

RESEARCH ARTICLE

Hybrid Aptamer/AgNps Modified Electrode for Target Detection of Methamphetamine

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ABSTRACT

A novel method for the determination of methamphetamine was investigated based on a glassy carbon electrode (GCE) treated by aptamer/ silver nanoparticles (AgNPs) hybrid layer. Methamphetamine specific aptamer and AgNPs was immobilized on the electrode surface. For this purpose, the functionalized sequence of the methamphetamine binding aptamer (MBA) was immobilized by the amine group and the covalent bond on the GCE. The binding of methamphetamine to its specific aptamer sequence results in a change in the aptamer spatial structure desired and, as a result, changes the electron transfer kinetic and oxidation peak position. Cyclic voltammetric (CV), differential pulse voltammetric (DPV) and electrochemical impedance spectroscopy (EIS) methods were used to study occurred changes in electrochemical behavior. Presence of silver nanoparticles in the assembly of the electrode improved response time and sensitivity. A linear response to methamphetamine in the 10 nM to 630 nM concentration range was obtained with the detection limit of 1.03 nM (at an S/N ratio of 3) and quantitation limit of 3.126 nM. The proposed sensor represented a promising platform for the selective and sensitive determination of methamphetamine.

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INTRODUCTION

Stimulants are substances that increase alertness and reduce physical and mental fatigue and cause ill-treatment, violence and addiction in arbitrarily uses [1]. Common types of stimuli include natural stimulants such as nicotine, caffeine, cocaine and chemical stimulants.

Chemical stimulants are divided into adjuvant such as methamphetamine, ecstasy and a drug category such as methylphenidate, dexamphetamine, modafinil, ephedrine, etc [2].

Methamphetamine (MTH) is a stimulant of the nerves, which directly affects the brain and creates joy and excitement in the individual. This causes the substance to be misused. MTH use reduces appetite [3], stereotypical behaviors [4], impaired behavior under stimulant control [5-7]. As well as

general sympathetic effects such as hyperthermia [8], increased blood pressure [9], and piloerection [10] are its further side effects.

Also, in consumers, the number of D₂ receptors is lower than that of normal people [11]. Given the increasing tendency to abuse and the fact that overdose uses of the MTH causes seizure, coma and even death, the provision of efficient diagnostic methods is of great importance.

So far, various methods for the detection of MTH including gas chromatography [12], Liquid chromatography [13-14], mass spectrometry [15] Immunoassay [16], high performance liquid chromatography [17], thin-layer chromatography [18], electrochemiluminescent [19], surface ionization [20], Raman spectroscopy [21], solid-phase micro-extraction [22], and surface ionization [23] have been used. These methods have limitation

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of extensive application in sensing elements such as complicated operation, low accuracy, non-specific, time consuming and interference with other materials [24].

Recently, the use of DNA for the detection of metal ions has been increased because of its good stability, high solubility in aqueous solution and high affinity binding toward some metal ions [25]. Aptamers are single standard nucleic acids that mainly selected through the SELEX (systematic evolution of ligands by exponential enrichment) process identify the metal ions using a similar approach of antibodies [26]. These chemically synthesized aptamers interact with metal ions by hydrogen bonding, van der Waals forces and hydrophobic accumulation and form particular structures [27]. For examples, the guanine (G)-rich oligonucleotide aptamer utilized for recognition of Pb^{2+} is converted into a G-quadruplex structure in the presence of lead ions [25].

However, the use of electrochemical biosensors is very attractive for medical diagnosis due to their high sensitivity, easy operation, low cost, portability, simple-to-construct and limitation of interference materials [28-29]. In the electrochemical biosensors, electrode was used to convert biological information into an electronic signal. In an aptamer based electrochemical biosensors, DNA sequence is used as a bio-element to measure the specimen. This specimen specifically linked to aptamer and consequently increases the sensitivity of the sensors for detection of different materials even in biological solutions [30-31].

Meanwhile, high specific surface area of nonmaterial enables immobilization of an enhanced amount of bioreceptor on electrode. Van der Waals forces have been used to conjugate the bio-specific entity onto such nanomaterials [32]. Therefore nano-biosensors preserve all specific properties of both, nanomaterial and biomolecule. Different nanoparticles such as carbon nanotubes [33-34], Au nanoparticles [35-36], Ag nanoparticles [37-38] and Pt nanoparticles [39-40] have been used in nano-biosensors to increase sensitivity and detection limit. But to date no attempts has been made to determine MTH, one of the most important stimulants, using electrochemical nano-biosensors [41].

In this work, for the first time a nano-aptasensor based on silver nanoparticles and apta METH has been treated, developed, optimized and used for electrochemical detection of methamphetamine.

Furthermore, the proposed nano-aptasensor has been employed for the determination of MTH in blood serum and urine samples.

Different electrochemical methods such as cyclic voltammetric (CV) and differential pulse voltammetric (DPV) and electrochemical impedance spectroscopy (EIS) methods used in this research to investigate electrochemical behavior of methamphetamine on GCE. By claiming the advantages of AgNps and methamphetamine-specific aptamers, our research attempt to introduce strong and reliable sensing method that lead us to the result of the rapid, portable MTH detection challenges.

EXPERIMENTAL

Chemicals

Glassy carbon electrode (GC)-2.0 mm diameter was used as working electrode in all electrochemical measurements. Deionized water was used to prepare all the solutions. The blood serum sample was prepared from a personal lab.

Silver nitrate, sodium chloride, potassium chloride, sodium borohydride, disodium hydrogen phosphate, potassium dihydrogen phosphate, N-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) MTH and were purchased from Sigma Aldrich in analytical grade and were used without further purification. Double distilled water (DDW) was used to prepare and dilute all the solutions. The following amino-functionalized aptamer (OD = 51) which has been invented for specific detection of MTH was obtained from Bioner-Germany according to Qiunan et al. (2014): 58-mer aptaMETH, single stranded DNA (5'-NH₂-(CH₂)₆-ACG GTT GCA AGT GGG ACT CTG GTA GGC TGG GTT AAT TTG G-3'). The aptamer was received as a lyophilized solution and was then diluted in 0.001 mL DDW. Serum samples were obtained from Danesh Pathology Center (DPT) in Tabriz.

Electrochemical analysis

The AUTOLB (model: PGSTAT302N) with the Nova 1.8 software package (Eco Chemie, the Netherlands) was used to perform electrochemical tests. The electrochemical three-electrode cell consists of a GCE as working electrode, a platinum wire as auxiliary electrode, and a saturated calomel electrode (SCE) as reference electrode were used for electrochemical measurements. All

experiments were carried out at room temperature. The electrochemical measurements were performed in a phosphate buffer solution (PBS) that contained Na_2HPO_4 (0.14 M), KH_2PO_4 (0.027 M), KCl (0.15 M) and NaCl (0.15 M). A continuous cyclic voltammetric (CV) sweep of 8 cycles with potential ranging from 0.2V to 1.4V versus SCE was performed at a scan rate of 50 mV/s in a PBS (pH= 7.4) and $\text{K}_4[\text{Fe}(\text{CN})_6]$ (0.5 mM) solution. The electrochemical impedance spectroscopy (EIS) was conducted at a single frequency (between 20 to 300 Hz). DPV measurements were carried out in 10mL of 0.01M PBS in pH 7.4 at room temperature.

Synthesis of silver nanoparticles

According to previous reports [38] 5.00 mL of silver nitrate (1.0 mM) aqueous solution was added dropwisely to 15.0 mL of sodium borohydride solution (2.0 mM) under vigorous stirring and room temperature. Following the addition of 2.0 mL of silver nitrate, the solution turned to light yellow. Ultimately, 0.06 g tri-sodium citrate was added to the resulting solution in order to minimize the aggregation of composed AgNPs. The sizes of the synthesized AgNPs were 25 ± 3 nm which has been characterized by transmission electron microscopy.

Electrochemical nano-aptasensor assembly

To have a smooth surface of the electrode α -Alumina slurry with diameter of 0.3 and 1.0 μm was used to sequentially polish of glassy carbon electrode. To eliminate the effects of previous treatments it was sonicated in ethanol and water (%50) for 5 min. Electrochemical activation of the electrode was conducted over potential range from 1.5 to 2.0 V by scan rate of 100 mV/S in PBS (pH 7.4). Thus, carboxylic acid groups were generated on the surface of GCE and activated by plunging in a solution containing 8 mM NHS and 5 mM EDC for 1 h. Then the electrode was completely rinsed with the buffer solution.

To prepare aptamer modified electrode, 20 μL of amino labeled aptamer solution (1 μM of aptamer in PBS with pH 7.4) was dropped on the electrode surface and kept upside-down. Then the electrode was covered by a plastic hat and held for 20 h at 4 °C. The modified electrode needs to wash with PBS solution for a couple of minutes before using. After that, the electrode was incubated in various concentrations of MTH for 1 hour and rinsed with PBS solution after incubation. At least, repeatedly

20 μL of amino labeled aptamer (1 μM) was dropped on the electrode to accomplish the sandwich format layers (GCE/MBA/MTH/MBA). Finally rinse with PBS solution was conducted to remove nonspecific adsorption of MBA (methamphetamine binding aptamer).

For construction of AgNps/MBA probe, firstly 30 μL of the solution including 8mM NHS and 5mM EDC in 0.1M PBS solution was dropped on the AgNPs to activate its citrate layers by carboxylic groups. Then 20 μL of apta-METH was dropped to the surface of Ag-NPs to attach the activated carboxylic groups. This construction (AgNPs/MBA) was then enforced to 20 μL MTH solution for 1 h. By dropping 20 μL of the secondary MBA layer into this solution, functionalization with the secondary MBA was completed.

A stock solution of 1 μM of MTH solution was prepared in blood serum and diluted with PBS solution (pH 7.4) to 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 nm. Then they were used to analyze presented nano-aptasensor.

RESULTS AND DISCUSSION

Sensing scheme

The nano-aptasensor combination with MTH is shown in scheme1 step by step. EDC (1-Ethyl-3- [3-dimethylaminopropyl] carbodiimide hydrochloride and sulfo-NHS were used to activate carboxylic groups on the surface of the GCE and AgNPs. An amine-reactive sulfo-NHS ester was formed during interaction with sulfo-NHS, which increase the efficiency of EDC-mediated coupling reactions. Higher surface area of silver nanoparticles introduces higher carboxyl groups to create in nanoparticles surface. Thus the number of aptamers connected to electrode and MTH molecules increase consequently. As well as, GCE/AgNPs conjugate could enhance the electron transfer between the redox probe and electrode surface and electrochemical signal of electrode [42]. Along with the addition of MTH, a conformational change of aptamer was occurred and aptamer/target conjugate was created with a greater binding constant. So, addition of AgNPs on the surface of electrode, leads to higher sensitivity of the modified nano-aptasensor toward MTH detection [43-44].

Function of the modified electrochemical nanoaptasensor

The electron transmission procedure and

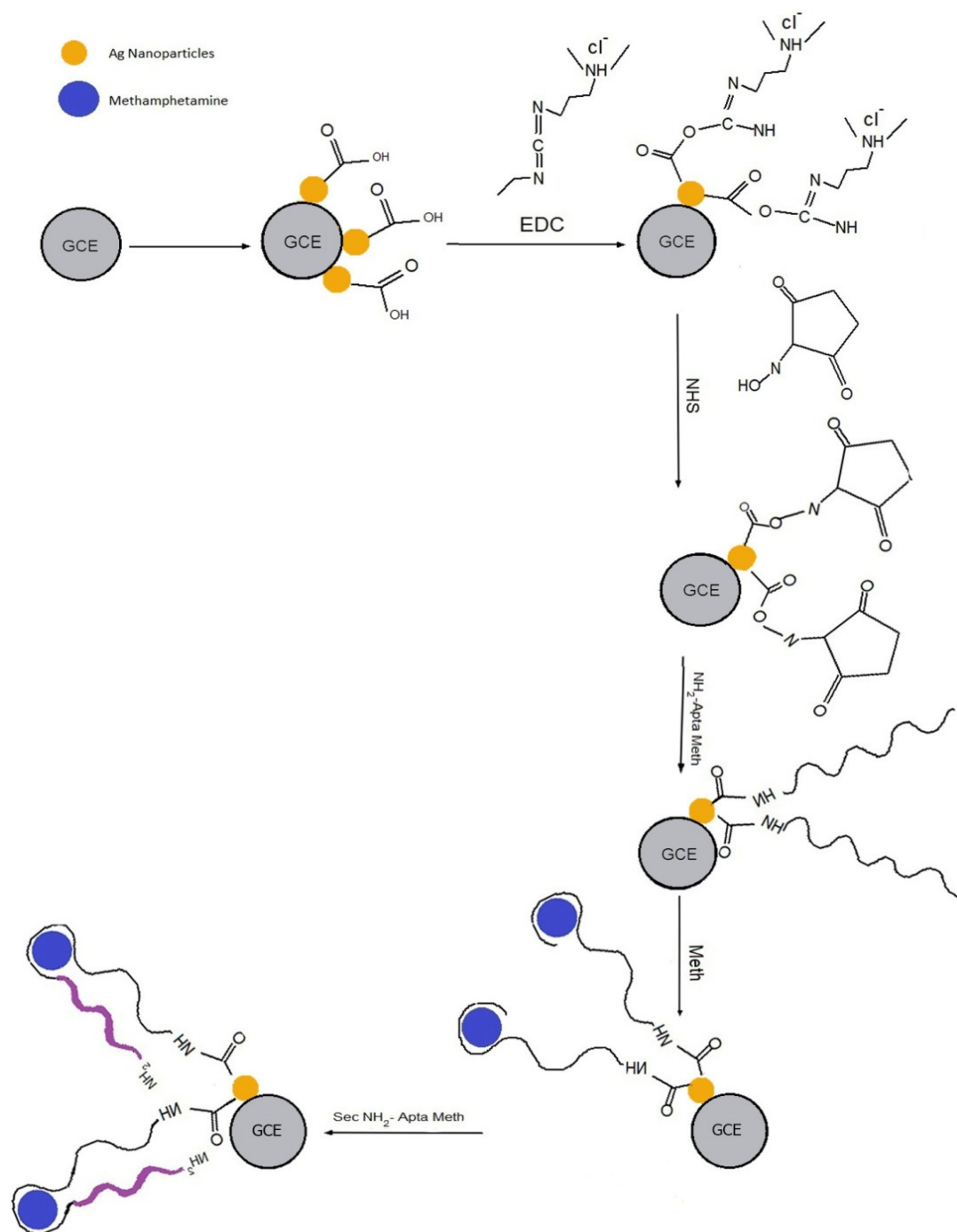


Fig. 1. Schematic description of MTH detection based on electrochemical nano-aptasensor.

sensing interface in modified nano-aptasensor was monitored using CV method. Electrochemical characteristics were studied in 0.01 M PBS (pH 7.4) containing 0.5 mM of KCl, EDC and NHS solution in presence and absence of MTH. As shown in

figure 2(A) a maximum redox peak was observed for AgNPs/GCE electrode which indicated high capability of electron transfer in electrode surface. After immobilization of aptamer probe on the electrode, the redox peak current was decreased in

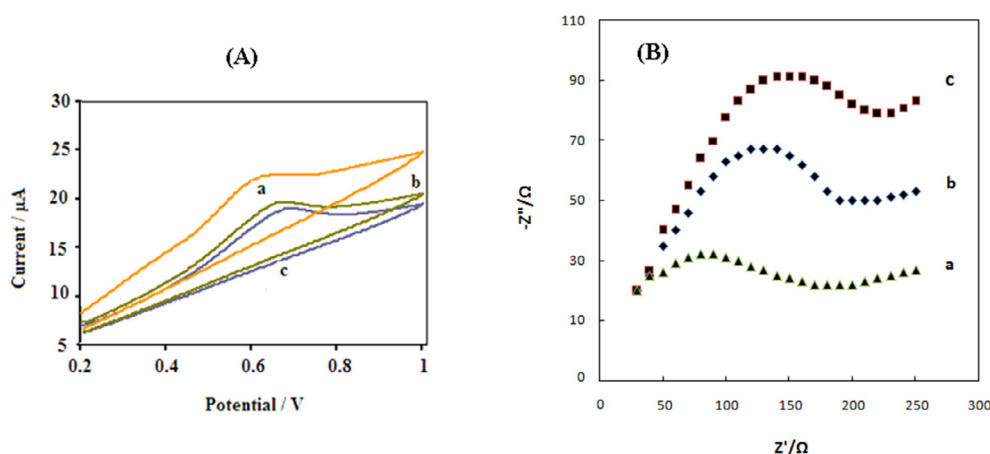


Fig. 2. CVs (A) and Nyquist plots (B) of different modified electrodes in 0.01M PBS (pH= 7.4) solution with 0.5 mM KCl. (a) GCE/AgNPs, (b) GCE/AgNPs/MBA, (c) GCE/AgNPs/MBA/MTH modified electrode.

figure 2(A) b. This indicated that modified aptamers blocked the electron transfer of the electroactive prob. In the presence of MTH, three-way junction between the aptamer and MTH was form and leads to voltametric peak response being further decreased (figure 2(A) c). In fact, the configuration of aptamer was changed in the presence of MTH and steric hindrance was increased in the surface of electrode.

EIS technique is a highly sensitive method for study the folding and surface properties of aptasensor. The magnitude of the electron transfer resistance could be altered in the presence of target due to the specific and unique three dimensional conformation of aptamer for specific target [45]. When the shape of aptamer changed, the charge transfer resistance increases consequently [46]. The folding capabilities of aptamer after binding to MTH verify high affinity of aptamer to its target. Figure 2B shows the Nyquist diagrams at GCE/AgNPs (curve a), after hybridization with aptamer (curve b) and after incubation of MTH (curve c). As it is observed, the R_{ct} increased for curve b and c due to the hindrance and repulsive effect on the electrode response. It is indicated that the aptamer has been successfully fixed on the surface of GCE electrode.

Optimization of experimental conditions pH

pH of the analyte is one of the main quantities affected the performance of MTH nano-aptasensor. 7-In the context of sensing and drug release, it would be especially advantageous to have aptamers

with affinity modulated by environmental pH. Ricci group designed a cocaine-binding aptamer that incorporates a pH-dependent triplex and were able to modulate the affinity of the aptamer for cocaine through pH changes. Thus the pH dependence affinity is one of the advantages of a aptasensor, which increase its specificity in detection [47].

Electrochemical behavior of the sensor was studied in 1 μM MTH and 0.01M PBS containing 0.5 mM KCl at pH values between 3.5 and 9.5 and scan rate of 50 mVs^{-1} (figure 3). It is observed that the anodic peak potential of proposed nano-aptasensor increased with increasing pH value up to 7.5 and decreased in further values. Thus, pH of 7.5 was selected as the optimum point of pH for MTH sensing.

Incubation time

The optimal reaction time for aptamer with MTH was chosen according to the current responses obtained after various incubation time. For this purpose, the effect of different incubation times 5 to 30 minutes on the current response in constant concentration of analyte was studied (figure 4). The results show that the current responses increase with incubation time up to 25 minutes and then tended to level off and reached a plateau. This indicated that 25 minutes was the efficient time for self-assembly of supramolecular complex of aptamer-target and confined by inherent association constant of the aptamer-target.

Effect of scan rate

Electrochemical behavior of MTH in solution

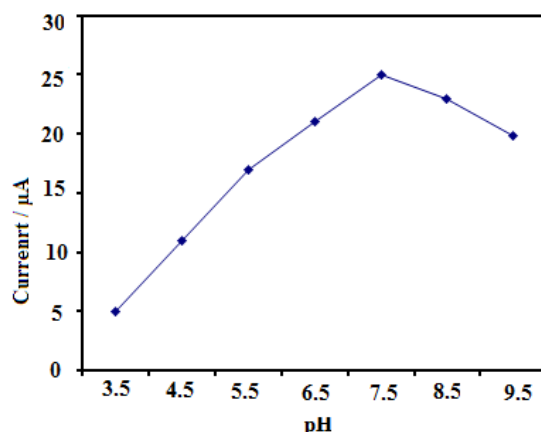


Fig. 3. DPV of 1 μM MTH in PBS containing 0.5 mM KCl and different pHs of solution.

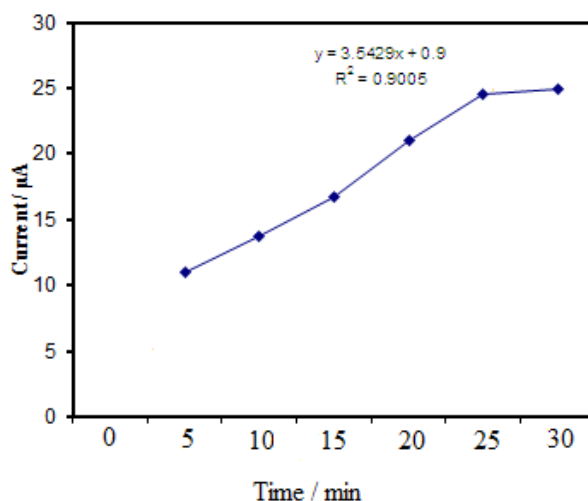


Fig. 4. (A) Effect of incubation time on GCE/MBA/MTH/MBA nano-aptasensor response.

can be obtained from the relationship between peak current and scan rate. Figure 5A shows voltammetric behavior of MTH at different scan rate investigated using CV method. It can be observed that the oxidation peak potential is positively shifted with scan rate and the peak current increased linearly with the increase of scan rate. The change in the intensity of cranial oxidation peak of MTH in terms of scan rate was linear with r square of 0.998 (figure 5B). Electrochemical behavior of MTH on the electrode surface was obtained from the relationship between square root of scan rate and peak current. A linear relationship in the range of 10–60 mV/s and potential range of 0.2–1V was observed for MTH, which is of a typical diffusion controlled reaction [48–49].

Analytical characteristic

Calibration curve of aptasensor

The DPV responses of the modified nano-aptasensor were recorded in different concentration of MTH under the optimum condition. The concentration dependence of MTH was measured in the range of 10 nM to 10 μM and presented in figure 6A. The calibration curve was obtained based on the information of figure 6A and shown in figure 6B. A linear response was observed in the range of 10 nM and 10 μM based on equation of $I_p(\mu\text{A}) = 2.9643X(\text{nM}) + 1.6107$, $R^2 = 0.9874$. The detection limit of 1.03 nM and quantitation limit of 3.126 nM were calculated using calibration curve. Accordingly, the proposed aptasensor are able to detection of MTH with high sensitivity and a low

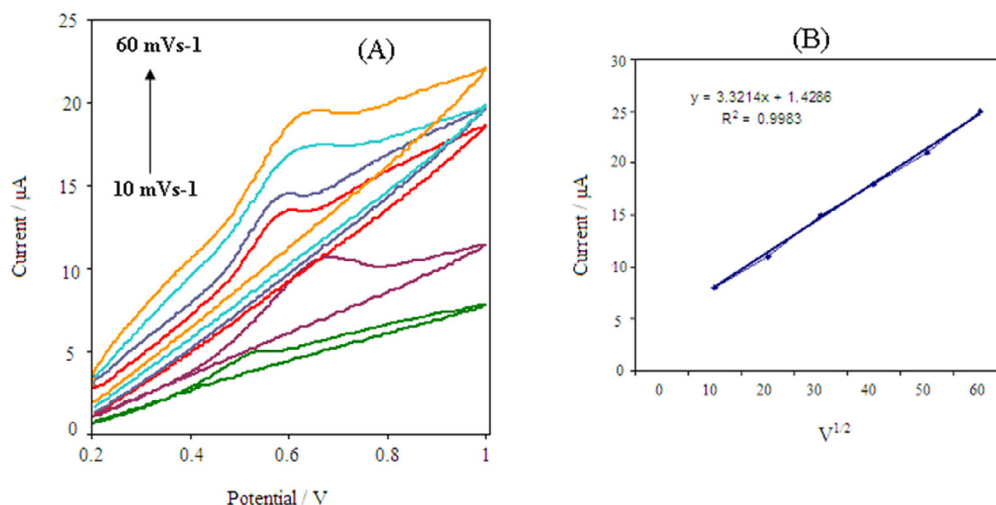


Fig. 5. CVs for the oxidation of MTH at different scan rates (1) 10 (2) 20 (3) 30 (4) 40 (5) 50 (6) 60 mVs⁻¹ (B) Changes in the intensity of cranial oxidation peak of MTH in terms of scan rate.

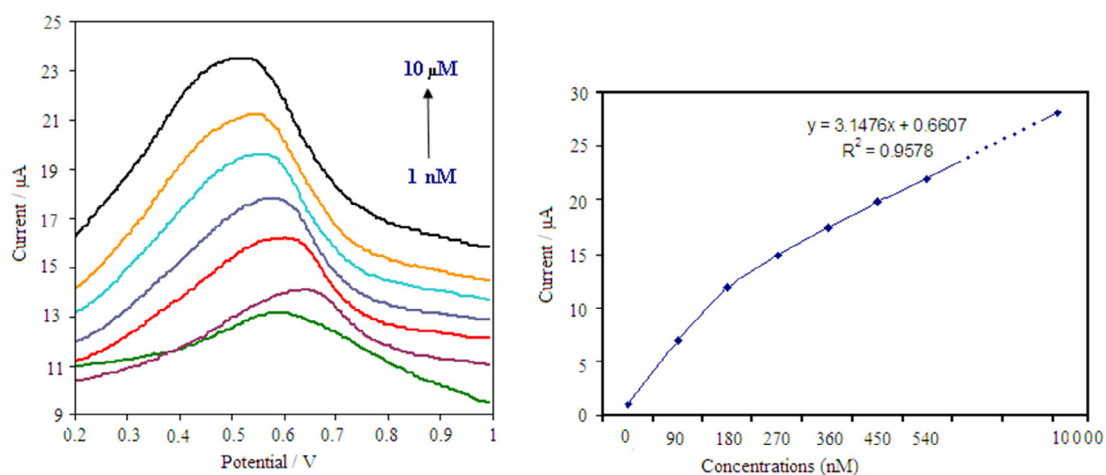


Fig. 6. DPV of MTH in the PBS and different concentration range of 10 nM to 10 μM, (B) concentration dependence of MTH.

detection limit.

The related performances of proposed nano-aptasensor were compared with other MTH aptasensors with various transduction methods including electrochemiluminescent, electrochemical, bioelectrochemical, colorimetric, fluorescent, gas chromatography (Table 1). The result demonstrated that the proposed electrochemical nano-aptasensor has a high sensitivity and fast analysis of MTH compared to the other recently reported methods. Label-free EIS methods offers advantages for miniaturization and integration into portable devices.

The excellent performance of the sensor

proposed for investigating specific urine samples demonstrates its potential clinical and forensic applications. Table 2. Presented the comparison of the analytical performance of the developed aptasensor with other reported methods for methamphetamine detection.

Specificity of aptasensor

The selectivity of a sensor to specific target is the main characteristics which facilitate its application in human serum with multiplicity of materials. The specificity of the proposed sensor was examined by DPV in the presence of MTH and two interfering agents: cocaine and codeine

Table 1. Analysis of MTH in human serum samples

Transduction method	LOD (nM)	LRD (nM)	Reference
Electrochemiluminescent	200	$500-10^6$	(50)
Electrochemical method	50	$70-80 \times 10^4$	(51)
Bioelectrochemical method	6000	$60 \times 10^3-67 \times 10^3$	(52)
Colorimetric and bare eye determination	820	$10^3-2 \times 10^4$	(53)
Fluorescent	50	$230-54 \times 10^3$	(54)
Gas chromatography	80	$270-230 \times 10^3$	(55)
Electrochemical aptasensor	1.03	$10-1 \times 10^4$	This method

Table 2. Analysis of MTH in human serum samples

Samples	Added (nM)	Found (nM)	Recovery (%)
Blood	1	280	98
	2	200	103
	3	250	100
Urine	1	280	99
	2	200	105
	3	250	101

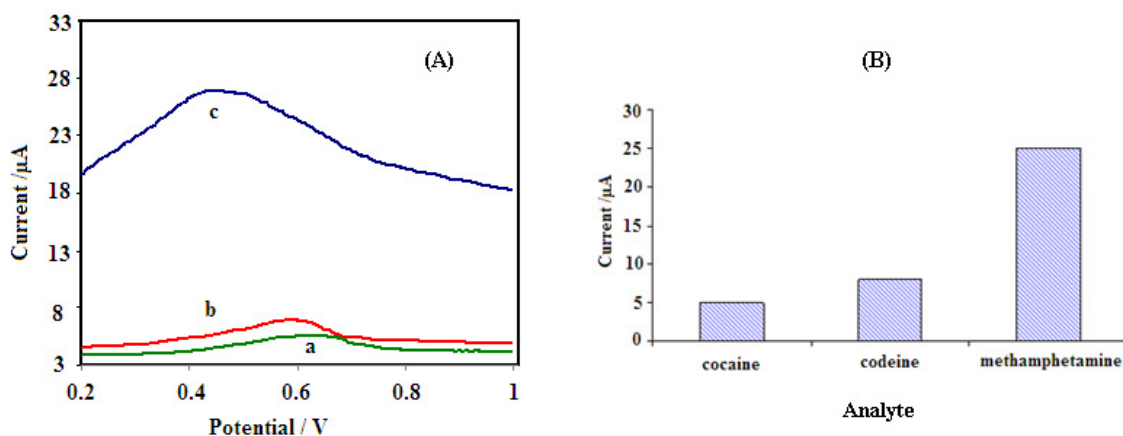


Fig. 7. (A) DPV peaks of the modified electrode in the presence of (a) cocaine (b) codeine and (c) MTH; (B) typical histogram of current intensity versus analyte type.

under optimized conditions. The result presented in figure 7(A)c indicated that an aptasensor incubated with 1 μM MTH produced a relatively high peak current response. The figure 7(A)b and fig.6(A)a, were obtained after incubation with 10 μM codeine and cocaine. Figure 7(B) shows typical histogram of current intensity versus analyte type for interferences. As it observed, the presence of interferences with higher concentration showed almost negligible electrochemical response. This indicated insignificant cross reactivity of proposed nano-aptasensor for other MTH metabolites and specific interaction by target molecule. Thus, modified nano-aptasensor with high specificity

toward MTH could be used in biological samples.

Reproducibility and stability of aptasensor

After incubation of five proposed nano-aptasensor in 1 nM MTH, the responses of current were similar with standard deviation of 4.01%, which demonstrates the good reproducibility of the aptasensor. Then the proposed nano-aptasensor was stored for 10 days and electrochemical measurement was conducted every 2 days for stability study. The current response with 87% of initial detection was obtained after storage time. That is confirming good stability of proposed nano-aptasensor during storage time. This decrease in

current response can be related to the adsorption of AgNPs on the amine groups of some compounds through Ag-NH₂ bond, which is very stable [56]. Thus, the reproducibility and stability of the nano-aptasensor were improved by combination of nano-aptasensor and silver nanoparticle [57].

Analysis in blood serum

In order to evaluate the effectiveness of the modified nano-aptasensor for determining MTH in biological samples, the sensor was used to MTH analysis in human blood serum by standard addition method. Detection was conducted in blood and urine serum, repeated for three times and presented in Table 2. The result of the recovery for the spiked samples was in the range of 98-105% which indicated that the detection procedures were free from interrupting by the serum samples matrix. Thus, the proposed nano-aptasensor has good potential for practical application of MTH analysis in real clinical samples.

CONCLUSION

In this paper, a novel label-free electrochemical nano-aptasensor for ultrasensitive detection of MTH was constructed. AgNPs and aptamer were directly immobilized on the activated GCE as a MTH capture. Different voltammetric techniques (CV, DPV and EIS) were used for the optimization and analysis. The effect of different kinetic parameters for electrochemical oxidation of MTH on modified nano-aptasensor was investigated. The limit of detection for MTH was improved as low as 1.03 nM compared with other reported sensors. It was attributed to that the silver nanoparticles could enhance the surface activation of the electrode to accelerate the aptamer combination with electrode. Moreover, the proposed nano-aptasensor was effective and applicable for detection of MTH in blood serum samples. Future work should focus on developing other nanomaterial-based sensors and integrating them into portable devices for in-situ testing, as well as investigating the potential application of this biosensor in other stimulant samples.

Despite high sensitivity and selectivity, these time-consuming techniques require expensive instrumentation and well-trained experts

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest related to the research, authorship, or

publication of this manuscript. All authors have disclosed any financial or personal relationships that could potentially influence or bias the work presented.

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