the cycles from 5 to 20 min were optimized. According to Figure 4D, the removal cycle was chosen as 17.

Rebinding Time

The cavities formed during the removal process were immersed in DBT solution and exposed to a thermal shaker for a certain period to bind the DBT. The 10^{-3} M DBT, the target molecule, was diluted to different concentrations. While the results are evaluated, the difference of peak currents (ΔI_2) which are obtained after the removal process and rebinding process were calculated. According to Figure 4E, the rebinding time was found as 20 min for MIP(DBT)/GCE.

Analytical Performances of MIP(DBT)/GCE and NIP-Based Electrochemical Sensors

Analytical applicability and performance evaluation of the MIP(DBT)/GCE sensor was conducted using the indirect method. Accordingly, the DBT concentration is not measured directly; instead, the correlated decreases in the current of the redox probe as a result of binding DBT solutions to MIP(DBT)/GCE at increasing concentrations are measured. While the calibration curve is obtained, the differences between the current values obtained after removal and after rebinding (ΔI) are plotted against increasing DBT concentrations (Figure 5A). The key point to note here is that, unlike the linear response obtained with MIP(DBT)/GCE, there is no logical relationship between concentration and ΔI values due to the absence of specific cavities in NIP. The regression equation of the resulting calibration curve between 1×10^{-13} and 1×10^{-12} M is as follows: $\Delta I\ (\mu A) = 7.72 \times 10^{13}\ (\mu A/M) \times C\ (M) + 20.76$ ($r = 0.992$). An overlay of the differential pulse voltammograms of the redox probe obtained after rebinding increasing DBT concentrations is shown in Figure 5B.

We used the following ICH-recommended formulae to establish the limit of detection (LOD) and the limit of quantification (LOQ) values: LOD = 3 × standard deviation/slope and LOQ = 10 × standard deviation/slope [22]. LOD and LOQ values, along with other relevant regression data, are reported in Table 2.

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

Another stage of analytical performance evaluation and validation is analysis in a biological environment. For this purpose, the MIP(DBT)/GCE sensor was tested in commercial samples of human serum to determine DBT. When DBT concentrations were plotted against ΔI values, as in the standard

solution, a linear response was obtained between 1×10^{-13} and 1×10^{-12} M (Figure 5C). Associated differential pulse voltammograms are included in Figure 5D. The equation for the calibration curve is ΔI (µA) = 5.79×10^{13} (µA/M) × C (M) + 18.81 (r = 0.995). The LOD and LOQ values were calculated as 0.033×10^{-13} M and 0.111×10^{-13} M, respectively. Other regression data are included in Table 2.

A recovery study was conducted to test the feasibility and accuracy of MIP(DBT)/GCE in the serum sample. For this purpose, two known concentrations of standard DBT solution were spiked into the serum sample. The recovery% and RSD% values found as a result of the relevant applications and calculations are in Table 3 and demonstrate the accuracy and applicability of the MIP(DBT)/GCE sensor.

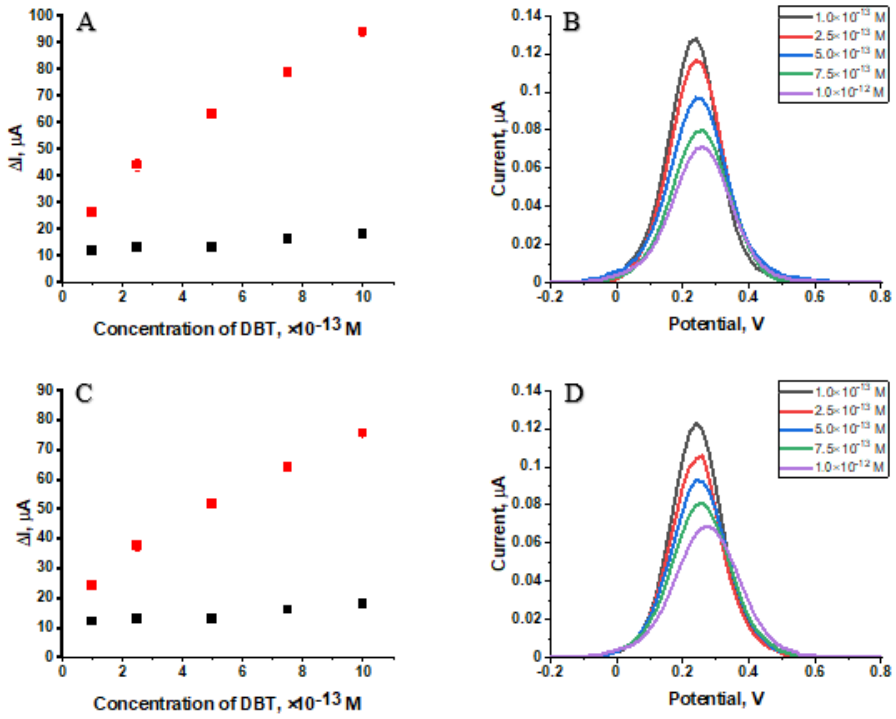

Figure 5. Calibration dependence on the MIP(DBT)/GCE for DBT in A) standard solution and C) commercial serum sample (red dots: MIP; black dots: NIP); differential pulse voltammograms obtained after rebinding increasing DBT concentrations in B) standard solution and D) commercial serum sample

Table 2. Analytical validation parameters for DBT determination on MIP(DBT)/GCE in standard solution and commercial human serum sample

	Standard solution	**Commercial human serum sample**
Linear range of DBT (M)	1×10^{-13}-1×10^{-12}	1×10^{-13}-1×10^{-12}
Slope (µA /10^{13} M)	7.717	5.791
S.E. of slope	0.397	0.235
Intercept (µA)	20.760	18.806
SE of intercept	2.742	1.273
Correlation coefficient (r)	0.992	0.995
LOD (M)	0.087×10^{-13}	0.033×10^{-13}
LOQ (M)	0.288×10^{-13}	0.111×10^{-13}
Repeatability of peak current (RSD%)*	0.756	0.270
Reproducibility of peak current (RSD%)*	1.544	1.650

* Each value is the mean of three experiments

Table 3. Recovery results of commercial human serum samples for DBT determination on MIP(DBT)/GCE

	Serum Samples#1	**Serum Samples#2**
Sample Concentration(M)	1.0×10^{-13}	2.5×10^{-13}
Spiked amount (M)	4.0×10^{-13}	2.5×10^{-13}
Found amount (M)*	4.98×10^{-13}	5.02×10^{-13}
Average recovery (%)*	99.68	100.32
RSD%	1.66	1.38
Bias%	-0.32	+0.32

* Each value is the mean of five experiments

Selectivity of MIP(DBT)/GCE and NIP-Based Electrochemical Sensors

Selectivity studies were performed with imprinting factor(IF) experiments and calculations. Since the cavities in the polymeric structures can recognize DBT in terms of shape, structure, and functional groups, it must be shown that molecules with a similar structure to the target molecule do not bind to the MIP(DBT)/GCE sensor. For this reason, catecholamine-derived compounds (carbidopa (CAR), levodopa (LEV), and dopamine (DOP)) shown in Figure 6 were chosen.

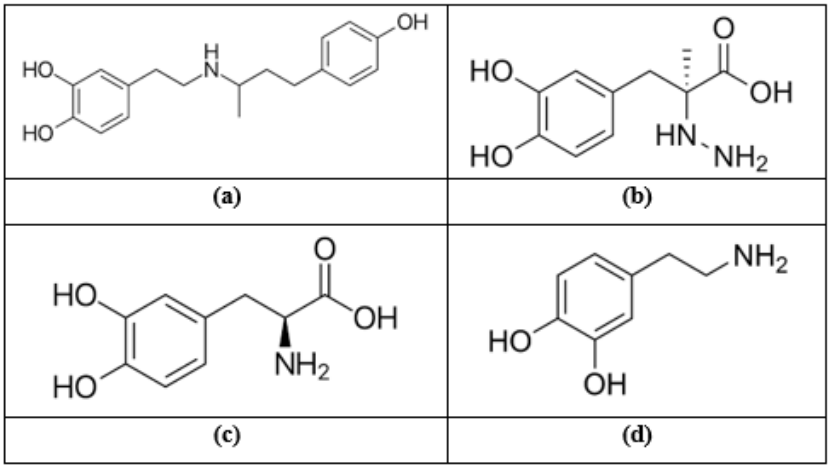

Figure 6. The molecular structures of the DBT(a), CAR(b), LEV(c) and DOP(d)

To calculate IF and IF' with the MIP(DBT)/GCE sensor using the ΔI_2 value, the equations below were employed. The results demonstrating the specificity of the sensor are presented in Table 4. It was emphasized that the sensor can determine DBT without being affected even in the presence of molecules that may affect selectivity at similar molecular structures.

$$IF_{(MIP)} = \frac{\Delta I_2(MIP)\ for\ DBT}{\Delta I_2(MIP)\ for\ other\ drug}$$

$$\mathrm{IF}_{(NIP)} = \frac{\Delta I_2(NIP)\ for\ DBT}{\Delta I_2(NIP)\ for\ other\ drug}$$

$$\mathrm{IF}' = \frac{IF_{(MIP)}}{IF_{(NIP)}}$$

Table 4. IF study results for MIP(DBT)/GCE sensor on DBT determination

Molecules	MIP/GCE		NIP/GCE		IF'$_{(MIP/NIP)}$
	$\Delta I_2/\mu A$	IF$_{(MIP)}$	$\Delta I_2/\mu A$	IF$_{(NIP)}$	
DBT	68.4	-	14.5	-	-
DOP	23.4	2.9	8.2	1.8	1.70
LEV	22.7	3.0	9.1	1.53	1.96
CAR	32.7	2.1	12.4	1.2	1.80

Interference Study of MIP Based Electrochemical Sensors

To evaluate the sensor's selectivity, studies are being carried out to analyze the DBT signal of the target molecule in the presence of potentially interfering substances (uric acid, ascorbic acid, paracetamol, Na_2SO_4, $MgCl_2$). DBT values were evaluated in the presence of interfering substances at different rates (1; 10; 100). The obtained recovery% and RSD% values for the sensor varied between 95.605– 103.431% and 0.57-1.98%, respectively (Table 5). These results demonstrate that the MIP(DBT)/GCE sensor can successfully detect DBT with interfering molecules and ions.

Table 5. Effect of interferents on the determination of DBT

Interferening Molecule/Ion	DBT: Interferent agents	Recovery of DBT (%)	RSD (%)*
Na^+	1:1	95.605	1.35
	1:10	98.716	1.03
	1:100	102.153	0.98
Cl^-	1:1	102.234	0.86
	1:10	96.182	0.57
	1:100	103.431	1.02
Mg^{2+}	1:1	102.234	0.86
	1:10	96.182	0.57
	1:100	103.431	1.02
SO_4^{2-}	1:1	95.605	1.35
	1:10	98.716	1.03
	1:100	102.153	0.98
Ascorbic acid	1:1	98.716	0.98
	1:10	98.364	1.57
	1:100	97.449	1.04
Paracetamol	1:1	101.531	0.79
	1:10	98.526	0.99
	1:100	101.305	1.79
Uric acid	1:1	102.153	1.65
	1:10	98.716	1.78
	1:100	96.935	1.98

*Each value is the mean of three experiments

Conclusion

This study explains the first MIP-based sensor for the electrochemical determination of DBT, a synthetic catecholamine. The contribution of the MIP approach used in this study and its advantage over existing studies is superior selectivity. EP was performed with the 4-ABA monomer selected in the MIP(DBT)/GCE sensor design. Critical parameters of the MIP process (number of cycles for EP, removal process, rebinding time, etc.) were optimized carefully and step by step. Morphological and electrochemical characterizations of the MIP(DBT)/GCE sensor, all stages of which were optimized, were performed with SEM, EDX, CV, and EIS methods, and the characteristic features of MIP were revealed. Analytical performance and validation assessment performed in both standard solution and commercial serum samples showed very good performance of the MIP(DBT)/GCE sensor, considering parameters such as sensitivity, accuracy, precision, and repeatability. In addition to all these, the effects of dopamine, levodopa, and carbidopa, molecules that are similar to DBT in terms of chemical structure and functional groups, were examined to reveal the selectivity, which is the most vital feature of the MIP-based approach. The high affinity of the MIP(DBT)/GCE sensor for DBT compared to these molecules is emphasized. Moreover, the MIP(DBT)/GCE sensor can selectively determine DBT without being affected by the possible interference effect of some molecules and ions commonly found in the body. In conclusion, this newly developed sensor is a very advantageous option for routine analysis of DBT with its remarkable selectivity.

AUTHOR CONTRIBUTIONS

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

CONFLICT OF INTEREST

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

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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