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Highly selective and sensitive adenosine aptasensor based on platinum nanoparticles as catalytical label for amplified detection of biorecognition events through H_2O_2 reduction

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ABSTRACT

Based on a combination of aptamer and platinum nanoparticles a new sensitive and selective adenosine aptasensor was fabricated. Functionalized thiol-terminated adenosine aptamer (5'-AGAGAACCTGGGG-GAGTATTGCGGAGGAAGGT-SH-3') with Pt Nanoparticles (Pt-NPs) was employed as highly catalytic label for electrochemical detection of adenosine based on electrocatalytic activity of Pt-NPs toward H_2O_2 reduction. Multiwalled carbon nanotubes/ionic liquid/chitosan (MWCNTs/IL/CHIT) nanocomposite was applied as the interface for covalent attachment of 3'-amine-terminated capture probe (3'-NH_2-$(CH_2)_6$-TCTCTTGGACCC-5'). The presence of Pt nanoparticles improvement the conductivity and performance characteristics of the biosensor as well as incensement in the loading amount of the aptamer DNA sequence. The interaction of adenosine with the aptamer causes the releasing of aptamer with PtNPs into solution which resulted in a decreasing of hydrogen peroxide reduction peak current. Sensitive quantitative detection of adenosine was achieved by monitoring the decrease of voltammetric responses of H_2O_2 peak current. The peak current of H_2O_2 decreased with increase in the concentration of adenosine over a range of 1–750 nM with detection limit 1 nM. In addition the proposed aptasensor showed excellent selectivity toward adenosine in compared to some other nucleosides such as guanosine, cytidine and uridine. The proposed aptasensor was successfully used to detect adenosine in human serum samples. The elimination of enzymes or antibodies for the amplified detection of adenosine and the use of platinum nanoparticles as inorganic catalytic label, are the advantage of the proposed aptasensor.

1. Introduction

Recently, development in the nanostructure material synthesis has made numerous impacts on a number of particular fields including photonics, nanoelectronics, biology and medicine (Daniel and Astruc., 2004), due to unique catalytical, electrical, magnetic, optical, chemical and other properties of nanomaterials. Nanomaterials can be coupled to biomolecules such as nucleic acids, antibodies or antigens as active units in bioanalytical systems. Therefore, biomaterial hybrid systems based on nanomaterials are broadly used in different biosensing applications (Teymourian et al., 2012a, 2012b; Teymourian et al., 2013; Khezrian et al., 2013; Navaee et al., 2012; Salimi et al., 2013; Sharifi et al., 2013; Sharifi et al., 2012; Saadati et al., 2012). Aptamers are short single-stranded functional nucleic acids (DNA or RNA molecules) that can interact with a specific analyte; they can also result in a conformation change or catalytic reaction (Lu and Liu., 2006; Lu and Liu., 2007). Aptasensors which are a new kind of biosensor are made based on the immobilization of an aptamer onto the electrode surface. They have been developed using different analytical methods (Fang et al., 2008; Yang et al., 2005; Nagatoishi et al., 2007; Basnar et al., 2006; Feng et al., 2008; Lu et al., 2008).

The amplified detection of biorecognition events is a very important factor in developing aptasensores. The coupling of enzymes as biocatalytic amplifying labels is a general paradigm in developing these sensing devices. Biological materials such as enzymes have disadvantages such the loss of activity after the first few cycles [Shi et al., 2007], and inherent thermal and environmental instability (Polsky et al., 2006). Recent progress in developing methods based on a combination of aptamers with a various number of nanomaterials shows that these novel bionanomaterials can be used as highly sensitive electrochemical markers for the detection of different analytes in electrochemical systems (Lee et al., 2010; Famulok and Mayer., 2006; Pavlov et al., 2004). Aptamers can be modified with different nanomaterials including; fluorescent nanomaterials e.g., Quantom dots (QDs) or semiconductor nanoparticles (Alivisatos., 1996), magnetic nanoparticles (Yan et al., 2009) and metallic nanoparticles (NPs) for bioimaging

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applications such as the detection of DNA or real time monitoring of hybridization procedures (Mitchell et al., 1999; Zhang et al., 2005). Furthermore, aptamers conjugated with nanomaterials such as platinum nanoparticles (Dhar et al., 2008) and Au–Ag nanorods (Huang et al., 2008) have also been applied in target drug delivery. Nanoparticles show unique properties and changes in surface area. Enhancements in the catalytic properties of metallic nanoparticles are associated with changes in surface area and reactivity. Palladium (Ason et al., 2007), silver, gold (Zhang et al., 2006), copper [Male et al., 2004] and platinum nanoparticles (PtNPs) (Murkarian et al., 2005) have great catalytic activity and are widely used to enhance the performance of modified electrodes. Therefore, a range of approaches have been used for the preparation of metallic nanoparticles such as co-precipitation of the appropriate metals (Cushing et al., 2004), sputtering (Kabiraj et al., 2006), the sonochemical (Park et al., 2005), microemulsion (Pal et al., 2007), UV-irradiation (Malik et al., 2001), sol–gel encapsulation and solvothermal (Burda et al., 2005) methods. The shapes of metal nanoparticles can be controlled by both kinetic and thermodynamic factors, which are dictated by intrinsic structural properties of metal and reaction systems such as solvents, reducing agents and surface stabilizers. The controlling of reaction conditions parameters for synthesis of nanoparticles including concentration, pH, reaction temperature and time are also important. Platinum nanoparticles are widely used in organic catalysis, hydrogen storage and electrocatalysis. They are active and selective electrocatalysts for various redox reactions and can provide new opportunities for electrocatalysis and serve as a bridge between analyte and electrode surfaces (Lewis, 1993; Lipkowski and Ross, 1998). Particularly, PtNPs have been demonstrated to lower efficiently the H_2O_2 oxidation/reduction overvoltage which is very important for biosensors (Miscoria et al., 2002).Furthermore, electrochemical amplification of DNA analysis by application of Pt Nanoparticle was also reported (Kwon and Bard, 2012).

Adenosine is the component of many biological cofactors and an endogenous modulator with potent vasodilator and antiarrhythmic activities which has received much attention due to its crucial signaling function in both the peripheral and the central nervous system (Spychala, 2000; Yan et al., 2009). It also plays a fundamental role in many biological processes such as energy generation and protein metabolism. A number of investigations have been used to examine adenosine biomedical significance as possible biomarkers for cancer [Yang et al., 2002]. Therefore, rapid measurement of adenosine in vivo is important. Although different detection methods reported in literature; however, more of them have some disadvantages such as low temporal resolution, expensive instrumentation, tissue damage from implantation of the relatively large robes and complex fabrication process (Dobolyi et al., 1998; Clapp-Lilly et al., 1999). So, a simple, rapid, sensitive and specific method for the detection of adenosine is a challenge.

Here, we study the application of thiolated aptamer functionalized with PtNPs as catalytic label, for measuring of adenosine. At first, in the absence of any usual protective agent, stable PtNPs in organic media with average diameter (1–3 nm), were prepared by heating platinum hydroxide colloids in ethylene glycol containing NaOH with pH > 12. The new aptasensor is fabricated based on covalent attachments of amine-terminated aptamer capture onto the nanocomposite formed from multiwalled-carbon nanotubes (MWCNTs), ionic liquids (ILs) and chitosan (CHIT) and the immobilization of the adenosine aptamer probe-functionalized Pt nanoparticles to the surface of the nanocomposite with DNA hybridization. Platinum nanoparticles were used as catalytic label for the reduction of H_2O_2. Using this system, sensitive quantitative detection of adenosine was realized by monitoring the differences of the voltammetric response of the system toward H_2O_2 reduction. In the presence of adenosine, the H_2O_2 reduction peak current decreases due to aptamer release from the capture probe and formation of a complex between the aptamer probe-functionalized Pt nanoparticles and adenosine. The proposed aptasensor was successfully used to detect adenosine in human serum samples and pharmaceutics products.

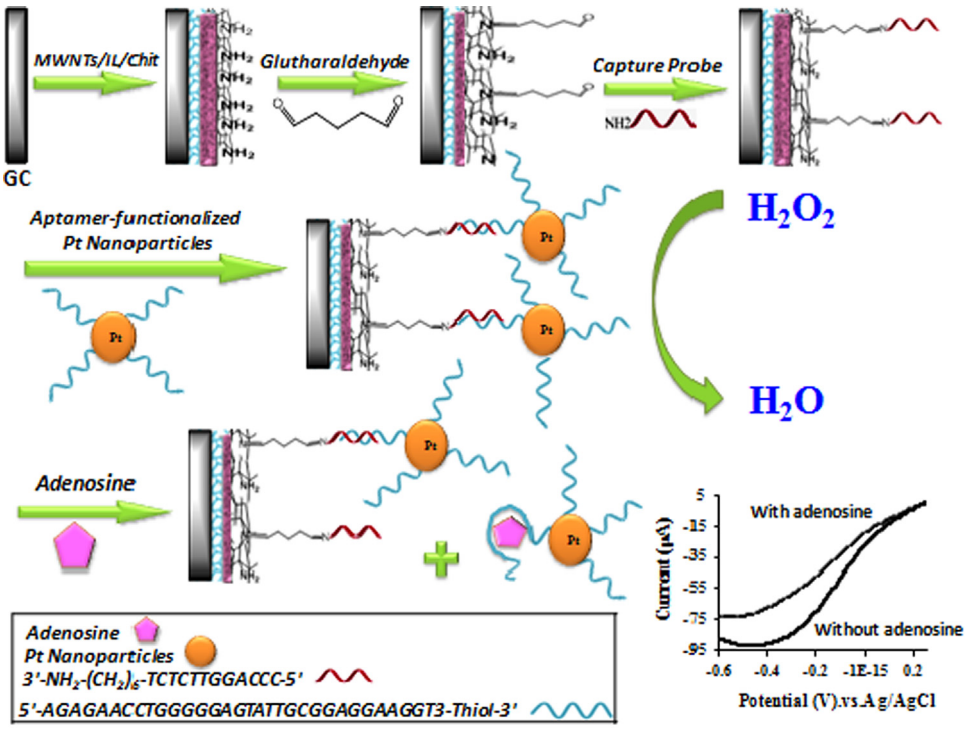
2. Experimental section

2.1. Materials and reagents

In the present study and based on the literature (Zou et al., 2007) the oligonucletides were designed, then synthesized by the Takapouzist Co. (Tehran, Iran). The sequences of 3'-amine-terminated (12 bases) and 3'-thiolated DNA probe (32 bases) as capture and detection probes were (3'-NH_2-$(CH_2)_6$-TCTCTTGGACCC-5') and (5'-AGAGAACCTGGGGGAGTATTGCGGAGGAAGGT-SH-3'), respectively. In this sequence Pt nanoparticles coupled with thiolated aptamer. Aptamer-functionalized PtNPs was designed to hybridize with the 12-base capture probe. 1-Butyl-1-methyl pyrolydinium bis (trifluoro-methyl-sulfonyl) imide [$C_{11}H_{20}F_6N_2O_4S_2$] (IL), H_2O_2, ethylene glycol, NaOH, chitosan (CHIT), glutaraldehyde (GLA), adenosine, cytidine, uridine, guanosine and K_2PtCl_6 were purchased from Sigma. Multiwalled carbon nanotubes (MWCNTs) with a purity of 95%, surface specific area of 480 $m^2\ g^{-1}$, diameter of 20–30 nm and 1 μm length were obtained from Nanolab (Brighton, MA). The other used chemicals were of analytical reagent grade and used without further purification. All sample solutions (adenosine, guanosine, cytidine, uridine) at various concentrations were prepared by dissolving analytes in PBS buffer solution pH 7.0. All DNA solutions were prepared by dissolving in distilled water. Phosphate buffer solution (PBS, 0.1 M, pH 7.4), prepared with disodium hydrogen phosphate and NaOH as the adjusting solution, was used as the supporting electrolyte throughout the electrochemical studies. The other chemicals were used without further purification. All aqueous solutions were prepared with ultra-pure water (≥ 18 MΩ cm) from a Milli-Q Plus system (Millipore). During the experiments, to avoid possible oxidation pure N_2 (99.999%) was passed through the solution. For determination of adenosine in the real sample, a stock solution of human blood serum was diluted 25 times with PBS buffer (0.1 M), and then analyzed under the same experimental conditions which described above.

2.2. Apparatus and measurements

All electrochemical measurements were carried out with a conventional three-electrode system comprising a modified glassy carbon electrode (GC) as the working electrode, an Ag/AgCl (3 M KCl) and a platinum wire as the reference and counter electrode, respectively. Cyclic voltammetry (CV) was performed with a Metrohm electrochemical system, 757 VA Computrace Metrohm (The Netherlands) equipped with 757 VA Computrace software. Surface morphology was examined using a Vega-Tesacn electron microscope (SEM). High-resolution transmission electron microscopy (HRTEM) photographs and EDS: Energy-dispersive X-ray spectra were taken on a Philips CM-200-FEG electron microscopy operated at an accelerating voltage of 200 kV with a magnification of 300,000 to 500,000. Samples for the HRTEM measurements were prepared by placing a drop of the PtNPs solution, appropriately diluted, onto a formvar carbon coated copper grid 300 mesh. Excess solution was dried in air. All electrochemical measurements were performed at an ambient temperature of 25.0 ± 0.1 °C.



Scheme 1. Schematic representation of different steps of aptasensor fabrication.

2.3. Preparation of platinum nanoparticles

Platinum nanoparticles were prepared according to the reported procedure [Wang et al., 2000]. A glycol solution of NaOH (50 ml, 0.5 M) was added to a glycol solution of K_2PtCl_6 (1.0 g, 1.93 mmol in 50 ml) with stirring to obtain a transparent yellow platinum hydroxide or oxide colloidal solution and the resulting solution was then heated at 160 °C for 3 h, under an atmosphere of N_2 to take away water and organic by products. A transparent dark-brown homogenous colloidal solution of Platinum metal nanoparticles (Pt: 3.76 g/L glycol, 19.3 mmol/L) was obtained without any precipitate which indicates the reduction of Pt precursors and the formation of Pt nanoparticles. After standing for several months in the obtained PtNPs solution, no precipitate was observed. The morphology of PtNPs was characterized by High-resolution transmission electron microscopy (HRTEM). For functionalization of PtNPs with adenosine aptamer, 20 μl PtNPs colloid was added to 20 μl thiolated-DNA aptamer, 10 μl NaCl (0.1 M) and 10 μl PBS (0.1 M) pH=7.4 and mixture stirred for 24 h at room temperature. PtNPs were bonded to the thiolated aptamer via covalent S-Pt bonds.

2.4. Preparation of sensing interface

A GC electrode was pre-treated through polishing sequentially with 0.03 μm alumina powder followed by ultrasonic cleaning with distilled water and ethanol for 5 min. Finally, the electrode was rinsed with distilled water and dried at room temperature. To form a homogeneous layer, the MWCNTs/IL/CHIT nanocomposite was coated on the electrode surface. MWCNTs/IL/CHIT nanocomposite was prepared based on our recently reported procedure (Salimi et al., 2013). After modification of GC electrode with MWCNTs/IL/CHIT nanocomposite, 10 μl glutaraldehyde (0.25% v/v) was dropped onto the surface of the modified electrode. After the electrode was rinsed with distilled water, 10 μl of 20 μM of 3'-amine-terminated capture probe (ssDNA1) was dropped onto the surface of the modified GC electrode and kept for 2 h at 4 °C. At the same time amine group of chitosan reacted with the cross-linking reagent glutaraldehyde by Schiff-base formation while the remaining aldehyde groups could be used to immobilize amine capped aptamers (Zhang J.Q. et al., 2010; Stadtherr et al., 2005). Then, 10 μl of 32-base adenosine aptamer-functionalized PtNP was added to the prepared electrode for 12 h at 4 °C to hybrid with the capture probe through DNA hybridization reaction. The GC electrode modified with MWCNTs-IL-CHIT/capture probe/aptamer-functionalized PtNPs is abbreviated as dsDNA-modified GC electrode. Afterwards, the dsDNA-modified GC electrode was immersed in deoxygenated PBS solution (0.1 M, pH 7.4) and H_2O_2. By this process platinum nanoparticles acted as catalytic label for the reduction of H_2O_2. To measure the adenosine, 10 μl of adenosine solutions in different concentrations were dropped onto the sensing surface and incubated 45 min. The sensing was found by monitoring the change of voltammetric responses of H_2O_2 reduction peak current. The application of PtNPs as a catalytic label was employed in the potential region where the electrocatalytic reduction of H_2O_2 proceeds. The results of electrochemical characterizations of the modified electrode were obtained using cyclic voltammetry in deoxygenated PBS solution after each step of modification. The aptasensor fabrication steps are shown in Scheme 1.

3. Results and discussion

3.1. Characterization of PtNPs

HRTEM photographs show the proper formation of PtNPs with a narrow size distribution from 1 nm to 3 nm. The average diameter of PtNPs was 1.3 nm (Fig. 1A,B). The TEM-SAED electron diffraction pattern verified the synthesized PtNPs were amorphous (Fig. 1C). Energy-dispersive X-ray (EDS) spectroscopy (Fig. 1D) also confirms the presence of Pt. The Cu signal and the K is signal concerned with the supporting copper grid and K_2PtCl_6, respectively. As reported in literature with decreasing the Pt particle size, the binding energy between the S atoms and PtNPs increasing (Yang et al.,2007). The very strong Pt-S covalent bond restricts Pt

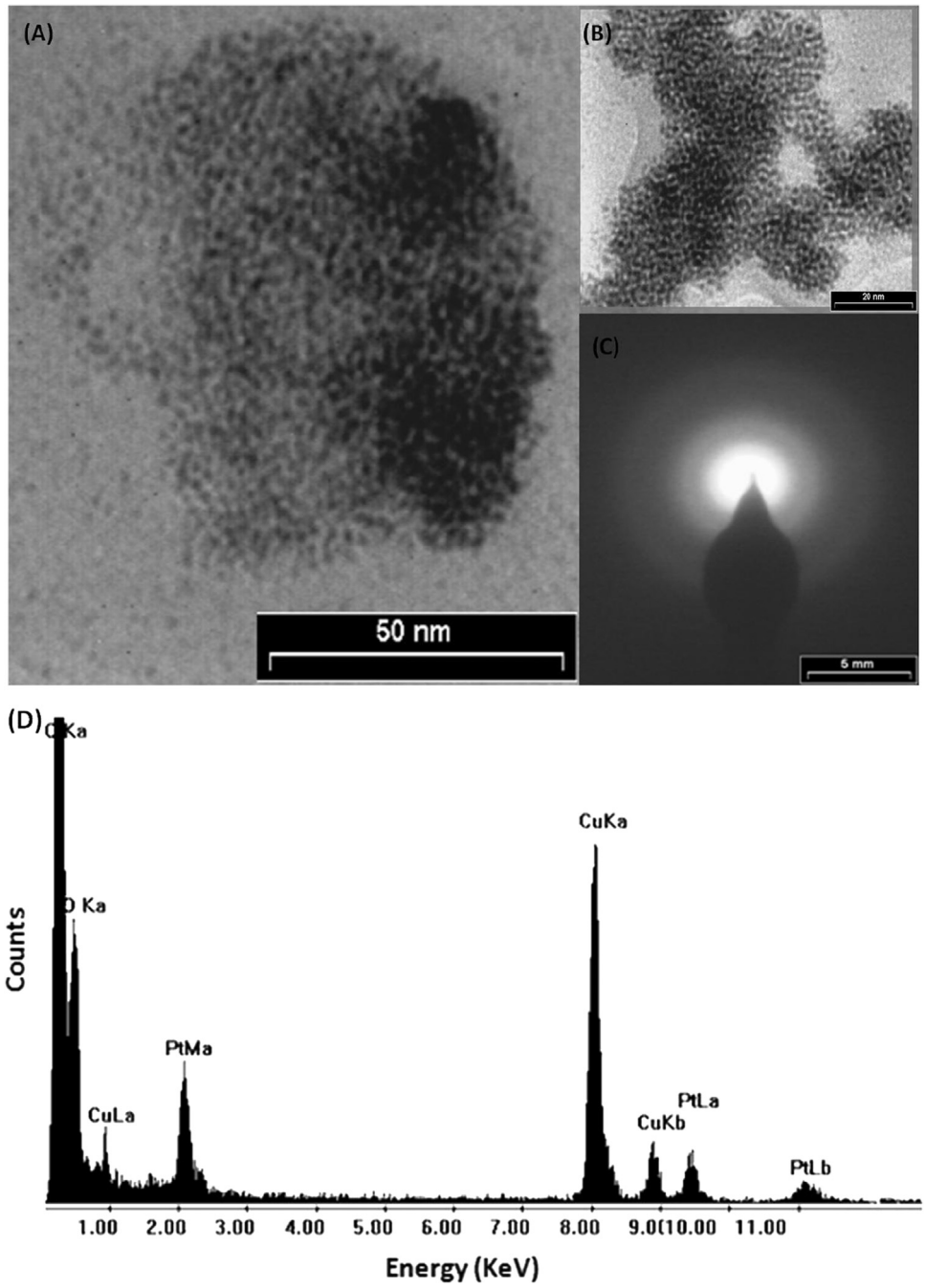


Fig. 1. (A and B) HRTEM photographs of the prepared Pt nanoparticles. (C) TEM-SAED electron diffraction pattern of the platinum nanoparticles and (D) Energy-dispersive X-ray spectra (EDS) of the platinum nanoparticles.

surface diffusion, so the PtNPs cannot coalesce into the larger atoms (Yong and Tadaoki, 2006). Due to the small size of the PtNPs, there is a strong interaction between the thiol groups and PtNPs and this is one of the advantages of the present study.

3.2. Electrochemical characteristics of the modified electrode

Fig. 2A illustrates the cyclic voltammograms of the bare GC electrode (curve a) and the PtNPs/dsDNA/ MWCNTs/IL/CHIT nanocomposite modified GC electrode in PBS pH=7.4 in the absence (curve b) and presence of 150 mM H_2O_2 (curve c) at scan rate 10 mVs^{-1}. As can be seen in the presence H_2O_2 a high-intensity cathodic and anodic peak current in the region is observed, which corresponds to the electrocatalytic activity of Pt-NPs toward reduction and oxidation of H_2O_2. The same result has been reported in literature [Polsky et al., 2006]. Therefore, in the presence of H_2O_2, immobilized PtNPs catalysis not only H_2O_2 reduction but also its oxidation at reduced overvoltage. Since the intensity of H_2O_2 reduction peak is more than its oxidation peak, the electrocatalytic reduction peak current was used as measuring signal. When the adenosine is dropped on the prepared aptasensor, the aptamer-functionalized PtNPs/adenosine complex is formed, which is more stable than the aptamer-functionalized PtNPs/DNA probe duplex; thus the interaction between adenosine and aptamer-functionalized PtNPs resulted in the separation of the aptamer probe from the electrode surface. So the PtNPs are released from the surface of the electrode and the reduction signal concerned with H_2O_2 decreases.

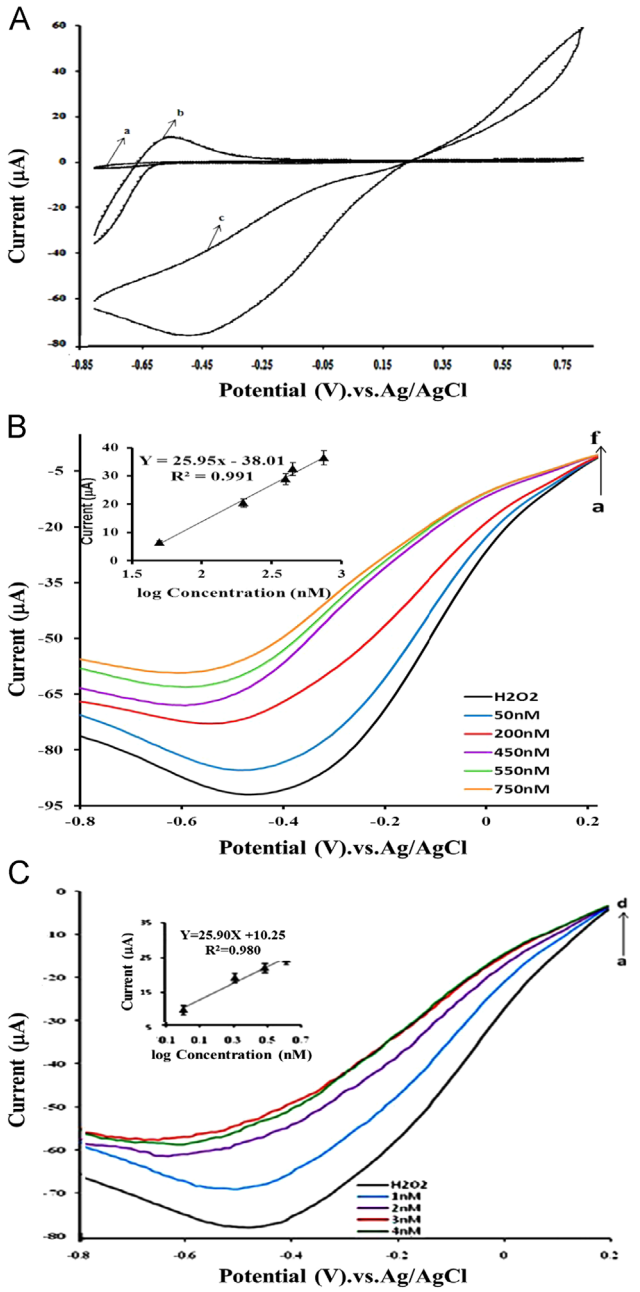


Fig. 2. (A) Recorded cyclic voltammograms of bare GC electrode in 0.1 M PBS (pH 7.4), (a) PtNP/dsDNA/MWCNTs/IL/CHIT nanocomposite modified GC electrode in the absence (b) and presence 150 mM of H_2O_2 (c) At scan rate of 10 mV s^{-1}.(B and C) Recorded voltammograms of Pt-NPdsDNA-modified GC electrode after dropping of different concentrations of adenosine, (B) 0.0, 50.250, 450, 550 and 750 nM and (C) 0.0, 1, 2, 3 and 4 nM in 0.1 M PBS (pH 7.4) containing 150 mM of H_2O_2 at scan rate of 10 mV s^{-1}. Insets: plots of reduction peak current vs. log $C_{adenosine}$ (nM).

Fig. 2B and C shows the voltammetric peak response of the aptasensor in the presence of various concentrations of adenosine. As can be seen with increasing adenosine concentrations at concentration range 1–750 nM, the reduction peak current of H_2O_2 decreases. This means that PtNPs are desorbed from the surface of electrode after reaction with the target adenosine. The plot of the H_2O_2 reduction peak current versus the logarithm of the adenosine concentration is linear over different concentration ranges. The linear equation at low concentrations (1–4 nM) is I/nA=25.90 log [adenosine]/nM+10.25 (R^2=0.980), while for higher concentration ranges of adenosine (50–750 nM) the relationship between peak current and adenosine concentration is I/nA=25.95 log [adenosine]/nM −38.01 (R^2=0.991). Based on S/N=3, the detection limit of 1.0 nM was obtained. Comparing the results obtained with this aptasensor to those reported in literature (Table 1), we could conclude that the detection limit and linear range for proposed aptasensor improved (Table 1).

3.3. Optimization of experimental conditions for aptasensor

In order to obtain the perfect assay performance of the proposed aptasensor, different experimental parameters such as hybridization time, incubation time and concentration of H_2O_2 were optimized. To fabricate a suitable aptasensor, achieving efficiency of hybridization is a very important factor. The incubation time for the interaction between12 bases capture probe (3'-NH_2-$(CH_2)_6$-TCTCTTGGACCC-5' and 32 bases (ssDNA1) adenosine aptamer functionalized PtNPs was experimentally investigated; results show, 4 h is the optimum incubation time. Furthermore, the effect of incubation time between adenosine and aptamer on the current response of the aptasensor was also investigated (Fig. 3A). As illustrated with increasing incubation time from 20 min to 45 min the aptasensor response increased, then starts to level off, therefore 45 min was used as optimum incubation time between aptamer and adenosine in measuring process, which in agreement with previously value for adenosine aptasensor (Shahdoost fard et al., 2013).

The concentration of H_2O_2 was also investigated as another factor that may influence the sensitivity of the adenosine aptasensor. In the absence of adenosine, the aptasensor was tested in 0.1 M PBS (pH 7.4) containing H_2O_2 with different concentrations of 50, 100, 150, 250, 400 mM and scan rate 20 mVs^{-1}. As illustrated in Fig. 3B, the voltammetric peak response increased rapidly with increase in the concentration of H_2O_2 to 150 mM, and then the surfaces of the modified electrode were ruined gradually. Therefore, 150 mM H_2O_2 was chosen as the optimum concentration.

The reproducibility of the aptasensor was estimated by six repetitive measurements of 10 nM of adenosine solution with the proposed system. The obtained signals with acceptable RSD of 3.5% indicated the reproducibility of results. Furthermore, for investigation the reproducibility of the aptasensor fabrication strategy, five PtNP/dsDNA/MWCNTs/IL/CHIT modified GC electrode prepared independently. The RSD of the prepared aptasensors responses toward 10 nM of adenosine was 4.5%, which indicated that the aptasensor fabrication strategy is reproducible.

We also performed the studies of the long-term storage stability of the proposed aptasensor by measuring current response of the system toward 10 nM of adenosine. When the aptasensor was stored at 4 °C, it retained 97% of its initial response after one week and 8% decrease of current was observed after 15 days. These results the covalent attachment of aptamer to the supported MWCNTs-IL-CHIT nanocomposite improved the robustness of the aptasensor and signal response stability.

3.4. Selectivity of aptaensor for adenosine detection

The selectivity of aptasensors is an important factor in evaluation of measuring process. The selectivity of the aptasensor was examined by incubation with cytidine, uridine and guanosine which belong to the nucleosides family and the results are shown in Fig. 4. As illustrated a no recognizable decrease in electrocatalytic reduction signal of H_2O_2 was observed in the presence of 1 μM of non target molecules such as cytidine, uridine and guanosine (Fig. 4A–C), while after dropping 1 μM of adenosine onto the surface of the modified electrode, the reduction current response decrease significantly (Fig. 4D). The results demonstrated that the developed strategy had sufficient selectivity to detect the interaction between the adenosine and the aptamer immobilized on the electrode surface. The data was obtained as shown in the bar chart in Fig. 4E.

Table 1
Analytical parameters for detection of adenosine with different aptasensors.

Electrode	Electrochemical Technique	Detection limit (nM)	Concentration range (nM)
Au/$Ru(bpy)_3^{2+}$–AuNPs[a] (Wang et al., 2010)	ECL[k]	5.0	10–100
Au/ $Ru(bpy)_3^{2+}$–SNPs[b] (Zhu et al., 2011)	ECL	0.03	0.1–5000
Au/SAM/HDT/AuNPs[c] (Feng et al., 2008)	DPV[l]	1.0	5–1000
Au/thiol/Aptamer(Wang et al., 2009)	DPV	10.0	20–2000
Au/AuNPs/DNA/AuNPs (Zhang et al., 2008)	CV[m]	0.18	0.5–4.0
Au/ polytyramine/AuNPs (Wu et al., 2007)	DPV	20.0	100–10000
Au/SAM of Aptamer/MB[d] (Yan et al., 2011)	DPV	10.0	10–1000
Au/ABEI[e]–AuNPs (Chai et al., 2012)	ECL	0.0022	0.005–5
GCE/ILs[f]/MWCNTs[g]/CHIT[h] (Shahdoost fard et al., 2013)	DPV	0.154	0.5–400
GCE/ILs[f]/MWCNTs[g]/CHIT[h] (This study)	LSV[m]	1	1–750
Non-Electrochemical Methods			
Aptamer-AuNPs(Zhang X. et al., 2010)	RLS[n]	1.8	6–115
Aptamer-AuNPs (Chen et al., 2010)	Fluoremetry	5.5	10–200
Aptamer-AuNPs (Zhang et al., 2012)	Fluoremetry	6.0	20–1800
Aptamer/ATMND[i] (Xiang et.al., 2009)	Fluoremetry	3400	0–25,000
Au/FET[j] (Goda and Miyahara, 2012)	Change in gate potential		10–1000

[a] Gold nanoparticles.
[b] Silica nanoparticles.
[c] Self-assembled monolayer of 1,6-hexanedithiol/gold nanoparticle.
[d] Methylene blue.
[e] N-(aminobutyl)–N-(ethylisoluminol).
[f] Ionic liquid.
[g] Multiwalled-Carbon nanotube.
[h] Chitosan.
[i] 2-amini-5,6,7-trimethyl-1,8,naphthyridine.
[j] Field effect transistor.
[k] Electrochemical luminescence.
[l] Differential pulse voltammetry.
[m] Linear sweep voltammetry.
[n] Resonance light scattering.

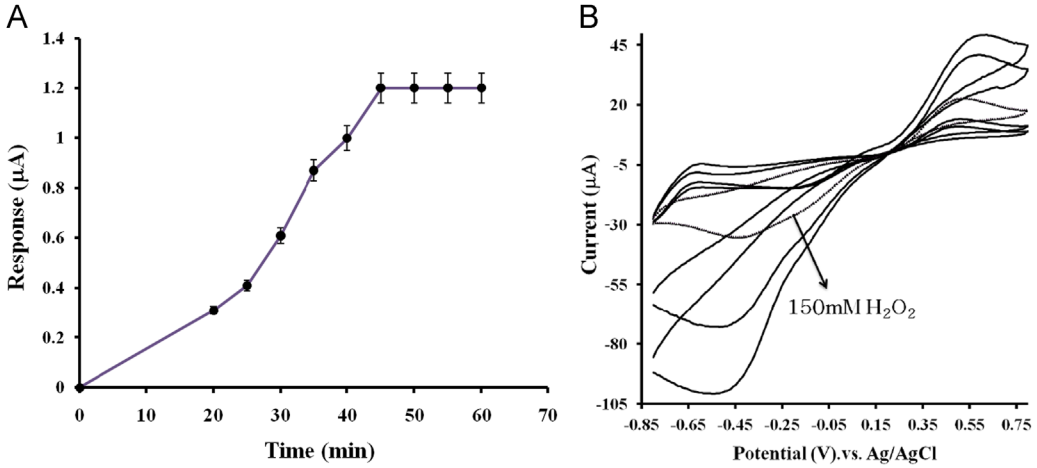


Fig. 3. (A) Influence of incubation time on the current response of the aptasensor. (B) Recorded Cyclic voltammograms of PtNP/dsDNA/ MWCNTs/IL/CHIT nanocomposite modified GC electrode at scan rate 10 mV s^{-1} in PBS pH=7.4 containing different concentration of H_2O_2: (a) 50, (b) 100, (c) 150, (d) 250 and (e) 400 mM.

3.5. Adenosine determination in real samples

The feasibility of the developed aptasensor for practical applications was studied by analyzing a clinical serum sample. The blood serum of a 47 year-old normal man was diluted 25 times with PBS buffer (0.1 M) and used as a real sample, and adenosine concentration was determined using standard addition method. The adenosine concentration in blood serum sample was 73 nM. This reported value for adenosine in normal human blood was in range 50 nM to 0.1 μM (Csoma et al., 2005). These results suggest that the serum did not change the molecular recognition property of the aptasensor. Furthermore, the proposed aptasensor was also used for the detection of adenosine in adenosine ampoules. The measured value for adenosine in this pharmaceutical product was about 3.03 (± 0.05) mg/mL, which is in congruence with the empirical concentration of 3 mg/mL. These results indicate the system presented in this study can be valid for the analysis of adenosine in pharmaceutical product and biological fluids.

4. Conclusion

Based on immobilization of (3'-NH_2-$(CH_2)_6$-TCTCTTGGACCC-5') as adenosine capture probe onto MWCNTs-IL-Chit and thiol-terminated adenosine aptamer (5'-AGAGAACCTGGGGGAGTATTGCGGAGGAAG-GT-SH-3') probe which functionalized with Pt Nanoparticles a sensitive and selective adenosine aptasensor was developed using hydrogen peroxide electrorcatalytic reduction peak current as measuring signal. The proposed system shows a stable and reproducible redox response

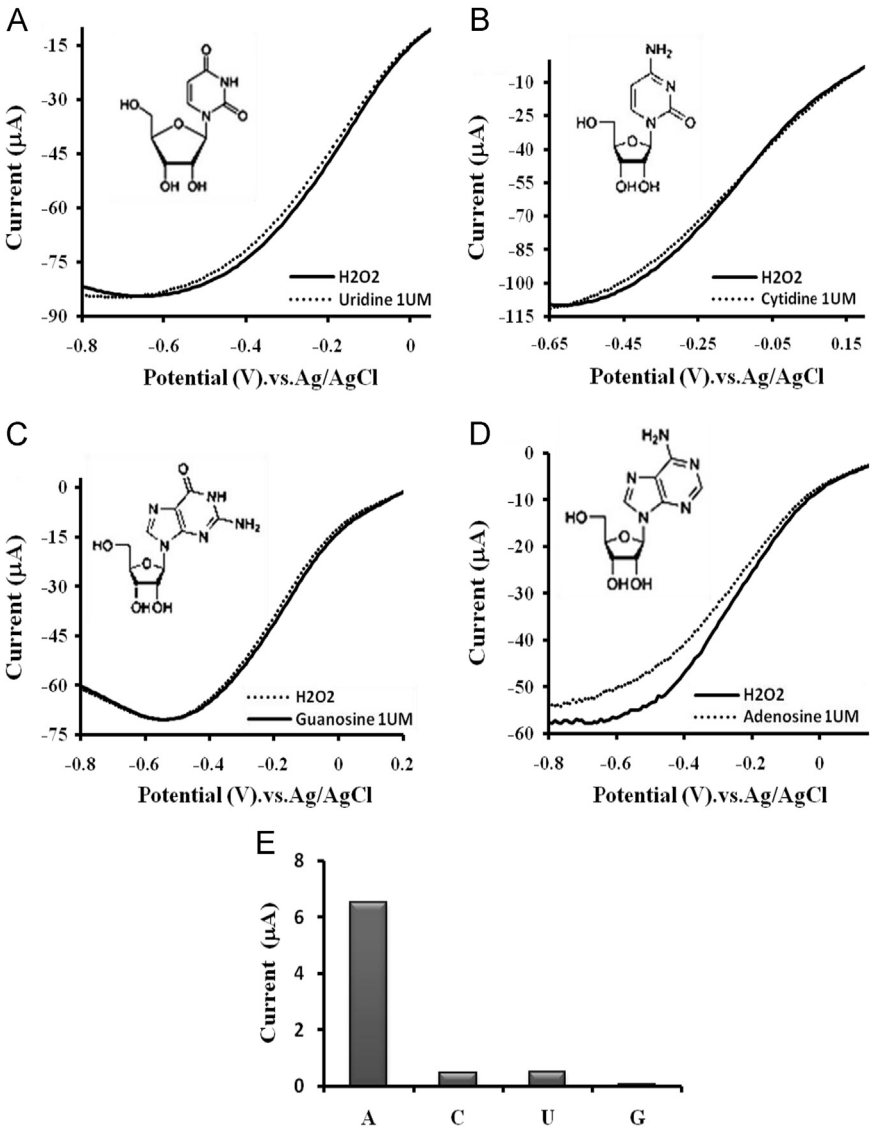


Fig. 4. Cyclic voltammetry responses of Pt-NPs-dsDNA-modified GC electrode after dropping of 1 μM uridine (A), 1 μM cytidine (B), 1 μM guanosine (C) and (D) 1 μM adenosine in 0.1 M PBS (pH 7.4) at scan rate of 10 mV s^{-1}, (E) Histogram of the change of aptasensor response after reaction with adenosine and other nucleoside family.

with long stability and high sensitivity toward hydrogen peroxide reduction. Platinum nanoparticles which functionalized with aptamer as redox labels instead of enzymes or other redox mediators toward electrochemical detection and covalent attachment of the capture probe on the MWCNTs/ILs/CHIT nanocomposite improved the applicability and sensitivity of the aptasensing process. The detection limit of the aptasensor was 1.0 nM and linear concentration range was more than 750 nM. Furthermore, the control experiments showed the high selectivity of this aptasensor due to the specific recognition between the adenosine and its aptamer in compared to other similarly nucleosides such as guanosine, cytidine and uridine. The proposed method has been successfully applied to the determination of adenosine in human blood serum and pharmaceutical formulations. Therefore, it is expected that this approach might have the potential for sensitive determination of other small molecules, and be beneficial in extending the application of the combination of aptamers with nanomaterials.

Acknowledgments

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