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Gr–Pt hybrid NP modified GCPE as label and indicator free electrochemical genosensor platform

Sinan Cemgil Sultan, Ülkü Anik*

Muğla Sıtkı Koçman University, Faculty of Science, Chemistry Department, 48000 Kötekli, Muğla Turkey

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ABSTRACT

Glassy carbon paste electrode (GCPE) was modified with graphene platinum hybrid nanoparticle (Gr–Pt hybrid NP) and used as a transducer for label and indicator free electrochemical genosensor. 22 mer oligonucleotides representing *Escherichia coli* bacteria were used as a model case. As far as it is known, this study is the first study where Gr–Pt hybrid NP was incorporated into GCPE and used for genosensor transducer. The extent of hybridization was determined by using differential pulse voltammetric signals of guanin oxidation. After the optimization of experimental parameters, analytical characteristics were investigated. The linear range was found between 1.5×10^{-7} and 2.25×10^{-6} M with the equation of $y=1.6566x-2.6161$ and R^2 of 0.9959. RSD and LOD were calculated as 4.2% ($n=6$) and 1.12×10^{-9} M respectively.

1. Introduction

In the last years different approaches have been conducted to improve the performance of electrochemical biosensors. In this sense, nanomaterials like metal oxide nanoparticles and carbon nanotubes (CNTs) have largely contributed to the success of new electrochemical biosensors due to their exceptional electronic properties, electric conductivity and catalytic effects [1–3]. Recently another carbon based nanomaterial, graphene (Gr) has attracted extensive attention due to excellent conductivity and electrocatalytic activity [4–8]. Gr is in sp^2 form two dimentional sheets. These sheets provide very suitable two dimentional environment for electron transfer and give fast electron transfer at the edges. For these reasons, Gr and another form of Gr, graphene oxide (Grox) based NP have been widely used for electroanalytical purposes.

Different strategies for modification of electrode surfaces with these nanostructures have been proposed. Also the usefulness to detect several analytes has been widely demonstrated [4–7]. Glassy carbon paste electrode (GCPE) is a kind of composite carbon electrode which shows better electrochemical performance compared to carbon paste electrodes. Due to its composite structure it can easily be prepared and renew just by mixing the proper amount of glassy carbon microparticles with mineral oil [9]. This electrode material has been extensively used in biosensor applications by our group [10–13]. Also as far as we know there is only one work that uses plain GCPE (without any nanomaterial inside) as DNA genosensor transducer [14].

For the design of an electrochemical genosensor, immobilization of a DNA probe onto nanomaterial modified electrode surfaces have received considerable attention. It is important to develop an assay which has low cost, high sensitivity and good selectivity. For this reason, in this work a label free and indicator free electrochemical genosensing of specific DNA hybridization by graphene–platinum hybrid nanoparticle (Gr–Pt hybrid NP) is introduced. There are variety of works that include different strategies for preparation of Gr–Pt hybrid NP in the literature. Herein we used more practical way for preparation of this hybrid NP and as it is known, this system is the first system where GCPE was modified with Gr–Pt hybrid NP [15–18].

By adding Pt-NP into Gr, it is aimed to combine Pt NPs electrocatalytic activity with attractive properties of Gr. By introducing this hybrid nanomaterial into the composite electrode structure, it is expected to obtain more effective and sensitive genosensor.

E. coli oligonucleotides were used as a model case and differential pulse voltammetric (DPV) guanin oxidation signals before and after hybridization at Gr–Pt hybrid NPs/GCPE surfaces were evaluated for direct anaysis. After the optimization of experimental working conditions like probe immobilization time, probe concentration and probe hybridization time, analytical characteristics were examined and limit of detection (LOD) value was calculated.

2. Experimental

2.1. Apparatus

The oxidation signal of guanine was investigated by using DPV with an AUTOLAB PGSTAT 12 electrochemical analysis system and

* Corresponding author. Tel.: +90 252 211 1503.
E-mail address: ulkuanik@yahoo.com (Ü. Anik).

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GPES software package (ECO CHEMIE Instruments B.V., The Netherlands). Sigma 3-16 pk was used for centrifugation. Bandelin sonorex was used for sonication. Gr–Pt hybrid NPs were dried with EV 018 (vacuum oven). Glass, metal and plastic materials were cleaned with OT 012 (autoclave). TEM images were recorded using JEOL-JEM 2100. Gr–Pt hybrid NPs modified GCPE was used as a working electrode. Ag/AgCl and platinum electrodes were used as reference and auxiliary electrode, respectively.

2.2. Chemicals

Glacial acetic acid, HCl, NaOH, NaCl, KH_2PO_4, H_2SO_4, H_2O_2, $NaNO_3$ and $KMnO_4$ were purchased from Merck. H_2PtCl_6 and ethylene glycol were purchased from Sigma. All of other chemicals were of analytical grade. Ultrapure distilled water was used in all solutions. All experiments were performed at room temperature.

Acetate buffer (ACB) of 0.5 M containing 20 mM NaCl (pH=4.8) and desired concentration of probe DNA was used as binding buffer. 0.05 M phosphate buffer solution (PBS) including 20 mM NaCl and 2 × saline-sodium citrate (2 × SSC) was used as hybridization buffer (pH=7.4). Finally PBS solution was used as a washing buffer in all the experiments.

Capture probes and target sequences in the form of lyophilized powder, were purchased from Ellia Biotech (Germany) with purification by HPLC. The base sequences of the oligonucleotides, were shown in Table 1.

The oligonucleotide stock solutions (1000 mg L^{-1}) were prepared with ultrapure water and kept frozen. The diluted solutions of the capture probes were prepared with the binding buffer and the target sequences were prepared with the hybridization buffer as described above.

2.3. Methods

The hybridization detections were performed by monitoring DPV signals of guanine oxidation. The procedure of the study consisted of the following steps; Gr–Pt hybrid NPs fabrication, modification of GCPE with Gr–Pt hybrid NPs, probe immobilization, hybridization, washing and electrochemical transduction.

2.3.1. Fabrication of Gr–Pt hybrid NPs

GrOx was prepared by modifiying the Hummers–Offeman method [19]. 1 g of graphite powder was added into 23 mL 98% H_2SO_4 solution and stirred at room temperature over a 24 h. After that, 100 mg of $NaNO_3$ was added into the mixture and stirred for 30 min. Then, 46 mL water was added into above mixture during a period of 25 min. Finally, 140 mL of water and 10 mL of 30% H_2O_2 were added into the mixture to stop the reaction. The unexploited graphite in the resulting mixture was removed by centrifugation.

A 10 mg portion of graphite oxide powder was dispersed in 10 mL of water by sonication for 1 h, forming stable GrOx colloid [20,21]. Then 20 mL of ethylene glycol and 0.5 mL of 0.01 M H_2PtCl_6 were added to the solution and stirred for 30 min. Subsequently, the mixture was put in an oil bath and heated at 100 °C for 6 h with magnetic stirring. The Gr–Pt hybrid NPs were centrifuged to seperated from the ethylene glycol solution and washed with deionized water five times. The resulting products were dried in a vacuum oven at 60 °C for 12 h [22].

Finally, the prepared Gr–Pt hybrid NPs were dispersed to 10 mg mL^{-1} in water by ultrasonication and stored at 4 °C when not used.

Table 1
Oligonucleotide sequences.

Oligonucleotide	Sequence
Probe sequence	5′-GGC-AGC-GGT-GAC-TAT-GGC-ACC-A-3′
Complementary sequence	5′-TGG-TGC-CAT-AGT-CAC-CGC-TGC-C-3′
Non-complementary sequence	5′-GCA-CCT-GAC-CAT-AGA-ACG-GT-3′

2.3.2. Modification of GCPE with Gr–Pt hybrid NPs

Glassy carbon paste was prepared in the usual way by hand-mixing glassy carbon powder and mineral oil in a 80:20 mass ratio. Then, 6.0 μL of prepared Gr–Pt hybrid NPs was added to glassy carbon paste and was mixed again. The resulting paste was then packed firmly into electrode cavity. The surface was polished on a weighing paper to smooth electrode's surface and rinsed carefully with doubly distilled water before use (Scheme 1a)

2.3.3. Fabrication of DNA biosensor

The immobilization of the ssDNA probe on the electrode surface was carried out as follows: 0.5 M 50 μL ACB solution (pH: 4.8) containing 1.5×10^{-6} M probe sequence was dropped on the surface of Gr–Pt hybrid NPs/GCPE for 60 min at room temperature. Then, the electrode was washed three times with a ACB (0.5 M pH:4.8) to remove any unimmobilized probe sequence. The hybridization was performed at room temperature by 50 μL hybridization buffer solution (2 × SSC) containing 2.25×10^{-6} M target sequence. This solution was dropped on the surface of probe immobilized Gr–Pt hybrid NPs/GCPE and waited for 30 min at room temperature. The electrode was rinsed three times with washing buffer solution including 2 × SSC to prevent the unspecific binding. The desgined geosensor's selectivity was performed by using a 20 mer non-complementary target sequence representing antrax bacteria. Fabrication of DNA biosensor is shown in Scheme 1b.

2.3.4. Electrochemical transduction

The oxidation signal of guanine was measured by using DPV in potential range between +0.75 V and +1.45 V (step potential: 7 mV; modulation amplitude: 50 mV; modulation time: 0.05 s; and interval time: 0.5 s)

All results reported in this paper represent the means of at least three measurements, and the error bars represent the corresponding standard deviation.

3. Result and discussion

A label free voltammetric nanogenosensor system has been developed for direct detection of DNA hybridization. For this purpose, GCPE was modified with Gr–Pt hybrid NPs and used as transducer. As far as we know, this system is the first transducer system where GCPE was modified with this hybrid NP. Also the preparation procedure is developed by our group simply by combining Hammers method (for the preparation of GrOx) and Pt NP preparation procedures. DNA hybridization detection was performed after interaction between target sequences at probe modified Gr–Pt hybrid NPs/GCPE surfaces based on guanine oxidation signals.

3.1. Characterization of prepared Gr–Pt hybrid NPs

Gr–Pt hybrid NPs were prepared according to the procedure given into experimental part. Then this hybrid nanomaterial was characterized by using TEM. Fig. 1a demonstrates the TEM images of 10 nm and Fig. 1b 20 nm scale. As can clearly be seen from the figure, Pt nanoparticles were distributed onto the graphene sheets. The presence of Pt into the structure increases the surface area as well as enhances the electrocatalytic effect of the developed nanomaterial. As a result, produced hybrid NP provides larger electrochemically active surface areas for the adsorption of biomolecules and effectively

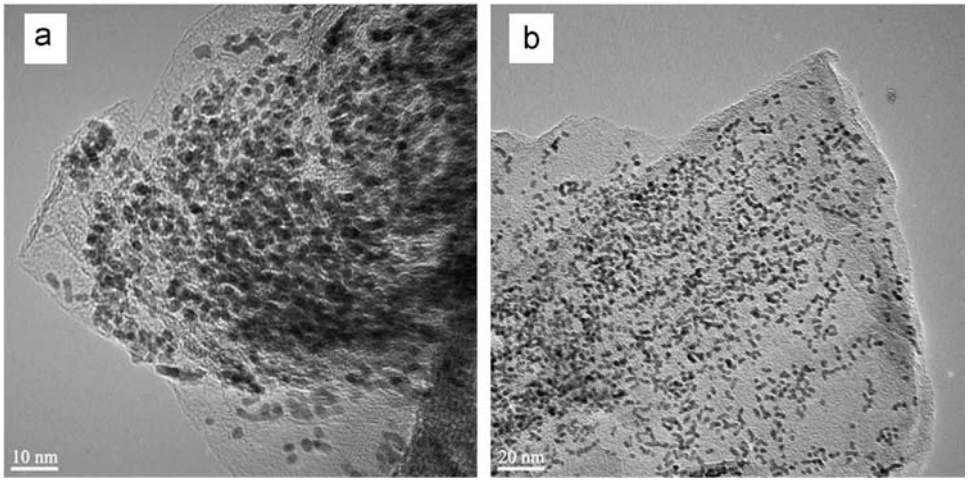


Fig. 1. TEM images of Gr–Pt hybrid NPs with (a) 10 nm and (b) 20 nm scale.

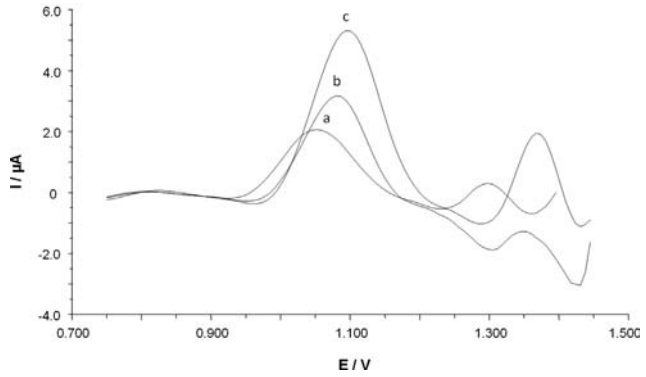


Fig. 2. DPVs of guanine oxidation at 1.5×10^{-6} M probe modified (a) plain GCPE, (b) 6 µL GrOx Nps modified, and (c) 6 µL Gr–Pt hybrid NPs modified composite GCPE. DPV parameters were as follows: 0.75–1.45 V; step potential: 7 mV; modulation amplitude: 50 mV; modulation time: 0.05 s; and interval time: 0.5 s.

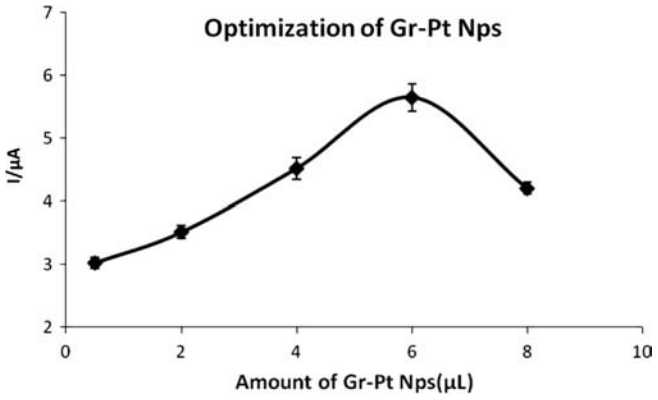


Fig. 3. Optimization of Gr–Pt hybrid NPs amount.

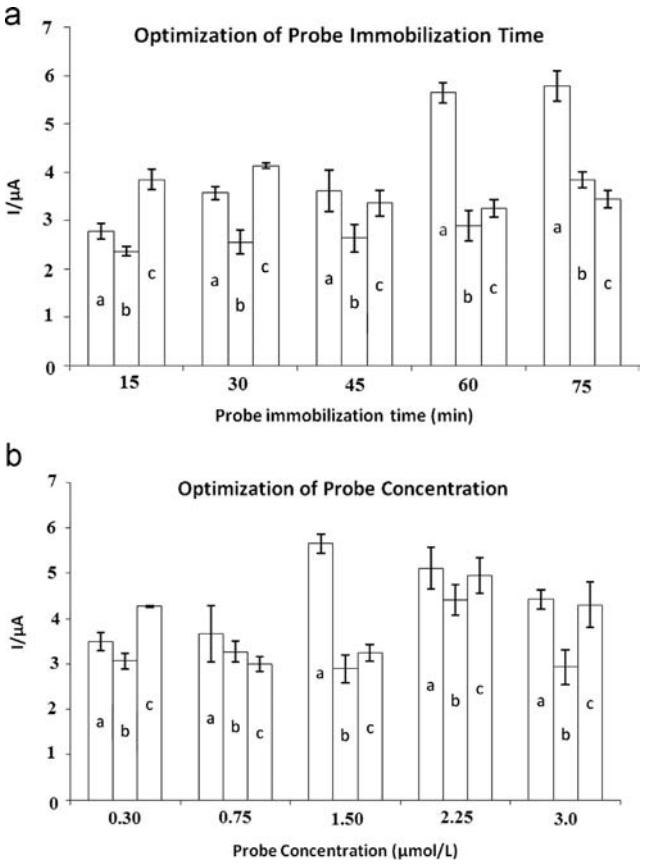


Fig. 4. Optimization of probe immobilization time, probe concentration. For the bars (a) probe, (b) hybridization with complementary target, and (c) hybridization with non-complementory target.

accelerate the electron transfer between electrode and detection molecules, which could lead to a more rapid and sensitive current response [23].

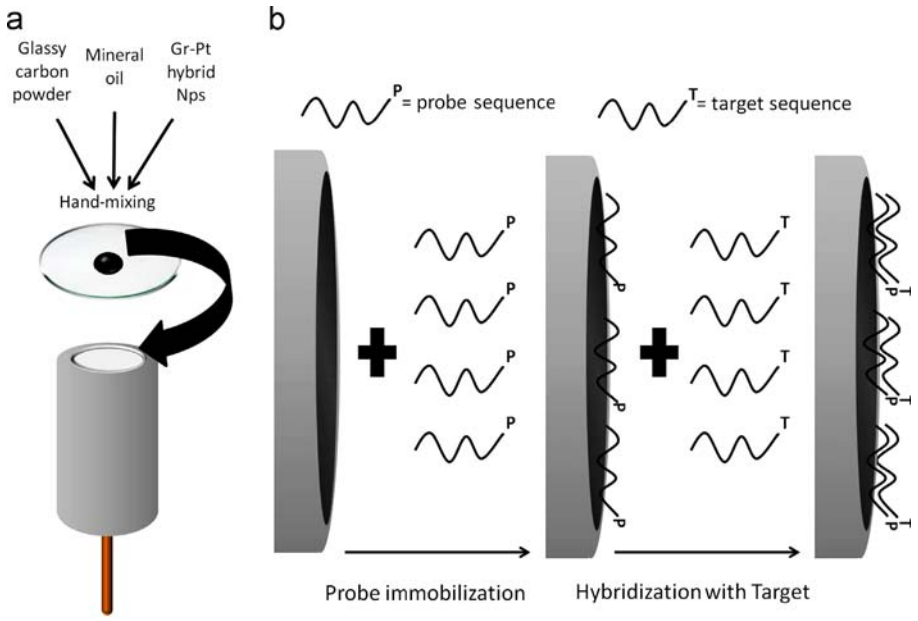
3.2. The effect of Gr–Pt hybrid NPs on the probe signal

Fig. 2 demonstrates the guanin oxidation voltammograms of plain GCPE (Fig. 2a), GrOx modified GCPE (Fig. 2b) and Gr–Pt hybrid NPs modified GCPE (Fig. 2c). As can be seen from the figure, modification of GCPE with GrOx and Gr–Pt hybrid NP increases the guanin oxidation signal significantly. Compared GrOx-NP modified electrode, it is obvious that hybrid NP provides higher current for the oxidation of guanin. This increase can be attributed to the combined effect of Gr and Pt NPs in the structure. As it is explained before, this structure provides larger surface area with enhanced electron transfer properties [24,25].

3.3. Optimization of the developed probe

3.3.1. Effect of Gr–Pt hybrid NPs amount

Optimization of nanomaterial amount is important since after a specific value current decrease is obtained due to its large surface



Scheme 1. (a) Preparation of the G–Pt hybrid NPs/GCPE and (b) fabrication of the DNA biosensor.

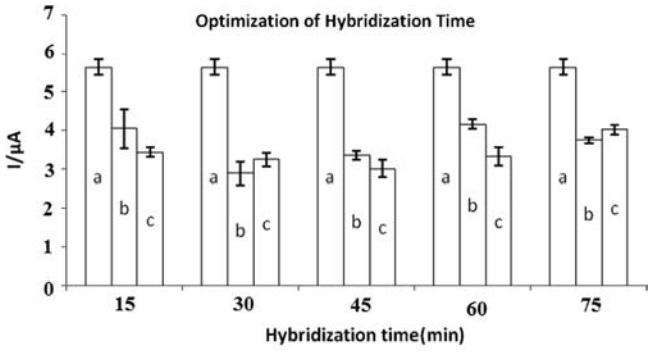


Fig. 5. Optimization of hybridization time, target concentration, For the bars (a) probe, (b) hybridization with complementary target, and (c) hybridization with non-complementary target.

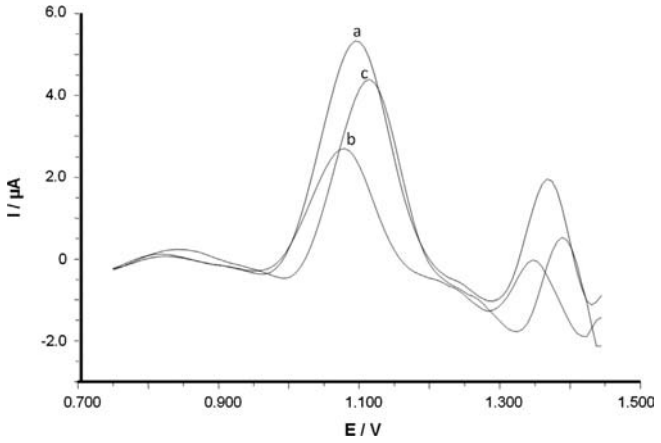


Fig. 6. DPV of guanine oxidation peak at 1.5×10^{-6} M probe sequence modified Gr–Pt hybrid NPs/GCPE (a) and after hybridization with 2.25×10^{-6} M complementary sequence (b), and 2.25×10^{-6} M non-complementary sequence (c). DPV parameters were as follows: 0.75–1.45 V; step potential: 7 mV; modulation amplitude: 50 mV; modulation time: 0.05 s; and interval time: 0.5 s.

areas. Because large areas can cause increments in backround current probably due to the capacitive current [1]. For this purpose, 0.5, 2.0, 4.0, 6.0 and 8.0 μL Gr–Pt hybrid NPs were put into the GCPE structure and tested by following the probe's guanine base current value (Fig. 3). Since best current value was obtained with 6.0 μL Gr–Pt hybrid NPs, further experiments were conducted by using this value.

3.3.2. *Optimization of probe working conditions*

Optimization of probe's working conditions were conducted in order to decide about working conditions that results with higher current for oligonucleotide oxidation signal. For this purpose, probe immobilization time, probe concentration and probe hybridization time were optimized.

3.3.3. *Effect of probe immobilization time and probe concentration*

15, 30, 45, 60 and 75 min were tried as probe immobilization times (Fig. 4A) under the working conditions of 6.0 μL Gr–Pt hybrid NPs, 1.5 μM probe concentration, 1.5 μM target concentration, 30 min target hybridization time and 2 × SSC hybridization solution. The current decrease between probe (Fig. 4A, a) and complementary target (Fig. 4A, b) hybridization and between noncomplementary target (Fig. 4A, c) and probe (Fig. 4A, a) hybridization were followed. As can be seen from the Fig. 4A, best results were observed at 60 min. For this reason, this value was utilized for further experiments. For the optimization of probe concentration 0.30, 0.75, 1.50, 2.25 and 3.00 μM probe amounts were immobilized onto the electrode surface (6.0 μL Gr–Pt hybrid NPs, 60 min probe immobilization time, 30 min target hybridization time, 1.5 μM target concentration, 2 × SSC hybridization solution). The similar current decrease as explained for Fig. 4B was followed. As can clearly be seen from the Fig. 4 B, optimum probe concentration was chosen as 1.5 μM, since more significant decrease was obtained when this amount was used.

3.3.4. *Effect of hybridization time*

15, 30, 45, 60 and 75 min were applied as target hybridization time to the system (6.0 μL Gr–Pt hybrid NPs, 60 min probe immobilization time, 1.5 μM probe concentration, 1.5 μM target concentration, 2 × SSC hybridization solution). 30 min was chosen

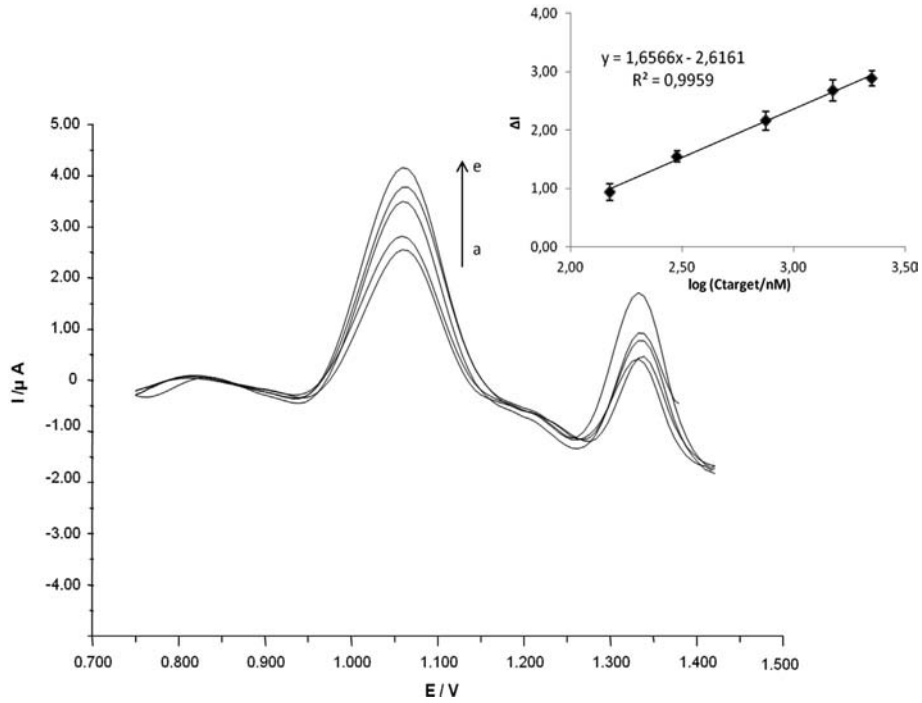


Fig. 7. DPV measurements were obtained after hibridization with (a) 2.25×10^{-6} M, (b) 1.5×10^{-6} M, (c) 7.5×10^{-7} M, (d) 3.00×10^{-7} M and (e) 1.5×10^{-7} M target concentration and calibration curve of ΔI ($I_{probe} - I_{hybridization\ with\ complementary\ target}$) current values after hybridization with different concentration of target sequence.

Table 2
Comparison with other indicator-free DNA biosensors.

Electrode	Linear range	LOD (M)	Reference
CNT-CHIT PGE	10–80 μg ml^{-1}	2.09×10^{-6}	[31]
PGE	0.1–10 μg ml^{-1}	2.00×10^{-8}	[32]
CNT modified PGE	2–30 μg ml^{-1}	1.60×10^{-8}	[33]
Au Nps coated PGE	10 nM–1.0 μM	6.90×10^{-9}	[34]
Gr–Pt hybrid NPs modified GCPE	0.15–2.25 μM (1–15 μg ml^{-1})	1.12×10^{-9}	Our work

as optimum target hybridization time considering obtained results (Fig. 5).

3.4. Selectivity of genosensor

The detection capability of developed genosensor under optimized conditions were then tested by using DPV before (a) and after hybridization with complementary (b) and non-complementary (c) target sequences (Fig. 6).

As can be seen from the figure, the current difference of complementary and noncomplementary differs enough to differentiate the two sequences. As a result it is clear that developed genosensor's selectivity is high enough to conduct the experiment.

3.5. Analytical characteristics

After the completion of optimization of experimental conditions, analytical characteristics were examined. As can be seen from Fig. 7, ΔI was linear with respect to logarithmic value of complementary target sequence over the range from 1.5×10^{-7} to 2.25×10^{-6} M. The regression equation was obtained as $y = 1.6566x - 2.6161$ (x was the logarithmic value of complementary target sequence, nM; y was ΔI, μA; $n = 3$) and a regression correlation coefficient (R^2) of the calibration line was 0.9959. The LOD was was also calculated based on $S/N = 3$ and found as 1.12×10^{-9} M. The relative standard deviation RSD was 4.2% for six successive renewals ($n = 6$).

Obviously it is possible to obtain better LOD values when an indicator involves in the genosensor structure [26–30]. However label and indicator free genosensors are more practical and easy to use systems. In order to show our system's performance, the comparison of developed system was made with nanomaterial included label and indicator free genosensors. The results are shown in Table 2. As can be seen from the Table, Gr–Pt hybrid NPs genosensor has the smallest LOD value. In terms of linear range again developed system has one of the wider range.

4. Conclusions

In this work Gr–Pt hybrid NP modified composite electrode, GCPE was used as a DNA genosensor transducer for the first time. 22 mer oligonucleotides representing *E. coli* bacteria were used as a model case. The linear range for developed genosensor was found between 1.5×10^{-7} M and 2.25×10^{-6} M with the LOD value of 1.12×10^{-9} M. Compared to nanomaterial based label and indicator free genosensords, developed system has lower LOD value with one of the wider linear range (Table 2). These results demonstrate that sensitive and effective genosensor was developed. Further studies continue in our lab for the production of various nanomaterial modified GCPE as genosensor transducer.

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