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Polypeptide Functional Surface for the Aptamer Immobilization: Electrochemical Cocaine Biosensing

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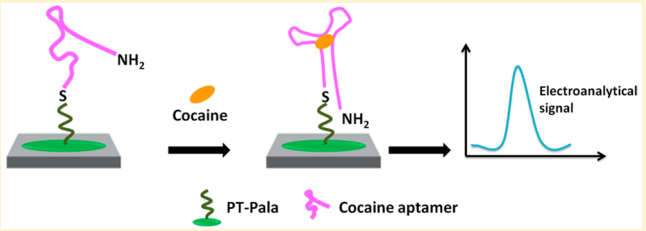
Supporting Information

ABSTRACT: Electroanalytical technologies as a beneficial subject of modern analytical chemistry can play an important role for abused drug analysis which is crucial for both legal and social respects. This article reports a novel aptamer-based biosensing procedure for cocaine analysis by combining the advantages of aptamers as selective recognition elements with the well-known advantages of biosensor systems such as the possibility of miniaturization and automation, easy fabrication and modification, low cost, and sensitivity. In order to construct the aptasensor platform, first, polythiophene bearing polyalanine homopeptide side chains (PT-Pala) was electrochemically coated onto the surface of an electrode and then cocaine aptamer was attached to the polymer via covalent conjugation chemistry. The stepwise modification of the surface was confirmed by electrochemical characterization. The designed biosensing system was applied for the detection of cocaine and its metabolite, benzoylecgonine (BE), which exhibited a linear correlation in the range from 2.5 up to 10 nM and 0.5 up to 50 μM for cocaine and BE, respectively. In order to expand its practical application, the proposed method was successfully tested for the analysis of synthetic biological fluids.



Rapid, sensitive, simple, and inexpensive procedures for drug-of-abuse detection are needed in the field of toxicology to serve for clinical diagnostics, drug trafficking, and law enforcements. Currently, immunoassay-based methods including radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) are generally used for forensic chemical analysis with a further confirmation by using more complex instrumental methodologies such as high-performance liquid chromatography (HPLC) and gas chromatography/mass spectrometry (GC/MS).[1,2] However, these detection methods exhibit some limitations including application with time-consuming procedures, expensive equipment, requirement of extensive sample preparation steps, in the case of immunoassays, obtainment of false positive and false negative results, and sometimes, lower sensitivity and selectivity. In order to overcome drawbacks observed with chromatographic and spectroscopic methods, electrochemical affinity biosensors have been introduced as promising alternative methods.[3,4] Among various biosensors, aptamer-based ones have attracted substantial attention in numerous fields such as biochemistry, analytical chemistry, and detection science. These types of biosensors are particularly useful for practical applications as they offer significant advantages such as specificity, short analysis time, low cost, and need little or no sample manipulation.[5]

Short and single-stranded oligonucleotides known as aptamers are integrated to biosensing technologies as biorecognition moieties in order to generate high selectivity to their target with strong specificity and affinity.[6−10] Moreover, aptamers engineered for the detection of their preselected analytes provide detection of substances ranging from small sizes to cells and exhibit considerable advantages such as a cheap and easy synthesis, lack of immunogenicity, high thermal stability, and reversible denaturation relative to antibodies. Due to unique properties of aptamers, their application for the development of new cost-effective sensing platforms provides achievement for quantitative detection of abused drugs with low detection limits.[11−13]

Among the worldwide addiction problems, cocaine addiction is a common and serious public health disorder; for this reason development of new detection tools to fight against its trafficking and use is required for the application in medical and toxicology laboratories.[14] Cocaine induces a sensation of

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euphoria via functioning as a powerful stimulant for the central nervous system,[15] and abuse of cocaine as a chronic relapsing disorder could elicit the serious side effects including anxiety, spread of human immunodeficiency, organ damage, and cardiac arrest.[3] Up to now, different analytical methodologies have been applied for detection of cocaine from chromatographic techniques[16−18] to electroanalytical analyses. In the past few years, electrochemistry-based ones have become more preferable in parallel to possibilities in biosensor technology such as miniaturization of electrodevices, flexibility, and automatization in manufacturing, and standardization of sensitive and low-cost methods.[19]

Herein, we describe the fabrication of an electrochemical aptasensor for the detection of the illicit and abused drug, cocaine. The proposed biosensing system combines the advantages of both aptamer and electroanalytical sensitivities and therefore is considered to be a potential tool to determine trace amount of cocaine in biological fluids. In order to construct the aptasensor, cocaine aptamer was covalently attached onto the surface of a polypeptide bearing conducting polymer, namely, polythiophene-*g*-polyalanine (PT-Pala), which was successfully synthesized, characterized, and applied as an immobilization matrix for the biomolecule conjugation in our previous work.[20] This conducting polymer was synthesized by ring-opening polymerization of the corresponding α-amino acids of *N*-carboxyanhydrides (NCAs). The "living" polymerization nature of this technique enables us to control molecular weight and terminal structure. In general, this technique is experimentally facile and can be applied to the fabrication of polypeptides on various matrixes through amino groups. Electrodeposition and surface modification of PT-Pala with the aptamer were evaluated by using cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and differential pulse voltammetry (DPV) techniques. Finally, cocaine analyses of the proposed aptasensor were confirmed by the HPLC method. After analytical characterization, its application to biological samples was tested by using synthetic urine and saliva.

EXPERIMENTAL SECTION

Reagents. The 32 base pair cocaine aptamer with 5′-NH_2 and 3′-SH_2 modification used in biosensor fabrication was obtained from Metabion International AG (Germany), and the sequence of custom-made oligonucleotides was as follows: 5′-C_6−NH_2−AGACAAGGAAAATCCTTCAATGAAGTGGGT-CG−SH_2-3′. Some addictive substances such as cocaine, methamphetamine, and benzoylecgonine (cocaine metabolite) were taken from Cerilliant (Cerilliant Corporation, Round Rock, TX, U.S.A.). Sulfosuccinimidyl 4-[*N*-maleimidomethyl]-cyclohexane-1-carboxylate (sulfo-SMCC) was purchased from Thermo Scientific (Pierce Biotechnology, Rockford, IL, U.S.A.). Bovine serum albumin (BSA), acetaminophenol, potassium hexacyanoferrate(III) [$K_3Fe(CN)_6$], and other chemical reagents were purchased from Sigma Chemical Company (St. Louis, MO, U.S.A.). All other chemicals were analytical grade.

Synthetic urine and saliva samples were prepared according to the Wilsenach et al. and Gal et al., and the compositions of synthetic urine and saline solutions are given in Supporting Information as Tables S1 and S2.[21,22]

Apparatus. The CV and DPV measurements were carried out with a PalmSens potentiostat (Palm Instruments, Houten, Netherlands). The EIS was performed with a CHI 6005 C electrochemical analyzer (CH Instruments Incorporated, Austin, TX, U.S.A.). The three-electrode system consisted of the glassy carbon electrode/gold electrode, platinum (Pt), and Ag/AgCl (3.0 M KCl, Metrohm, Switzerland) electrodes used as working, counter, and reference electrode, respectively. CH instruments analysis software was fitted with a Randle's equivalent circuit in order to analyze the EIS experimental data.[23] All measurements were conducted at room temperature.

Electrodeposition of T-Pala and Aptamer Immobilization. PT-Pala film was formed via electrodeposition onto a glassy carbon electrode (GCE) according to the previously described procedure.[20] Electrochemical modification was realized by depositing T-Pala at +1.6 V for 2.5 min in a solution containing 0.1 M sodium dodecyl sulfate (SDS) and 30:70 water−acetonitrile. Afterward, the conducting polymer-coated electrode was subjected to sulfo-SMCC chemistry for covalent attachment of thiolated cocaine aptamer. For this fabrication step, aptamer was dissolved in 5.0 mM $MgCl_2$ containing sodium phosphate buffer (10 mM, pH 7.0). According to conjugation procedure, the aptamer and heterobifunctional cross-linker sulfo-SMCC were dropped onto the surface of the PT-Pala-coated electrode and incubated at room temperature for 2 h at 1000 rpm.[24] Finally, the fabricated aptasensor (hereafter denoted as GCE/PT-Pala/aptamer) was rinsed with ultrapure water in order to remove unbound aptamers. Step-by-step modification of aptasensor surface was confirmed by electrochemical characterization using CV and EIS.

Moreover, another aptasensor was constructed by direct immobilization of the aptamer onto the gold electrode by self-assembled monolayer (SAM) formation. Prior to the aptamer immobilization, the gold electrode was polished with 0.05 μm alumina slurry prior to sonication in ethanol/distilled water (1:1). Second, gold electrodes were activated by applying a voltage range between +0.5 and +1.5 V (scan rate, 50 mV/s) via the CV technique. Then, the aptamer solution was prepared as mentioned before and directly applied to the electrode surface. Then, the electrode denoted as gold/aptamer was used for the comparison test for the GCE/PT-Pala/aptamer surface.

Cocaine and Benzoylecgonine (BE) Analysis by Aptasensor. In order to construct calibration curve for both cocaine and its metabolite benzoylecgonine (BE), the aptasensors were allowed to interact with a 10 μL droplet of either cocaine or BE analytes from the stock solutions (33 μM for cocaine and 3.5 mM for BE) with different concentrations, for 1 h at room temperature. Afterward, DPV measurements were conducted to evaluate analytical performance of the aptasensors. BE assay procedure was applied to confirm all analytical characteristics including repeatability assay, selectivity testing, and analysis in synthetic biological matrix. In addition, CV and EIS techniques were carried out to report analytical characterization of the aptasensor. Electrochemical signals were obtained in a background solution of $Fe(CN)_6^{3-/4-}$ (5.0 mM) in 0.1 M KCl. Electrochemical measurements by applying different techniques were performed under the following conditions: the voltage scanned from −0.4 to +0.6 V (for bare GCE) and −0.2 to +1.0 V (for PT-Pala-coated GCE) for DPV measurements; potential range from −0.4 to +0.8 V at a scan rate of 50 mV/s for CV measurements; a frequency range from 0.03 Hz to 10 kHz at +0.18 V for EIS measurements.

HPLC Analysis. The chromatographic separation and analysis of BE were carried out using Agilent HPLC with a DAD detector system (Santa Clara, CA, U.S.A.), and the

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elution of the peaks in the chromatogram was performed with an Eclipse XDB-C18 column of 5.0 μm particle size, 4.6 mm × 150 mm. The mobile phase, which consists of a mixture of (A) 0.2% (v/v) aqueous phosphoric acid and (B) acetonitrile (80:20, v/v), had a flow rate of 1.00 mL/min. The detector wavelength was set at 225 nm. The injection volume was 20 μL, and the column temperature was maintained at 35 °C. The stock solutions of BE (10 μg/mL) was prepared in methanol. Different concentrations of working solutions for HPLC analysis were prepared by dilution from this solution with methanol.

Confirmatory test was applied to complex samples by using the proposed method; the synthetic saliva and urine samples were used as extraction solution for determining matrix interferences and corresponding recovery of the spiked BE. The samples were prepared freshly before analysis. BE added samples were injected chromatographic system without needing extraction procedures.

RESULTS AND DISCUSSION

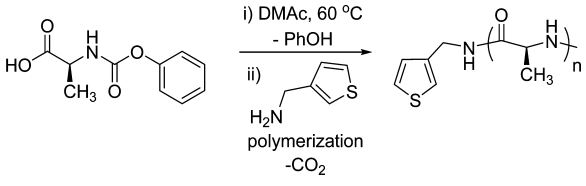
The critical strategy of our electrochemical sensing system for cocaine detection comprises a novel fabrication protocol by combining a conducting polymer and a cocaine-specific aptamer. The obtained conjugated polymer, PT-Pala, provides not only an excellent matrix with good conductivity but also appropriate surface for aptamer attachment with good orientation due to the presence of peptide side chains. Moreover, this sensing interface has improved the performance characteristics of the aptasensor. In addition, another important aspect of our methodology is the conformational change based target-binding mechanism of the aptamer, which plays a key role in measurable electrochemical signals. Because selective binding of cocaine to the aptamer induces the folding of aptamer into a three-way junction form, electron-transfer characteristics change during the electrochemical reactions in the presence of the redox probe, $Fe(CN)_6^{3-/4-}$.

Although aptamer-based cocaine detection studies were performed with various reaction mechanisms via enzymes,[25] nanoparticles,[26] and other labeling agents,[27] these strategies suffer from the complex fabrication processes. As for our constructed surface, a label-free approach is adopted. Thus, aptamer-folding property provides an interface by generating a compact structure due to the effect of analyte addition as a result of aptamer−small molecule interaction. Therefore, this fact prevents the electron transfer of redox probe compared to the unfolded aptamer structure on the surface in the absence of cocaine.

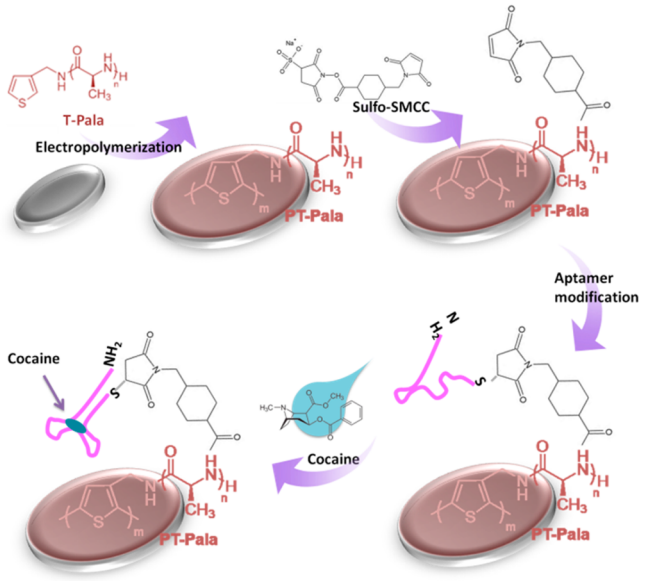
Thiophene functional polypeptide macromonomer (T-Pala) was prepared directly through in situ NCA formation and polymerization processes (Scheme 1).

The obtained macromonomer (M_n = 1140 g mol^{-1}, M_w/M_n = 1.6) was then used in the subsequent electropolymerization process on the electrode surface. Schematic representation of electrochemical PT-Pala formation and further modification by the aptamer immobilization via sulfo-SMCC cross-linker is given in Scheme 2.

Scheme 1. Synthesis of Thiophene Functional Polypeptide Macromonomer (T-Pala)



Scheme 2. Schematic Representation of PT-Pala Electrodeposition and Further Modification by the Aptamer Immobilization and Biosensing of Analytes



Surface modification was characterized by recording CV and EIS to confirm whether sensing interfaces were formed successfully. According to the CV technique, the stepwise voltammetric behavior of the aptasensor is given in Figure 1.

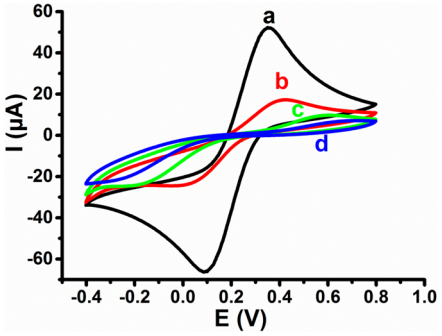


Figure 1. Cyclic voltammograms of (a) bare GCE, (b) GCE/PT-Pala, (c) GCE/PT-Pala/aptamer, (d) GCE/PT-Pala/aptamer/BE (BE: 25 μM); measurements were carried out in 50 mM sodium phosphate buffer (pH 7.0) in the presence of $[Fe(CN)_6]^{3-/4-}$ (5.0 mM) and 0.1 M KCl.

The CV of unmodified GCE with sharp oxidation and reduction peaks presents its typical curve in the presence of redox mediator (Figure 1a). Following the electropolymerization of T-Pala, a dramatic decrease occurred as the proof of PT-Pala covering of the bare electrode. In addition, the charge and thickness of electropolymerized PT-Pala film was monitored via chronoamperometric electrodeposition. These values were recorded as 9.72 mC and 216 nm, respectively. It appears that the high charge-transfer capacity of redox mediator was hindered by those modification steps as a result of surface

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covering. Furthermore, covalent binding of −SH-capped aptamer to PT-Pala that is bearing terminal amino groups was enabled with the heterobifunctional cross-linker sulfo-SMCC. After aptamer conjugation onto the PT-Pala-modified surface, redox peaks of the mediator exhibited a drop compared to the PT-Pala-coated electrode. In the final step, to demonstrate the BE capturing, 25 μM of metabolite standard (BE) was added onto the aptamer-coated surface and the signal responses were compared with the obtained signals from the aptamer and polymer-covered surfaces. The decrease of peak currents and shift of the peak potentials illustrate the certain modification of the GCE surface, successfully. In general, the anodic peak currents were obtained as 51.86 μA (peak-to-peak separation of +0.24 V) for bare GCE, 17.05 μA (peak-to-peak separation of +0.388 V) for GCE/PT-Pala, 9.68 μA (peak-to-peak separation of 0.79) for GCE/PT-Pala/aptamer, and 6.87 μA (peak-to-peak separation of 0.95) for GCE/PT-Pala/aptamer/BE surfaces.

Among the most proper analyte detection technologies, EIS has moved forward for the sample detection for lower concentrations and to measure electrode/electrolyte interface and electrode surface kinetics.[28,29] Besides the CV technique, EIS also gives an idea about the modification of electrocatalytic interfaces, precisely. To interpret an impedimetric data conclusively, the EIS curve, surface characteristics, and circuit design are the most important elements to perform a reliable EIS simulation.[30] Herein, the Nyquist plots, which create a semicircle defined as resistance of electron transfer, present the Randle's equivalent circuit. All the EIS data was fitted according to this circuit design containing solution resistance (R_s), Warburg impedance (Z_w) resulting from the diffusion of the $[Fe(CN)_6]^{3-/4-}$ redox probe, the double layer capacitance (C_{dl}), and the charge-transfer resistance (R_{ct}). According to the R_{ct} results of each modification step, an increase can be seen from the Figure 2. This increase was observed in both cocaine and its metabolite BE, respectively. The corresponding R_{ct} values of Figure 2A are 1345.9 Ω for bare GCE, 7576.8 Ω for GCE/PT-Pala, 35942 Ω for GCE/PT-Pala/aptamer, and 76829.7 Ω for GCE/PT-Pala/aptamer/BE (BE: 25 μM). Furthermore, another electrode was prepared for the cocaine binding to aptasensor, separately. The R_{ct} values of this system are as follows: 730 Ω for bare GCE, 5358 Ω for GCE/PT-Pala, 10400 Ω for GCE/PT-Pala/aptamer, and 14049 Ω for GCE/PT-Pala/aptamer/cocaine (cocaine, 0.5 μM).

Analytical Performance of the Aptasensor. Aptamer-based detection technologies have been widely used in the recognition and capture of cocaine with high selectivity and availability of aptamers for commercial purposes.[31] Additionally, aptamer-based sensors have been constructed with signal amplifiers such as fluorescent nanoparticles, magnetic nanoparticles, etc.[32−34] In this study, we evaluated the performance of an aptamer-based cocaine sensor with a conducting polymer called PT-Pala, which deposited onto GCE surfaces chronoamperometrically. Several colorimetric or electrochemical studies mainly mention only cocaine detection. However, BE, known as an analgesic for muscle pain and the primary metabolite of cocaine,[35,36] was utilized to create a platform for the analysis of cocaine metabolite in urine or saliva samples. In order to evaluate analytical performance of GCE/PT-Pala/aptamer electrode, the DPV technique was applied to the electrodes with varying concentrations of BE and cocaine. The current signals were recorded after each step of the modification process. Hence, the electrochemical signal difference before and after analyte addition was calculated by using the same electrode. Initially, different cocaine standards were added onto the aptamer-modified electrodes in the range of 1.0−10 nM (given as logarithm of 1000−10 000 pM), and a calibration curve between 2.5 [as the point of 3.4 (log pM)] and 10 [as the point of 4 (log pM)] nM was obtained with a linear equation of $y = 1.01x - 3.24$ ($R^2 = 0.998$) (Figure 3). Subsequently, BE standards were prepared and applied to the GCE/PT-Pala/aptamer surfaces in the range of 0.5−50 [2.7−4.7 (log pM)] μM. Therefore, another calibration curve

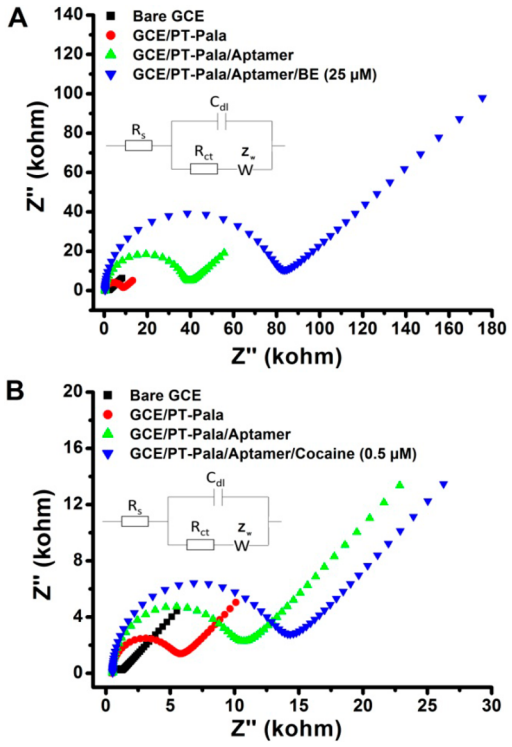

Figure 2. Nyquist diagrams of modified surfaces. Measurements were carried out in 50 mM sodium phosphate buffer (pH 7.0) in the presence of $[Fe(CN)_6]^{3-/4-}$ (5.0 mM) and 0.1 M KCl. Nyquist plots were fitted according to Randle's equivalent circuit which was placed into the graphs.

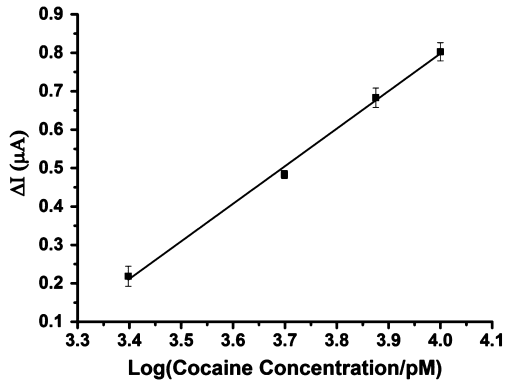

Figure 3. Calibration curve of GCE/PT-Pala/aptamer/cocaine with the concentrations of 2.5, 5.0, 7.5, and 10 nM generated by the DPV method. The concentrations of cocaine standards were transformed into picomolar from nanomolar to enable a logarithmic-based linearity curve. (All experimental steps were the same as mentioned above. Error bars shows ±SD.)

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between 0.5 and 50 μM related to metabolite (BE) was obtained with a linear equation of $y = 0.797x - 1.51$ ($R^2 = 0.984$) (Figure 4).

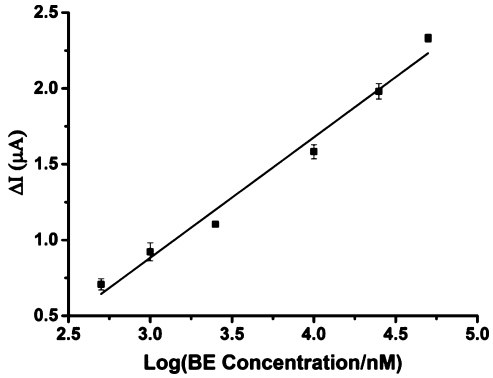

Figure 4. Calibration curve of GCE/PT-Pala/aptamer/BE with the concentrations of 0.5, 1.0, 2.5, 10, 25, and 50 μM generated by the DPV method. The concentrations of BE standards were transformed into nanomolar from micromolar to enable a logarithmic-based linearity curve. (All experimental steps were the same as mentioned above. Error bars shows ±SD.)

Additionally, it is known that aptamers with pendant −SH group are also functional biostructures for the direct binding of the biomolecules to the gold electrodes via generating a SAM.[37,38] Thus, we further investigated the role of the presence of the polymer coating and the efficiency of GCE/PT-Pala/aptamer biofilm in comparison with gold/aptamer surface for the capturing of BE metabolite. For this aim, another aptasensor was prepared by formation of SAM of the aptamer via −SH groups. Figure S1 illustrates the comparative linearity curves of both aptasensor platforms (GCE/PT-Pala/aptamer and gold/aptamer). By using a gold/aptamer surface, a linearity between 0.5 and 10 μM was created and an equation was obtained as $y = 0.883x - 2.194$ ($R^2 = 0.974$) (given in Supporting Information). Within this result, it can be claimed that PT-Pala conducting polymer film increased the analytical performance of the proposed aptasensor in comparison with direct binding by −SH−aptamer to a gold surface. Moreover, GCE/PT-Pala/aptamer biofilm was found more effective in the detection of cocaine metabolite in the way of linearity and coefficient of regression.

Other analytical parameters such as repeatability and limit of detection (LOD) related to the GCE/PT-Pala/aptamer surface were carried out. After the linearity part, the rest of the study was maintained with cocaine metabolite BE as the presence of metabolic residue following the cocaine uptake. The repeatability was calculated with six consecutive measurements and ±SD and coefficient of variation (CV) were calculated as 0.024 and 2.25%, respectively. After that, the LOD was calculated according to $3Sb/m$ formula, and this value of aptasensor for BE application was found as 1.5 nM ($n = 3$). The general analytical performance of the aptasensor is summarized in Table 1. Further studies related to cocaine detection are shown in Table 2 as electrochemical and other methods.

The selectivity of the GCE/PT-Pala/aptamer electrode was investigated with possible interfering agents such as methamphetamine, codeine, BSA, and 3-acetamidophenol. Among those interfering agents, methamphetamine as the addictive stimulant drug and codeine as another opiate are picked, which have an effect upon central nervous system. Besides, 3-acetamidophenol as an electroactive species is generally applied to the biosensing platforms.[39] BSA was used as a possible structure in body fluids. BE and all interfering agents except BSA were applied to the electrodes as 0.5 μM. As shown in Figure 5, the proposed aptasensor platform hardly showed any change in the current signals obtained by DPV. This control experiment proved that the sensing platform was highly specific to BE.

Table 1. Analytical Parameters of GCE/PT-Pala/Aptamer/BE Electrode

	values
linear range (μM)	0.5−50
slope [μA log C (nM)$^{-1}$]	0.797
intercept	1.51
SE of intercept[a]	0.195
SE of slope[a]	0.051
correlation coefficient	0.984
limit of detection (nM)	1.5
repeatability (±SD)	0.024
coefficient of variation	2.25%

[a]SE: standard error.

Sample Application and Confirmation of the Aptasensor. In the final part, the constructed aptasensor was applied to synthetic urine and saliva samples which contain a known amount of BE. Standard addition of BE (2.5 μM) was initially performed, and the corresponding results were compared with those obtained using known concentrations. Values of 94.8% ± 8.8% recovery ($n = 3$) for synthetic urine and 94.16% ± 3.24% for synthetic saliva were achieved. Besides, the confirmation of this experiment was further evaluated via the HPLC method using DAD. Initially, the calibration solutions containing BE in the concentrations range of 50−5000 ng/mL were prepared from the stock solutions. The linear equation was calculated as $y = 0.024x + 0.202$ with $R^2 = 0.999$. LOD and the limit of quantification (LOQ) for BE were found as 4.62 and 15.38 ng/mL, respectively. Calculations were also performed by using BE (2.5 μM which equals to 725 ng/mL) added samples, and those were injected to the HPLC system. Data are given as mean ±SD for the samples. BE concentrations were calculated for urine and saliva as 725.27 ± 3.17 and 741 ± 1.28 ng/mL, respectively. As a result, the developed aptasensor has a good efficiency in the detection of cocaine metabolite with the confirmation of a certain HPLC method.

CONCLUSION

An aptasensor platform was constructed with the electrodeposition of thiophene macromonomer bearing polypeptides. Subsequent conjugation of the cocaine aptamer onto the obtained PT-Pala film was accomplished with a heterobifunctional cross-linker, and all these modification steps were proved with electrochemical techniques such as CV and EIS. Besides advantageous one-step fabrication of the functional immobilization platform on the electroactive surface, the aptasensor enables us to detect trace amounts of cocaine with linear range of 2.5−10 nM and BE with linear range of 0.5−50 μM. Furthermore, in order to make a comparison, cocaine aptamer was directly coated onto gold electrode via SAM, and it was observed that conducting polymer-based aptasensor has greater sensitivity in compared to the gold/aptamer electrode. The repeatability and LOD of the aptasensor platform gave

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Table 2. Comparison of Aptamer-Based Cocaine Biosensors in the Literature

electrode	electrochemical detection method	concn range (nM)	limit of detection (nM)	ref
GE[a]/Fc[b]−aptamer	DPV[c]	200−5000	97	40
SPCE[d]/graphene/AuNP/aptamer	DPV	1.0−500	1.0	41
GE/TPF_{DNA}[e]/aptamer	FIS[f]	1.0−2000	0.21	42
SPGE[g]/CS[h]− aptamer/SWNTs[i]	DPV	0.1−50	0.105	6
GCE[j]/MWCNTs[k]/IL[l]/Chit[m]/AgNP−aptamer	DPV	2.0−2500	0.15	5
	Non-electrochemical Methods			
GE/AuNPs−aptamer	SPR[n]	1000−1000000	1000	43
aptamer-functionalized microbeads on a microfluidic platform	FRET[o]	1.0−100000	0.01	44
GO[p]− ICSDA[q]/SG[r]−aptamer	fluorescence	200−100000	190	45
GE/aptamer/$Ru(bpy)_2(dcbpy)NHS$[s]	ECL[t]	5.0−300	1.0	46
MC[u]/aptamer	isothermal titration calorimetry	25000−500000	5000	47
MNPs[v]−NH_2/AG4[w]−aptamer	colorimetric	100−20000	50	48
GCE/PT-Pala/aptamer	DPV	2.5−10 for cocaine 500−50000 for BE	1.5 for BE	this work

[a]Gold electrode. [b]Ferrocene. [c]Differential pulse voltammetry. [d]Screen-printed carbon electrode. [e]Triangular pyramid frustum nanostructure. [f]Faradaic impedance response. [g]Screen-printed gold electrode. [h]Complementary strand. [i]Single-walled carbon nanotubes. [j]Glassy carbon electrode. [k]Multiwalled carbon nanotubes. [l]Ionic liquid. [m]Chitosan. [n]Surface plasmon resonance. [o]Fluorescence resonance energy transfer. [p]Graphene oxide. [q]Isothermal circular strand-displacement amplification. [r]SYBR Green I dye. [s]Ruthenium bis(2,20-bipyridine) (2,20-bipyridine-4,40-dicarboxylic acid)-*N*-hydroxysuccinimide ester. [t]Electrogenerated chemiluminescence. [u]Micromechanical cantilever. [v]Magnetic nanoparticles. [w]G-riched strand.

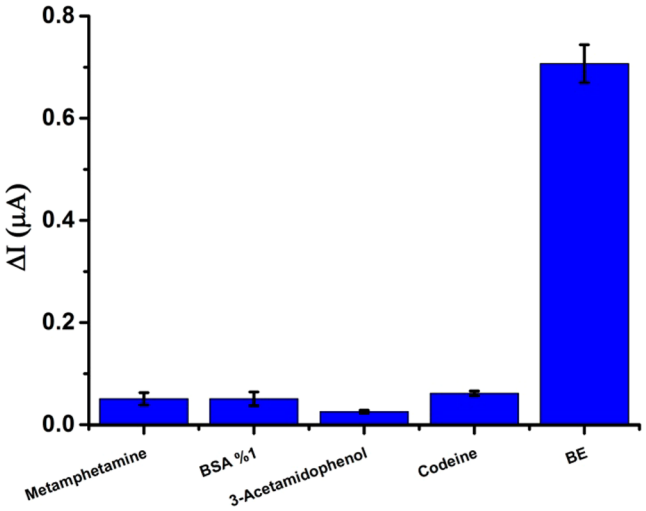

Figure 5. Influence of interfering agents such as BSA (1.0%), 0.5 μM of metamphetamine, 3-acetamidophenol, and codeine. (Error bars shows ±SD.)

satisfactory results, and no interference as well as sample matrix effect was seen in the presence of BE. In the final step, we have applied the synthetic urine and saliva samples containing BE, and the results were inspiring when come to think of confirmation via HPLC as a certain chromatographic method.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.6b00760.

Compositions of synthetic urine and saliva samples and calibration curves of gold/aptamer/BE and GCE/PT-Pala/aptamer/BE (PDF)

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Notes

The authors declare no competing financial interest.

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