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A novel electrochemical DNA biosensor based on a modified magnetic bar carbon paste electrode with Fe_3O_4NPs-reduced graphene oxide/PANHS nanocomposite

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ABSTRACT

In this study, we have designed a label free DNA biosensor based on a magnetic bar carbon paste electrode (MBCPE) modified with nanomaterial of Fe_3O_4/reduced graphene oxide (Fe_3O_4NP-RGO) as a composite and 1-pyrenebutyric acid-*N*- hydroxysuccinimide ester (PANHS) as a linker for detection of DNA sequences. Probe (*BRCA1* 5382 insC mutation detection) strands were immobilized on the MBCPE/Fe_3O_4-RGO/PANHS electrode for the exact incubation time. The characterization of the modified electrode was studied using different techniques such as scanning electron microscopy (SEM), infrared spectroscopy (IR), vibrating sample magnetometer (VSM), electrochemical impedance spectroscopy (EIS) and cyclic voltammetry methods. Some experimental parameters such as immobilization time of probe DNA, time and temperature of hybridization process were investigated. Under the optimum conditions, the immobilization of the probe and its hybridization with the target DNA (Complementary DNA) were tested. This DNA biosensor revealed a good linear relationship between ΔR_{ct} and logarithm of the complementary target DNA concentration ranging from 1.0×10^{-18} mol L^{-1} to 1.0×10^{-8} mol L^{-1} with a correlation coefficient of 0.9935 and a detection limit of 2.8×10^{-19} mol L^{-1}. In addition, the mentioned biosensor was satisfactorily applied for discriminating of complementary sequences from non-complementary sequences. The constructed biosensor (MBCPE/Fe_3O_4-RGO/PANHS/ssDNA) with high sensitivity, selectivity, stability, reproducibility and low cost can be used for detection of *BRCA1* 5382 insC mutation.

1. Introduction

Nowadays, various nanomaterials have been fabricated for increasing the sensing performance of electrochemical biosensors by developing nanotechnology. Nanotechnology has indicated a key role in the fabrication of DNA biosensors. Some researchers such as Wang and his coworkers have used different nanomaterials for fabrication of novel electrochemical biosensors based on different platforms [1,2]. As known, it has been paid high attention to magnetic beads (MBs) in the recent years for electrochemical bases [3,4]. One of the most important components to make magnetic beads is iron oxide nanoparticle (Fe_3O_4NPs) which can be enhanced the surface area of electrode. According to the mentioned property, magnetic nanoparticles have been applied to modify the surface of electrodes [5,6].

Graphene nanosheets as a single atom with two-dimensional sheet of sp^2-bonded carbon, is the other nanomaterial which has been used in this research. The unique structure of graphene can lead to some properties such as good biocompatibility, high electrocatalytic and large specific surface area. So, it has been applied in fabrication of electrochemical sensors [7,8]. Recently, the electrochemical reduction process of graphene oxide (GO) on the surface of electrode has been offered. This process can be performed with different techniques such as cyclic voltammetry or potentiostatic method [9]. In this work, the Fe_3O_4-RGO nanocomposite was synthesized through a simple approach while reduction of GO to RGO and in situ formation of Fe_3O_4NPs on graphene sheets were performed in a one-step reaction [10].

In addition to nanomaterials, some linkers have been used in designing some electrochemical biosensors. One of the applied linkers is PANHS (1- pyrenebutyric acid-*N*-hydroxysuccinimide ester). PANHS, as an activating reagent for carboxylic acids, is found in organic chemistry or biochemistry courses. This material contains an anchor group and a terminal group with hydrophobic π system group (a scaffold molecule) which has strong attachment to the base plane of the carbon sheets through π-stacking [11]. PANHS due to possessing succinimide ester group can bind to nucleophilic substitution by amine groups on the proteins strongly [12,13]. Using PANHS can cause decreasing electrode preparation (no need to use NHS/EDC (1-ethyl-3-(3-dimethyl amino propyl) carbo-diimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS) to active carboxylic acid groups).

As known, a malignant tumor consists a group of cancer cells that can spread to circumambient tissues and breast cancer as a malignant

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tumor which starts in the cells of breast, occurs in women more than men but men can get it, too. The BRCA1 5382 insC mutation increases the risk of getting breast cancer ten times and ovarian cancer twenty times, compared with the general population. The detection of cancer in initial stages and analysis of gene sequences in diagnostic tests are so important. Breast cancer can be detected by different methods [14] such as mammography and biopsy. Due to importance of cancer detection in the initial stages, different DNA biosensors based on various detection strategies have been developed [15–18]. Among the mentioned biosensors, electrochemical biosensors have been improved effectively, due to some advantages such as being highly sensitive, selective and rapid [19–22].

In our recent works [19,23–25], some electrochemical biosensors have been constructed. In the reference [23], fabrication of a modified glassy carbon electrode with gold nanoparticles and RGO for breast cancer determination, was investigated. In other references some DNA label free biosensors were fabricated based on glassy carbon electrode modified with graphene nanosheets and MWCNTs, for detection of amylogenin.

In the present paper, we reported a new electrochemical biosensor based on a modified magnetic bar carbon paste electrode which is named MBCPE/Fe_3O_4-RGO/PANHS/ssDNA. To fabricate this biosensor, at first the designed magnet bar was placed on a carbon paste electrode (MBCPE), and consequently the nanocomposite of Fe_3O_4-RGO with high magnetic property and the linker of PANHS were placed on the electrode surface, respectively. The modified magnetic bar carbon paste electrode with nanoparticles of Fe_3O_4-RGO and PANHS as a linker, possess some benefits such as providing a suitable base for attaching the DNA strands and consequently occurring a better hybridization process at the electrode surface. Using the designed electrode (MBCPE/Fe_3O_4-RGO/PANHS) leads to increasing in the accessible surface of the electrode and consequently obtaining a low detection limit and wide linear range by electrochemical impedance spectroscopy (EIS) technique (2.8×10^{-19} mol L^{-1} and 1.0×10^{-18} mol L^{-1}–1.0×10^{-8} mol L^{-1}, respectively). The limit of detection and linear range by differential plus voltammetry (DPV) technique for mentioned electrode were obtained (2.1×10^{-13} mol L^{-1} and 1.0×10^{-6} mol L^{-1}–1.0×10^{-12} mol L^{-1}, respectively), too. Moreover, under the optimum conditions the responses of complementary sequences and non-complementary sequences were tested.

Briefly, the advantages of this paper are making electrochemical biosensor without label, using nano-magnetic composite of Fe_3O_4-RGO in the fabrication of MBCPE/Fe_3O_4-RGO/PANHS biosensor which is lead to increasing in the accessible surface of the electrode to improve linear range and detection limit. Low cost, no need to use NHS/EDC for activation of carboxylic acid groups, possessing high stability and selectivity for detecting complementary and non-complementary of *BRCA1 5382* sequences. The designed approach for immobilization of probe DNA and hybridization with target oligonucleotides is illustrated in Scheme 1.

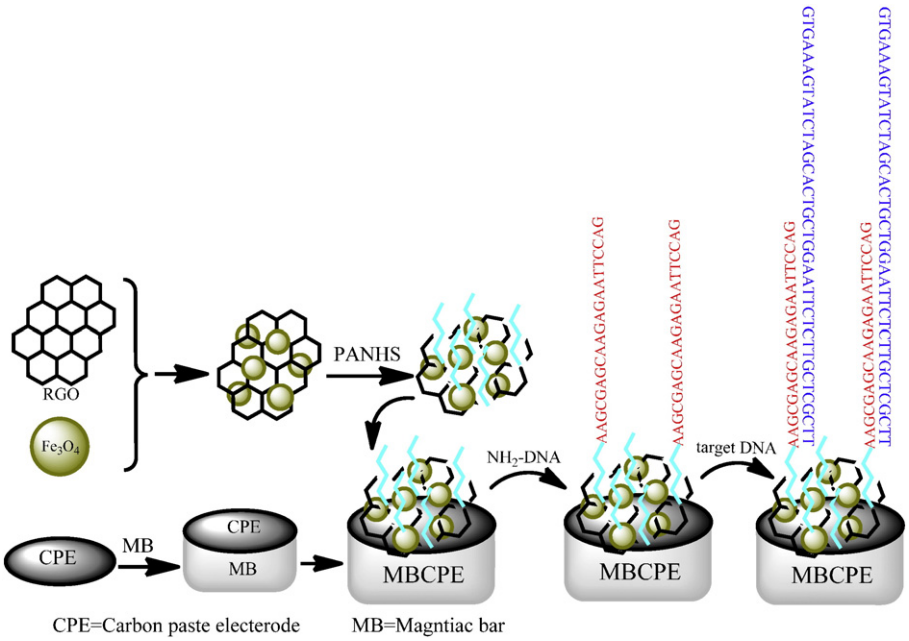
2. Experimental

2.1. Reagents and apparatus

Specific primers and probes were designed based on breast cancer obtained from Gen Bank database. The stock solution of primers was prepared by dissolving in water to form 1 mg mL^{-1} and kept frozen at −20 °C. Tween 20 (Polyoxy ethylenesorbitan monolaurate), potassium ferrocyanide, potassium ferricyanide, graphite, 1-pyrenebutyric acid-*N*-hydroxysuccinimide ester (PANHS) and other applied chemical reagents were purchased from Merck or Sigma company. All solutions were prepared with deionized water (DI: 18 MΩ cm^{-1} resistivity). Graphene oxide (GO) nanosheets were prepared according to the papers [25,26]. The sequences of probe and complementary were as follows:

Probe sequence (ssDNA = P1):
5′-AAGCGAGCAAGAGAATTCCAG-3′.
Complementary sequence (dsDNA = P1C):
5′-GTGAAAGTATCTAGCACTGCTGGAATTCTCTTGCTCGCTT-3′.
Non-complementary sequence (noncom dsDNA = P1nC):
5′-TGTGAAAGTATCTAGCACTGTGGGAATTCTCTTGCTCGCT-3′.

Electrochemical measurements were carried out with Autolab potentiostat/galvanostat (model PGSTAT 302 N, Eco Chemic, Utrecht, Netherlands) and NOVA 1.7 software at laboratory temperature. The components of applied three-electrode system were a magnetic bar carbon paste, an Ag/AgCl (1.0 mol L^{-1} KCl) and a platinum wire as a working, reference and auxiliary electrodes, respectively. The pH measurements were examined using a Metrohm model 691 pH/mV meter. FT-IR experiments were performed by a spectrometer (VERTEX



Scheme 1. Schematic representation of the modified electrochemical biosensor based on MBCPE/Fe_3O_4-RGO/PANHS platform.

70, Bruker Optics, Ettlingen, Germany) equipped with a deuterated triglycine sulfate (DTGS) detector. The SEM was performed respectively with a TESCAN instrument model VEGA3.

2.2. Synthesis of Fe_3O_4-RGO composite

Fe_3O_4-RGO was synthesized according to reference [27]. Briefly, at first Fe_3O_4NPs was prepared by dissolving 4 mmol $FeCl_3$ and 2 mmol $FeCl_2 \cdot 4H_2O$ in 50 mL deionized water. To obtain a uniform solution, the mixture was stirred vigorously by mechanical stirring. 0.2 g of graphene oxide (GO) in 50 mL of water was ultra-sonicated for 30 min. The mixed water solution of $FeCl_3$ and $FeCl_2$ was added slowly to the GO solution and subsequently, temperature of this solution was increased to 90 °C and 2.5 mL of 25% (*w*/*w*) NH_3 was added quickly while kept under nitrogen atmosphere. GO is reduced to reduced graphene oxide (RGO) chemically by adding hydrazine hydrate (450 μL 80%) which was added slowly and stirred for 4 h at 90 °C. The formed precipitate was collected by a permanent magnet and washed three times with water/ethanol solution and dried in vacuum at 70 °C.

2.3. Preparation of MBCPE/Fe_3O_4-RGO/PANHS/ssDNA

For preparation of a magnetic bar carbon paste electrode (MBCPE), 0.500 g graphite powder, Paraffin (Dc 350, Merck), a glass tube (ca. 3 mm i.d. and 10 cm long), a copper wire and a magnetic bar were used. Carbon paste was prepared by hand mixing of graphite powder and Paraffin using a mortar and pestle. This paste was then packed into the glass tube and inserted a magnetic bar into the carbon paste to provide a magnetic field. An electrical contact was performed by pushing a piece of copper wire into the down side of the glass tube. A 20 μL of homogeneously dispersed solution of Fe_3O_4-RGO (0.1 g mL^{-1}) was then pipetted onto the surface of MBCPE and was dried under ambient condition. After that, 20 μL of PANHS solution (3 μM) was dropped on the surface of electrode for 3 h in a saturated chamber with DMF vapor. For immobilization of DNA probe on the electrode surface, 10 μL of DNA probe (ssDNA) solution (1 μmol L^{-1}) was dropped on the electrode surface for 30 min in a wet chamber to fabricate MBCPE/Fe_3O_4-RGO/PANHS/ssDNA. The electrode was then kept in a 0.1% Tween solution at room temperature for 30 min to remove non-specific adsorption of DNA and fill the non-covered areas of MBCPE/Fe_3O_4-RGO/PANHS/ssDNA biosensor by DNA molecules. All of the electrode preparation processes were followed using CV and EIS techniques.

2.4. Hybridization procedure

Hybridization reaction was performed by putting 100 μL of the Tris-HCl (0.1 mol L^{-1}, pH = 7.4) buffer solution containing a proper concentration of the complementary target DNA on the MBCPE/Fe_3O_4-RGO/PANHS/ssDNA surface for 50 min at 35 °C. After the hybridization, the electrode was rinsed with large amount of the Tris-HCl (0.1 mol L^{-1}, pH = 7.4) buffer and deionization water to remove the unhybridized ssDNA. The chain hybridization was studied by using DPV and EIS methods.

3. Results and discussion

3.1. Characterization of the fabricated modified electrode

3.1.1. Characterization by SEM and FT-IR techniques

In this study, the morphology investigation of bare MBCPE and MBCPE/Fe_3O_4-RGO was studied by SEM (Fig. 1). In Fig. 1A, the SEM image of MBCPE, does not reveal any spherical or amorphous structure. Fig. 1B shows the SEM image of Fe_3O_4NPs, as shown in this figure the Fe_3O_4 nanoparticles have spherical shape and modification of electrode surface with these nanoparticles increases the electrode surface. Also, the SEM image reveals that the dimension of magnetic iron oxide nanoparticles (Fe_3O_4NPs) has a size range of 50–140 nm (D1 and D2 in Fig.1B). Fig. 1C shows the SEM image of RGO which contains nanosheets of graphene with petal-like appearance, sharp edges with random directions to the substrate, which can be resulted in forming a nest-like porous structure with a large surface area. Fig. 1D is related to Fe_3O_4-RGO indicating a homogeneous distribution of Fe_3O_4 particles in the Fe_3O_4-RGO composite and decreasing the size of Fe_3O_4 particles (L1, L2, L3 and L4 reveal size of Fe_3O_4NPs in Fe_3O_4-RGO composite). So, the modification of MBCPE with Fe_3O_4-RGO increases the loading amount of DNA probes that can cause the improvement of immobilization and hybridization processes.

Another technique for characterization of the designed biosensor was FT-IR method. The FT-IR spectra of Fe_3O_4NPs, GO, RGO and Fe_3O_4-RGO were shown in Fig. 2A. The IR spectrum of Fe_3O_4NPs (curve a of Fig. 2A) shows a weak peak at 3500–3300 cm^{-1} corresponds to O-H stretch [28]. Curve b in Fig. 2A indicates IR spectrum of GO, as it can be seen a C═O stretch at 1720–1690 cm^{-1}, a strong O-H stretch at 3500–3300 cm^{-1} and the band located at 1200–1250 cm^{-1} is related to the symmetric stretching of CO form COOH group indicating GO was sensitized properly [25]. On the other hand, FT-IR of RGO shows absence of the peaks at 1720–1690 indicating removal of the carboxyl groups attached to the basal graphene layer (curve c of Fig. 2A). As the results show, while the hydrazine treatment reduces the oxygenated groups in GO restoring a large fraction of the electrical conductivity, it does not remove them completely [25]. The spectra of Fe_3O_4-RGO (curve d of Fig. 2A) shows a peak at 1200–1250 cm^{-1} and 3500–3300 cm^{-1} corresponding to C-O and OH stretching vibrations, respectively. The same peaks with slight shift to lower wavenumber are observed in RGO, indicating that the Fe_3O_4 Nanoparticles are precipitated on the RGO. As a result, FT-IR spectra of Fig. 2A showed that fabrication of Fe_3O_4-RGO was done properly.

The magnetic properties of magnetic nanoparticles before and after adsorption of RGO were studied by vibrating sample magnetometer (VSM). According to Fig. 2B, the curve of magnetic hysteresis loop of Fe_3O_4 and Fe_3O_4NPs-RGO nanomaterial are both in S shape and the saturation magnetization value of the Fe_3O_4NPs-RGO nanomaterial is 57.4 emu g^{-1} which is slightly lower than that of pure Fe_3O_4 (64.7 emu g^{-1}). Thus, the saturation magnetization of Fe_3O_4NPs-RGO nanoparticles is adequate to ensure its convenient separation from solutions by external magnetic field.

3.1.2. Characterization by cyclic voltammetry and EIS techniques

EIS and CV techniques were also used to follow the fabrication process of the modified biosensor. All experiments were performed with a 0.5 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ (1:1) in a PBS (0.1 mol L^{-1}) as a redox probe solution. Fig. 3 reveals the CVs of different electrodes in a 0.5 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ solution at a scan rate of 100 mVs^{-1}. As shown in Fig. 3, a clear increase in the peak current (i_p) of MBCPE/Fe_3O_4-RGO electrode is observed compared with the cyclic voltammogram of bare MBCPE (curves a and b). This increase in peak current is probably due to the unique properties of RGO such as its huge surface, high electric transfer kinetics and excellent conductivity, in addition using nanoparticles of Fe_3O_4 can cause increasing in the accessible surface of the electrode [29]. After addition of PANHS to the surface of electrode, peak current decreases and peak potential difference (ΔE_p) increases due to preventing $[Fe(CN)_6]^{3-/4-}$ ions from reaching to the electrode surface (curve c). By immobilization of probe strands on the electrode surface, peak current declines and peak potential difference (ΔE_p) increases. Because the repulsion of $[Fe(CN)_6]^{3-/4-}$ by the negative charged phosphate backbone of probe DNA and consequently preventing $[Fe(CN)_6]^{3-/4-}$ ions from reaching to the surface of electrode can be occurred (curve d) [30].

EIS signals for different modification steps were in good agreement with the obtained results by CV experiments. As shown in Fig. 4, by modification of bare electrode with Fe_3O_4-RGO, the surface of electrode is improved and due to high conductivity of RGO and its high surface

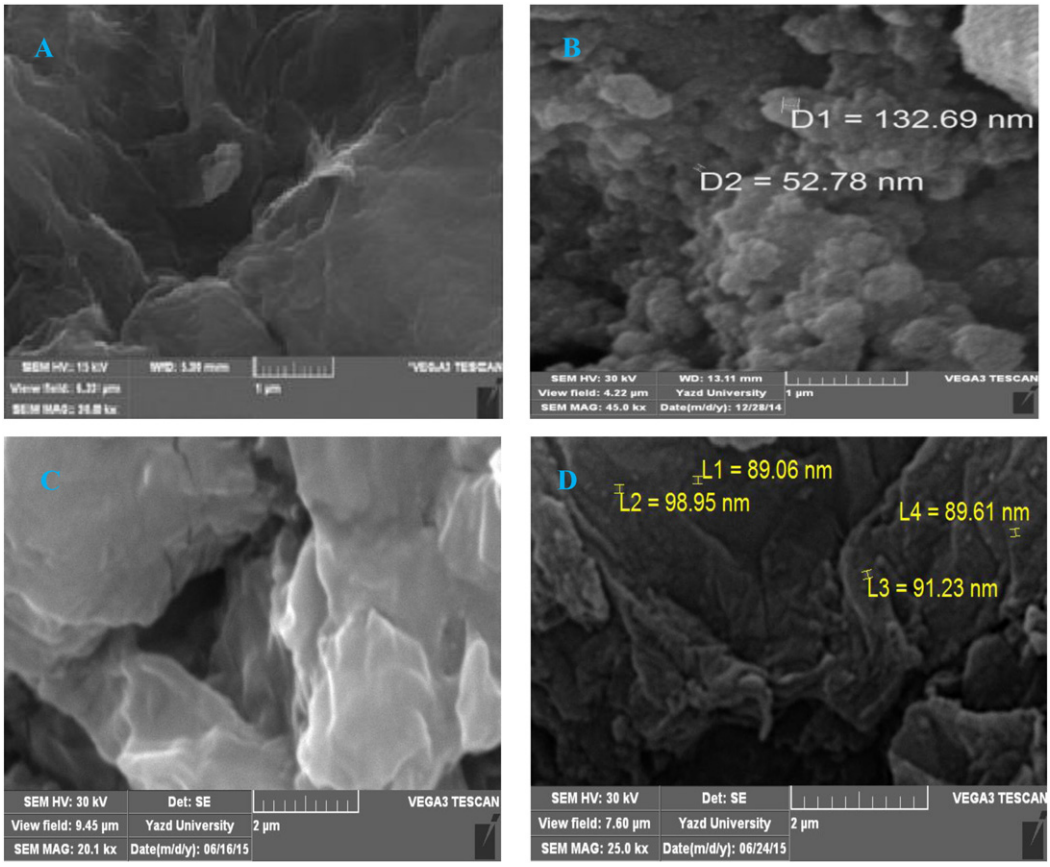


Fig. 1. SEM images of (A) bare MBCPE, (B) Fe_3O_4NPs, (C) RGO (D) Fe_3O_4-RGO.

area, R_{ct} is decreased compared with bare MBCPE (Fig. 4 curves a and b) [25]. By adding PANHS on the surface of electrode, R_{ct} is increased because the electrode surface is covered by PANHS and preventing $[Fe(CN)_6]^{3-/4-}$ ions from reaching to the electrode surface occurs (curve c). The amount of R_{ct} is increased by immobilization of DNA probe on the MBCPE/Fe_3O_4-RGO/PANHS surface (Fig. 4, curve d) [25]. Generally, CV and EIS technique of Fig. 3 and Fig. 4 indicate that the fabrication processes of electrode (MBCPE/Fe_3O_4-RGO/PANHS/ssDNA) has been done properly.

3.2. Optimization of experimental conditions

3.2.1. Investigation of concentration of PANHS and immobilization time of probe DNA

The concentration of PANHS suspension on the MBCPE/Fe_3O_4-RGO electrode surface was optimized (Fig. 5A). According to Fig. 5A, the optimum concentration of PANHS suspension was selected to be 3 μM. As Fig. 5A shows with increasing the concentration of PANHS up to 3 μM, ΔR_{ct} values ($\Delta R_{ct} = R_{final} - R_{initial}$ where R_{final} and $R_{initial}$ are (charge transfer resistance of MBCPE/Fe_3O_4-RGO in 0.5 mM of $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl solution) with and without PANHS, respectively) are increased and then are constant. The obtained results reveal that by increasing the concentration of PANHS suspension up to 3 μmol L^{-1}, the total surface of MBCPE/Fe_3O_4-RGO electrode is saturated and more than this amount (3 μmol L^{-1}), does not influence on the surface of designed electrode strongly.

The immobilization amount of probe is an important factor which effects on the sensitivity of DNA biosensor, so the effect of immobilization time was investigated on the electrochemical signal. The different times of probe immobilization time on the electrode surface from 1 h to 16 h were tested. It was obtained that by increasing time, ΔR_{ct} increases significantly (while $(\Delta R_{ct} = (R_{ct})_{final} - (R_{ct})_{initial})$ and $(R_{ct})_{initial}$, is the R_{ct} before probe immobilization and $(R_{ct})_{final,}$ is R_{ct} after probe immobilization). ΔR_{ct} starts to be constant after 6 h (Fig. 5B) and this is probably due to the full coverage of the electrode surface by the DNA probe after 6 h. Thus, an immobilization time of 6 h was chosen for further experiments.

3.2.2. Effect of time and temperature of hybridization

Time and temperature of hybridization process are other main parameters which have to be optimized. Hybridization time was changed from 5 min to 1 h while keeping hybridization temperature and complementary target DNA concentration constant at 25 °C and 1×10^{-14} mol L^{-1}, respectively (Fig. 5C). In this test, the impedance values of target strands were used as a function of hybridization time. As shown in Fig. 5C, 50 min was obtained as an optimal hybridization time.

The last parameter for optimization was temperature of hybridization. The obtained results in Fig. 5D reveal that ΔR_{ct} values are increased by increasing temperature up to 35 °C and rapidly decreased by increasing temperature. This phenomenon can be related to removing some molecules from the electrode surface in higher temperature and consequently reduction of ΔR_{ct}, so the temperature of 35 °C was used as the optimum hybridization temperature.

3.3. Calibration investigation on the modified electrode

Fig. 6 shows EIS responses of MBCPE/Fe_3O_4-RGO/PANHS/ssDNA biosensor versus the various concentrations of complementary target DNA. According to this figure, with increasing the concentrations of complementary target DNA, R_{ct} increases. Inset A of Fig. 6 reveals the used Randles equivalent circuit for EIS measurements. As known, in this equivalent circuit, R_s is the electrolyte resistance, W_{01}, is Warburg impedance resulting from the ions diffusion, CPE is a constant phase

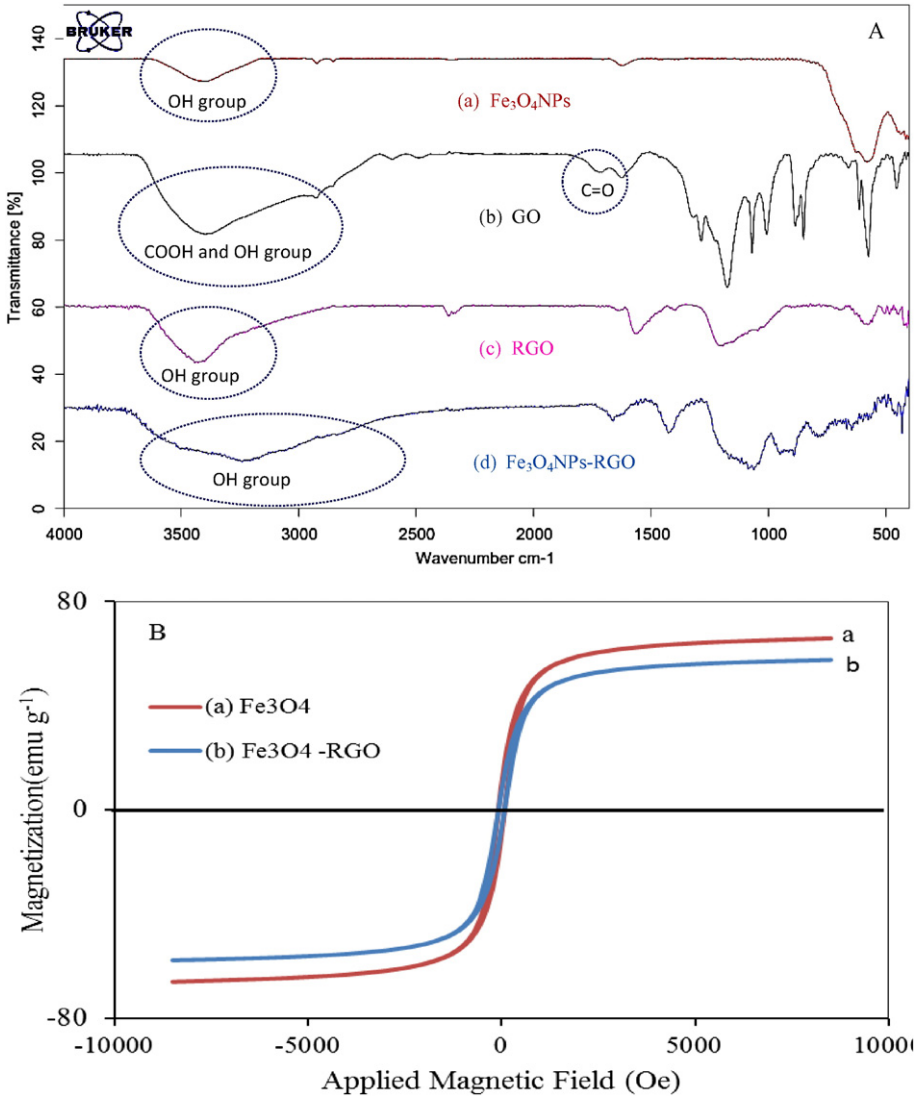


Fig. 2. (A) FT-IR spectra of (a) Fe_3O_4NPs, (b) GO (c) RGO (d) Fe_3O_4-RGO. (B) Magnetization curves of (a) Fe_3O_4 and (b) Fe_3O_4-RGO.

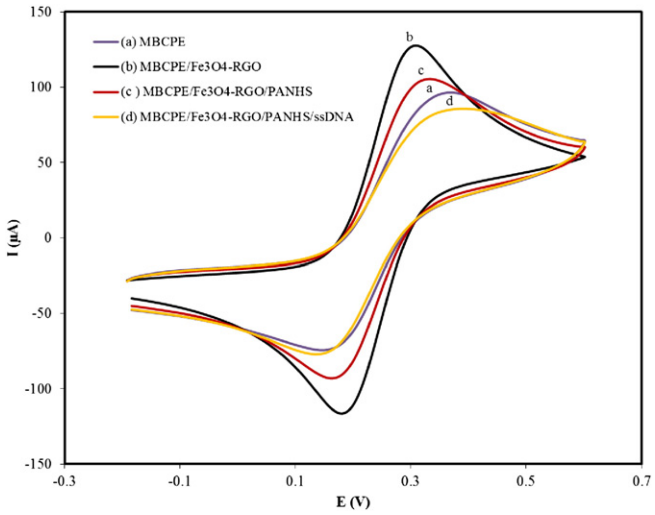


Fig. 3. Cyclic voltammograms of different electrodes in a solution containing 0.5 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ in 0.1 mol L^{-1} KCl at (a) MBCPE, (b) MBCPE/Fe_3O_4-RGO, (c) MBCPE/Fe_3O_4-RGO/PANHS (d) MBCPE/Fe_3O_4-RGO/PANHS/ssDNA.

element and R_{ct} is the electron transfer resistance. We used the recorded R_{ct} value for each experimental steps, because electron transfer process between the redox indicator solution of $[Fe(CN)_6]^{3-/4-}$ and

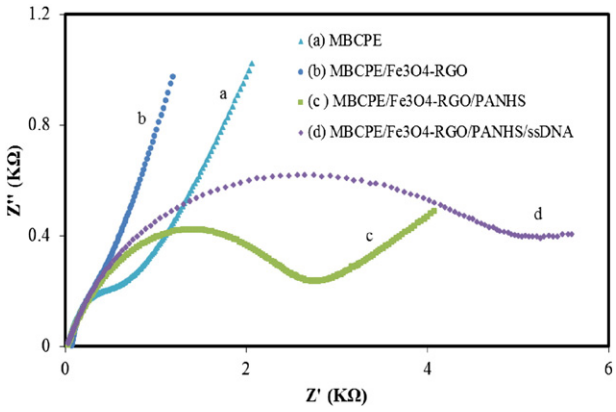


Fig. 4. Nyquist diagrams in a solution containing 0.5 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ and 0.1 mol L^{-1} KCl at (a) MBCPE, (b) MBCPE/Fe_3O_4-RGO, (c) MBCPE/Fe_3O_4-RGO/PANHS (d) MBCPE/Fe_3O_4-RGO/PANHS/ssDNA. EIS conditions: ac potential 5 mV, frequency range 10 kHz to 0.1 Hz.

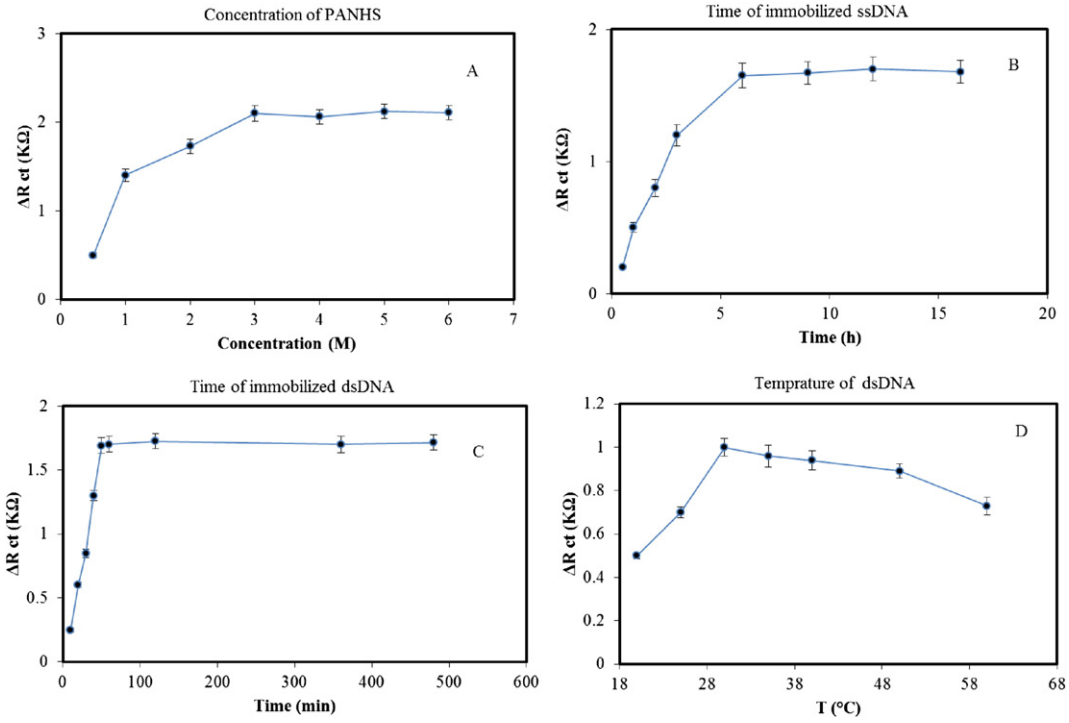


Fig. 5. The optimization of (A) concentration of PANHS (B) immobilization time (C) the time of hybridization (D) the temperature of hybridization.

electrode surface has been influenced by electrode modification and hybridization processes.

The obtained results in inset B of Fig. 6 (which repeated every test three times and the average values were reported) show that for MBCPE/Fe_3O_4-RGO/PANHS electrode ΔR_{ct} versus concentration of complementary DNA strands is constituted from 1.0×10^{-18} mol L^{-1} to 1.0×10^{-8} mol L^{-1} with a regression equation of $\Delta R_{ct} = 0.334$ Log C (mol L^{-1}) + 6.57 ($R^2 = 0.9935$). The detection limit based on $3s_{bl}$ (s_{bl} was the relative standard deviation of 10 replicate measurements of blank solution) was obtained to be 2.8×10^{-19} mol L^{-1}.

Fig. 7 shows DPV responses of the fabricated biosensor (MBCPE/Fe_3O_4-RGO/PANHS/ssDNA) through the addition of different concentrations of complementary DNA strands. According to this figure, with increasing the concentrations of target, current (I) values are decreased, this behavior can be explained by the fact that more active sites of surface are occupied by complementary DNA strands. The obtained results for MBCPE/Fe_3O_4-RGO/PANHS/ssDNA biosensor exhibited that ΔI versus different concentration of complementary DNA strands is evaluated from 1.0×10^{-12} to 1.0×10^{-6} mol L^{-1} with a regression equation of $\Delta I = 2.45$ Log C (mol L^{-1}) + 34.8 ($R^2 = 0.978$) and the limit of detection based on $3s_{bl}$ was obtained to be 2.1×10^{-13} mol L^{-1} (see the inset A of Fig. 7).

The obtained results revealed that using Fe_3O_4-RGO and PANHS due to their unique properties lead to an enhancement in sensitivity of the fabricated biosensor. Some characteristics of the proposed electrode were compared with similar reported electrodes [13,23,25,30–33] in Table 1. According to this table, obtained detection limit and linear

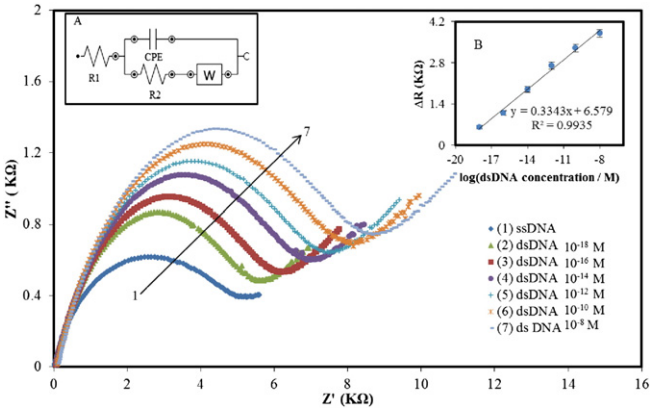


Fig. 6. EIS signals at ssDNA immobilized and after hybridization with its complementary with different concentrations (1.0×10^{-18} mol L^{-1}–1.0×10^{-8} mol L^{-1}), Inset A: The applied equivalent circuit for impedance data in the presence of redox couples Inset B: Plots of the dependence of ΔR_{ct} versus the different concentrations of target for MBCPE/Fe_3O_4-RGO/PANHS/ssDNA.

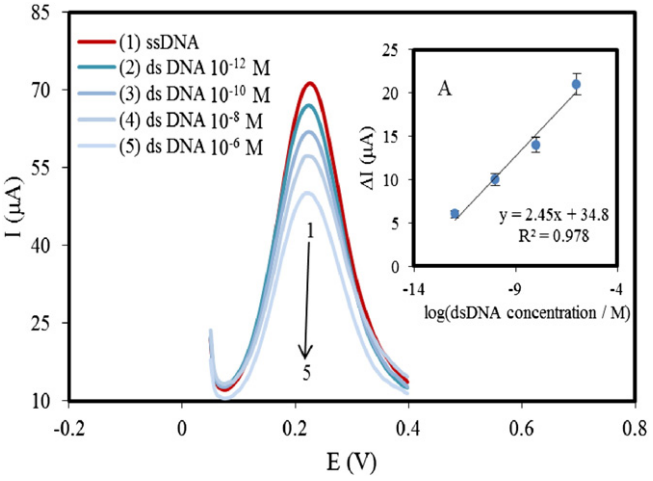


Fig. 7. Differential pulse voltammograms recorded at MBCPE/Fe_3O_4-RGO/PANHS/ssDNA and after hybridization with the complementary DNA with different concentrations (1.0×10^{-12} mol L^{-1}–1.0×10^{-6} mol L^{-1}), Inset A: Plot of the dependence of ΔI versus the different concentrations of target DNA.

Table 1
Comparison of analytical performances of several electrochemical DNA sensors.

DNA sensor	Detection technique	Linear range (M)	Detection limit (M)	Ref
GR-PBA/Au	SPR	1.0×10^{-15}–1.0×10^{-12}	3.8×10^{-16}	[13]
AuNPs/RGO/GCE	EIS	3.0×10^{-20}–1.0×10^{-7}	1.0×10^{-20}	[23]
RGO/GCE	EIS	1.0×10^{-20}–1.0×10^{-14}	3.2×10^{-21}	[25]
MWCNTs/GCE	CV	2.0×10^{-10}–5.0×10^{-8}	1.0×10^{-10}	[30]
DNA/AuNP/cys/PGA	DPV	0.09×10^{-9}- 4.8×10^{-9}	0.042×10^{-9}	[31]
DNA/polyaniline/GCE	DPV	22.5×10^{-9}–2.25×10^{-12}	1.0×10^{-12}	[32]
ssDNA/RGO/GCE	DPV	1.0×10^{-13}–1.0×10^{-7}	1.58×10^{-13}	[33]
MBCPE/Fe_3O_4-RGO/PANHS	EIS	1.0×10^{-18}–1.0×10^{-8}	2.8×10^{-19}	This work

(PICA): poly(indole-5-carboxylic acid).
GNs: graphene nano sheets.
(GR): graphene.
(PBA): pyrenebutyric acid.
(AuNP/cys/PGA): gold nanoparticles/cysteamine/poly(glutamic acid).

range in this work for MBCPE/Fe_3O_4-RGO/PANHS biosensor, are comparable and in some cases better than other works [23,25].

3.4. The selectivity and analytical performance of the modified DNA sensor

Recognition of differences between the targets DNA from others is defined as the selectivity of biosensor. Selectivity of the MBCPE/Fe_3O_4-RGO/PANHS/ssDNA biosensor was tested by using prepared modified electrode to hybridize with different kinds of DNA sequences (target P1C and non-complementary P1nC). The results indicated that the designed biosensor has different EIS responses after hybridization with complementary and non-complementary DNA sequences with the same concentration (1.0×10^{-14} mol L^{-1}). MBCPE/Fe_3O_4-RGO/PANHS/ssDNA biosensor revealed high signal with P1C sequence ($\Delta R_{ct} = 1.9$ kΩ for P1C $= 1.0 \times 10^{-14}$ mol L^{-1}) and a small change in EIS response for P1nC sequence ($\Delta R_{ct} = 0.2$ kΩ for P1nC $= 1.0 \times 10^{-14}$ mol L^{-1}). So, the obtained results indicate that selectivity of the designed electrode is excellent and it can be suggested that the fabricated biosensor applies in the next works to detect *BRCA1* mutation in real samples.

3.5. Repeatability, reproducibility and stability of the DNA sensors

The electrode capability for the generation of a repeatable response is defined as the repeatability of sensor. The hybridization process of MBCPE/Fe_3O_4-RGO/PANHS/ssDNA was performed with 1.0×10^{-14} mol L^{-1} target DNA for five times and a deviation percentage of relative standard of 4.2% was obtained. For reproducibility investigations, three independently MBCPE/Fe_3O_4-RGO/PANHS/ssDNA sensors were prepared and three fabricated MBCPE/Fe_3O_4-RGO/PANHS/ssDNA were hybridized with 1.0×10^{-14} mol L^{-1} target DNA. The results showed the ΔR_{ct} values ($\Delta R_{ct} = 2.00$, 1.99 and 1.83 kΩ) with a relative standard deviation of 4.4% ($n = 3$) indicating a satisfactory reproducibility of this electrochemical biosensor.

In addition, the stability of the designed biosensor was investigated, too. When the fabricated biosensor was stored in a 0.1 mol L^{-1} of phosphate buffer solution (pH 7.4) at 4 °C for 4 days, the response of biosensor was retained about 94% of its primary response. According to the obtained results, the stability of this fabricated DNA biosensor is appropriate.

3.6. Genome DNA detection

Detection of genomic samples is important for the analysis of clinical samples and genetic organisms. Measurements of complementary DNA strains in the genome samples were examined by EIS technique. According to the results in Table 2, at first, ssDNA was immobilized on the three electrode surfaces of MBCPE/Fe_3O_4-RGO/PANHS. Then, prepared MBCPE/Fe_3O_4-RGO/PANHS/ssDNA electrodes were immersed in different spiked 1.0×10^{-10} mol L^{-1}, 1.0×10^{-12} mol L^{-1}, 1.0×10^{-14} mol L^{-1} target DNAs solutions of genomic samples which were extracted from blood using a DNA extraction kit and were employed as the target. The EIS responses revealed that, by immersing the probe in a genome sample solution, R_{ct} was increased indicating the hybridization of various ds-DNA strands with the probe sequences. The obtained recovery percentages revealed that the designed biosensor has a good accuracy for BRCA1 detection (each measurement was repeated at least five times using different electrodes). At last, the electrodes were immersed in a buffer solution at 94 °C for 10 min to dissociate the double strands. After denaturation, the target DNAs were eliminated, and the R_{ct} values approximately decreased to the initial values. Therefore, according to the obtained recovery percentages, this method was successful in the detection of the spiked target DNA sequences in the genomic DNA solution, and the regeneration of the designed biosensor was very good.

4. Conclusion

In this research, we explained the construction of a label-free electrochemical biosensor for BRCA1 mutation detection, based on a modified magnetic bar carbon paste electrode with Fe_3O_4-RGO nanoparticles and PANHS. At first, Fe_3O_4-RGO nanoparticles were synthesized and then the synthetized nanoparticles composite was adsorbed onto the designed magnetic bar carbon paste electrode (MBCPE) by physical method. Some advantages of this electrochemical biosensor due to its unique structure are as follows: Using PANHS leads to decreasing electrode preparation, possessing an excellent selectivity for determination

Table 2
The obtained recovery percentages of three constructed biosensor for the determination of target DNA after hybridization with various spiked complementary DNA in the genome samples by EIS technique ($n = 5$).

Sensor	R_{ct} before hybridization (kΩ)	Added target DNA concentration (mol L^{-1})	R_{ct} after hybridization	Obtained target DNA concentration (mol L^{-1})	Recovery percentages (%)	R_{ct} after denaturation (kΩ)
1	5.50 ± 0.20	1.0×10^{-14}	7.40 ± 0.23	0.95×10^{-14}	95	5.62 ± 0.20
2	5.51 ± 0.25	1.0×10^{-12}	8.08 ± 0.20	1.05×10^{-12}	105	5.63 ± 0.23
3	5.61 ± 0.22	1.0×10^{-10}	8.84 ± 0.21	0.94×10^{-10}	96	5.46 ± 0.25

of breast cancer mutation using EIS and DPV techniques, being fast, non-expensive and easy fabrication. Under optimum conditions, for MBCPE/Fe_3O_4-RGO/PANHS/ssDNA electrochemical biosensor a detection limit of 2.8×10^{-19} mol L^{-1} and a linear range (1.0×10^{-18} mol L^{-1} to 1.0×10^{-8} mol L^{-1}) were obtained. Also, this designed biosensor with high sensitivity was applied successfully for discrimination of complementary and non-complementary sequences of *BRCA1 5382 insC*. We are hopeful that our present work leads to improving novel sensors in medical field for detection of some disease which are difficult to cure such as cancers.

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