Progress in Chemical and Biochemical Research

Journal homepage: www.pcbiochemres.com

Original Research Article

A Simple Specific Dopamine Aptasensor Based on Partially Reduced Graphene Oxide–AuNPs composite

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ARTICLE INFO

Article history

Submitted: 2023-01-14
Revised: 2023-02-21
Accepted: 2023-03-26
Available online: 2023-04-05
Manuscript ID: PCBR-2301-1245
DOI: 10.22034/pcbr.2023.381280.1245

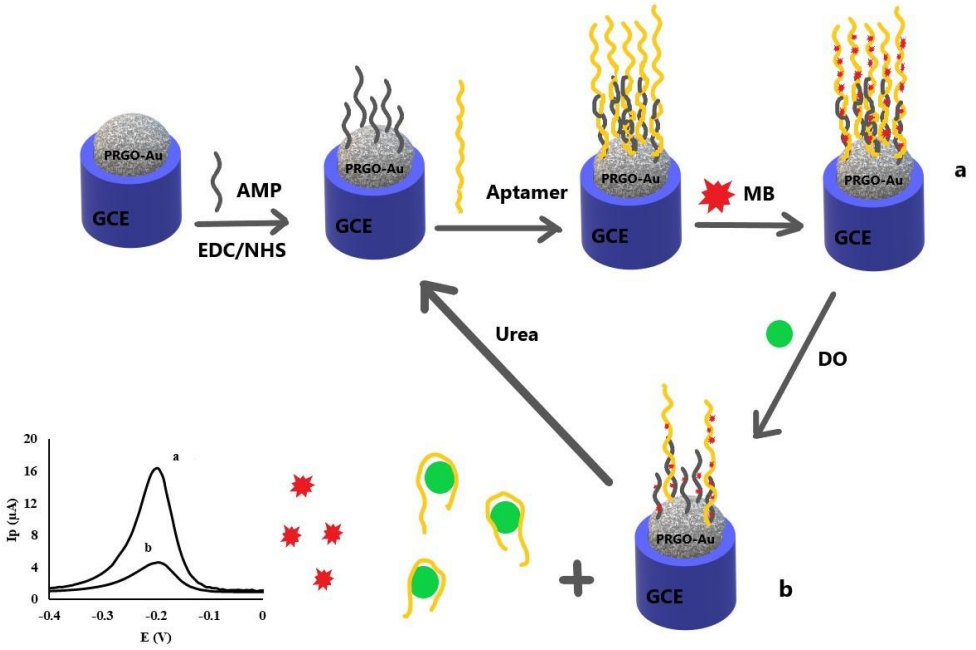
KEYWORDS

Nanocomposite
Parkinson
Electrochemical Aptasensor
Graphene Oxide

ABSTRACT

Lack of dopamine, which is a neurotransmitter in the brain, causes diseases such as Parkinson. Therefore, in order to diagnose and prevent these diseases, it is important to accurately measure the amount of dopamine. Aptasensor is one of the most sensitive and selective measuring tools for this purpose. In this research, a modified electrochemical sensor (Apt-AMP/AuNPs-PRGO/GCE) by nanocomposite was designed for highly accurate and selective measurement of dopamine. The results of the experiment using FTIR, SEM, and CV methods show a very favourable modification of the electron surface. Methylene blue dye was used as an indicator in this experiment and the maximum concentration and interaction time for this dye were optimized at 50 µM and 15 minutes. The electrode designed using the DPV method was able to identify and measure dopamine with a detection limit of 120 pM and a very high sensitivity compared with other compounds with the same structure.

GRAPHICAL ABSTRACT



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1. Introduction

Synthetic single-stranded DNA or RNA oligonucleotides produced by SELEX (systematic evolution of ligands by exponential enrichment) from a pool containing a huge number of random sequences are known as aptamers [1]. Like antibodies, these molecules show high binding affinity and outstanding selectivity. Although they have several advantages compared with antibodies. For example, aptamers are cheaper to synthesize than antibodies, the selection process is done *in vitro*, and they are smaller in size. They are also inherently more resistant to degradation and can be reversibly denatured. Therefore, it may be better than antibodies in similar fields [2].

In recent decades, aptasensors have been a powerful molecular recognition tool in drug delivery and design, especially analytical assays [3]. A unique feature of the aptasensor is being selective and specific for a large variety of molecules like proteins, enzymes, antigens, antibiotics, toxins, drugs, viruses, and whole cells. Various techniques, including colorimetry, fluorescence measurements, surface plasmon resonance (SPR), quartz crystal microbalances (QCM), and electrochemical techniques, have been used to detect binding events between aptamers and specific targets [4-7]. Compared with these methods, electrochemical aptasensors have benefits such as fast response, high sensitivity, simplicity, and reusability. These preferences make them an ideal sensor for detecting a variety of small targets[8,9].

Dopamine is a neurotransmitter in the brain that belongs to the catecholamine and phenethylamine families. Dopaminergic signalling is associated with reward-motivated behaviour and motor control and impairment in its function causing to make many diseases. For example, degenerative Parkinson's disease is caused by the loss of dopamine-secreting neurons, leading to motor impairment [10]. Therefore, concerning the mentioned points about dopamine, its precise level in the body is critical.

Graphene is one of the nanostructures which has attracted special attention because of its outstanding properties including electronic, optical, thermal, and mechanical properties [11]. Some results indicate that graphene has workability problems because of the irreversibly restacking of layers; this phenomenon is due to πi-πi interactions and Van der Waals forces.

There are two reasons behind the addition of conducting nanostructures like gold nanoparticles. The first reason is to avoid the graphene sheet from agglomerating in the dry state, and the other is to improve the catalytic properties through synergistic effect [12,13]. There are two main methods for synthesizing Au nanoparticles (AuNP) and graphene sheet composites. The first approach uses an intermediate linker between AuNP and graphene [14]. The main bug of this method is the reduced conductivity of composite as a result of the presence of insulating material. The second method is the simultaneous reduction of graphene oxide and Au salt for the *in-situ* synthesis of graphene-AuNP composites.

Cui and Zhang measured epinephrine levels using an electrochemical sensor modified with a graphene-AuNP composite[15]. The second method presents some challenges, such as the morphology, distribution of synthesized AuNP, and the need to control the reduction process. However, the main problem is the lack of adequate processing power. Inappropriate workability is due to the physical form of the resulting composite, but biosensor applications require dispersed materials. In this approach, as mentioned above, graphene oxide sheets should not be interbedded with metal nanoparticles [16].

Here, we used the method developed by Kazami *et al.*[17]. In this approach, by reducing graphene oxide under optimal conditions, graphene sheets are made, preserving both their conductivity and

some properties that enable aptamers to communicate with electrodes. Stable colloids can be formed by maintaining the functional groups, and AuNP and PRGO can be mixed uniformLy without polymer additives. As an added benefit, colloidal stability also makes PRGO-AuNP more easy to process and cast into glassy carbon electrodes (GCE). In this study, an exact method was used to synthesize AuNPs and PRGO by reducing in HAuCl4 with sodium citrate, separately. Electron transfer kinetics is enhanced by the reaction between the amino portion of the aptamer and the carboxyl group of PRGO.

2. Materials and methods

2.1. Reagents

Dopamine (DO), hydrazinium hydroxide (N_2H_4), and graphite powder were acquired from Sigma-Aldrich Company and used without further measures. Other chemicals were bought from the Merck Company. All the solutions were made with double distilled water. All the experiments were done at room temperature. The specific sequence of aptamer was obtained through the others' paper [18]. All synthetic oligonucleotides were supplied by Takapoozist (Iran), prepared with TE buffer (pH 8.0), and kept at 4 °C. Phosphate buffer solution (PBS) containing 0.1 $mol.L^{-1}$ NaCl (0.01 $mol.L^{-1}$, pH 7.4 PBS) was utilized as the supporting electrolyte. The base sequences of aptamer probe and capture probe (AMP) were as follows, respectively:

5′CCTGCCACGCTCCGCAAGCTTAGGGTTACGCCTG CAGCGATTCTTGATCGCGCTGCTGGTAATCCTTCT TTAAGCTTGGCACCCGCATCGT3′.

5′NH2-$(CH_2)_6$-ACGATGCGGGTGCCAAGCTT3′.

2.2. Apparatus and methods

Autolab Type 204 potentiostat/galvanostat was used for voltammetric measurements. A modified glassy carbon electrode (Metrohm electrode 3 mm diameter) as working electrode, an Ag/AgCl electrode as reference electrode, and Pt wire as a counter electrode, were the three electrodes in this system. The lambda 25 UV-Vis spectrophotometer (Perkin Elmer Lambda 25) was used for recording the absorption spectra. The synthesized PRGO and AuNPs were characterized by FTIR spectroscopy (model ALPHA, Bruker, Germany), FE-SEM (model MIRA III, TESCAN Co., Czech).

2.3. Synthesis of GO and PRGO

The known modified Hummer method was used to synthesize the Graphene oxide [19]. Briefly speaking, a mild pre-oxidation was carried out in this condition when graphite powder (1 g) and $NaNO_3$ (0.5 g) were mixed in H_2SO_4 and stirred for 30 minutes. The next step is forming a paste by adding 4 g of $KMnO_4$ to the abovementioned solution (T: 30 °C, during time: 120 min). To produce graphite oxide, 200 mL of H_2O and 17 mL of H_2O_2 (30 wt.%) solution were added to the mixture for complete oxidation. After centrifuging and rinsing with water and 1.0 M HCl (3 times), this product was applied to create a brown suspension.

For the last step of the synthesis, the dialysis process was applied for the complete elimination of remaining salts and acids. Next, the purified-graphite oxide suspension was dispersed in water to make 0.05 wt% dispersion and exfoliated to GO by ultrasonication for 30 min. The acquired brown dispersion was then introduced to a centrifuge (30 min, 3,000 r.p.m.) to eliminate any unexfoliated graphite oxide.

The second part is chemical reduction of graphene oxide to prepare the partially reduced graphene oxide (PRGO) [20]. Briefly, 85 mL of graphene oxide dispersion (0.5 mg/mL) reacted with N_2H_4 (0.06 mL, 55% wt. in water). Then, to convert the carboxylic acid groups into the carboxylate form, ammonia solution (28% wt. in water) was added to graphene oxide. After that, the solution was put on a magnetic stirrer (10 min) and a water bath (95 °C, 1 h), respectively. The final stage is removal the black precipitates to obtain the stable PRGO dispersion.

2.4. Gold nanoparticles synthesis and making its composite with PRGO

Detailed information about this section can be found in the supplementary material.

2.5. Fabrication steps of Aptamer/AMP/ PRGO–AuNP /GCE aptasensor

Detailed information about fabrication steps section can be found in the supplementary material.

For determination stage, the fabricated electrode was initially incubated with a defined concentration of methylene blue (MB) (60 µM) for 30 min to accumulate the MB indicator. After washing the electrode with PBS, it was incubated with different concentrations of Dopamine. Differential pulse voltammetry technique was applied to detect the decrease in MB accumulation on electrode surface.

3. Results and Discussion

3.1. Characterization of PRGO-AuNPs

One of the ways to detect and identify the presence of various functional groups in the structure of compounds is FT-IR spectroscopic technique. FT-IR results can be found in the supplementary material. The UV-Vis absorption method was also applied for GO's reduction process. The C-C band π–π* transition reflected in the absorption peak around 230 nm characterizes conjugated carbon atoms. A absorption peak of 268 nm is indicated in Figure 1 indicating reduced graphene oxide with maintained conjugated bonds in carbon structure[21]. Previously reported more red shifts up to 280 nm for a maximum absorption peak for completely reduced graphene oxide, this product can be therefore viewed as incomplete reduced GO with a partial restoration of the conjugated bonds coupled with a partial retention of oxygen functional groups [21].

In our final sample, DNA strains can find suitable locations on conjugated bonds and remaining functional groups. Reduced GO due to its high stability has prominent properties such as good processability, considerable electrical behaviour, and making a uniform solution with a diversity of compounds. UV-Vis spectroscopy was applied to characterize the synthesized AuNPs and PRGO-AuNP colloids. A plasmonic band around 540 nm confirms the presence of a colloid solution of spherical non-aggregated AuNPs (Figure 1C) [22].

Furthermore, PRGO and AuNPs exhibit distinctive absorbance bands (526 nm and 275nm for AuNPs and PRGO, respectively). As a result of a little more reduction of PRGO after exposure to sodium citrate, the distinguished band may shift from 256 to 264 nm.

The morphology analysis of the PRGO-AuNP was done by SEM (Figure 2). SEM results illustrate, to some extent, the homogeneous distributing of AuNPs on the PRGO surface.

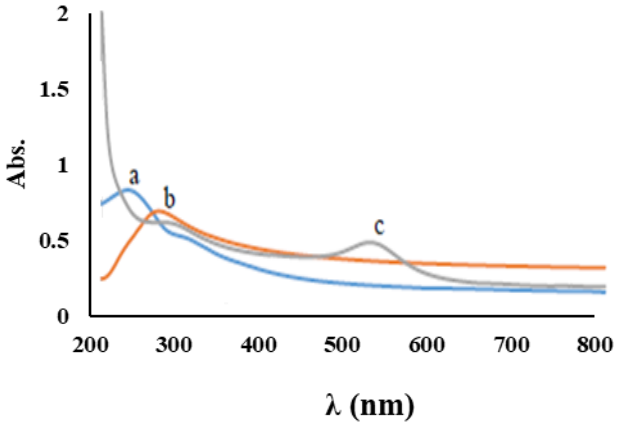

Fig. 1. UV-Vis spectrum of A) GO, B) PRGO, and C) AuNPs-PRGO composite.

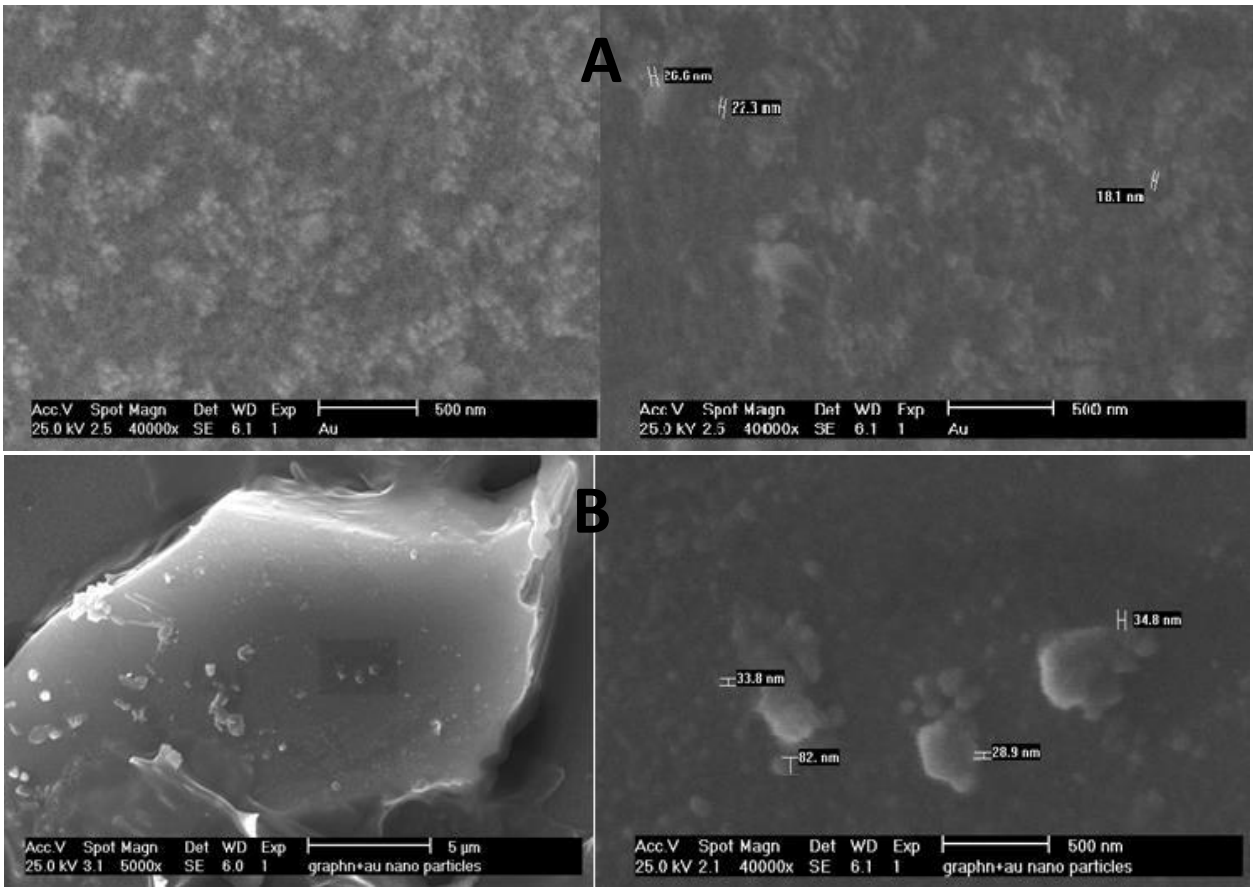

Figure 2. SEM images of (A) AuNPs and (B) AuNPs-PRGO composite

3.2. Characterization of the distinctive layers on electrode surface by electrochemistry

Analysing the properties of the modified electrode surfaces by Cyclic Voltammetry (CV) is a powerful method to confirm the modifying stages. This method relies on different charge transfer values for different modified electrodes which reflect the surface properties throughout the aptasensor construction.

As illustrated in Figure 3, the step-wise modification stages of the aptasensor were studied by the CV method in 5.0 mM $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl. A CV curve (curve a) was recorded of bare electrode and its reversible redox behaviour was observed. When GO is added to bare electrodes, the CV current is decreased (curve b), shows that negative charge of oxygen atoms in functional groups inhibits electron transfer between electrodes and solutions. By modifying the electrode with PRGO, the peak current of $[Fe(CN)_6]^{3-/4-}$ has amplified compared with the state (b). In PRGO, electron transfer on the electrode surface became easier due to the regeneration of some functional groups (curve c).

By introducing gold nanoparticles into the electrode surface, the surface area of the electrode increased, allowing faster and easier electron transfer. The peak current increased significantly (curve d) by modifying the electrode with PRGO-AuNPs. Due to the facilitation of electron transfer, we can also see a slight shift towards the more negative potential. This phenomenon contributes to the excellent conductivity performance of the gold nanoparticle and its synergic effect. Following this, the CV of the AMP/Au-PRGO/GCE (curve e) decreased marginally because the DNA and the $[Fe(CN)_6]^{-4/-3}$ with negative charge are repelled by each other.

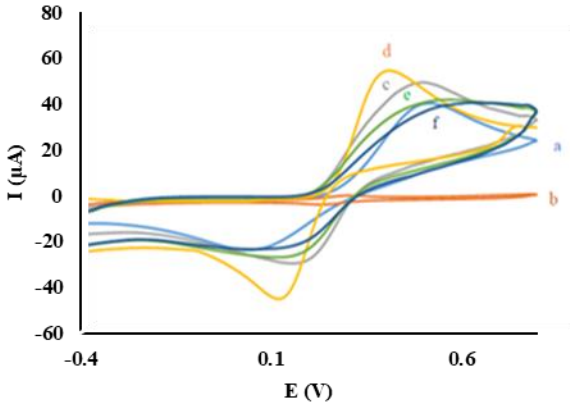

Fig. 3. Cyclic voltammetry of: (a) Unmodified electrode, (b) GO modified electrode, (c) PRGO modified electrode, (d) PRGO-AuNPs modified electrode, (e) AuNPs-PRGO/AMP modified electrode, and (f) modified electrode with AuNPs-PRGO/AMP/Aptamer (5 mM $[Fe(CN)_6]^{-4/-3}$ and 0.1 M potassium chloride solution (scan rate = 0.1 U/s)).

Following hybridization between aptamer and AMP on the surface, more decrease in current of peak was found. This observation is a result of repulsion between negatively charged aptamer and AMP (curve f).

3.3. Setting up the best experimental conditions

To investigate the hybridization process between aptamer and AMP at the biosensor surface, methylene blue is used as an indicator like DNA biosensors[23]. Methylene blue is placed inside the holes and grooves of two hybridized strands by intercalation, and by examining its electrochemical behaviour before and after adding analyte, we will be able to measure the amount of the added analyte. The intercalation bonding includes the compounds insertion (mainly the aromatic ring of molecules) between two DNA strands and the opening of two strands. In single-stranded DNA, the MB binds to the end of the free bases of guanine and causes MB peak appears (Figure 4) [24, 25].

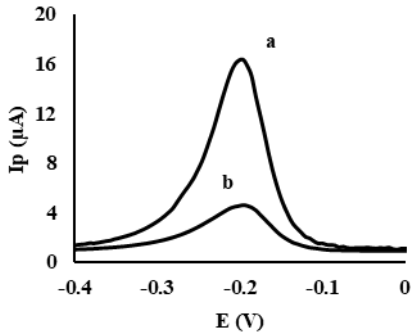

Fig. 4. DPV of MB on modified electrode (A) before and (B) after of dopamine addition.

There are several parameters that affect peak current, including MB. To eliminate the effect of MB concentration, the electrochemical peak of this compound in different concentrations on the modified electrode surface is determined. A steady increase in current intensity is observed with increasing MB concentrations as demonstrated in Figure 5, but afterward, the intensity does not increase. Likewise, the current changes in different incubation times were checked, and it was seen that after 15 minutes, the current intensity was almost constant. Therefore, the MB concentration of 50 µM and an incubation time of 15 minutes were chosen as optimal values.

In the next step, the incubation time and temperature with dopamine were investigated. The modified electrode (Apt-AMP/AuNPs-PRGO/GCE) was placed in an MB solution with a concentration of 50 µM for 15 minutes to accumulate MB on two strands. The electrode was washed to remove excessive MB, and then placed in a dopamine solution of various concentrations, and the response signal was recorded. It was seen that decreasing trend continued until 60 minutes and since then remained constant. Therefore, the incubation time with dopamine was chosen at 60 minutes. In the same way, the incubator's temperature was also checked, and the optimal temperature of 40 °C was selected (Figure 6).

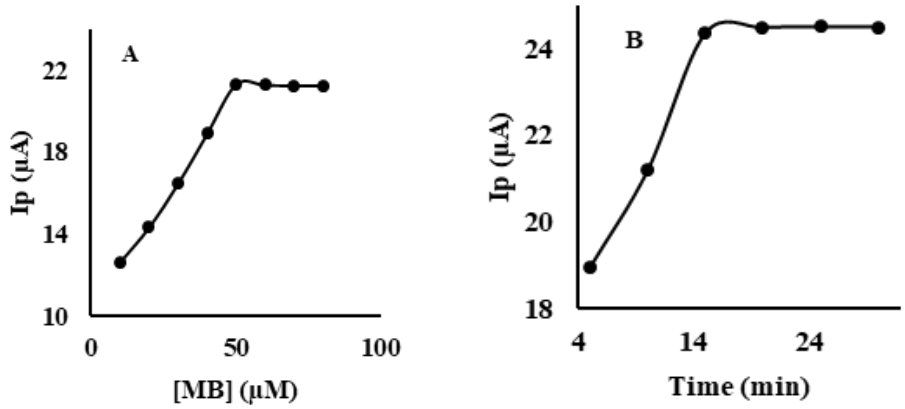

Fig. 5. Investigating the effect of different concentrations of methylene blue (A) and different incubation times (B).

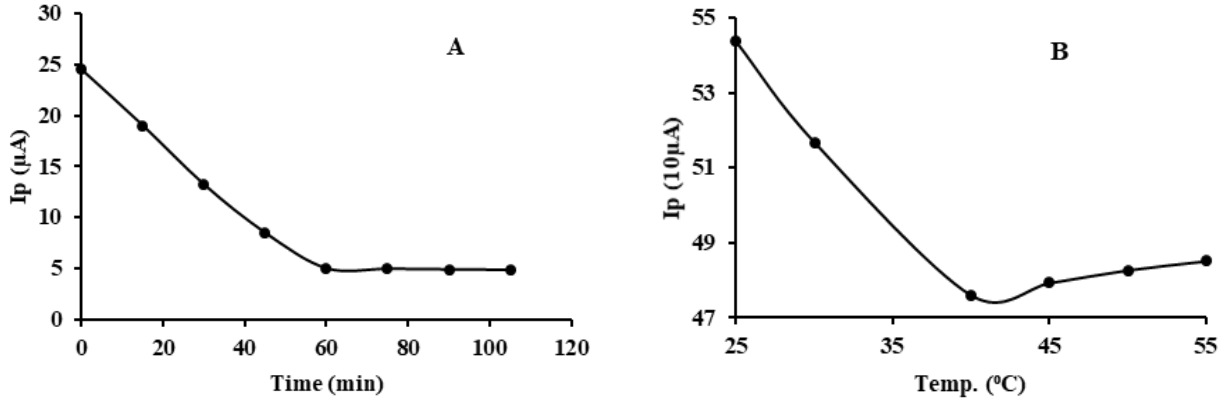

Fig. 6. Optimizing the test conditions, (A) the incubation time with dopamine (and B) the incubation temperature ([MB] = [DO] = 50 µM).

3.4. Sensitivity for dopamine detection

After incubation with different dopamine concentrations, biosensor sensitivity was tested under optimal conditions. Figure 7 depicts that peak current of DPV has decreased as the dopamine concentration has increased. Aptamer binds to dopamine and simultaneously detached from AMP. It is due to these phenomena that methylene blue content on the electrode surface is reduced and DPV current is decreased. In the range of 0.2-0.5 nM, a good linear relevance between peak current and dopamine concentration was found and the value of calculated limit of detection (LOD) 120 pM (S/N=3) was achieved.

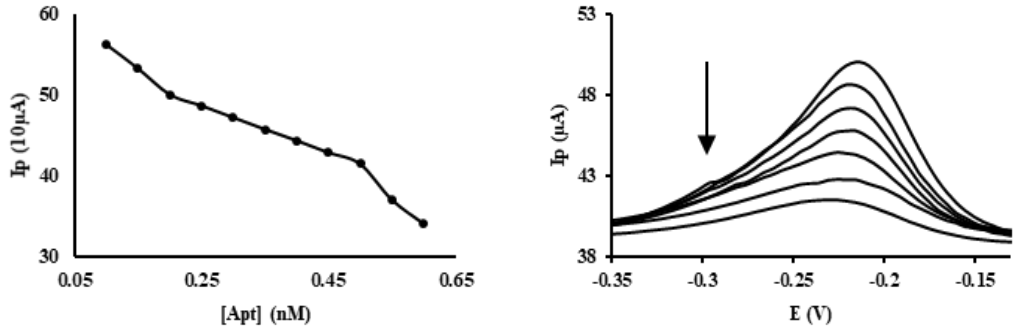

Fig. 7. DPV curve of modified electrode in different concentrations of Dopamine in optimum condition

3.5. Selectivity, reproducibility, and stability of aptasensor

To determine how specific the aptasensor is in detecting dopamine, when similar compounds are present, its selectivity is measured, compounds such as norepinephrine, L-dopamine, ascorbic acid, and epinephrine, whose concentrations are up to 250 times higher than dopamine's concentration. According to the results (Table 1), a significant change in the signal only occurred following dopamine addition, while there is no noticeable change due to other interferences. According to these results, dopamine does not bind to aptamer through non-specific surface adsorption, but through specific recognition.

Sensor reusability is one of the critical parameters in practical use of sensors. We used urea to disrupt the duplex between aptamer and AMP in this work. After the incubation stage with MB and dopamine, the electrode was placed in urea solution for half an hour and used again. After repeating the above steps for twenty times, the signal flow decreased by 3.95% compared with the first time. The figure exhibits the peak current of DPV biosensor after immersion in 20 nM dopamine, 50 µM methylene blue, and 8 M urea solutions in five regeneration cycles [23].

To ensure reproducibility, six electrodes were modified in the same way, their electrochemical response was taken and the results had a relative standard deviation (RSD) of 2.13%.

To determine the electrode stability, consecutive cyclic voltammetry peaks were taken from the modified electrode in methylene blue solution, which showed a decrease of 4.9% after 100 repetitions. In addition, the electrode showed a good response signal in 20 nM dopamine solution after being kept ten days at refrigerator temperature.

4. Conclusion

One of the most important aspects of practical application of chemistry is very accurate measurement of compounds and drugs in the body. This is achieved by designing and manufacturing highly sensitive sensors. Concerning high selectivity and very low detection limits, nano-biosensors are ideal for this purpose.

Nano biosensors are a good choice for this purpose due to their high selectivity and very low detection limit. In this research, with the help of partially reduced graphene oxide-AuNPs nanocomposite, we made an aptasensor (with the MB indicator) to detect dopamine was obtained with a detection limit of 120 pM. Our designed sensor is able to identify and measure dopamine among similar structures with good selectivity. Electrochemical methods, spectroscopy, and SEM imaging were used to confirm the electrode modification.

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HOW TO CITE THIS ARTICLE
Hamideh Roshanfekr. A Simple Specific Dopamine Aptasensor Based on Partially Reduced Graphene Oxide–AuNPs composite . Prog. Chem. Biochem. Res, 6(1) (2023) 61-70

DOI: 10.22034/pcbr.2023.381280.1245
URL: http://www.pcbiochemres.com/article_169238.html