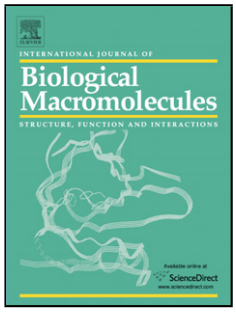

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PII: S0141-8130(16)30499-8
DOI: http://dx.doi.org/doi:10.1016/j.ijbiomac.2016.05.087
Reference: BIOMAC 6148

To appear in: *International Journal of Biological Macromolecules*

Received date: 22-10-2015
Revised date: 22-5-2016
Accepted date: 25-5-2016

Please cite this article as: Ezat Hamidi-Asl, Jahan Bakhsh Raoof, Nahid Naghizadeh, Haleh Akhavan-Niaki, Reza Ojani, Ali Banihashemi, A bimetallic nanocomposite modified genosensor for recognition and determination of thalassemia gene, International Journal of Biological Macromolecules http://dx.doi.org/10.1016/j.ijbiomac.2016.05.087

A bimetallic nanocomposite modified genosensor for recognition and determination of thalassemia gene

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Highlights

- New genosensor for recognition of thalassemia geneusing methylene blue as indicator
- Using of bimetallic nanocomposite modified carbon paste electrode as genosensor
- CPE modified by 15% (w/w) of Ag plus Pt nanoparticles
- Using polymerase chain reaction (PCR) as real sample

Abstract:

The main roles of DNA in the cells are to maintain and properly express genetic information. It is important to have analytical methods capable of fast and sensitive detection of DNA damage. DNA hybridization sensors are well suited for diagnostics and other purposes, including determination of bacteria and viruses. Beta thalassemias (βth) are due to mutations in the β-globin gene. In this study, an electrochemical biosensor which detects the sequences related to the β- globin gene issued from real samples amplified by polymerase chain reaction (PCR) is described for the first time. The biosensor relies on the immobilization of 20-mer single stranded oligonucleotide (probe) related to βth sequence on the carbon paste electrode (CPE) modified by 15% silver (Ag) and platinum (Pt) nanoparticles to prepare the bimetallic nanocomposite electrode and hybridization of this oligonucleotide with its complementary sequence (target). The extent of hybridization between the probe and target sequences was shown by using linear sweep voltammetry (LSV) with methylene blue (MB) as hybridization indicator. The selectivity of sensor was investigated using PCR samples containing non-complementary oligonucleotides. The detection limit of biosensor was calculated about 470.0 pg/µL.

Keywords: Biosensor; Bimetallic nanoparticles; Methylene blue; β-Thalassemia; Polymerase chain reaction.

1. Introduction

The easy fabrication of DNA surfaces which are reproducible, stable and selective to complementary DNA sequences is crucial in the development of emerging biotechnologies such as DNA chips and simple diagnostic devices for detecting a few sequences of target DNA [1]. Biosensors are small devices employing biochemical molecular recognition properties as basis for selective analysis. Due to their specificity, speed, portability and low cost, biosensors offer exciting opportunities for numerous decentralized clinical applications [2].

Recently our research group has published several articles regarding DNA biosensors modified by mono nanoparticles such as gold, platinum or silver [3-6]. However, the use of binary nanoparticles for improving the electrochemical sensing devices is an extremely promising [7]. As the addition of the second metal lead to variation in particle size, shape, surface morphology, chemical and physical properties. Multilayer of conductive nanoparticles give rise to porous, high surface area electrode, where the local microenvironment of metallic nanoparticles can be controlled by crosslinking elements and causes the specific and selective interactions with substrates [8, 9].

In addition to direct electrochemistry of DNA (using intrinsic DNA electroactivity and surface activity), indirect approaches have been introduced in DNA electroanalysis and DNA biosensor technology [10]. To improve the sensitivity of electrochemical assays and to achieve better, more reliable analysis, there is a great demand for labels with higher specific activity. The most commonly used labels for electrochemical sensors to date have been enzymes and small electroactive indicator molecules [11]. A number of formats for electrochemical detection are described in the literature, including hybridization with probes conjugated to a redox active label

[12-16]. Methylene blue (MB), an organic dye which belongs to the phenothiazine family, has been widely used in DNA hybridization detection as an electrochemical indicator [17-24].

Meric et al. described the use of MB as hybridization indicator for electrochemical detection of Hepatits B and TT virus in the surface of carbon paste electrode [25]. Pournaghi-Azar et al. used pencil graphite electrode and MB as electroactive label for the sense of strand of interleukine-2 gene [26]. Raoof et al. described development of an electrochemical biosensor based on peptide nucleic acid (PNA) probe for detection of target DNA sequence and single nucleotide mutation in p53 tumor suppressor gene corresponding oligonucleotide using methylene blue (MB) as an electrochemical indicator [27]. Huan et al. utilized Muts protein and MB marker for mutation recognition in β-thalassemia gene on to the gold electrode [28].

Hemoglobin (Hb) is the essential oxygen transporting protein in animal kingdom. In human, the major component of red cells in postnatal life consists of 2α- and β- globin chain (HbA). Globins are encoded by 1β and 2α genes located on the short arm of chromosome 11 and 16, respectively. Thalassemia is recessively inherited disorders characterized by a quantitative reduction of the α- or β-globin genes expression leading to anemia [29]. B-thalassemia is monogenic diseases which are transmitted most frequently in an autosome recessive way [30]. The disease appears generally during the first year of life. It manifests with severe anemia (Hb < 7g/dL) requiring regular blood transfusion [31].

Beta thalassemias are due to mutations in the β- globin gene. The DNA biosensor showed here was proved to detect β- globin gene efficiently on the modified carbon paste electrode (CPE) with silver and platinum nanoparticles modified carbon paste electrode (MCPE) by indirect method. We used MB as electroactive indicator and polymerase chain reaction (PCR) products

of real samples. No literature has been recorded using similar procedure for detection of the thalassemia gene PCR products originating from real blood samples.

2. Experimental

2.1. Reagents and materials

MB was analytical grade and was purchased from Merck. A 20-mer oligonucleotide corresponding to sense strand of human β-thalassemia gene (IVSII-1) was used as the probe and its complementary (CIVSII-1) oligonucleotide corresponding to antisense strand of human β-thalassemia gene was used as target DNA. Both probe and target DNA were supplied as lyophilized powder by Bioneer Company, South Korea with the following sequences:

Probe DNA (IVSII-1): 5′-ACTTCAGGATGAGTCTATGG-3′

Complementary DNA (CIVSII-1): 5′-CCATAGACTCATCCTGAAGT-3′

The PCR amplified DNAs (0.45 ng/mL) obtained from real blood samples were donated by Genetic Laboratory of Amirkola Children Hospital and were prepared as reported elsewhere [33, 34]. Two sets of PCR products were used in this study. One corresponding to the β-globin gene and complementary to the oligonucleotides described above and another non-complementary sequence (A and B) amplified by PCR. Their sequences are as follows:

A (Fc): 5′-TGG GGA TGG AGA ACT-3′

B (α2 IVS-I):5′-CAC TCT TCT GGT CCC CAC AGACT-3′

All oligonucleotides stock solution (100 μM) were prepared with TE buffer solution (10 mM Tris-HCl, 1.0 mM EDTA, pH 8.00) and kept frozen. More diluted solutions of oligonucleotides

were prepared using 0.50 M acetate buffer solution (pH 4.80) containing 20mM NaCl. The stock solution of MB (1.0 mM) was prepared using distilled and sterilized water. H_2PtCl_6 and $AgNO_3$ were purchased from Merck Company. Other chemicals were of analytical grade. The distilled and sterilized water was used in all solution preparations. Each measurement consists of the immobilization cycle carried on a fresh MCPE surface. All the experimental procedures were performed at room temperature in an electrochemical cell.

2.2. Instrumentation

Electrochemical experiments were performed by AUTOLAB PGSTAT 30 electrochemical analysis system; GPES 4.9 and FRA 4.9 software package (Eco Chemie, Netherlands). All electrochemical measurements were carried out using conventional three-electrode cell.

MCPE or CPE was used as working electrodes (surface area of 0.03 cm^2) and Pt wire served as auxiliary electrode. All potentials are reported against an Ag│AgCl│KCl(3M) reference electrode. All parameters in procedure were optimized before and published elsewhere [32]. Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM) were taken using Tescan Vega II XMU and Nanosurf easyscan 2, alternatively.

2.3. Procedure

2.3.1. Synthesis of nanoparticles

Platinum nanoparticles (NPt) were prepared according to the literature [35,36]. Briefly, 60 mL of a 2.0 mM aqueous H_2PtCl_6 solution were mixed with 3 mL of 50mM aqueous sodium citrate solution, then under vigorous stirring, 7 mL 120 mM aqueous $NaBH_4$ solution were added drop wise.

Silver nanoparticles (NAg) were prepared by a chemical reduction method reported in literature [37]. In this method, $AgNO_3$ (17.0 mg) was dissolved in 100 mL water in a 250 mL tri-neck flask. The solution was heated to boiling with a hemisphere heating mantle under vigorous magnetic stirring. After boiling for 2 minutes, an aqueous solution of sodium citrate (35 mM, 10 mL) was rapidly added to the flask. The solution gradually turned yellow within a few minutes, indicating the formation of Ag nanoparticles. The solution was kept boiling for an additional 6 minutes. After that, the heating mantle was removed, and the solution was allowed to cool.

For taking AFM picture, the sample was prepared by putting a drop of stock solution of nanoparticles on the surface of a lam. After drying the drop, the lam was scanned by Easyscan 2 Flex. The particle size was determined using Nanosurf easy Scan 2 software and most of the particles size was between 10 to 20 nm.

2.3.2. Preparation of MCPE

CPE was prepared according to the literature [38]. For fabrication of silver nanoparticles modified carbon paste electrode, sufficient Ag nanoparticles were added to carbon paste to obtain modified CPE containing different percentages of Ag nanoparticles (Ag-MCPE). A portion of the resulting paste was packed into the bottom of a glass tube. The electrical connection was implemented via copper wire fitted into the glass tube. The surface of the resulting electrode was smooth on a weighing paper and rinsed carefully with distilled water. The procedure for preparation of Pt-MCPE and Ag/Pt-MCPE has been just similar to the preparation of Ag-MCPE.

2.3.3. Electrochemical activation of the working electrode

The electrochemical pretreatment of the surface of electrode was performed on the optimized potential of 1.80 V vs. SCE for 5 min in 0.50 M acetate buffer solution (pH 4.80) containing 20 mM of NaCl without stirring.

2.3.4. Immobilization of the probe on the working electrode

After activation, for immobilization of the probe on the electrode, it was immersed in 0.50 M acetate buffer solution (pH 4.8) containing 1.0 µM probe and 20 mM of NaCl. Afterward, the 0.5 V vs. Ag│AgCl│KCl(3M) was applied on the electrode for 5 min in the stirred solution (200 rpm) at room temperature. Then the electrode was rinsed with sterilized and deionized water.

2.3.5. Hybridization

The hybridization was performed by dipping the probe modified electrode into the stirred hybridization solution (0.5 M acetate buffer pH 4.8) containing DNA target and 20 mM aqueous solution of NaCl for 5 min, while the electrode potential was kept at 0.50 V vs. Ag│AgCl│KCl(3M). The electrode was rinsed with sterilized and deionized water to remove the non-hybridized DNA.

PCR products should be pretreated before using in hybridization step. Therefore, the diluted PCR product was denatured by heating in a water bath at 95 ^{0}C for 5 min and immersed in an ice bath for 2 min. After that, it can be used as a target.

2.3.6. MB accumulation on the working electrode

Following immobilization of the single stranded DNA (ssDNA) probe on MCPE, MB was accumulated on the probe by dipping the electrode into MB (20 µM) for 5 min without stirring

and applying any potential to the working electrode. The same strategy was performed for the accumulation of MB on the bare CPE and surface hybrid of MCPE with complementary and noncomplementary DNA.

2.3.7. Voltammetric measurements

The reduction of accumulated MB was studied using linear sweep voltammetry (LSV) at MCPE in 20mM of Tris-HCl buffer (pH7.00) at potential between 0.3 to -0.8 V vs. Ag │AgCl │KCl(3M). Respective measurements were carried out after renewing the electrode by cutting and polishing the electrode surface.

2.3.8. Impedance measurement

Impedance measurements were carried out in phosphate buffer solution (pH 7.00) containing 10.0 mM $K_4Fe(CN)_6$ / $K_3Fe(CN)_6$ at a potential 0.18 V vs. Ag │AgCl │KCl(3M). The data are displayed in the complex plane; Nyquist plots (Z" vs. Z', Z" = imaginary impedance and Z' = real impedance).

2.4. PCR amplification

PCR amplification was conducted on human genomic DNA extracted from blood sample using DNA purification kit (DNPTMKit, CinnaGen Inc.). Briefly, 200 µl of blood was centrifuged at 3000 rpm for 10 min to separate cells from serum. The precipitated cells were lysed by suspending in lysis solution and incubation at 37 °C for 20 min. To precipitate genomic DNA, precipitation solution was added; the tube was placed at 20 °C for another 20 min and then centrifuged at 14000 rpm for 20 min at 4 °C. Then, supernatant was poured off, DNA pellet was washed with washing buffer and then air dried. The extracted DNA was finally solved in solvent

buffer and kept frozen at -20°C. PCR amplification of non-complementary oligonucleotide corresponding to antisense strand of human β-thalassemia gene fragment was conducted in the presence of human genomic DNA, as the template DNA using A (Fc): 5′-TGG GGA TGG AGA ACT-3′ and B (α2 IVS-I):5′-CAC TCT TCT GGT CCC CAC AGACT-3′.

3. Results and discussion

The schematic diagram of the electrochemical DNA biosensor based on silver and platinum nanoparticles for DNA hybridization detection using MB indicator was illustrated in Fig. 1.

Fig. 1

3.1. Characterization of synthesized nanoparticles and MCPEs

As shown in Fig. 2(A), AFM was used for characterization of synthesized nanoparticles. The average size of Ag (a) and Pt (b) nanoparticles are 16.2 and 10.7 nm, respectively. The SEM images of surface polished MCPEs were shown in Fig. 2(B). As can be seen, Ag (c), Pt (d) and Ag/Pt (e) with a grain size of several nanometers pervaded in the surface of MCPE.

Fig. 2

To investigate the role of nanoparticles in the matrix of electrode, CPEs were prepared by different modifiers and the electrochemical impedance spectroscopy (EIS) was used for preliminary investigation. Figure 3A shows the electrochemical impedance spectra at bare CPE (a) and modified one with 15% of Pt nanoparticles (b), Ag nanoparticles (c) and bimetallic Ag/Pt (d) in solution of 10 mM $[Fe(CN)_6]^{3-/4-}$ redox system (1:1), 0.1 M KCl, pH 7.00. The high frequency section shows an arc with a charge transfer resistance (Rct) of 1771.12 Ω, 1299.88 Ω,

314.69 Ω and 202.45 Ω for curves (a), (b), (c) and (d), respectively. It is clear that a lower Rct is observed at bimetallic nanocomposite modified carbon paste electrode.

For determining the optimized percentage of bimetallic nanocomposite, carbon paste was modified by different percentage of silver/platinum nanoparticles. Then, the EIS has been taken from CPE and modified ones with different percentage of modification by Au/Pt nanoparticles (Fig.3B). The Rct is 1771.12 Ω (a), 1760.21 Ω (b), 1324.86 Ω (c), 769.32 Ω (d), 202.45 Ω (e), 765.35 (f) and (g) 1046.04 Ω for CPE, 3%, 5%, 10%, 15%, 20% and 30% Ag/Pt-MCPE, respectively. As seen, the lower Rct belongs to 15% modification of carbon paste electrode with Ag/Pt nanoparticles. Therefore, it can be concluded that this percent has the best function between the modified electrodes. As EIS illustrates the barrier properties of the surface of working electrode [39], probably metal nanoparticles aggregate in the modification at percent higher than 15 %. Therefore, the unique properties of nano materials are ruined and the charge transfer resistance at the electrode surface is increased.

Fig. 3

3.2. Preconcentration of MB at 15% Ag/Pt-MCPE

For investigation of accumulation and interaction of MB with DNA on 15% Ag/Pt-MCPE, linear sweep voltammetry (LSV) was selected as simple and sensitive electrochemical technique. LSV was performed in 20 mM Tris–HCl buffer solution (pH 7.0) containing 20 mM NaCl at scan rate: 5 mV s^{-1}. Figure 4 shows the linear sweep voltammetry (LSV) signal of MCPE before (a) and after accumulation of MB (b) and probe immobilized MCPE after accumulation of MB (c). MB was preconcentrated on the electrode as described in section 2.3.6. As seen, the reduction signal of MB at the MCPE (89 µA) is lower than the probe modified electrode (140 µA). This

observation clearly confirmed that MB has strong affinity for ssDNA and a considerable amount of MB accumulates on the probe modified MCPE surface; therefore, the interaction of MB with ssDNA could be electrochemically detected.

Fig. 4

3.3. Immobilization of DNA

Figures 5A and 5B show the LSV and histogram of MB reduction signals obtained after immobilization of probe (1.0 µM) on the surface of different modified CPE. The reduction currents are 9.10 µA ± 0.10 (a), 23.00 µA ± 0.15 (b), 101.00µA± 0.43 (c), and 140.00 µA ±0.05 (d) at the surface of CPE, 15% Pt-MCPE, 15% Ag-MCPE, and 15% Ag/Pt-MCPE, respectively. As can be seen, the immobilized probe on the modified carbon paste electrode with 15% bimetallic of nano-silver and nano-platinum particles have a high level of proficiency in DNA biosensor. Therefore, bimetallic nanocomposite led to a significantly improved electrical signal in biosensor's performance.

Fig. 5

Fig. 6A and 6B illustrate the LSV signals and the histogram of MB reduction signal (Ipa) after probe (1.0 µM) immobilization on the surface of MCPE modified with different percentage of nano particles, respectively. The LSV's reduction currents are 15.00 µA ± 0.06 (a), 22.00 µA ± 0.01 (b), 78.00 µA ± 0.07 (c), 140.00 µA ± 0.05 (d), 100.00 µA ± 0.7 (e), and 59 µA ± 0.1 (f) at the surface of modified CPE by 3%, 5%, 10%, 15%, 20%, and 30% Ag/Pt nanoparticles, respectively. As seen, the activity of the electrode improved when the modification of CPE exceeded by 3% of nano bimetallic until reached to its maximum value at 15% and then decreased at more percent modification rates. When the combination of CPE with nanoparticles

was higher than 15%, the performance of the MCPE was decreased. As mentioned earlier, the higher percent mixture of nanoparticles/CPE can cause either the decline the conductivity of the electrode or the removal of the active site of the working electrode surface.

Fig. 6

3.4. Hybridization

The new biosensor relies on the electrochemical transduction of the hybridization reaction between the short complementary βth oligonucleotides. The detection of hybridization is accomplished by using MB, where strong association with the immobilized ssDNA segment leads to significantly enhanced voltammetric indicator peak, thus, reflects the extent of the hybrid formation. As can be seen in Fig. 7, the reduction currents of MB in the LSV study are µ140.00µA ± 0.05 (a) and 28.00 µA ± 0.02 for the probe before and after hybridization with complementary DNA, respectively. The concentration of the probe and its complementary sequence is 1.0 µM. The detection method, therefore, involves monitoring the MB reduction by LSV, which is enhanced in the presence of ssDNA due to the interaction of MB with guanine bases. Because of the accessibility of the unbound guanine bases in the ssDNA and probe or probably due to a steric inhibition of the reducible groups of MB peaked between the bulky double helix of the DNA hybrids, with the help of the enhanced difference between the Voltammetric signals [40-42].

Fig. 7

The difference between MB signals on the probe MCPE studied in the absence and presence of complementary PCR products. This is the first βth biosensor that its performance is evaluated on PCR products amplified from human DNA samples. The results are very promising. Figure 8A

shows LSV signals of probe (1.0 µM) immobilized on the different working electrodes before (a, b, c) and after hybridization with PCR samples containing complementary sequences (a', b', c'). In this Figure, curve a, b and c are representatives of 15% Ag/Pt-MCPE, 15% Ag-MCPE and 15% Pt-MCPE, respectively. Figure 8B depicts the histogram of LSV signals of MB reduction before and after hybridization with complementary PCR products (1.25 ng/µL). As seen, the difference between MB signals before and after hybridization with complementary DNA from PCR products (ΔI) are nearly 64.00 µA ± 0.02, 5.00 µA ± 0.01 and 10.00 µA ± 0.10 for 15% Ag/Pt-MCPE, 15% Ag-MCPE and 15% Pt-MCPE, respectively. It can be concluded that the bimetallic nanocomposite modified CPE has the best ability to detect hybridization between probe and its complementary PCR product.

Fig. 8

3.6. Selectivity Study

In order to study the selectivity of the proposed biosensor based on MB electroactivity, the probe modified electrode was treated with some noncomplementary PCR products amplified with Fc and α2 IVS-I (Fig. 9: curves b and c). As seen in Fig. 9, the interaction between these non-complementary PCR products and the immobilized probe (curve a) did not lead to a significant decrease in MB signal, due to negligible hybridization.

The DNA sensor selectivity was also investigated in PCR samples containing both complementary and non-complementary sequences (curves d and e). As illustrated, the interactions between βth and Fc or α2 IVS-I in the mixture solution have less effect on the hybridization event between probe and target PCR (curve f). PCR products concentration is 1.25 ng/µL in all samples.

These results further confirmed that this DNA sensor selectively responds to the target DNA and that MB signal could be addressed to the hybridization extent of the probe with the interacted DNA.

Fig. 9

3.7. Investigation of detection limit

The diagnostic performance of the sensor was studied by hybridization investigation between probes (1.0 µM) with different concentrations of complementary PCR sample (0.625 ng/µL to 12.5 ng/µL). The difference between MB reduction signal on the probe-MCPE in the absence and presence of complementary PCR, ΔI, is increased with increasing the complementary PCR concentration and leveled off at concentration12.5 ng/µL (Fig. 10). Thus at this concentration, all of the available probe strands on the electrode surface were involved in hybridization event. The detection limit was calculated based on three times standard deviation of the blank divided by the slope of the calibration curve and given about 470.0 pg/µL.

Fig. 10

4. Conclusion

The integration of nanotechnology with biology and electrochemistry is expected to produce major advances in the field of electrochemical biosensors. Recent progress has led to the development of functional nanoparticles that are linked to biological molecules, such as peptides, proteins and nucleic acids. The present study demonstrated a new approach toward advanced development of highly sensitive electrochemical assay of β-thalassemia in PCR sample by using bimetallic nanocomposite modified carbon paste electrode prepared by mixing of 15% Ag and Pt

nanoparticles. MB was chosen as the DNA hybridization indicator and displayed enhanced hybridization signals. A good selectivity for the biosensor might have promising future in the detection of target ssDNA. Ag/Pt nanoparticles provided a powerful signal that could be used to detect and ensure the sensitivity of proposed biosensor. The sensitivity of biosensor could be enhanced greatly. The successful discrimination between complementary PCR and non-complementary PCR samples was displayed. This strategy could be extended to develop various electrochemical biosensors for detection and quantification of much kind of biocompound.

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Figure captions

Fig. 1. Schematic illustration of the sensing process of the amplifying system.

Fig. 2. (A) AFM images of silver (a) and platinum (b) nanoparticles; (B) SEM images of Ag-MCPE (c), Pt-MCPE (d) and Ag/Pt-MCPE (e).

Fig. 3. (A) Nyquist plots in solution of 10 mM $[Fe(CN)_6]^{3-/4-}$(1:1), in the 0.1 M KCl, pH 7.00 solution at the surface of CPE (a), 15% Pt-MCPE (b), 15% Ag-MCPE(c) and 15% Ag/ Pt-MCPE (d); (B) Nyquist plots of 1.0 mM $[Fe(CN)_6]^{3-/4-}$ (1:1), in the 0.1 M KCl, pH 7.00 solution at the surface of CPE (a),3% Ag/Pt-MCPE (b), 5% Ag/Pt-MCPE (c), 10% Ag/Pt-MCPE (d), 15% Ag/Pt-MCPE (e), 20% Ag/Pt-MCPE (f) and 30% Ag/Pt-MCPE (g).

Fig. 4. linear sweep voltammetry of 15% Ag/Pt-MCPE before (a) and after accumulation of MB (b) and probe immobilized MCPE after accumulation of MB (c) in 20 mM Tris–HCl buffer solution (pH 7.0) containing 20 mM NaCl, scan rate 5 mV/s.

Fig. 5. (A) linear sweep voltammograms and (B) Histogram of MB reduction current after immobilization of probe at the surface of CPE (a), 15% Pt-MCPE (b), 15% Ag-MCPE (c) and 15% Ag/Pt-MCPE (d) in 20 mM Tris–HCl buffer solution (pH 7.0) containing 20 mM NaCl, scan rate 5 mV/s, oligonucleotide and MB concentration was 1.0 μM and 20 μM, respectively.

Fig. 6. (A) linear sweep voltammograms and (B) Histogram of MB reduction current signal after immobilization of probe at the surface of various percent of nanocomposite modified carbon paste electrode: 3% Ag/Pt-MCPE (a), 5% Ag/Pt-MCPE (b), 10% Ag/Pt-MCPE (c), 15% Ag/Pt-MCPE (d), 20% Ag/Pt-MCPE (e) and 30% Ag/Pt-MCPE (f) in 20 mM Tris–HCl buffer solution

(pH 7.0) containing 20 mM NaCl, scan rate 5 mV/s. Oligonucleotide and MB concentration were 1.0 µM and 20 µM, respectively.

Fig. 7. linear sweep voltammograms of probe immobilized onto 15% Ag/Pt-MCPE before hybridization (a) and after hybridization with CIVSII-1, as complementary target DNA (b) in 20 mM Tris–HCl buffer solution (pH 7.0) containing 20 mM NaCl, scan rate: 5 mV/s, probe and CIVSII-1is concentration 1.0 µM.

Fig. 8. (A) linear sweep voltammograms and (B) Histogram of MB reduction current signal of probe immobilized at the surface of 15% Ag/Pt-MCPE (a), 15% Ag-MCPE (b), 15% Pt-MCPE (c) before hybridization and after hybridization with complementary DNA plasmid (a', b', c') in 20 mM Tris–HCl buffer solution (pH 7.0) containing 20 mM NaCl, scan rate: 5 mV/s, DNA probe concentration 1.0 µM, complementary PCR (1.25 ng/µL).

Fig. 9. Linear Sweep voltammograms of probe immobilized at the surface of 15% Ag/Pt-MCPE before hybridization (a) and after hybridization with noncomplementory PCR Fc (b), α2 IVS-I (c), mixture of Fc and complementary PCR (d), mixture of α2 IVS-I and complementary PCR (e), and after hybridization with complementary PCR (f) in 20 mM Tris–HCl buffer solution (pH 7.0) containing 20 mM NaCl, scan rate: 5 mV/s, Concentration of DNA probe: 1.0 µM, DNA plasmid: 1.25 ng/µL.

Fig. 10. Plot of ΔI (difference between the LSV signals of MB on the probe-MCPE in the presence and absence of complementary PCR) vs complementary PCR concentration. Experimental and solution conditions as mentioned in Fig. 9.

Figures:

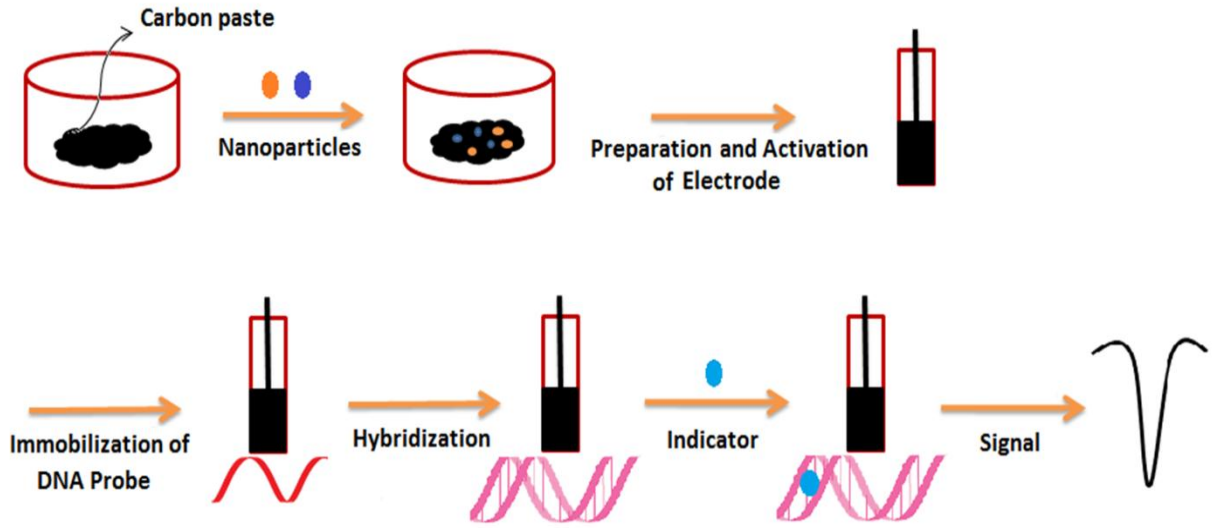


Fig. 1

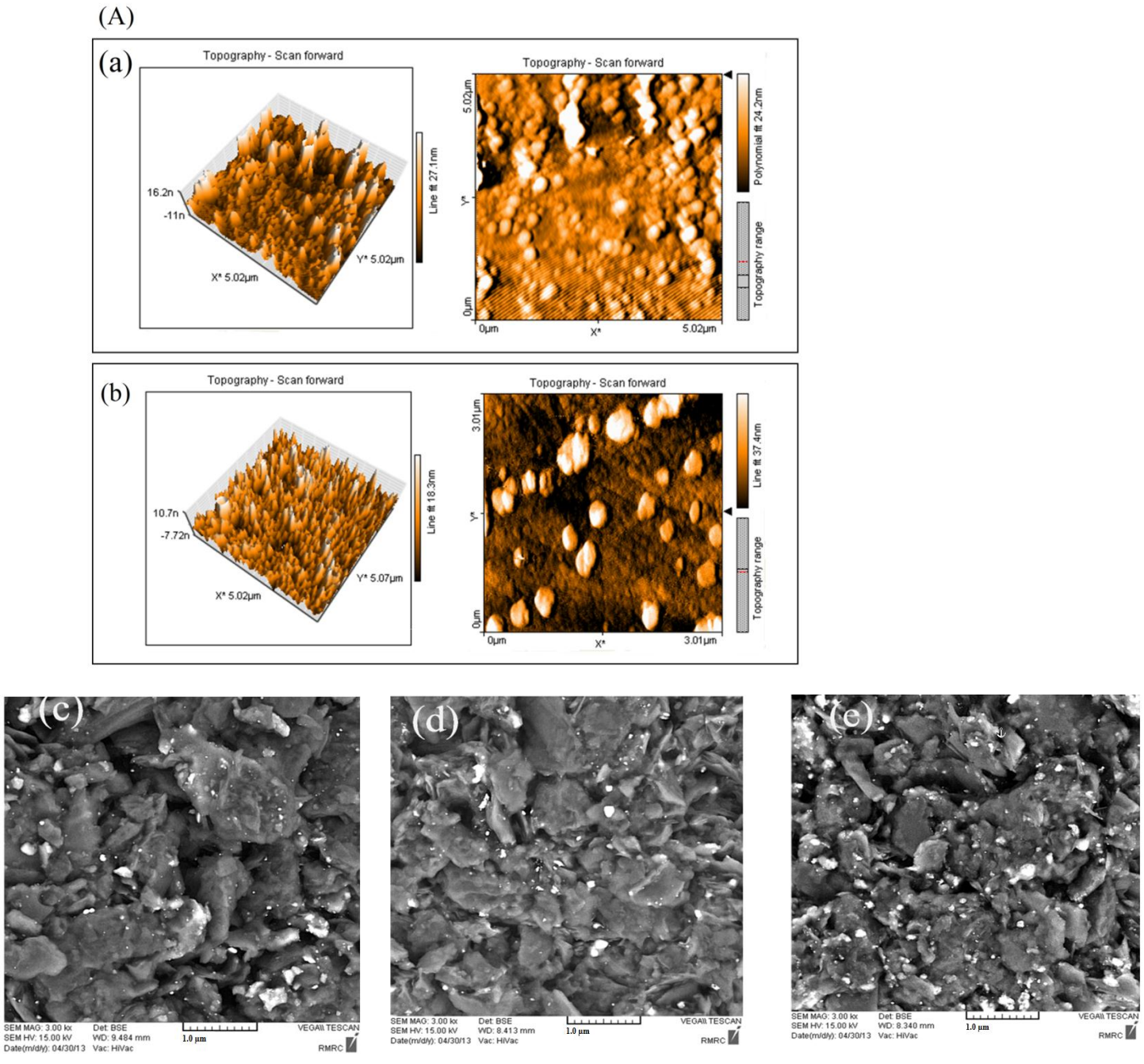
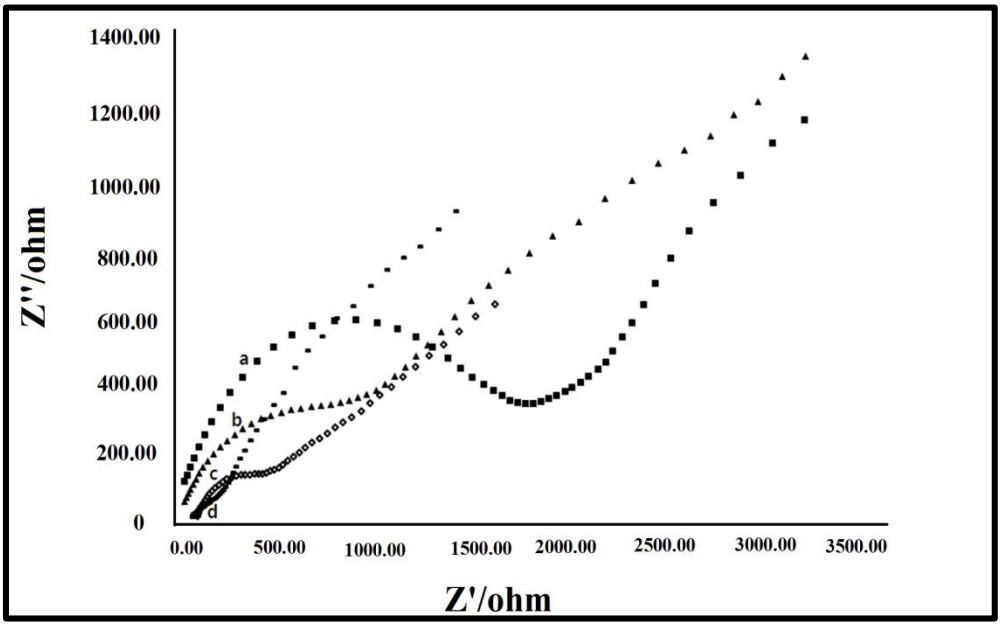


Fig. 2

(A)



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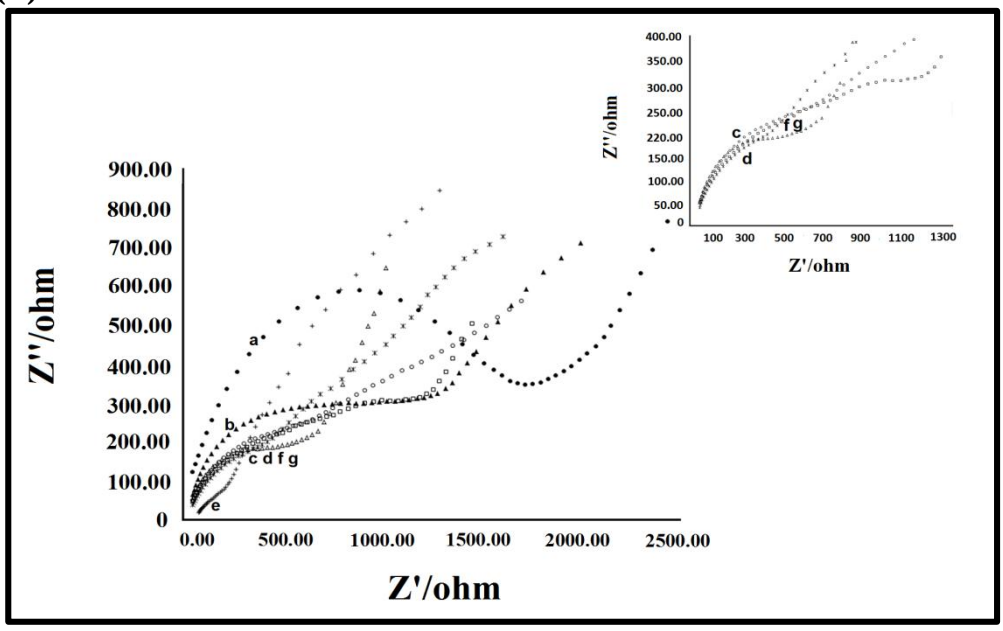
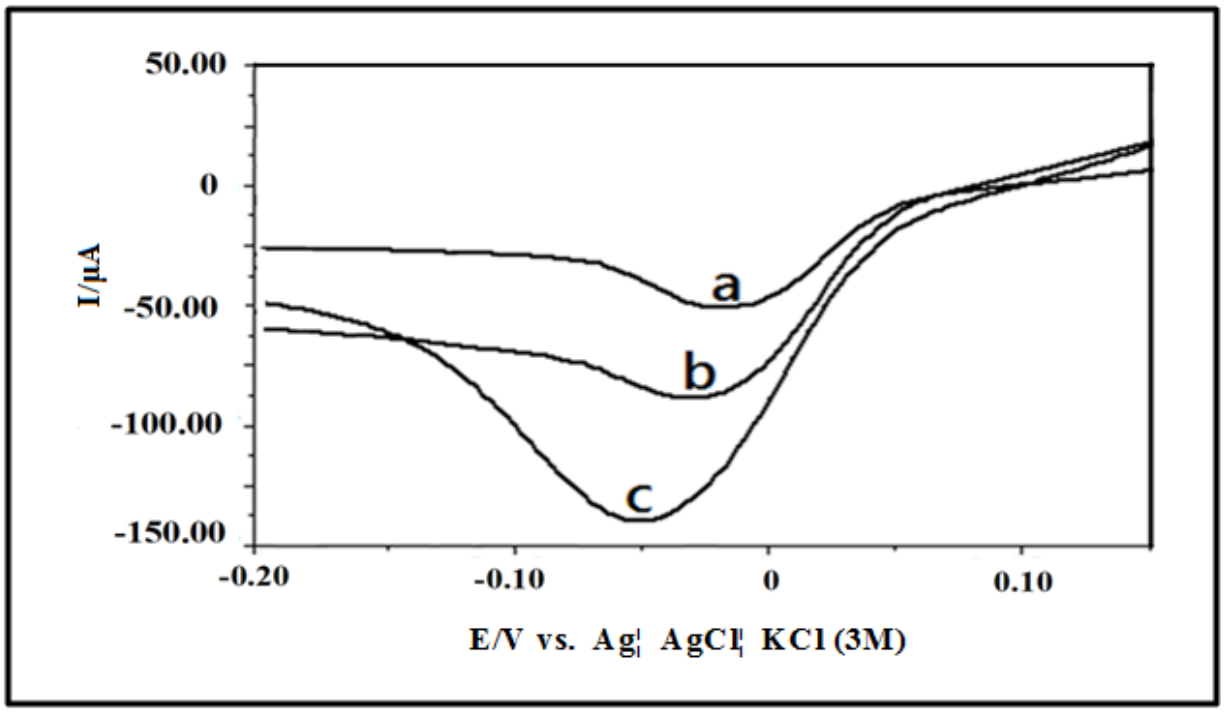


Fig. 3



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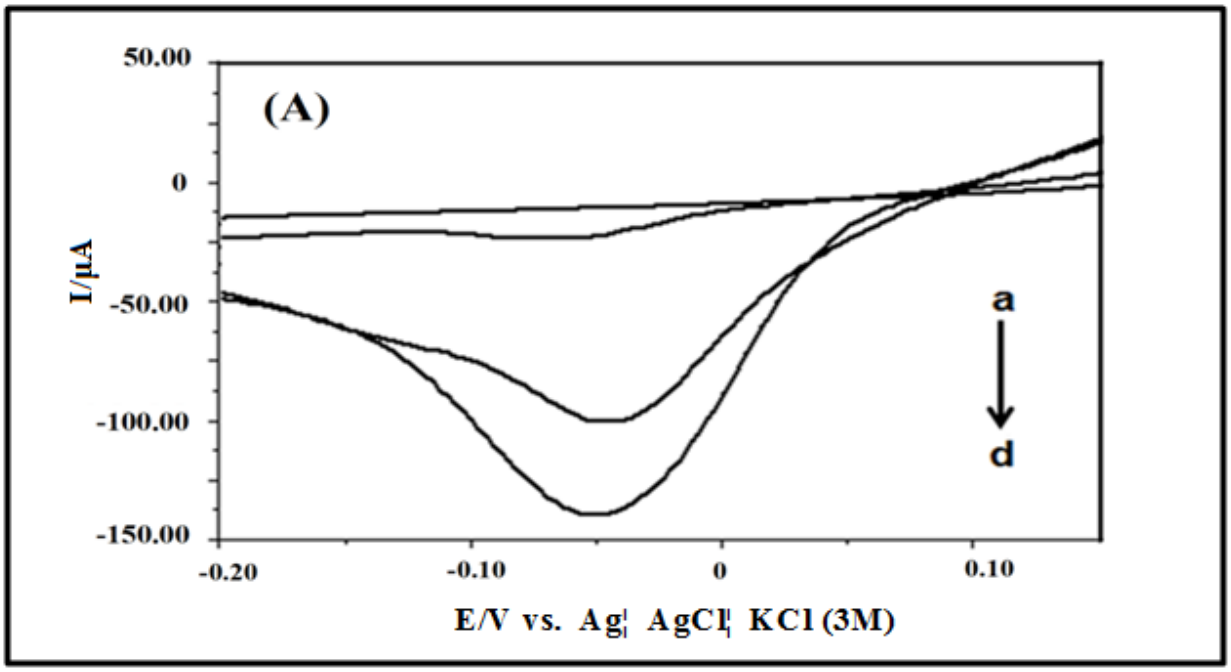


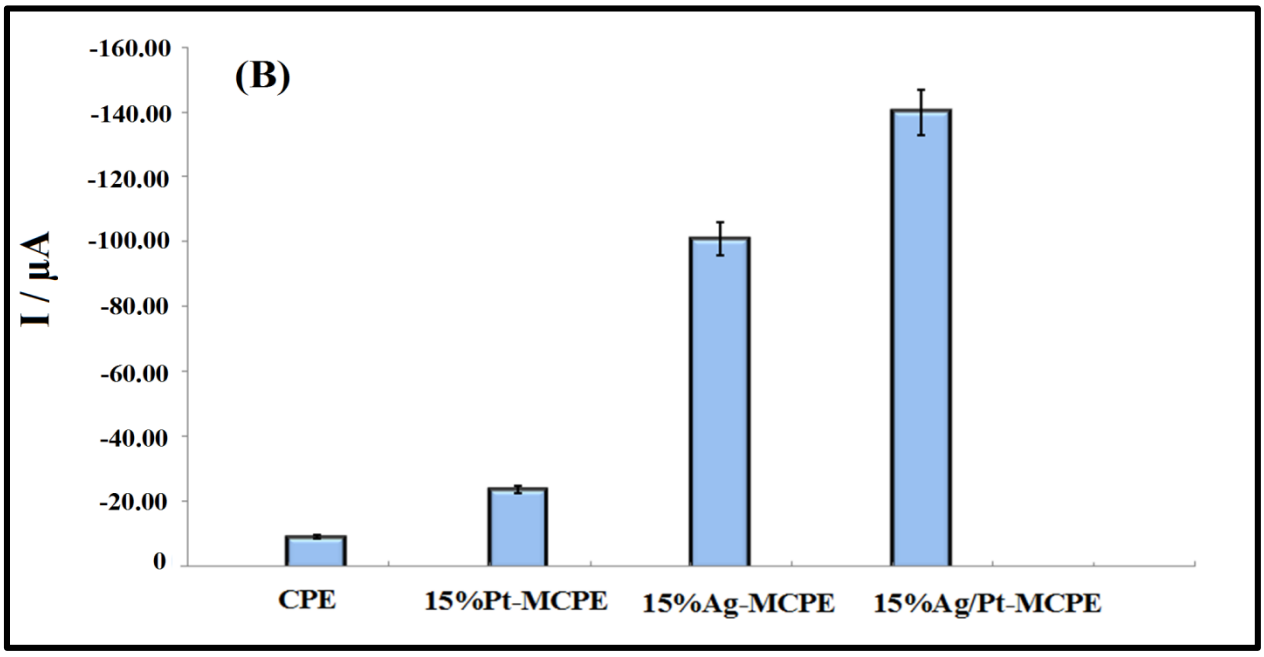


Fig. 5

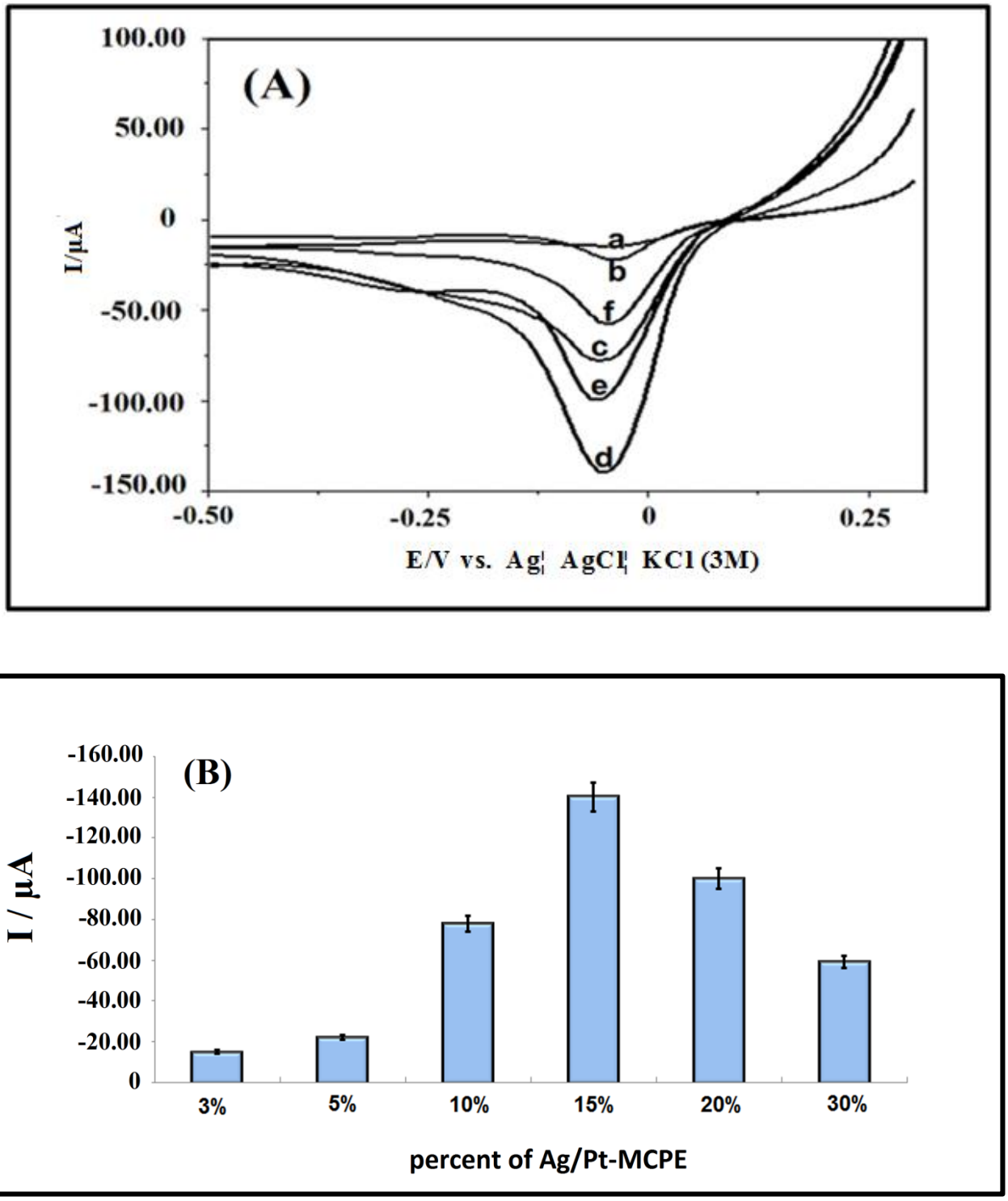


Fig. 6

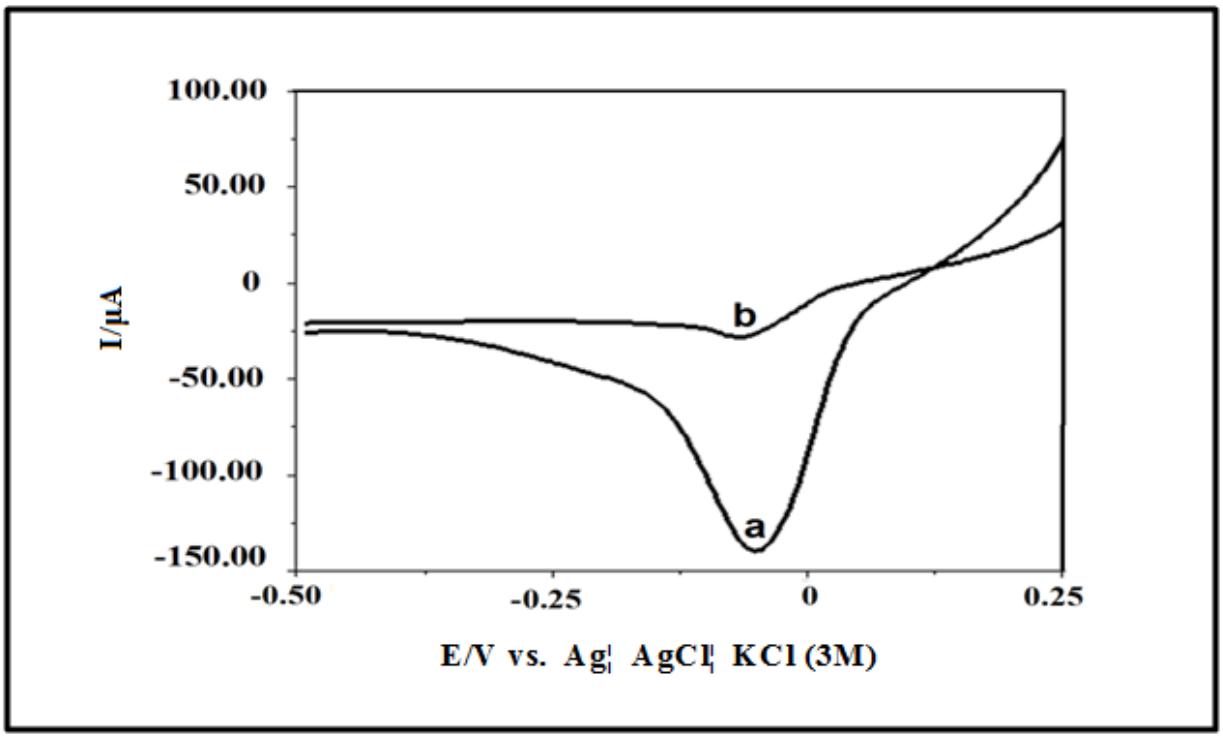
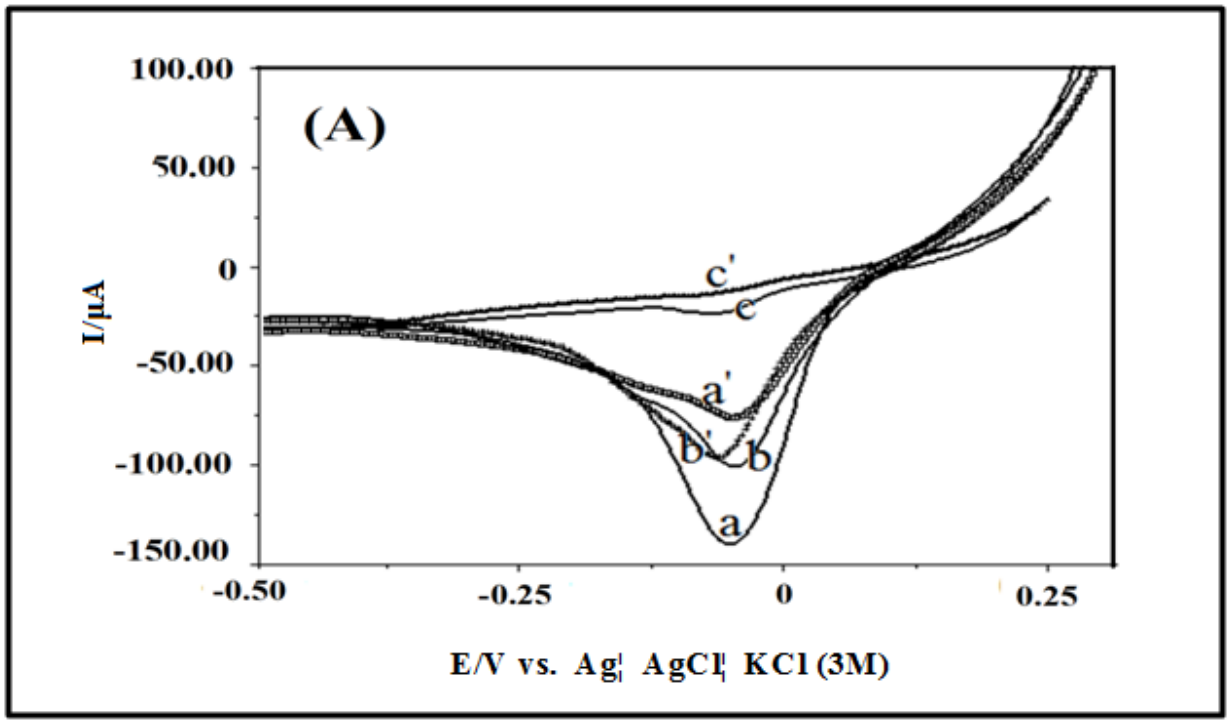


Fig. 7



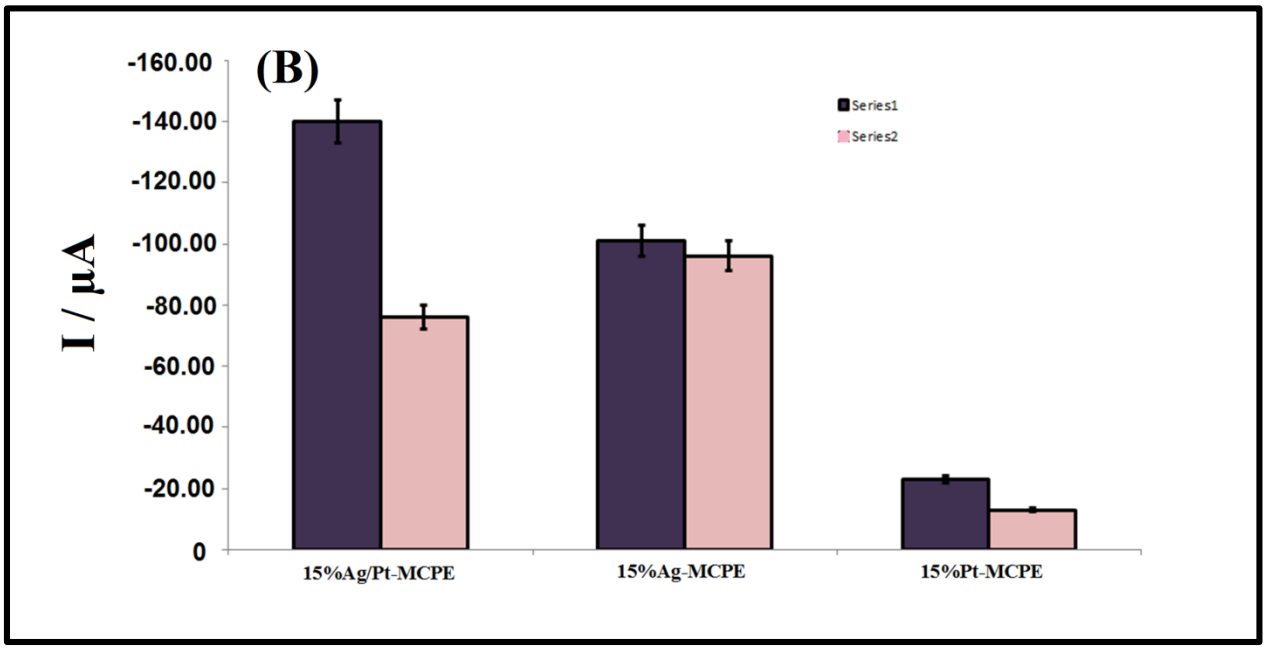


Fig. 8

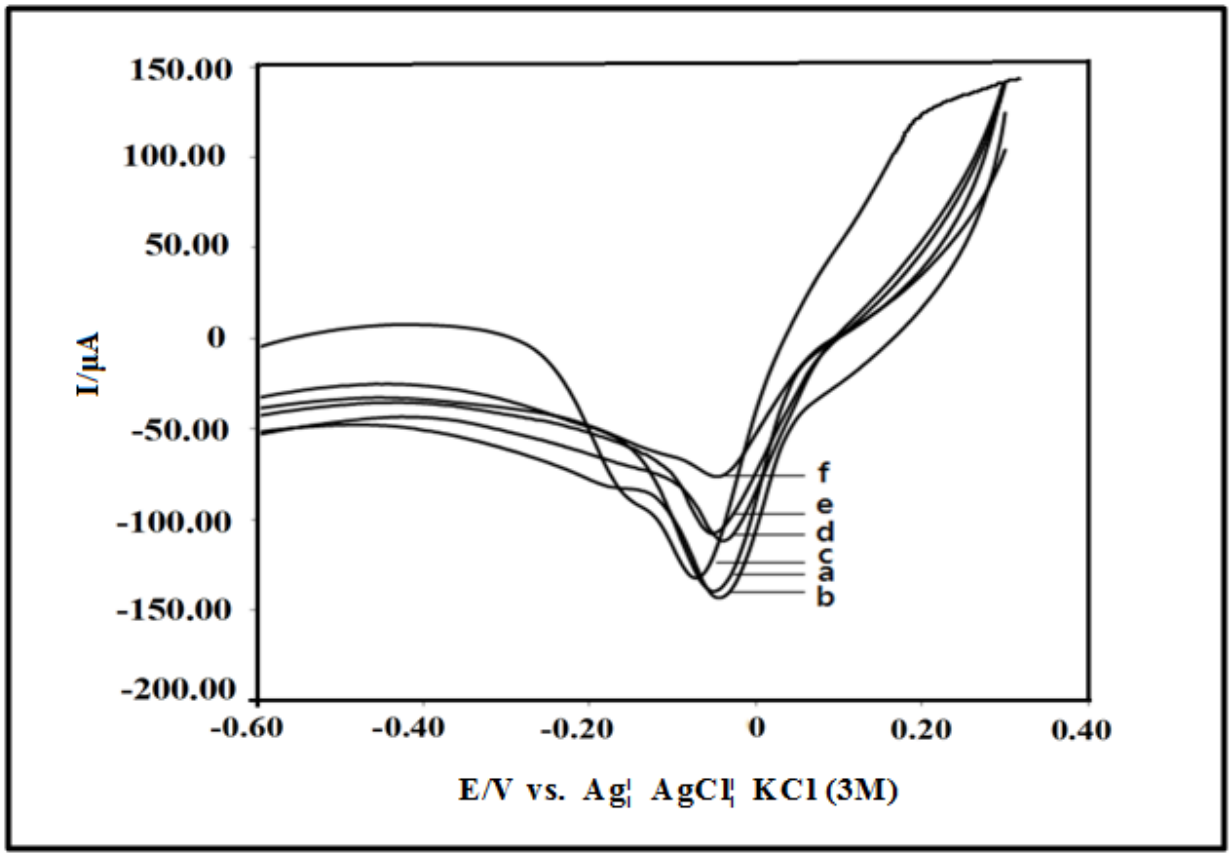


Fig. 9

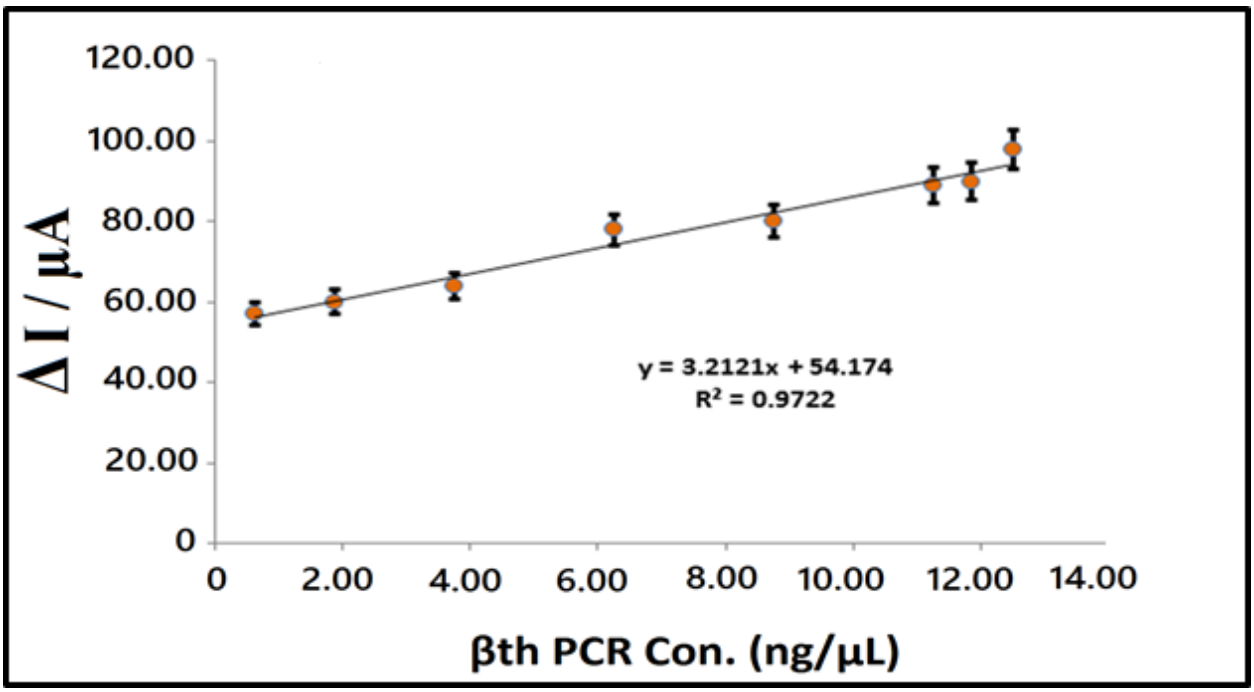


Fig. 10