Applicability of the Dendrimer-quantum Dot (Den-QD) Bioconjugate as a Novel Nanocomposite for Signal Amplification in the Fabrication of Cocaine Aptasensor

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A selective aptasensor was developed using the electrochemical transduction method for the ultrasensitive detection of cocaine. In this method, dendrimer-quantum dot (Den-QD) bioconjugate was utilized as a specific nanocomposite to efficiently fabricate the aptasensor. CdTe QD, which carries highly significant properties, was immobilized on the surface of a glassy carbon electrode (GCE), and polyamidoamine (PAMAM) dendrimer (Den) was covalently attached to the carboxyl-terminated surface of the CdTe QDs. This clever combination of QD and Den provides a highly stable matrix for the increased loading of aptamer (Apt) as a cocaine molecular receptor *via* covalent attachment. The Apt was functionalized with AuNPs leading to the amplification of the electrochemical signal. In this study, upon the incubation of cocaine on the aptasensor surface, the peak current of the redox probe decreased due to the hindered electron transfer reaction on the sensing surface. This study shows that the proposed aptasensor can detect cocaine by electrochemical impedance spectroscopy (EIS) technique at a linear range (5.00×10^{-3}-6.00 nM) and a capability of detection down to 1.60 pM. The excellent specificity of this sensing system is demonstrated using some common analgesic drugs, and finally, the proposed aptasensor was successfully used to measure cocaine in the human serum sample. The proposed methodology may hold great promise for the design of other aptasensors and immunosensors based on the use of the Den-QD bioconjugate as a nanocomposite with high performance.

Keywords: Aptasensor, Dendrimer, Quantum Dot, Bioconjugate, Cocaine

INTRODUCTION

Today, cocaine is one of the most dangerous and illegally available abused drugs because of its instantaneous and overwhelming effects on the central nervous system [1,2]. The latest statistic data have indicated that approximately 17 million people have used cocaine at least once in 2011, which is equivalent to 0.37% of the global population at aged 15-64 [3]. Therefore, the quantification of the trace amounts of cocaine is very important for law enforcement and clinical medicine. Up until now, as shown in Table 1, several methods such as non-electrochemical and electrochemical methods have been introduced for the detection of cocaine. Despite their advantages, speed, and selectivity of most of these methods have been unsatisfactory. Therefore, it seems necessary to develop a rapid, and economical approach for the ultrasensitive and selective measurement of cocaine.

Dendrimers (Dens), as regular tree-like and highly-branched macromolecules, are an unrivaled class of polymeric materials [4]. Unlike classical polymers, they have nonpareil properties such as globular shape and defined size, multivalency, great uniformity, internal void spaces, high ligand density, high drug loading capacity, and high functionalized surface [5,6]. These nano-scaled polymeric architectures are receiving considerable attention for applications in many fields such as chemicals and biological fields because of their numerous terminal groups that can be functionalized and conjugated with nanoparticles (NPs) and molecules of interest [7-11]. Furthermore, because of their small sizes, they can form attachments with biomolecules such as aptamer (Apt). Apt molecular

Table 1. Analytical Performance of the Cocaine Developed Aptasensor with some other Methods

Method	LOD (nM)	Linear Range (nM)	Ref.
Non-electrochemical Methods			
GC	3.29×10^2	1.60×10^6-3.29×10^8	[37]
LC-MS	16.48	16.48-988.94	[38]
Capillary electrophoresis	6.60	NR	[39]
Electrochemical methods			
Fluorescence	1.25×10^4	1.25×10^4-15.00×10^6	[40]
Colorimetric	10^4	NR	[41]
Absorbance	5.00×10^2	NR	[42]
ECL	1.00	5.00-3.00×10^2	[43]
CV	33.00	1.00×10^2-10^6	[44]
DPV	1.00	1.00-5.00×10^2	[45]
SWV	500	1.00×10^3-1.50×10^3	[46]
EIA	5.00×10^2	5.00×10^2-10^6	[47]
EMPAS	9.00×10^2	2.00×10^3-5.00×10^4	[48]
DPV	0.15	2.00-2.50×10^3	[32]
EIS	1.60×10^{-3}	5.00×10^{-3}-6.00	Present study

GC Gas Chromatography; *NR* not reported; *LC-MS* Liquide chromatography/Mass spectroscopy; *ECL* Electrogenerated chemiluminescence; *SWV* Square-wave voltametry; *EIA* Enzyme immonoassay; *EMPAS* Electromagnetic piezoelectric acoustic sensor.

receptors are artificial DNA or RNA oligonucleotides which can be used for basic research [12-15]. Apt-Den attachments combine the advantageous features of entities and have many applications [16-18].

Quantum dots (QDs) are a unique class of semiconductor nanoparticles which offer some advantages in many aspects because of their unique properties such as magnetic, optical and electrical properties [19,20]. Because of the their small size, they are similar to biological macromolecules such as proteins, nucleic acid, and Apts and can form conjugates with them [21]. Also, they hve a large surface area which can absorb more Apt molecules onto the electrode surface and produce a larger electrochemical signal [22]. Furthermore**,** they have been found to play the role of an efficient electron-conducting tunnel in electron transfer which gives rise to their potential use in a variety of fields [19]. CdTe QD as a well-known example of the QDs has received increasing attention because of its several advantages, and has been used in various fields such as electrode modifying systems [23]. Although functionalized

QDs show excellent electrochemical properties, their use in electrochemical systems for analytic purposes is at the onset [24]. The utilizing benefits of QDs and Dens, combined with those of Apt manifested in the preparation of Den-QDs bioconjugate as a specific platform, can give rise to many exciting material properties and enjoy great potential in various applications [25]. Generally, there are seven major advantages of the Den-QD bioconjugate in the attachment of an Apt: 1) QDs have been found to lead to an increase in the surface area [22,26]; 2) The specificity of Apts ensures a targeted detection or binding, which is the most important factor in the detection of biomolecules [27,28]; 3) The ease of synthesis and modification makes Apts attractive in deferent kinds of practical applications; 4) Flexible three dimensional (3D) structure and other inherent desired properties of Dens which allows to use them in almost every area of research; 5) The clever combination of Dens and Apts increases the detection signal level of aptasensors when compared with their use in isolation [29]; 6) QDs show excellent electrochemical properties and their use in biosensing systems for analytic purposes are at the onset [24]; 7) The Apt-Den-QD attachment strengthens the properties of each of them individually and increases their range of applications. Although a limited number of researchers have reported methods for the application of the Apt-Den-QD attachment in various fields [25], the electrochemical sensing applications of this bioconjugate have rarely been reported.

To the best of our knowledge, this paper is the first report based on the combination of the advantages of CdTe QDs, Den and Apt for utilizing Den-QD bioconjugate as an efficient nanocomposite in the electrochemical aptasensor for the diagnosis of a target. The results of this combination are the CdTe QDs size effects and increase in the surface area to the volume ratio of GCE [22]. Also, because of the multiplicity of the functional groups on the Den surface, the targeted loading of Apt as a cocaine molecular receptor is increased remarkably on the surface of GCE [22]. Based on this protocol, thioglycolic acid (TGA) capped CdTe QDs (TGA/CdTe) is immobilized on the GCE surface and the polyamidoamine Den of the fourth generation (PAMAM G4) is covalently attached to the carboxyl groups on the CdTe QDs *via* amide coupling. Finally, the 5′-NH$_2$-3′-AuNPs terminated Apt as the cocaine specific Apt is covalently attached to the amine groups on the surface of Den via terephthalaldehyde as the linker agent. The presence of AuNPs at the end of the Apt can provide electrochemical signal improvement. Cocaine incubation and the formation of the Apt/cocaine complex on the modified electrode surface change the electron transfer characteristics of $[Fe(CN)_6]^{3-/4-}$ as the redox probe; this can be monitored by the electrochemical impedance spectroscopy (EIS) techniques. Due to the use of Den-QD bioconjugate as an efficient nanocomposite and the covalent attachment of the cocaine specific Apt onto the GCE surface, this strategy is ultra-sensitive and selective. The elimination of enzymes or antibodies and the use of the Apt-Den-QD attachment as a unique platform, which is a covalent attachment on the electrode surface, are some advantages of the proposed aptasensor.

EXPRIMENTAL

Chemicals and Reagents

The cocaine Apt sequence was purchased from the Bioneer company (South Korea), coupled with 5′-amino modified containing a space (6CH$_2$) on the one end and 3′-thiol on the other end of the Apt: 5′-C$_6$-NH$_2$-AGACAAGGAAAATCCTTCAATGAAGTGGGTCG-SH$_2$-3′. Polyamidoamine (Den PAMAM G4) 10% in methanol, analytical grade tellurium powder, CdSO$_4$.8H$_2$O, TGA, N-hydroxysuccinimide (NHS), sodium borohydride (NaBH$_4$), C$_8$H$_{17}$N$_3$HCl (EDC HCl), Na$_3$C$_6$H$_5$O$_7$.2H$_2$O, sodium valproate, sodium diclofenac (DCF), codeine (COD) and acetaminophen (APAP) were purchased from Sigma-Aldrich and used without further purification. Other addictive substances such as cocaine, pethidine, and methadone (MTD) were taken from the center of narcotics prevention and the sanitation ministry, respectively (Tehran, Iran). All other reagents were obtained from Merk or Fluka. All drugs and addictive substances solutions with various concentrations were prepared by direct dissolution in a 0.10 M phosphate buffer solution (PBS) (pH: 7.40).

For analysis of the real sample, healthy people's serum specimen from four men provided by a local clinical laboratory and subjected to ultrafiltration by loading into a centrifugal filtration tube at 5000 rpm (30min). Afterward, the serum samples were diluted 5 times with PBS (0.10 M,

pH 7.40) at ratio 1:1. Twelve samples were prepared by spiking different concentrations of the cocaine solutions into the blood serums.

Apparatus and Electrochemical Measurements

EIS, DPV and cyclic voltammetry (CV) experiments were performed on a μ-AUTOLAB electrochemical system type III and FRA2 board computer controlled Potentiostat/ Galvanostat (Eco-Chemie, Switzerland) driven with NOVA software which was in conjunction with a conventional three-electrode system. A GCE with Apt-Den employed as the working electrode and a platinum wire as a counter electrode. All potentials were referred to an Ag/AgCl/KCl (3.00 M) electrode as a reference electrode. For all experiments, a solution containing 5.00 mM $K_3Fe(CN_6)$/ $K_4Fe(CN_6)$ and 0.50 M KCl (at a ratio of 1:1:1) as an electrolyte solution was applied. The EIS analysis was performed in a frequency range between 0.10 Hz and 100 kHz with a modulation voltage of 5.00 mV. The DPV measurements were recorded with common parameters: 5.00 mV potential steps, 25 mV modulation amplitude, 0.50 s interval time and 50.00 ms modulation time. UV-Vis absorption spectra of the synthesized AuNPs was recorded using a VARIAN 300Bio CARY UV-Vis spectrophotometer in the range of 190-800 nm. TEM image was recorded by a Hitachi H-800 electron microscope at 80 kV and a Vega-Tescan electron microscope. Furthermore, for study AFM (atomic force microscopic) image of the synthesis CdTe QDs, the AFM apparatus model nanosurf Mobile S software version 1.8 at operating mode of dynamic force and noncontact scan type was used.

Preparation of the 5′-NH$_2$-3′-AuNPs-terminated Apt

Our previous results demonstrated that two factors should be considered for the successful functionalization of the 5′-NH$_2$-3′-SH$_2$ terminated Apt with AuNPs and the formation of thiol-Au covalent bonds on the end of the Apt; 1) The nanoparticle size of Au should be small because, in small size, there is a strong interaction between the thiol groups in the Apt and AuNPs and thus sensitivity is increased; 2) The synthetic AuNPs chemical environment must be such that the Apt will not be destroyed and remain natural with a pH of about 7.00-7.40. Thus, based on the

applied method in our previous work [30], AuNPs were synthesized with some modifications [31]. In brief, in a 1 l round-bottom flask equipped with a condenser, 500.00 ml HAuCl$_4$ (0.01% w/v) was heated -while being stirred -to boiling point. To this solution, 7.50 ml of an aqueous solution of Na$_3$C$_6$H$_5$O$_7$.2H$_2$O (1% w/v) was added quickly. Finally, the color of the solution turned to blue and then changed to red-violet. Boiling continued for an additional 10.00 min. When the color of the obtained solution had turned to deep red, the heat was removed. After 15 min of vigorous stirring, a homogenous transparent red solution of AuNPs was obtained without any precipitate. The synthesized AuNPs was stored in a brown glass bottle at 4 °C when it was not in use. To functionalize the cocaine specific Apt with the AuNPs, a mixture containing PBS (0.10 M, pH 7.40), NaCl (0.10 M), 5′-NH$_2$-3′-SH$_2$ terminated Apt (0.50 μM) and the obtained AuNPs colloid at ratio 1:1:2:2 was prepared and stirred for 24 h (at room temperature). Thus, the small size of AuNPs was well bonded to the 3′-SH$_2$ terminated Apt *via* covalent S-Au bonds and was used for subsequent experiments.

Preparation of TGA/CdTe QDs

The syntheses of TGA/CdTe QDs was carried out by a wet chemical method based on a previously described method [26]. In a typical procedure, 278.80 mg NaBH$_4$ and 7.00 ml water are deaerated with argon and stirred for 30 min (in a two-necked flask). Afterward, under the condition in which stirring is accompanied by the blowing of a steady draft of argon, 90.00 mg tellurium powder is added, completely dissolved, and storage at 0 °C for 15 min. Then, the obtained colorless suspension is filtered. Finally, 42.00 ml water is added to the resulting solution and deaerated with argon and stirred for 120 min. The prepared fresh NaHTe is cooled to room temperature. Meanwhile, in a three-necked flask, 294.60 mg CdSO$_4$.8H$_2$O is dissolved in 176.00 ml water and 200.00 μL of 14.09 M TGA is added. The pH is then adjusted to 10.20 (by using concentrated NaOH solution) and the resulting solution is stirred under argon (for 30 min) followed by an injection of 49.90 ml freshly prepared NaHTe, and finally, the mixture is refluxed under argon (60 min). Afterward, the color of the resulting mixture changes to orange, demonstrating the growth of the synthesized nanocrystals. To prepare the CdTe QDs, the

obtained solution is refluxed under nitrogen at 100 °C. The reflux time may be adjusted to control the size of the resulting CdTe QDs [26].

Aptasensor Assembling as an Electrochemical Nanotool

First, 10.00 µl of TGA capped CdTe QDs was cast on the GCE surface and air-dried freely overnight to form a CdTe QDs/GCE (in darkness). A mixture of EDC: NHS at a ratio of 1:1 (75.00 mM: 15.00 mM) was dropped onto the CdTe QDs/GCE surface as a coupling agent to activate the carboxyl-terminated of CdTe QDs [22]. 5.00 µl of Den was allowed to dry under an N_2 stream and then diluted to 100.00 µl with PBS with pH = 7.40. 15.00 µl of the prepared Den solution was dropped on the surface of CdTe QD/GCE to enable the covalent binding between Den and the activated -COOH groups of the CdTe QDs (2 h). This time was optimized in accordance with the literature [29]. Thus, Den-QD bioconjugate as a nanocomposite was prepared and covalently immobilized to the electrode surface. In order to covalently attach the 5′-NH_2-3′-AuNPs terminated Apt on the amine groups of the immobilized Dens, 10.00 µl terephthalaldehyde aqueous solution (2.52 × 10^{-2} M) was added and 90 min as an optimum time was used for formation of the amide coupling [18]. Subsequently, 2.00 µl cocaine specific Apt (0.50 µM) was dropped and the amine-terminated end of the Apt was covalently attached to the aldehyde group of the terephthalaldehyde *via* amide linking. After each step, excess unreacted molecules were eliminated by washing the electrode several times with double distilled water and PBS buffer. The obtained GCE, modified with 5′-NH_2-3′-AuNPs terminated Apt/Den/CdTe QD, was denoted as Apt/Den/CdTe QD/GCE and stored at 4 °C until use. The aptasensor fabrication procedure is shown in Scheme 1. 2.00 µl of cocaine solution was added at the desired concentration on the aptasensor surface and incubated for 45 min at 4 °C. This optimal incubation time was based on our investigations in previous work [32]. The incubation time was necessary for the preparation of the modified electrode. After this time, the cocaine measurement will be done quickly. The detection of cocaine target levels was performed by measuring the increase in the value of R_{ct} in EIS induced by incubation of cocaine.

RESULTS AND DISCUSSION

Investigation of the Characterization of the Prepared AuNPs

For the strong attachment of AuNPs to the thiolated end of 5′-NH_2-3′-SH_2 terminated Apt, the synthesis of small size AuNPs is preferable. The small size of AuNPs leads to the strong interaction with the SH_2 groups at the end of the Apt, which is one of the advantages of the present study; therefore, the electrochemical signal is improved. This agrees well with the results previously obtained [30]. We used a TEM image to prove the synthesis of AuNPs. As shown in Fig. 1A, the presence of the spherical AuNPs with an average diameter of less than 8 nm indicates their proper formation of AuNPs. Also, as shown in Fig. 1B, the absorbance peak which is approximately 520 nm, is additional evidence confirming the AuNP synthesis. Furthermore, the determination of the size and concentration of the prepared AuNPs can be inferred from the UV-Vis spectra [33].

Study of the Synthesized Water Soluble CdTe QDs

Obviously, the use of Apt-Den-QD bioconjugate as a specific platform plays a vital role in the fabrication of the developed aptasensor. It has been found that the use CdTe QD layer for the modification of the electrode surface not only remarkably increases the surface area but also provides a quite suitable interface for the subsequent conjugation of biomolecules [22]. Thus, the presence of COOH groups related to the CdTe QD layer on the surface of GCE leads to covalent attachment of the Den with high density on the electrode surface (*via* amide coupling). Therefore, more Apt bonds with Den (*via* terephthalaldehyde as the linker agent) and cocaine are detected with more sensitivity. The study of the morphology and structure of TGA-stabilized CdTe QDs prepared in the aqueous phase gives relevant information concerning the synthesis of these nanoparticles. As can be seen in Fig. 2A, the absorbance peak related to the UV-Vis spectrum of the prepared CdTe QDs, which is approximately 470 nm, can be a confirmation of the synthesis of this sample. The size and concentration of the CdTe QDs can be found by the UV-Vis spectra [26]. We used AFM to study the morphology of these nanoparticles. As shown in Fig. 2B, there exist different geometrical
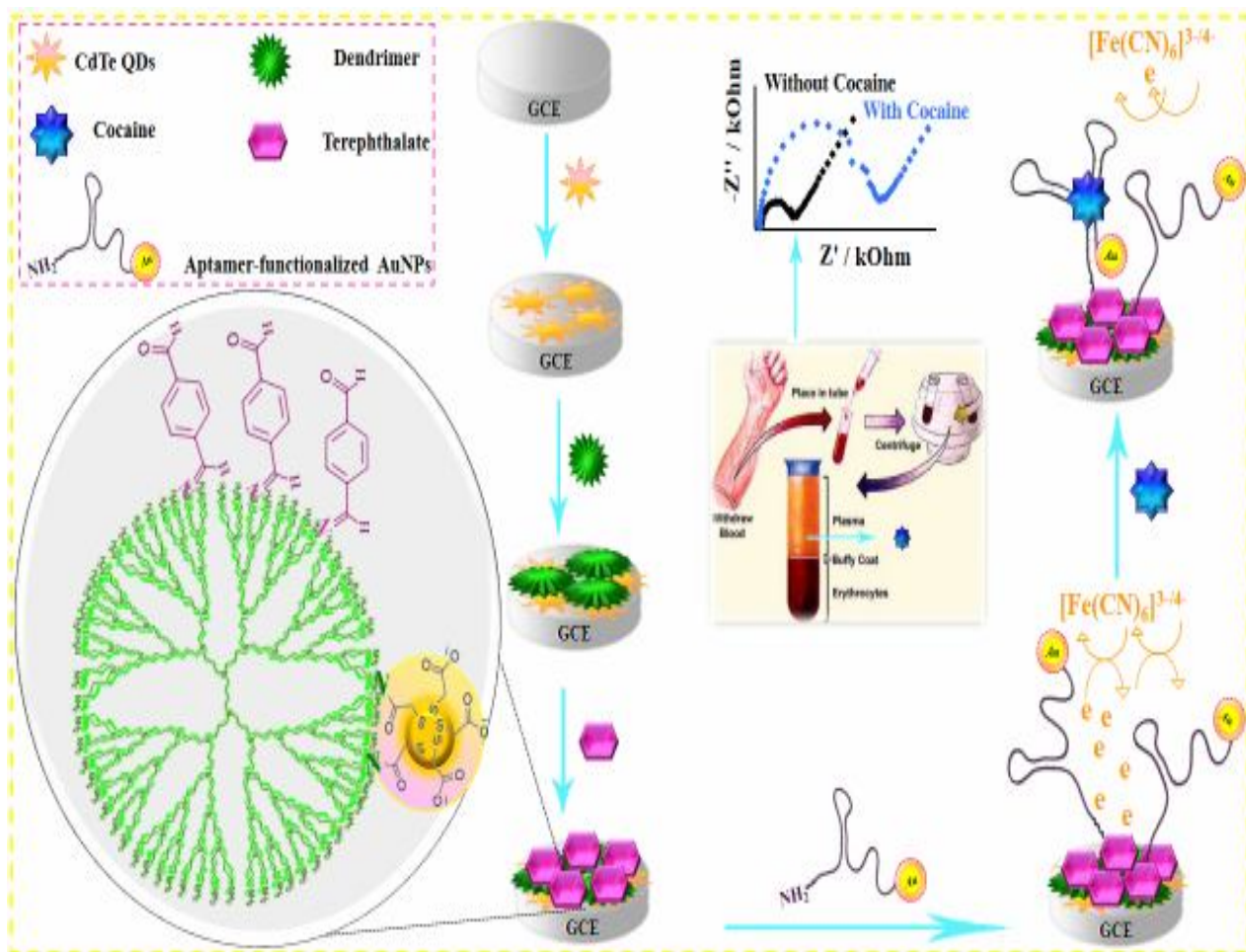
Scheme 1. Schematic illustration of electrochemical aptasensor for cocaine detection
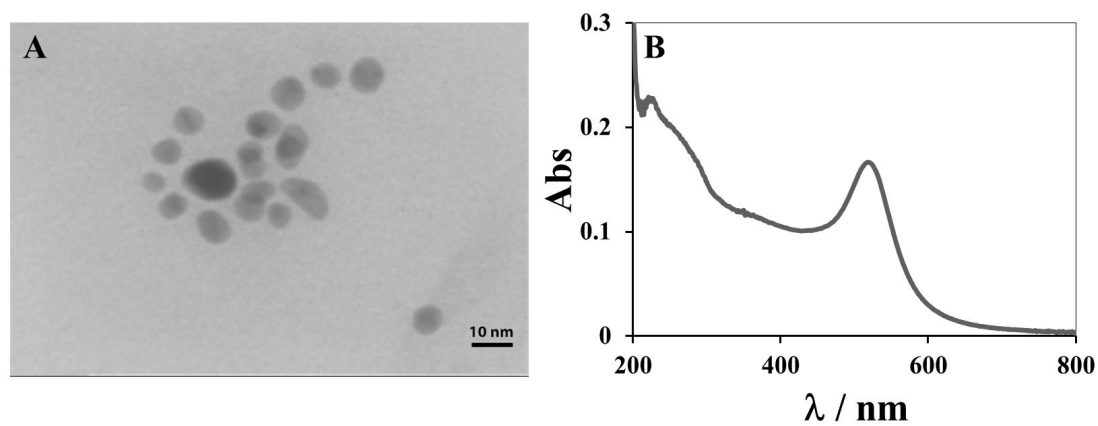

Fig. 1. (A) TEM image and (B) UV-Vis adsorption spectra of the synthetic AuNPs.
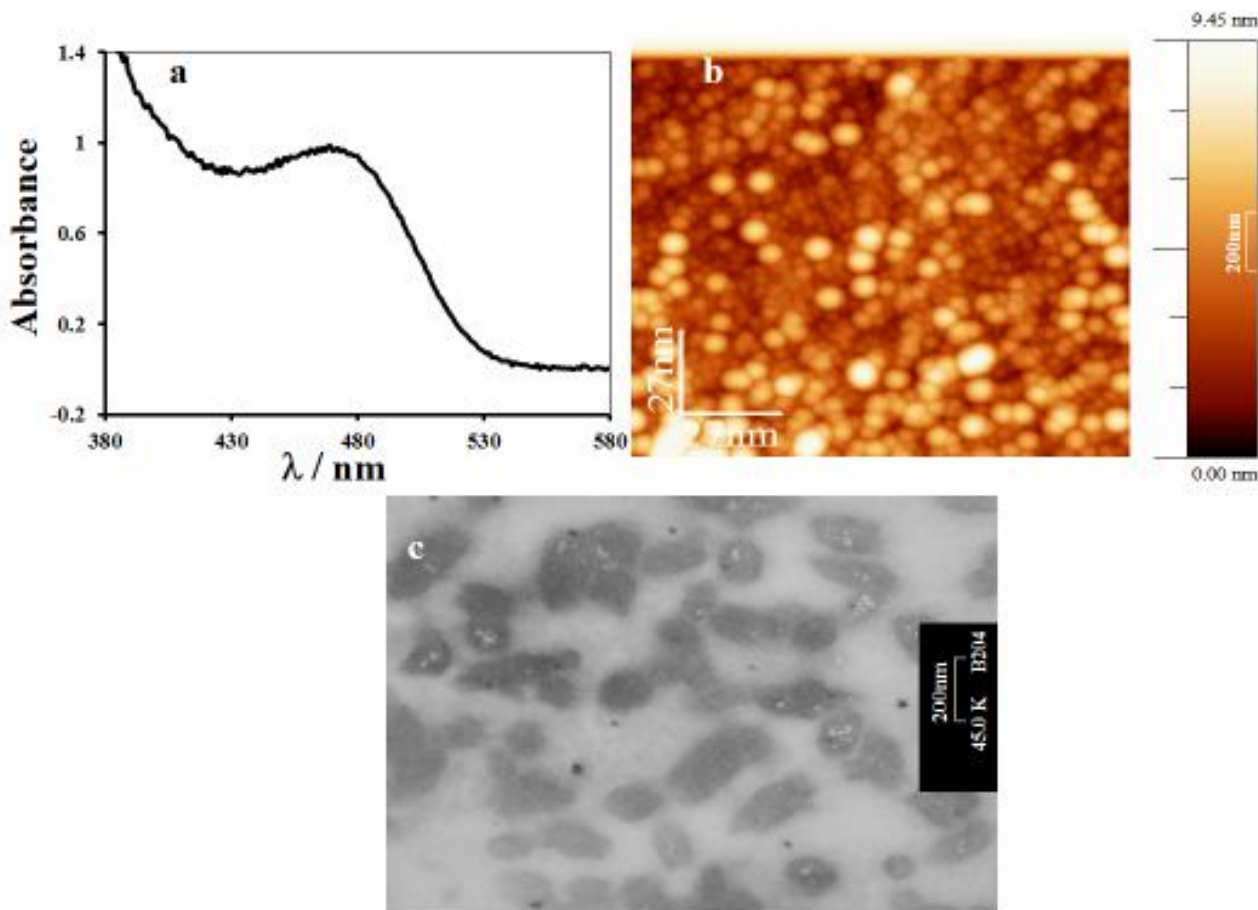
Fig. 2. (A) TEM image, (B) AFM image and (C) UV-Vis spectra of the prepared CdTe QDs.

shapes of the CdTe QD particles, which can lead to a better immobilization of Den on the surfaces of CdTe QD particles. Furthermore, the recorded TEM image indicated the synthesized CdTe QD nanoparticles with sizes less than 200 nm (Fig. 2C). All of these tests showed that CdTe QDs were successfully synthesized and were capable of loading biomolecules [22].

FT-IR Study of the Apt-Den-QD Attachment

We used the FT-IR technique to investigate the formation of Den-QD bioconjugate as the sensing layer and the attachment of the Apt to it. Figure 3 shows the FT-IR spectra related to each of the substrates such as CdTe QD, Den, terephthalaldehyde, and the Apt for the verification of layer by layer attachment. As shown in Fig. 3A, the absorbance bands at 1637.31 and 3452.85 cm^{-1} are ascribed

to the C=O and O-H stretching vibrations related to the COOH group in the TGA in CdTe QDs. As indicated in Fig. 3B, the absorbance band at 3463.75 cm^{-1} is related to NH_2 groups in Den. The formation of the amide coupling and the attachment of Den to TGA in CdTe QDs are proved by comparing the stretching vibrations of N-H in both Fig. 3B (N-H: 3463.78 cm^{-1}) and Fig. 3E (N-H: 3400.72 cm^{-1}). The existence of terephthalaldehyde as a linker to be attached to the surface of Den is proved by the bands at 1400-1638.22 cm^{-1},which is related to C=C bands in the aromatic ring (in this range, 1637 cm^{-1} is related to C=O band, 2829.82 cm^{-1} and 2829.95 cm^{-1} are related to aldehyde C-H, and 3472.04 cm^{-1} is related to O-H in H_2O as a solvent) assigned to the stretching vibrations (Fig. 3C). The formation of the imine coupling between NH_2 group in Den and aldehyde group in the terephthalaldehyde is confirmed by 3400.55 cm^{-1} (Fig.

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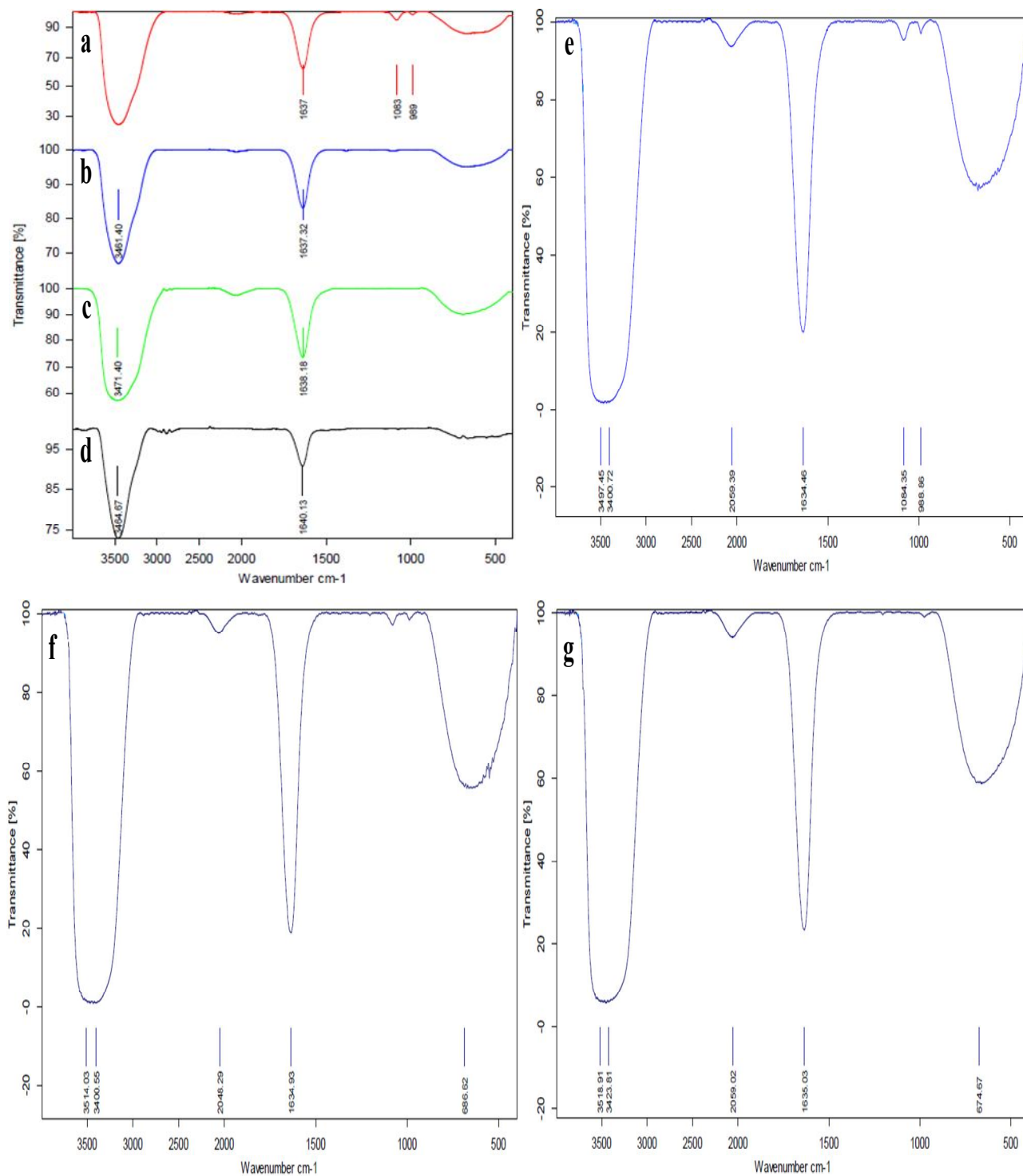

Fig. 3. FT-IR spectra related to (A) CdTe QDs, (B) Den, (C) terephthalaldehyde and (D) Apt, interactions between (E) CdTe QDs and Den, (F) Den and terephthalaldehyde and (G) terephthalaldehyde and Apt.
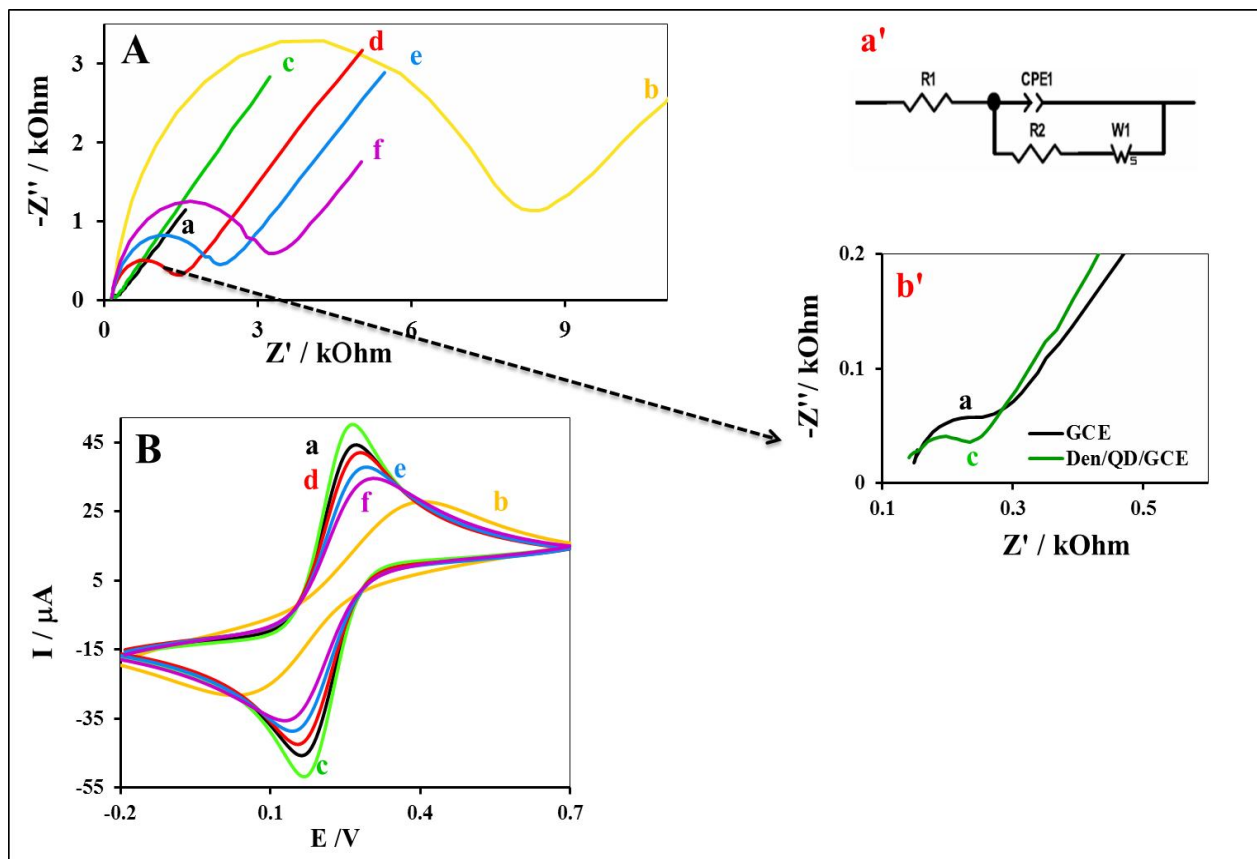
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Fig. 4. (A): Nyquist curves of the Recorded modified electrode for each steps: GCE (a), CdTe QDs/GCE (b), Den/CdTe QDs/CE (c), Apt/Terephthalaldehyde/Den /CdTe QDs/GCE (d), the modified GCE after incubation with 0.005 M cocaine, and (e) the modified GCE after incubation with 0.05 M of the cocaine (f). Inset a′ is the equivalent circuit. (B) Recorded CVs for the different steps of the modified electrode (scan rate 50 mV s^{-1}).
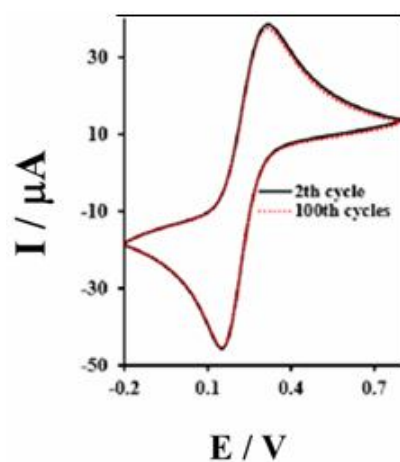

Fig. 5. Investigation of the stability of the cocaine aptasensor after 2th cycle and 100th cycles.

3F). As shown in Figure 3D, the spectra of the Apt contain the bands at 2985.28 and 2947.14 cm^{-1} related to aliphatic C-H, 1640.13 cm^{-1} related to the C=O band, and 3464.67 cm^{-1} related to NH$_2$ group. The attachment of the Apt to Den by the terephthalaldehyde as a linker is verified by comparing the stretching vibrations in the spectra in Fig. 3G and Fig. 3D. The formation of the imine coupling between NH$_2$ group in the Apt and aldehyde group in the terephthalaldehyde is confirmed by 3423.81 cm^{-1} in Figure 3G.

Electrochemical Study of the Sensing Principle of the Proposed Aptasensor

After selecting Den-QD bioconjugate as the most appropriate platform for the fabrication of the aptasensor, the electrochemistry based EIS technique was applied for demonstration the progressive formation of the sensing layer. The strategy for the attachment of the sensing layer relies on the immobilization of CdTe QDs that allows the covalent bond of Dens to be utilized as a means for the signal enhancement and consequently the anchoring of the Apt molecules. The EIS experiments confirmed this layer-by-layer immobilization by changes in the value of the charge transfer resistance (R$_{ct}$). These changes are related to the hindering of the electronic communication between the redox markers and electrode interface which are indicated as semicircles in the Nyquist plot. A big semicircle in the Nyquist plot corresponding to CdTe QD immobilization is the result of repulsion between the negative charge related to the TGA in the CdTe QDs and the indicator [Fe(CN)$_6$]$^{3-/4-}$ [18,26]. So, the electrons from the redox marker diffuse more slowly (Fig. 4A, curve b). In the next step, adding Den to the surface of CdTe QDs/GCE decreased the R$_{ct}$ value because of the presence of -NH$_2$ groups in Den which were attached to the activated -COOH groups of CdTe QDs (curve c). This signal improvement was most likely due to the positive surface charge of Dens that allowed the closer proximity of the redox probe anions to the surface of the aptasensor; this leads to the more efficient charge transfer. Evidently, the used Den molecules which have 64 primary amino groups on their surface can facilitate the attachment of more Apts on the sensing surface [34,35]. By introducing 5′-NH$_2$-3′-AuNPs terminated Apt to the surface of Den/CdTe QD/GCE, the electronic transfer between the redox

probe and sensing surface was decreased (curve d). This behavior was due to the negative charge of the phosphate groups on the Apt; also the negative charge related to AuNPs on the end of the Apt leads to a greater repulsion of the electrode surface by the redox anions, and the electron transfer was more prohibited [30]. When cocaine, as the target, was incubated onto the surface of the modified electrode, the Apt conformation was switched to form a three-way junction complex [36] which increased the steric hindrance on the surface of the electrode. Therefore, the diffusion of the redox probe on the modified electrode surface was inhibited (curve e). With an increase in the concentration of cocaine, the diameter of the semicircle increased (curve f) which indicated the specific attachment of cocaine to the Apt on the electrode surface.

In order to examine the most suitable approach for the sensing layer to be used as the immobilization platform for Apts against cocaine, the CV technique was applied on the different surfaces of the electrode: 1) Bare GCE, 2) CdTe QD/GCE, 3) Den/CdTe QD/GCE, 4) Apt/ Den/CdTe QD/GCE and 5) the incubated cocaine on the modified surface. The potential variation in the anodic and cathodic peaks ($\Delta E = E_p^a - E_p^c$) of the CV plots related to different steps were investigated. According to Fig. 4B, for a bare GCE in [Fe(CN)$_6$]$^{3-/4-}$ as the electrolyte solution, the ΔE value is 96.00 mV. By adding CdTe QD, due to the repulsion between the negative charges related to the TGA capped CdTe QDs and the redox probe, electron transfer is inhibited. Thus, the ΔE value is increased to 351.00 mV and the current value is decreased. This behavior indicates that the CdTe QDs layer is well attached on the surface of the GCE. The immobilization of Den on the surface of CdTe QD/GCE with $\Delta E = 99.00$ mV, which makes the redox markers diffuse more rapidly, indicates the obvious increment in the oxidation and reduction currents of the redox marker [34,35]. By covalent attachment of the Apt to the surface of Den/CdTe QD/GCE *via* terephthalaldehyde as the linker, due to the negative charges of the phosphate groups and the AuNPs functionalized Apt, the electron transfer is prohibited. Therefore, the ΔE value is increased to 126.00 mV and the current is decreased. The incubation of cocaine as the target on the aptasensor surface leads to the Apt conformation switch. Thus, because of the created steric/conformational restrictions on the sensing surface, the
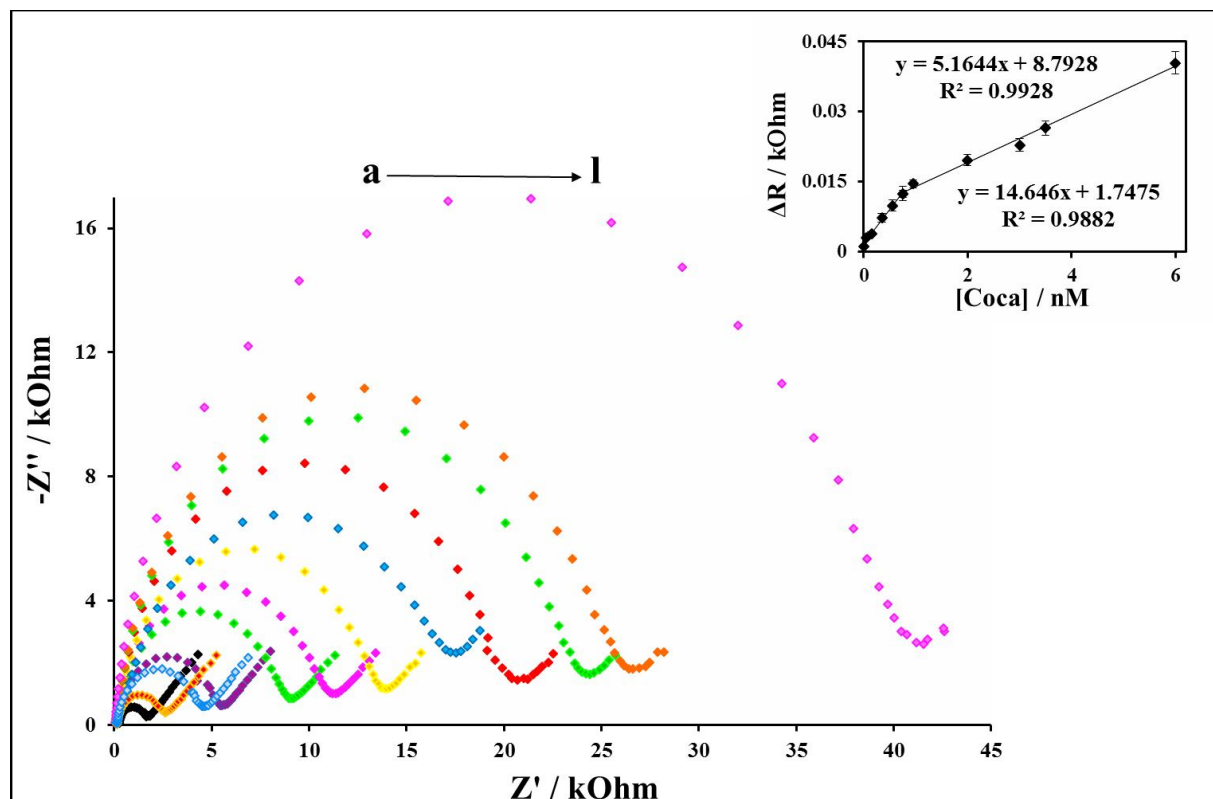
Fig. 6. EIS results for the developed aptasensor incubated with different concentrations of cocaine (from a to l: 0, 5.00×10^{-3}, 0.05, 0.15, 0.35, 0.55, 0.75, 0.95, 2.00, 3.00, 3.50, 6.00 nM). Inset is the corresponding calibration curves between the obtained signal and the cocaine concentration (data obtained were the averages of three measurements).

electron transfer decreases and, consequently, the ΔE value increases. Stability of the cocaine aptasensor was also studied via the CV technique. The voltammetric behavior of the modified electrode in the electrolyte solution was very stable and it was found to have reserved its initial efficacy (98%) after 100 repetitive cycles in one continuous operation (Fig. 5); the electrode surface fouling did not occur. Additionally, the long-term stability of the aptasensor was evaluated. The results showed that the peak height and peak potential of Apt/Den/CdTe QD/GCE as the modified electrode remained nearly unchanged and the sensing layer retained more than 97% of its performance after 12 days storage at 4 °C (data not shown). High stability of the proposed nanotool demonstrated that Den-QD bioconjugate is well-stabilized as an integrated sensing interface.

Investigation of the Analytical Performance of the Aptasensor for the Electrochemical Detection of Cocaine

To evaluate the efficiency of the fabricated electrochemical nanotool, under optimal conditions, the analytical calibration was performed. Due to the high ability of the EIS technique for probing the interfacial properties at the electrode surface and the possibility of performing label-free detections, the EIS measurement was applied as an efficient alternative detection system to confirm the analytical performance of the proposed system. Figure 6 displays the Nyquist plots obtained at the modified electrode after being incubated with different concentrations of cocaine. As can be seen in Fig. 6, when the cocaine concentration increases, the R_{ct} increases accordingly, due
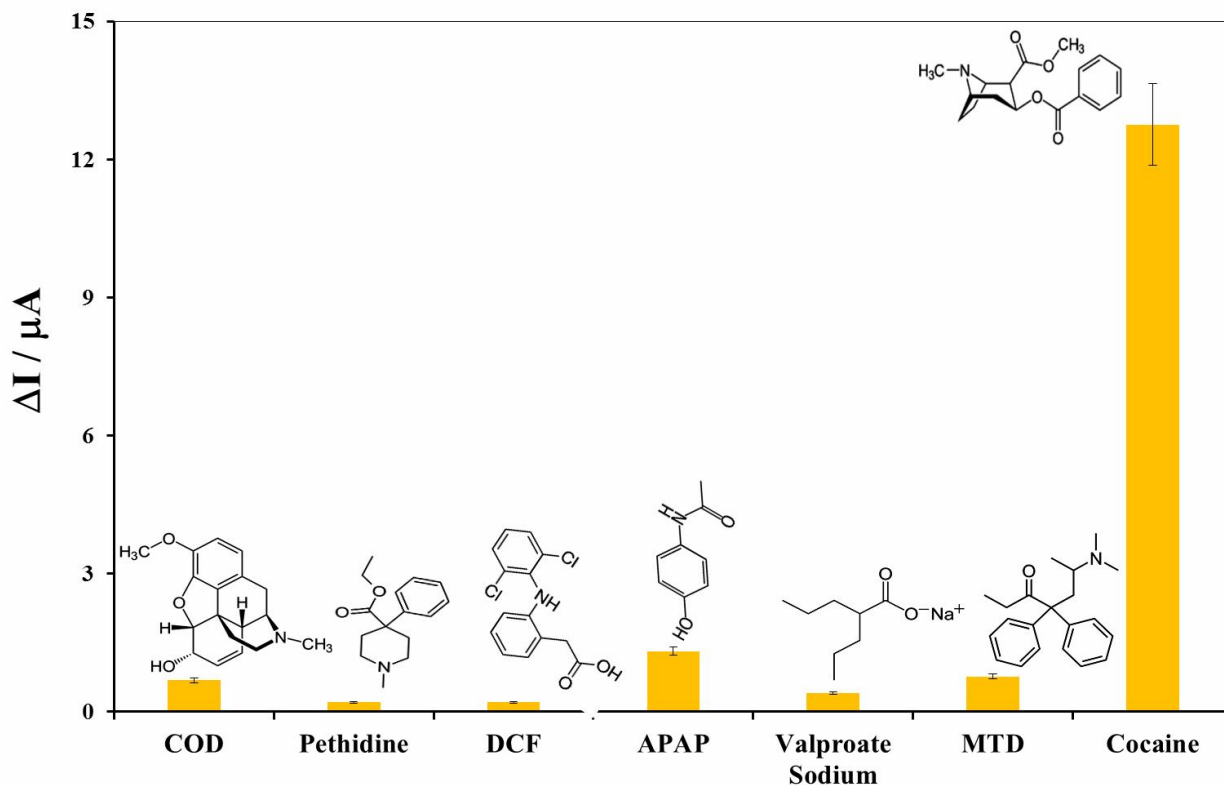
Fig. 7. Bar chart of the changes of the proposed aptasensor response after incubation with cocaine and other common drugs of COD, pethidine, DCF, APAP, Valproate sodium, and MTD with 10^5-fold higher concentration than that of the cocaine.

Table 2. Cocaine Detection in Twelve Spiked Blood Serums, as Three Categories of Four Samples, that Developed Aptasensor with Three Concentrations of Cocaine (Means of Three Different Determinations)

Serum sample (means of four samples)	Added (nM)	Founded (nM)	Recovery (%)
1	1.00	0.98 ± 0.03	98.00
2	2.00	2.02 ± 0.02	101.00
3	6.00	6.03 ± 0.04	100.50

to the formation of an increasing number of cocaine/Apt conjugates, with the result of enhanced hindering of the electron transfer reaction of the redox probe on the aptasensor surface. A good linear correlation existed between the value of the aptasensor response and the concentration of cocaine over the range of 5×10^{-3} nM to

6.00 nM with two equations: it was ΔR_{ct} (Ω) = 14.646 [cocaine] (nM) + 1.747 (R^2 = 0.9928) for low concentrations and ΔR_{ct} (Ω) = 5.164 [cocaine] (nM) + 8.792 (R^2 = 0.9882) for high concentrations (inset of Fig. 6). The capability of detection was estimated to be 1.60 pM based on the signal-to-noise ratio of 3 and by determining the RSD from three measurements and sensitive enough for cocaine detection in the clinical samples. This behavior may be due to the fact that by increasing the cocaine concentrations, more AuNPs functionalized Apt is folded and more Apt/cocaine complex is formed on the modified electrode surface. Thus, the AuNP negative charge at the end of the Apt causes the inhibition of the electron transfer from the [Fe(CN)$_6$]$^{3-/4-}$ anions as the redox probe to the electrode surface. These results clearly indicate that the proposed aptasensor can be used as an efficient ultrasensitive impedimetric nanotool for cocaine detection. By comparing the results obtained from the proposed aptasensor to those of other methods reported in the literature (Table 1), we conclude that the fabricated aptasensor, an electrochemical nanotool with having Den-QD nanocomposite as a suitable platform, displayed excellent analytical performance, which can be ascribed to the following reasons: (a) Unlike the adsorption or the electrostatic interactions between other receptors and their substrates, the used cocaine receptor in this method is the Apt, which has many inherent properties especially as regards cocaine. (b) Utilizing CdTe QD and Den in the proposed nanocomposite with the described strategy facilitated the covalent attachment of the cocaine specific Apt on the GCE surface and greatly enhanced the electrochemical signal of the redox probe. (c) The presence of the small size synthesized AuNPs leads to the improvement of the electrochemical signal.

Investigation of Reproducibility, Repeatability, and Selectivity of the Aptasensor and Real Sample Analysis

To study the reproducibility of the aptasensor and the reliability of the fabrication procedure, five modified electrodes were independently tested with the concentration of 1.00 nM cocaine in applying optimum conditions. The results showed acceptable reproducibility (relative standard

deviation (RSD) of about 3%) and good precision of the proposed aptasensor. Furthermore, to evaluate the repeatability of the fabricated aptasensor, the EIS data related to the modified electrode, incubated with cocaine in the same way, was recorded. This manner was repeated six times. A relative standard deviation (RSD) <4% was achieved for 1.00 nM cocaine. The basic factor in preparing of the good reproducibility, acceptable repeatability and high performance of the fabricated aptasensor can possibly contribute to the strong interaction between QD-Den bioconjugate as a nanocomposite and Apt on the electrode surface.

Selectivity is an important factor in the practical sensing of biosensors. The selectivity of the aptasensor was evaluated by conducting assays of other common analgesic drugs including APAP, MTD, pethidine, COD, DCF and valproate sodium under the same experimental conditions. The bar chart of the selective response related to the DPV technique of the developed nanotool is shown in Fig. 7. Obviously, the changes in the electrochemical response induced by the non-specific binding related to the addition of these interfering drugs are much lower than those induced by the specific binding of cocaine (even at a 10^5-fold higher concentration than that of cocaine). The specificity of this aptasensor relies on the highly specific and strong interactions occurring between cocaine and the Apt which is covalently attached to the sensing layer. This is also another advantage of the proposed nanotool.

In order to estimate the applicability of the methodology, the proposed nanotool was applied to measure the different concentrations of cocaine in the spiked human blood serums from four men. 2.00 µl of the solutions containing different concentrations of 1.00, 2.00 and 6.00 nM of cocaine were prepared separately and dropped on the surface of the modified electrode independently of the others. Twelve of the spiked blood serums, as three categories of four samples, were analyzed under the same experimental conditions described above using the EIS technique and standard addition method. For investigation of their repeatability, each sample was tested three times. The average quantities of cocaine in these serum samples were calculated and recoveries of different concentrations of cocaine were also

investigated. The obtained recoveries for spiked cocaine concentrations were 98.00, 101.00 and 100.50, respectively. As listed in Table 2, these are in good agreement with real values. It is well known that the basic factor contributing to the high sensitivity and selectivity of the presented sensing system is the use of Den-QD bioconjugate as a specific platform with unique properties and the covalent attachment of the Apt to it, that all occur on the electrode surface. So, the developed aptasensor might be a processing system as a reliable sensor for the detection of cocaine in the real samples.

CONCLUSIONS

In this study, we used the advantages of CdTe QDs and Dens and prepared a Den-QD bioconjugate as a promising transduction platform on the electrode surface to fabricate an ultrasensitive aptasensor. The presence of Den, due to the abundant reactive amine groups on its surface in the bioconjugate, functions as a signal amplifier to facilitate the immobilization of the Apt on its surface. Our results demonstrated that the proposed nanocomposite markedly enhances properties such as the surface-to-volume ratio, electrochemical signal, target-capturing ability, and specificity. The designed nanotool exhibited an extremely low capability of detection and the spiked serum sample was used as the model target to demonstrate the feasibility of the proposed methodology as a proof of concept. This method is specifically able ro detect cocaine at picomolar to nanomolar concentrations, and is not affected by the other common analgesic drugs. The developed aptasensor has been fabricated to offer a specific, sensitive, and convenient method for measuring cocaine as a model system; it also possesses the potential to be applied for the detection of other targets. Further attempts for the fabrication of other aptasensors based on nanocomposites containing other nanomaterials with higher efficiency to measure other analytes are underway in our laboratory.

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REFERENCES

[1] N.J. Ronkainen, H.B. Halsall, W.R. Heineman, Chem. Soc. Rev. 39 (2010) 1747.
[2] K. Kerman, M. Kobayashi, E. Tamiya, Meas. Sci. Technol. 15 (2004) R1.
[3] S.F. Lapachinske, G.G. Okai, Ad. Santos. AVd. Bairros, M. Yonamine, Forensic Sci Int. 247 (2015) 48.
[4] E.W. Buhleier, W, Wehner, F. Vogtle, Synthesis 9 (1978) 155.
[5] S. Svenson, D.A. Tomalia, Adv. Drug Deliv. Rev. 57 (2005) 2106.
[6] D.A. Tomalia, J.M.J. Frechet, John Wiley & Sons, Ltd; Chichester. 2002.
[7] I.Y. Goryacheva, E.S. Speranskaya, V.V. Goftman, D. Tang, S.D. Saeger, Trends Anal. Chem. 66 (2015) 53.
[8] A. Korani. A. Salimi, Biosens. Bioelectron. 50 (2013) 186.
[9] M. Fischer, F. Vgtle, Angew. Chem. Int. Ed. 38 (1999) 884.
[10] R.W.J. Scott, H. Ye, R.R. Hnriquez, R.M. Crooks, Chem. Mater. 15 (2003) 3873.
[11] L. MacKay, J. Frechet, F. Szoka, Nat. Biotechnol. 23 (2005) 1517.
[12] Sh. Jahanbani, A. Benvidi, Biosens. Bioelectron. 85 (2016) 553.
[13] A. Benvidi, S. Yazdanparast, M. Rezaeinasab, M. Dehghan Tezerjani, S. Abbasi, Electroanal. Chem. 808 (2018) 311.
[14] A. Benvidi, M. Dehghan Tezerjani, S.M. Moshtaghiun, M. Mazloum-Ardakani, Microchim. Acta 183 (2016) 1797.
[15] F. Shahdost-fard, M. Roushani, Biosens. Bioelectron. 87 (2017) 724.
[16] R. Duncan, L. Izzo, Adv. Drug Deliv. Rev. 57 (2005) 2215.

[17] F. Shahdost-fard, M. Roushani, Microchim. Acta 185 (2018) 214.

[18] F. Shahdost-fard, M. Roushani, Mater. Sci. Eng. C 75 (2017) 1091

[19] Y.G. Zheng, S.J. Gao, J.Y. Ying, Adv. Mater. 19 (2007) 376.

[20] X. Hu, R. Wang, Y. Ding, X. Zhang, W. Jin, Talanta 80 (2010) 1737.

[21] X. Gao, L. Yang, J.A. Petros, F.F. Marshall, J.W. Simons, S. Nie, Curr. Opin. Biotechnol. 16 (2005) 63.

[22] Y. Li, M. Han, H. Bai, Y. Wu, Zh. Dai, J. Bao, Electrochim. Acta 56 (2011) 7058.

[23] L. Hua, H. Han, H. Chen, Electrochim. Acta 54 (2009) 1389.

[24] J. Aldana, Y.A. Wang, X. Peng, J. Am. Chem. Soc. 123 (2001) 8844.

[25] Z. Li, P. Huang, R. He, J. Lin, S. Yang, X. Zhang, Q. Ren, D. Cui, Mater. Lett. 64 (2010) 375.

[26] M. Roushani, M. Shamsipur, H.R. Rajabi, Electroanal. Chem. 712 (2014) 19.

[27] F. Shahdost-fard, A. Salimi, E. Sharifi, A. Korani, Biosens. Bioelectron. 48 (2013) 100.

[28] M.Y. Ho, N. D'Souza, P. Migliorato, Anal. Chem. 84 (2012) 4245.

[29] A. Miodek, G. Castillo, T. Hianik, H. Korri-Youssoufi, Anal. Chem. 85 (2013) 7704.

[30] M. Roushani, F. Shahdost-fard, Anal. Chim. Acta 853 (2015) 214.

[31] G. Frens, Nature Phys. Sci. 241 (1973) 20.

[32] M. Roushani, F. Shahdost-fard, Sens. Actuators B: Chem. 207 (2015) 764.

[33] W. Haiss, J. Thanh, D.G. Aveyard, Fernig. Anal. Chem. 79 (2007) 4215.

[34] G. Castillo, K. Spinella, A. Poturnayova, M.

Snejdarkova, L. Mosiello, T. Hianik, Food Control 52 (2015) 9.

[35] B. Kavosi, R. Hallaj, H. Teymoourian, A. Salimi, Biosens. Bioelectron. 59 (2014) 389.

[36] M.F. Huang, Y.C. Kuo, C.C. Huang, H.T. Chang, Anal. Chem. 76 (2004) 192.

[37] S.F. Lapachinske, G. Gonc, A. Okai, A.D. Santos, A.V.D. Bairros, M. Yonamine, Forensic. Sci. Int. 247 (2015) 48.

[38] F.B. D'Avila, A.G. Pereira, F.R. Salazar, P.L. Ferreira, C.R. Salazar, R.P. Limberger, P.E. Froehlich, J. Pharm. Biomed. Anal. 103 (2015) 67.

[39] J.L. Costa, A.R. Morrone, R.R. Resende, A.A.D.A Chasin, M.F.M. Tavares, J. Chromatogr. B 945-946 (2014) 84.

[40] M.N. Stojanovic, D.W. Landry, J. Am. Chem. Soc. 124 (2002) 9678.

[41] J.E. Liu, Y. Lu, Angew. Chem. Int. Ed. Engl. 45 (2006) 90.

[42] Z. Zuo, Y. Xiao, K.W. Plaxco, J. Am. Chem. Soc. 131 (2009) 6944.

[43] W.S. Sutherland, J.D. Winefordner, J. Colloid Interface Sci. 148 (1992) 129.

[44] Y. Went, H. Pei, Y. Wan, Y. Su, Q. Huang, S. Song, C. Fan, Anal. Chem. 83 (2011) 7418.

[45] B. Jiang, M. Wang, Y. Chen, J. Xie, Y. Xiang, Biosens. Bioelectron. 32 (2012) 305.

[46] X.X. Li, H.L. Qi, H.L. Shen, Q. Gao, C.Z. Zhang, Electroanalysis 20 (2008) 1475.

[47] R. Freeman, E. Sharon, R. Tel-Vered, I. Willner, J. Am. Chem. Soc. 131 (2009) 5028.

[48] M.A.D. Neves, C. Blaszykowski, S. Bokhari, M. Thompson, Biosens. Bioelectron. 72 (2015) 383.