J Appl Electrochem (2010) 40:2039–2050
DOI 10.1007/s10800-010-0185-8

ORIGINAL PAPER

Characterization of poly(vinylferrocenium) coated surfaces and their applications in DNA sensor technology

Filiz Kuralay · Arzum Erdem · Serdar Abacı · Haluk Özyörük · Attila Yıldız

Received: 1 April 2010 / Accepted: 5 September 2010 / Published online: 19 September 2010

Abstract Poly(vinylferrocenium) (PVF^+) modified gold (Au) electrode was developed in this study for the electrochemical sensing of deoxyribonucleic acid (DNA) hybridization based on the oxidation signals of polymer and guanine, and also for the electrochemical investigation of interaction of anticancer drug, mitomycin C (MC) and DNA immobilized onto PVF^+ modified Au electrode. PVF^+ modified Au electrode was prepared by electrooxidation of poly(vinylferrocene) PVF at +0.7 V versus Ag/AgCl reference electrode. The polymer modified electrode and DNA immobilized polymer modified electrode were characterized by X-ray photoelectron (XPS), Fourier transform infrared-attenuated total reflentance (FTIR-ATR) and alternating current (AC) impedance spectroscopy. For application studies, differential pulse voltammetry (DPV) technique was used.

Keywords Poly(vinylferrocenium) modified electrode · Electrochemical DNA biosensor · DNA hybridization · Guanine · DNA–drug interaction · Mitomycin C

1 Introduction

The detection of deoxyribonucleic acid (DNA) is very important for various applications such as diagnosis of infectious diseases, genetic mutations, drug discovery, environmental science, forensics and food technology [1, 2]. Biosensors are promising alternative for faster, cheaper, and simpler DNA analysis [3]. Electrochemical transducers were commonly used for detection of DNA [4–7]. Modern electrochemical DNA bioassays provide remarkable sensitivity, compatibility with modern microfabrication technologies. Inherent miniaturization, low cost, minimal power requirements, and independence of sample turbidity are their other advantages [8].

DNA detection rely on methods such as; the intrinsic electrochemical properties of DNA (the oxidation of purine bases), accumulation of redox compounds at the DNA-modified electrode surface, electrochemical amplifications with nanoparticles and combination of magnetic separation [9–13]. Electrochemical devices based on DNA-mediated charge transport chemistry were widely reported [14, 15]. Applications of electroactive polymers received considerable recent attention for DNA detection [16, 17]. The use of polymers offers several advantages in the construction of DNA biosensors due to their cheapness and simple production. Polymer films can be deposited on various types of substrates easily [18].

Electrochemical DNA hybridization biosensors combine the base-pair recognition of nucleic acid probes with the advantages of electrochemical methods. They can be used to differentiate one base mismatch sequences from the complementary target sequences sensitively. These biosensors also do not require any additional preparation step [19, 20]. Arora et al. [20] studied DNA hybridization using polypyrrole-polyvinyl sulfonate modified Pt electrode. Garnier et al. [21] used electroactive pyrrole-based copolymer, poly[pyrrole-COOH, pyrrole-ODN_{probe}] deposited on Au microelectrode for detecting complementary oligonucleotide (ODN). Electrochemically fabricated polyaniline

F. Kuralay (✉) · S. Abacı · H. Özyörük · A. Yıldız
Department of Chemistry, Faculty of Science,
Hacettepe University, 06532 Beytepe, Ankara, Turkey
e-mail: filizkur@hacettepe.edu.tr

A. Erdem (✉)
Analytical Chemistry Department, Faculty of Pharmacy,
Ege University, 35100 Bornova, Izmir, Turkey
e-mail: arzum.erdem@ege.edu.tr

nanowire-modified electrode on glassy carbon electrode for voltammetric detection of DNA hybridization was examined using methylene blue (MB) as an indicator by Zhu et al. [22]. Poly(indole-6-carboxylic acid) was studied to discriminate one-base mismatched and noncomplementary oligonucleotide (ODN) sequences [23]. Electrochemical sensing of DNA hybridization with electroactive ferrocene functionalized polypyrrole was reported by Korri-Youssoufi et al. [24].

The interaction of anticancer drugs with DNA is an important topic for studies in drug discovery and pharmaceutical development processes. Li et al. [25] studied interaction of echinomycin with guanine on the surface of glassy carbon electrode. Impedance sensing of specific DNA anticancer drug, netropsin was investigated using gold disk electrode [26]. Wang et al. [27] performed interaction of daunomycin with DNA in solution and at the surface of carbon paste electrode. The biomolecular binding behavior of dacarbazine in the presence of nano-titanium dioxide was studied on glassy carbon electrode by Song et al. [28]. Electrochemical investigation of interaction between mitomycin C (MC) and DNA was examined in a novel drug-delivery system using pencil graphite electrode by Karadeniz et al. [29].

In this work, PVF^+ modified gold (Au) electrode was developed for the electrochemical sensing of DNA based on the oxidation signals of polymer and guanine and also for the electrochemical investigation of interaction of MC and DNA immobilized onto PVF^+ modified Au electrode. Characterization of the polymer and DNA immobilized polymer modified electrodes were studied with X-ray photoelectron (XPS), Fourier transform infrared-attenuated total reflentance (FTIR-ATR) and alternating current (AC) impedance spectroscopy. Differential pulse voltammetry (DPV) technique was used in sensor application studies. The DNA hybridization was studied using thiol linked probe in the presence of target, mismatch (MM) and non-complementary (NC) sequences. In order to obtain more sensitive electrochemical signals probe ODN concentration and target ODN concentration were examined. The interaction of MC and DNA immobilized onto polymer modified electrode was investigated in the presence/absence of MC. MC concentration and MC interaction time with DNA were examined to obtain optimum analytical conditions. AC impedance spectroscopy was also used for monitoring MC–DNA interaction.

In our previous works, this polymer was used for DNA hybridization using specific oligonucleotides (ODNs) [30–32]. However, in this work, besides different characterization methods, combination of Au electrode and PVF^+ was carried out in DNA hybridization sensing technology for the first time. The electrochemical signal of guanine was obtained at low potential with enhanced current in this work compared to our earlier works [30–32]. Also, to the best of our knowledge, there is no data in the literature about the investigation of interaction between MC and DNA immobilized onto polymer coated electrodes. The interaction of MC–DNA was monitored easily with a good sensitivity using this modified electrode.

2 Experimental

2.1 Apparatus

Electrochemical studies were carried out with CH Instruments System, Model 660 B. XPS spectra were recorded on a SPECS ESCA (Berlin, GERMANY) system with Mg/Al dual anode using Mg Kα radiation. FTIR-ATR spectra were performed with Perkin Elmer FT-IR System Spectrum-BX.

A gold (Au) (Aldrich) disc working electrode (area: 7.85×10^{-3} cm^2), a saturated calomel reference electrode (SCE) (BAS, USA) and a Pt (Aldrich) counter electrode were used. Ag/AgCl (Aldrich) reference electrode was used for the electrooxidation of polymer performed in methylene chloride. XPS spectra and FTIR-ATR studies were performed with Pt foil (Aldrich) electrode (0.5 cm × 0.5 cm).

2.2 Reagents

$NaH_2PO_4 \cdot 2H_2O$, $Na_2HPO_4 \cdot 2H_2O$ and NaOH were purchased from Merck. Methylene chloride was obtained from Riedel-de-Haen. $NaClO_4$ was purchased from Sigma-Aldrich. Vinylferrocene was purchased from Aldrich. 2,2′-Azo-bis(2-methyl-propionitrile) (AIBN) was Alfa. CH_3COONa and CH_3COOH were purchased from Fluka.

Fish sperm DNA (fsDNA) was obtained from Serva. Calf thymus double-stranded, single-stranded DNA (ds/ssDNA) and MC were purchased from Sigma. The 20-23 mer oligonucleotides (ODNs) were purchased as lyophilized powder from TIB MOLBIOL (Germany). Their base sequences are given below:

Thiol linked probe: 5′-SH-AATACCACATCATCCATATA
Target: 5′-TATATGGATGATGTGGTATT
Mismatch (MM): 5′-TATGTGGATGATGTGGTATT
Noncomplementary (NC) (23-mer): 5′-AATACCTGTATTCCTCGCCTGTC

Other chemicals were in analytical reagent grade and they were supplied from Sigma and Merck.

2.2.1 The preparation of solutions

Phosphate buffer solution (50 mM PBS, pH: 7.0) containing 0.1 M $NaClO_4$ was prepared from $NaH_2PO_4 \cdot 2H_2O$ and

$Na_2HPO_4{\cdot}2H_2O$ using triple distilled water. Acetate buffer solution (50 mM ABS, pH: 4.80) was prepared from CH_3COONa and CH_3COOH. DNA, ODN and MC stock solutions were prepared with ultrapure tri-distilled water and kept frozen. The diluted solutions of DNA/ODN and MC were prepared by using PBS containing 20 mM NaCl.

2.3 Supporting electrolytes

Tetra-*n*-butyl ammonium perchlorate (TBAP) was used as a supporting electrolyte of the polymer solution, in non-aqueous medium [30]. PBS and ABS were used as the supporting electrolytes in aqueous medium.

2.4 Chemical polymerization of vinylferrocene

Poly(vinyferrocene) (PVF) was prepared by the chemical polymerization of vinylferrocene with AIBN initiator [30].

2.5 Preparation of polymer solution

1.0 mg mL^{-1} PVF polymer solution was prepared in methylene chloride/TBAP solvent/supporting electrolyte system.

2.6 Procedure

All the experiments were done at room temperature. Each test was repeated three times, and the average values were presented in the histograms with the error bars.

2.6.1 The preparation of poly(vinylferrocenium) (PVF^+) modified Au electrode by potential-controlled coulometry

The PVF^+ modified Au electrode was prepared electrochemically by electrooxidation at +0.7 V versus Ag/AgCl reference electrode in PVF solution. +0.7 V was set in all our previous measurements as the optimized potential for the oxidation of PVF to PVF^+. Perchlorate ions (ClO_4^-) which was in the structure of TBAP inserted into the polymeric structure as a counter ion [31].

The thicknesses of $PVF^+ClO_4^-$ films were controlled by the charge passed during the electroprecipitation. This charge was considered as an indication of polymeric film thickness. A charge of 1×10^{-3} C corresponded to 1.32×10^{-6} mol of the oxidized PVF per cm^2 with a dry thickness of 300 μm, and about 3×10^5 layers [32].

Polymer modified electrode was waited in buffer solution at the given experimental parameter all the time for checking if the change was due to DNA immobilization or not.

2.6.2 The preparation of DNA immobilized PVF^+ modified electrode

The preparation of DNA immobilized electrode was accomplished by immersing the PVF^+ modified electrode into ds/ssDNA solution. ODN immobilized polymer electrodes were prepared dropping the ODN solutions onto the polymer modified Au electrode surface and kept for 1 h. For hybridization studies, target, NC and MM ODN solutions were dropped onto the probe immobilized polymer modified electrode and kept for 1 h. After immobilization of nucleic acid onto the polymer electrode, the electrode was washed using buffer solution for 10 s. These conditions were the optimized working conditions according to our earlier studies [30, 31].

2.6.3 Interaction of MC with dsDNA at PVF^+ modified electrode

MC solution was dropped onto the dsDNA immobilized polymer modified Au electrode and kept for 20 min. After interaction of MC with DNA at the polymer modified electrode, the electrode was washed with buffer solution for 10 s.

2.6.4 Voltammetric transduction

The cyclic voltammograms of the polymer modified and unmodified Au electrodes were measured between −0.1 V and +1.0 V versus SCE at 100 mV s^{-1} in 50 mM PBS containing 0.1 M $NaClO_4$.

The oxidation signals of polymer and guanine were measured by using DPV scanning between +0.0 V and +1.4 versus SCE at pulse amplitude of 50 mV in ABS. Oxidation signals of probe immobilized polymer, target, NC and MM immobilized probe modified polymer were measured using DPV at the same parameters reported above.

The electrochemical behavior of dsDNA immobilized polymer electrode was measured in 50 mM PBS containing 0.1 M $NaClO_4$ in the absence/presence of MC by using DPV scanning between +0.0 V and +1.0 V versus SCE at pulse amplitude of 50 mV.

2.6.5 Impedance measurements

AC impedance measurements were carried out at the open-circuit value; +0.4 V and the frequency was varied over the range 10^5–10^{-2} Hz with amplitude of 5 mV in PBS.

3 Results and discussion

3.1 XPS spectra

Figure 1a–c shows the wide energy range of XPS spectra of the polymer modified, ssDNA immobilized polymer modified and dsDNA immobilized polymer modified electrodes, respectively. The main peaks were assigned to C, O, Cl and Fe elements and data indicated that these elements existed on the $PVF^{+}ClO_4^{-}$ film (Fig. 1a). Similarly, the main peaks for C, O, P and N, indicated that these elements existed on the ss/dsDNA immobilized polymer film. The existence of P and N peaks on the XPS spectra of ss/dsDNA immobilized polymer film proved the immobilization of DNA onto the polymer film (Fig. 1b, c). Thus, it was concluded that ss/dsDNA could be immobilized onto

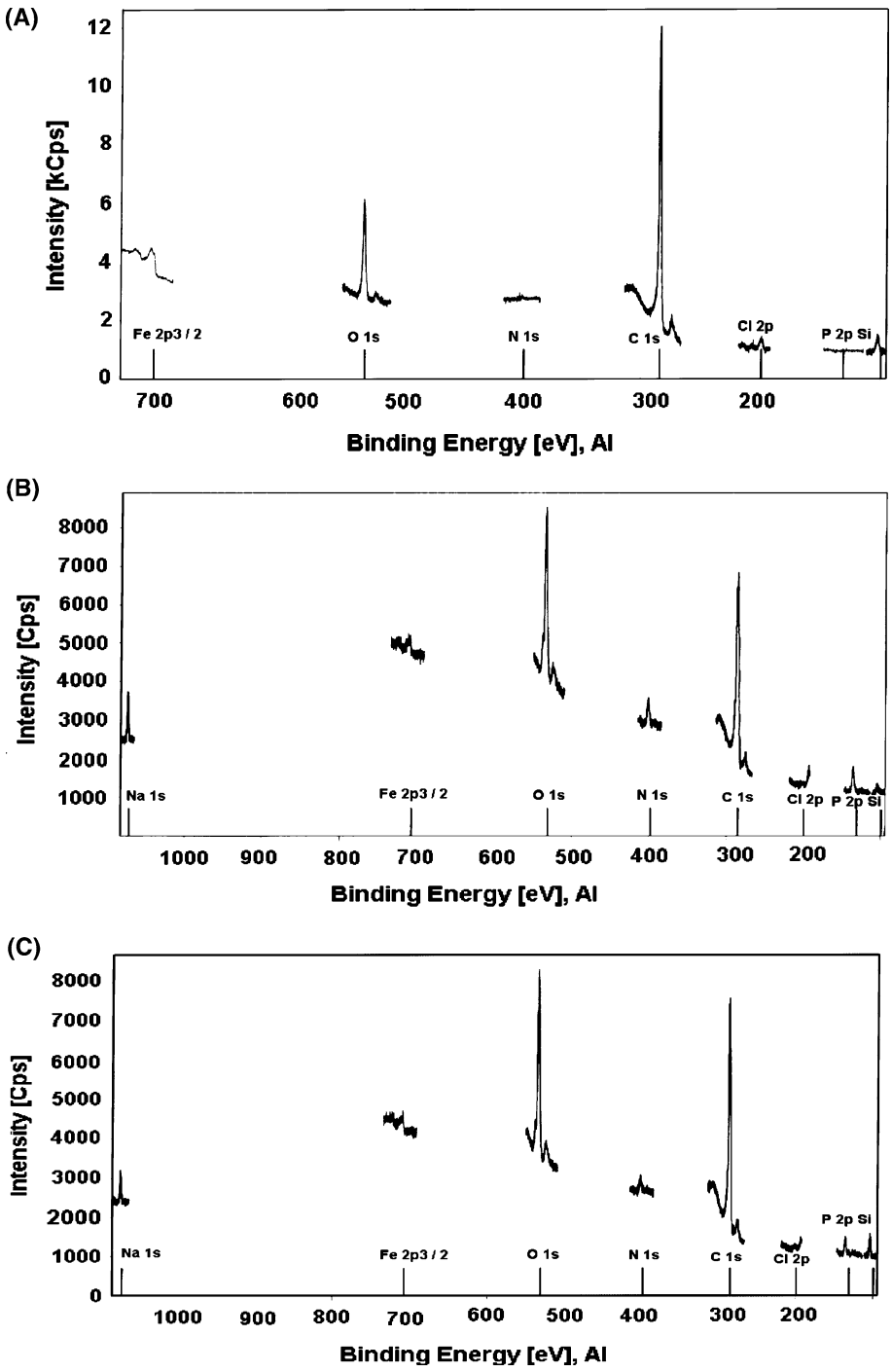

Fig. 1 XPS spectra of **a** polymer modified electrode, **b** ssDNA immobilized polymer modified electrode after immersion into 2500 μg mL^{-1} ssDNA solution, **c** dsDNA immobilized polymer modified electrode after immersion into 2500 μg mL^{-1} dsDNA solution

the polymer modified electrode [33]. It was also found that, the interaction of polymer with dsDNA was better than the interaction of polymer with ssDNA (4.6% N, 2.2% P for dsDNA immobilized polymer electrode; 3.3% N, 2.0% P for ssDNA immobilized polymer electrode) [30].

3.2 FTIR-ATR spectroscopy

FTIR-ATR spectra of polymer film and dsDNA immobilized polymer film are given in Fig. 2a and b, respectively. The peaks in the spectrum of $PVF^{+}ClO_4^{-}$ that appeared about 623 and 1093 cm^{-1} were attributed to ClO_4^{-} ion in the polymer structure. The intensity of the peaks that belong to ClO_4^{-} decreased due to the anion exchange occurred between counter anion and negatively charged biomolecule (Fig. 2b) [34]. According to the FTIR-ATR spectra of polymer film and dsDNA immobilized polymer film (respectively, shown in Fig. 2a, b), the peak at around 957.14 cm^{-1} can be attributed to ferrocene [35].

3.3 AC impedance spectroscopy

AC impedance spectroscopy was also used to identify and differentiate the immobilization of ss/dsDNA on the

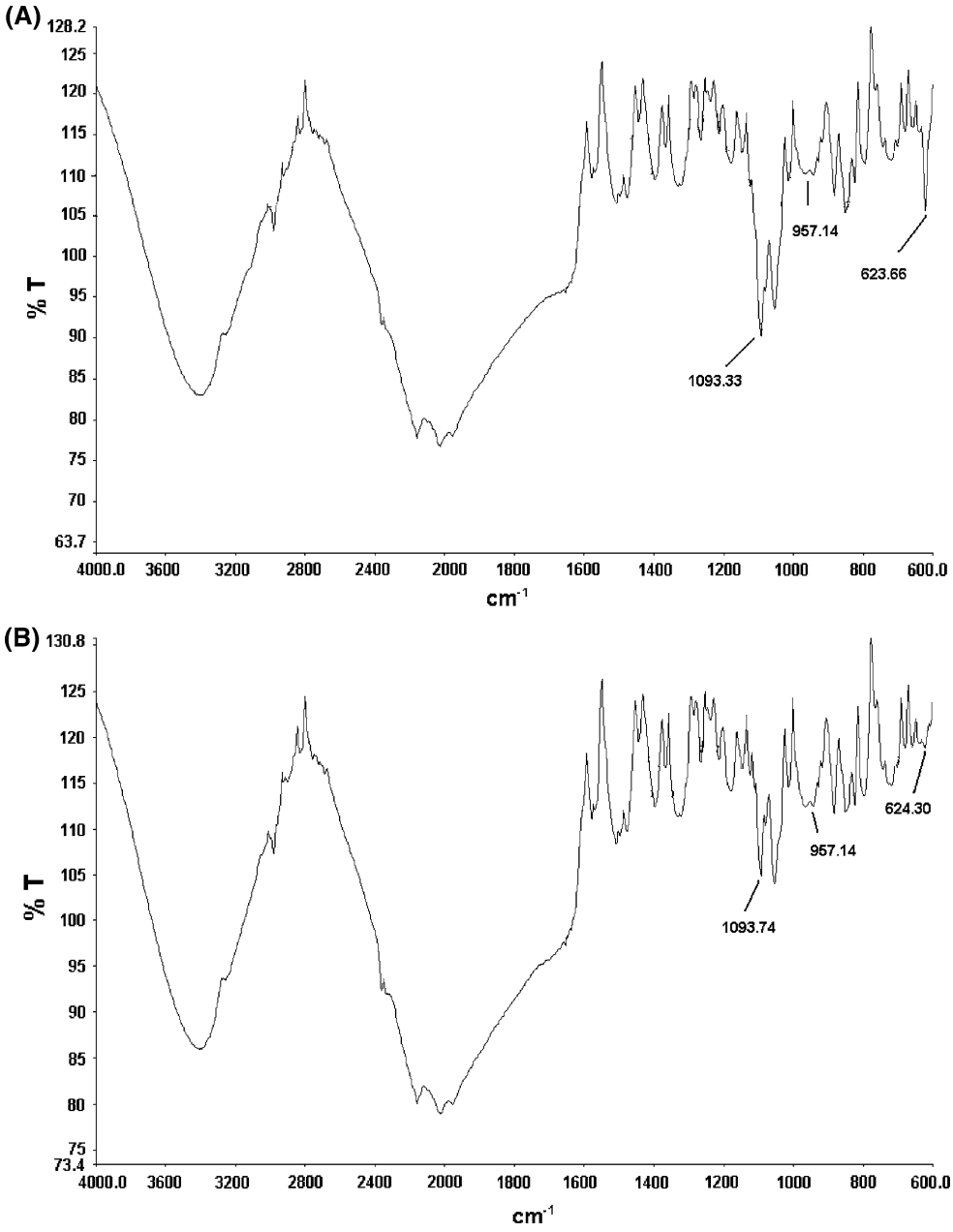

Fig. 2 FTIR-ATR spectra of **a** polymer modified electrode, **b** dsDNA immobilized polymer modified electrode. Other conditions are same in Fig. 1

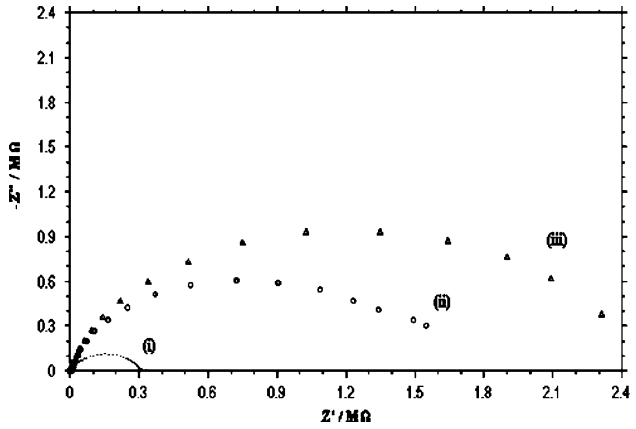

Fig. 3 AC impedance spectra of (*i*) polymer modified electrode, (*ii*) ssDNA immobilized polymer modified electrode, (*iii*) dsDNA immobilized polymer modified electrode. AC measurement: at the open-circuit value; +0.4 V between 10^5 and 10^{-2} Hz frequency range with amplitude of 5 mV in PBS. Other conditions are same in Fig. 1

polymer modified electrode. The semicircle portion at higher frequencies correspond to the charge-transfer limited process and linear portion seen at lower frequencies may be ascribed to the diffusion process in the Nyquist plot of impedance spectra [36]. The diameter of the semicircle represents the charge-transfer resistance (R_{ct}) at the electrode surface [37]. Figure 3 shows the impedance spectra of polymer modified electrode, ssDNA immobilized polymer modified electrode and dsDNA immobilized polymer electrode in PBS, respectively. There was an increase at the R_{ct} values after DNA immobilization onto the surface of the polymer modified electrode. This result indicated the enhanced resistance to the charge-transfer occured at the electrode surface. These results were in a good agreement with the results obtained by XPS and FTIR-ATR studies.

3.4 Electrochemical studies

The cyclic voltammogram (CV) of $PVF^+ClO_4^-$ film in PBS containing 0.1 M $NaClO_4$ is presented in Fig. 4a-i. The oxidation of PVF to PVF^+ was observed at +0.38 V and the reduction of PVF^+ to PVF was measured as +0.22 V versus SCE. These peaks are assigned to ferrocenium/ferrocene redox couple [30–32]. The cyclic voltammetric behavior of unmodifed Au electrode is also given in Fig. 4a-ii.

After characterization of PVF^+ coated surfaces, their applications in DNA biosensor technology were investigated using Au electrodes. Firstly, DNA hybridization was performed on thiol linked ODN immobilized polymer modified electrode in the presence of target, mismatch (MM) and noncomplementary (NC) sequences. Then, the electrochemical investigation of interaction of MC and DNA immobilized onto PVF^+ modified Au electrode was explored.

Figure 4b and c shows the DPVs of $PVF^+ClO_4^-$ and ss/dsDNA immobilized $PVF^+ClO_4^-$ modified electrodes in PBS containing 0.1 M $NaClO_4$ and ABS, respectively. The oxidation peak of the polymer was observed at +0.34 V by using PBS (pH = 7.0) (Fig. 4b-i). After ss/dsDNA immobilization, a significant decrease in the oxidation peak current of the polymer was observed similar to earlier results [19, 30–32, 38, 39]. A small peak was observed at about +0.92 V, which reflected the oxidation signal of guanine in the DPV of ssDNA immobilized polymer modified electrode (Fig. 4b-ii). There was also a small peak observed at about +0.91 V, which reflected the oxidation signal of guanine in the DPV of dsDNA immobilized polymer modified electrode (Fig. 4b-iii) [40].

The oxidation peak of the polymer was obtained at +0.38 V in ABS (pH = 4.8) (Fig. 4c-i). The peaks at +0.98 V in the DPV of dsDNA immobilized polymer modified electrode (Fig. 4c-iii) and +1.01 V in the DPV of ssDNA immobilized polymer modified electrode (Fig. 4c-ii) in ABS were assigned to the electroactive DNA base, guanine. The DPV of unmodified Au electrode is also given in Fig. 4c-iv. There was a interaction of DNA biomolecule with positively charged matrix at both pH values.

3.4.1 DNA hybridization on PVF^+ modified Au electrode

DNA hybridization was carried out using this biosensing method. Firstly, the effect of thiol linked ODN concentration on the oxidation signals of polymer was studied in various ODN concentration from 12.5 to 200 μg mL^{-1}. The oxidation peak current decreased gradually and then levelled off, when the concentration of ODN was 175 μg mL^{-1} (Fig. 5a). The optimum immobilization concentration for 20-mer thiol linked ODN was found as 175 μg mL^{-1}. The DPVs at three different concentrations of thiol linked ODN are shown in Fig. 5b.

The changes in the oxidation peak current of the polymer were monitored before and after DNA hybridization in the presence of thiol linked probe alone and the hybridization between probe and target/NC/MM sequences (Fig. 6a-i, ii, iii, iv, respectively). The polymer signal decreased as a result of DNA hybridization between probe and its complementary sequence (target) (Fig. 6a-i, ii). Due to the specific binding of thiol linked probe with its complementary (target) at the polymer electrode, there was a significant decrease around 53.7% at polymer signal in the

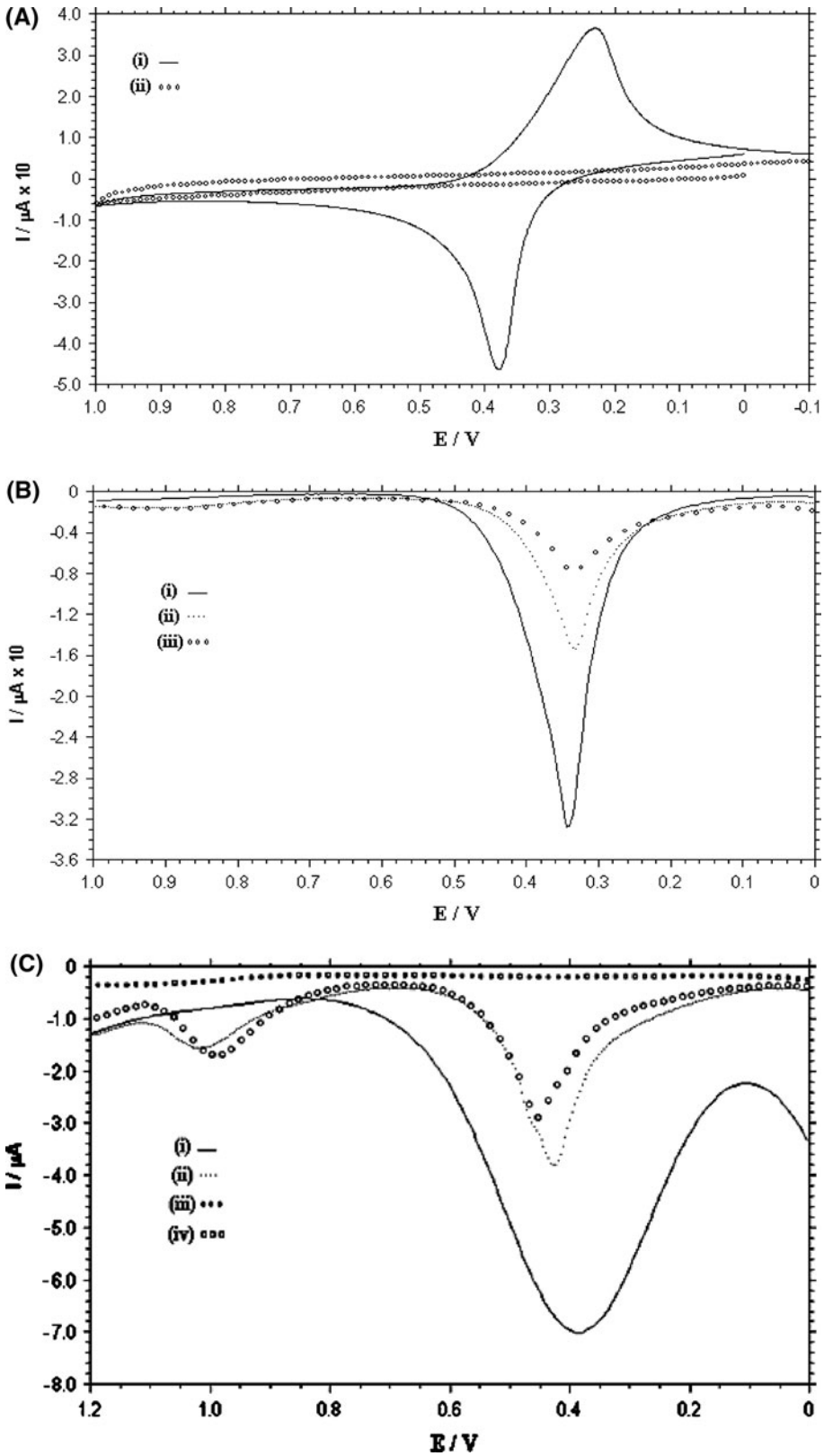

Fig. 4 **a** CVs of (*i*) polymer film, (*ii*) unmodified electrode in 50 mM PBS containing 0.1 M $NaClO_4$. DPVs of (*i*) polymer film, (*ii*) ssDNA immobilized polymer film, (*iii*) dsDNA immobilized polymer film (**b**) in 50 mM PBS containing 0.1 M $NaClO_4$ (**c**) in ABS. CV measurement: at 100 mV s^{-1} scan rate between −0.1 V and +1.0 V. DPV measurement: at 50 mV pulse amplitude between +0.0 V and +1.4 V. (1.0 mC polymeric film thickness, 1000 µg mL^{-1} dsDNA, 1 h immobilization time)

presence of full match DNA hybridization. A small decrease at polymer signal was obtained as 2.2 and 10.2%, respectively in the case of hybridization between probe and NC, or probe and MM similar to the results obtained in the literature (Fig. 6a-iii, iv) [37–39]. In addition, the selectivity of the modified electrode was tested in a mixture of

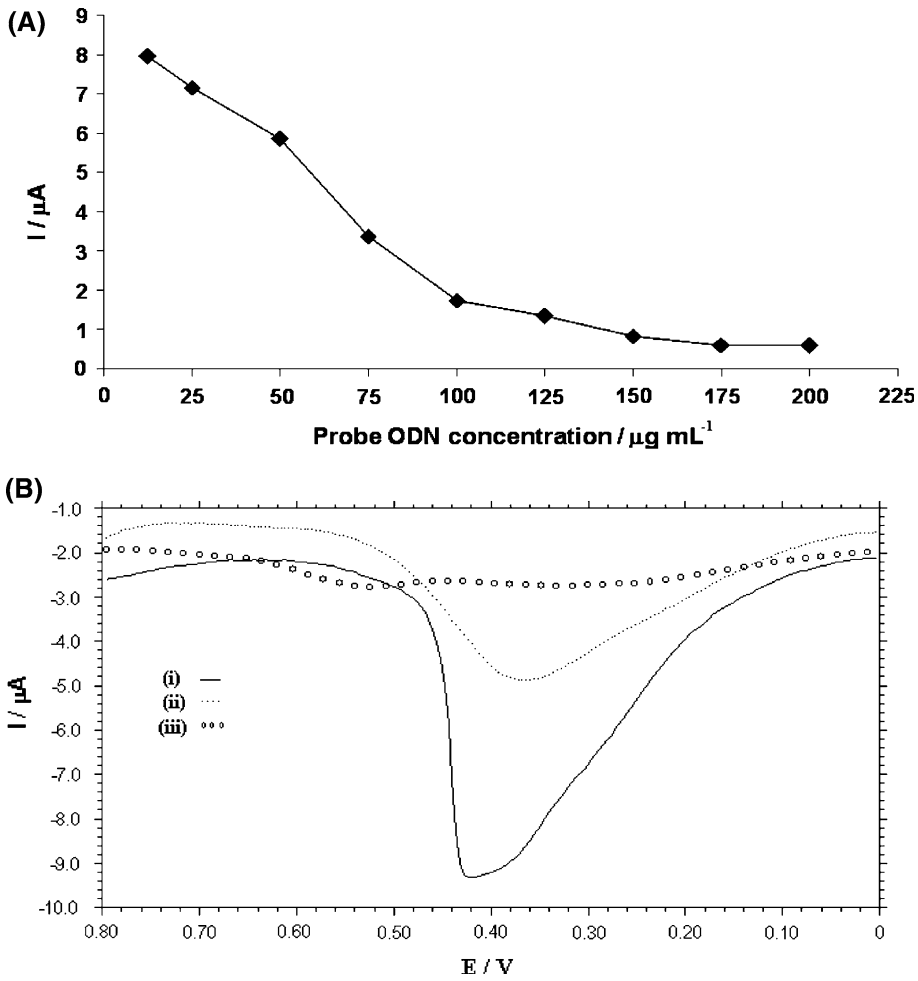

Fig. 5 **a** The effect of thiol linked ODN in various concentrations on the response of polymer. **b** DPVs of (*i*) 25 µg mL^{-1} thiol linked ODN, (*ii*) 75 µg mL^{-1} thiol linked ODN, (*iii*) 150 µg mL^{-1} thiol linked ODN. Other conditions are same in Fig. 4

target, NC and MM. When the probe encountered its complementary sequence (target) in the mixture, the current signal due to the polymer decreased parallel to the results above (not shown). According to all these data, the possibility for nonspecific adsorption at the surface of the polymer modified electrode was sufficiently low.

The effect of target concentration on the oxidation signals of polymer and guanine was also studied in various target concentration from 12.5 to 125 µg mL^{-1}. The polymer signal decreased gradually and then levelled off, when the concentration of target was increased to 75 µg mL^{-1} (Fig. 6b shown with P). However, the guanine oxidation signal increased and then levelled off (Fig. 6b shown with G). The optimum immobilization concentration for 20-mer thiol linked ODN was found as 75 µg mL^{-1}. The DPVs at three different concentrations of target ODN are shown in Fig. 6c. The peaks at about +0.90 V were attributed to guanine oxidation [41]. As seen from this figure, oxidation of guanine enhanced with increasing target concentration. It is also clear that oxidation potential of guanine shifted with increasing target concentration. This result indicated more interaction of DNA with Fe(II/III) redox couple which showed electrocatalytic effect [41].

This electrochemical method for DNA hybridization showed a good selectivity and high sensitivity. The level of nonspecific immobilization on these polymer modified electrode was sufficiently low. It was easy to distinquish DNA hybridization.

3.4.2 MC–DNA interaction at the surface of PVF^{+} modified Au electrode

DPVs of dsDNA immobilized polymer modified Au electrode before (Fig. 6a-i) and after interaction with 10 µg mL^{-1} (Fig. 7a-ii) and 25 µg mL^{-1} (Fig. 7a-iii) MC are given in Fig. 7. Oxidation peak current of guanine at +0.92 V decreased after the interaction process between MC and DNA (Fig. 7b-ii, iii). This decrease was attributed to the binding of MC to DNA by shielding of oxidizable group of guanine [13]. The oxidation peak current of the polymer increased due to the interaction of MC with

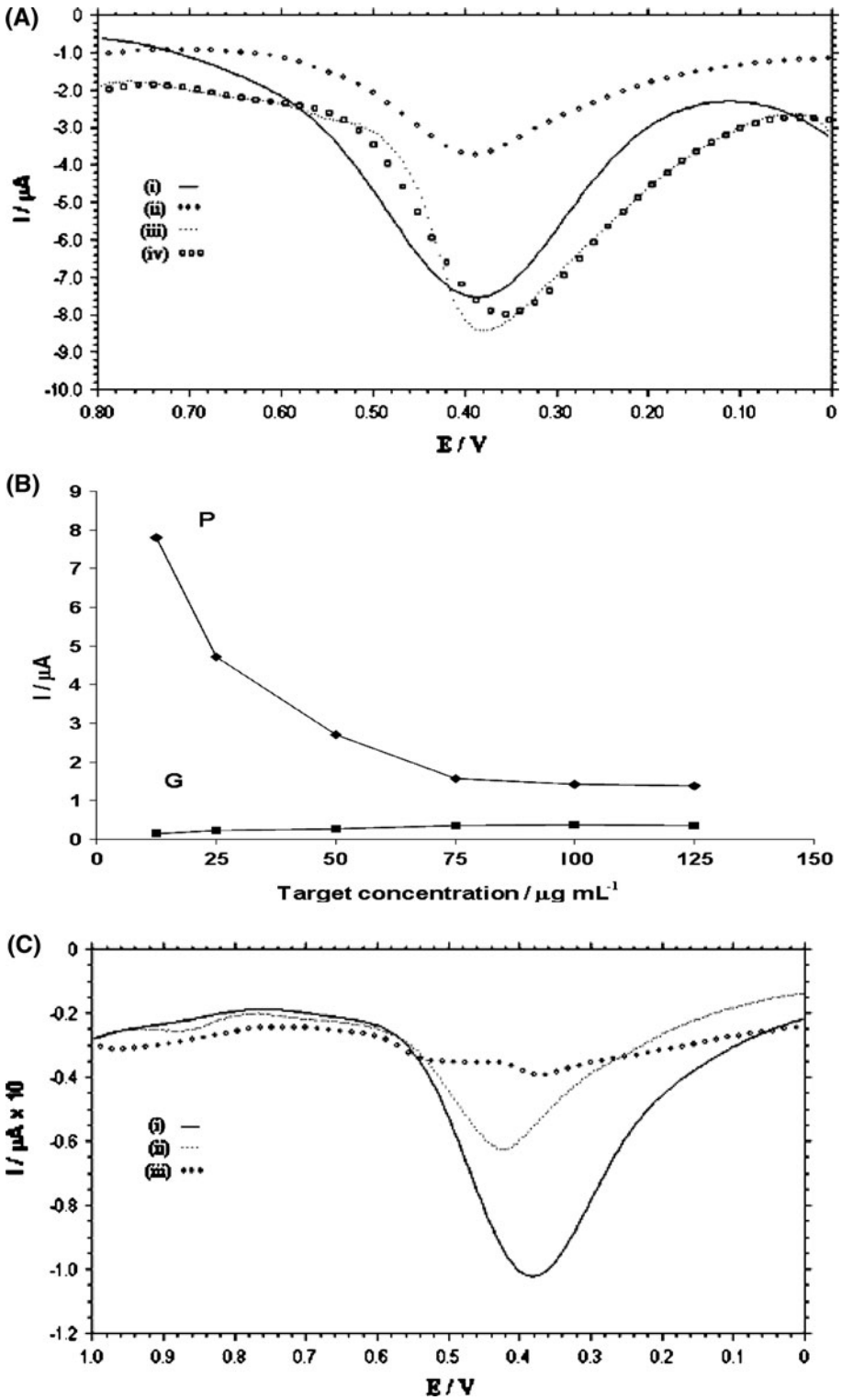

Fig. 6 **a** DPVs showing the oxidation signal of the polymer (*i*) 50 µg mL^{-1} probe alone, (*ii*) after hybridization between probe and 50 µg mL^{-1} complementary, (*iii*) interaction between probe and 50 µg mL^{-1} NC, (iv) interaction between probe and 50 µg mL^{-1} MM in ABS. **b** The effect of target in various concentrations on the response of PVF^{+} and guanine. Plot showing both oxidation signals of polymer (*P*) and guanine (*G*). **c** DPVs of (*i*) 12.5 µg mL^{-1} target ODN, (*ii*) 25 µg mL^{-1} target ODN, (*iii*) 75 µg mL^{-1} target ODN. Other conditions are same in Fig. 4

dsDNA immobilized onto the polymer modified Au electrode.

The effects of experimental parameters, such as MC concentration and MC interaction time with dsDNA were also studied to find the optimum analytical conditions. In order to investigate the effect of MC concentration on the response of this biosensing system, dsDNA immobilized $PVF^{+}ClO_4^{-}$ coated electrodes were interacted with MC in various concentrations. In Fig. 8a, the changes at the magnitude of guanine oxidation signals are presented in the

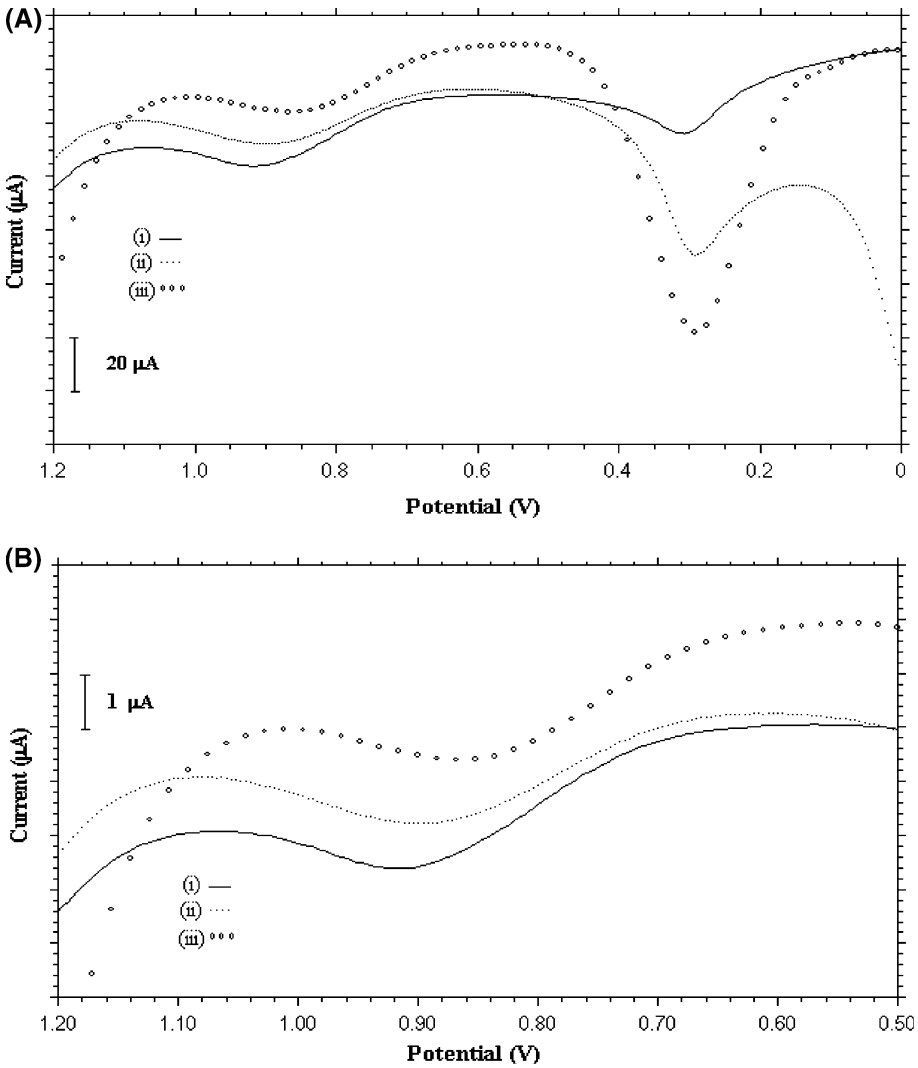

Fig. 7 DPVs of dsDNA immobilized polymer modified Au electrode (**a**, **b**) (*i*) before MC interaction, (*ii*) after interaction with 10 μg mL^{-1} MC, (*iii*) after interaction with 25 μg mL^{-1} MC in PBS containing 0.1 M $NaClO_4$. DPV measurement: at 50 mV pulse amplitude between 0 V and +1.2 V (1000 μg mL^{-1} dsDNA concentration, 20 min interaction time of MC). Other conditions are same in Fig. 4

absence and presence of the interaction of 10, 25, 50 and 75 μg mL^{-1} MC with dsDNA immobilized polymer modified Au electrode. A gradual decrease of the guanine oxidation signal was observed after the interaction of MC with dsDNA. After 50 μg mL^{-1} MC concentration value the decrease at the guanine peak current remained almost constant. The optimum concentration of MC was then chosen as 50 μg mL^{-1} for this study using dsDNA immobilized $PVF^{+}ClO_4^{-}$ modified Au electrode. After the interaction of MC, a decrease of 35% was obtained at guanine oxidation signal which was a comparable decreasing value compared to earlier reports [13, 42, 43].

The effect of interaction time of MC with dsDNA immobilized onto polymer modified Au electrode on guanine oxidation signal was studied to find the optimum interaction time of MC and dsDNA. A gradual decrease of guanine oxidation signal was observed with increasing interaction time of MC by using polymer modified Au electrode (Fig. 8b). After 20 min interaction time of MC no appreciable change was obtained in the oxidation peak current of guanine. A series of three repetitive DPV measurements of the interaction at 50 μg mL^{-1} concentration level of MC with dsDNA immobilized onto the polymer modified Au electrode in 20 min of MC interaction time resulted in reproducible results such as a mean response of 1053 nA with a relative standard deviation of 34.5 ($n = 3$). The detection limit estimated from S/N = 3 corresponded to 90 ng mL^{-1} for MC using dsDNA immobilized polymer modified Au electrode. The polymer modified Au electrode for MC–DNA interaction showed a linearity ($R^2 = 0.973$) to MC in a high concentration range of 10 to 50 μg mL^{-1}.

AC impedance spectroscopy was also used to differentiate $PVF^{+}ClO_4^{-}$ modified Au electrode, dsDNA immobilized $PVF^{+}ClO_4^{-}$ modified Au electrode and dsDNA immobilized $PVF^{+}ClO_4^{-}$ modified Au electrode after interaction with 50 μg mL^{-1} MC (Fig. 9-i, ii, iii, respectively). R_{ct} value decreased after interaction of dsDNA immobilized onto polymer Au electrode with MC due to

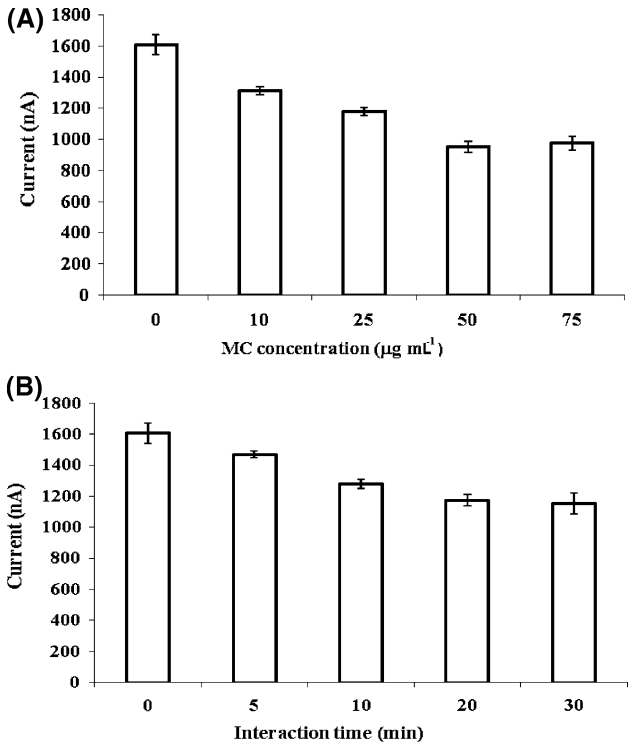

Fig. 8 **a** The effect of MC concentration on the response of guanine oxidation signal using dsDNA immobilized polymer modified Au electrode. **b** The effect of interaction time of MC with dsDNA immobilized onto polymer modified Au electrode on guanine oxidation signal. Other conditions are same in Figs. 4 and 7

the binding of MC to DNA (Fig. 9-ii to iii) [13]. These results were found in a good agreement with the results obtained by using DPV techniques.

These results showed that PVF^+ modified Au electrode could be used for the effective detection of DNA-MC interaction. The detection limit estimated for MC using dsDNA immobilized polymer modified Au electrode has comparability to early MC–DNA interaction studies [42]. The modified Au electrode provided a high concentration range of MC with a good linearity.

4 Conclusions

The characterization of polymer modified electrodes and their applications in DNA hybridization and drug–DNA interaction at Au electrode were investigated in this work. Detection of DNA hybridization was performed with a good selectivity and high sensitivity. The level of non-specific immobilization on this polymer modified electrode was sufficiently low. The enhanced guanine signal was obtained at lower potentials when compared with our earlier results [30–32]. This DNA sensing method based on $PVF^+ClO_4^-$ modified Au electrode provided comparable results with other DNA detection techniques based on polymer electrodes [9, 37, 44–48]. The redox polymer, $PVF^+ClO_4^-$ provided an appropriate interface for the development of electrochemical DNA biosensor. There was no need for an extra redox indicator to monitor the electrochemical behavior of polymer electrode and DNA immobilized polymer electrode.

To the best of our knowledge, for the first time in the literature DNA immobilized polymer modified Au electrode was used for the electrochemical investigation of the interaction of the anticancer drug MC with DNA. The redox polymer, $PVF^+ClO_4^-$ provided an appropriate interface for this purpose with the advantage of cheap, easy and fast preparation. Compared with other drug-DNA interaction techniques based on electrochemistry [25–29, 42, 43], these polymer electrodes can provide simple detection scheme for drug-DNA interaction with a satisfactory detection limit in a linear dynamic range.

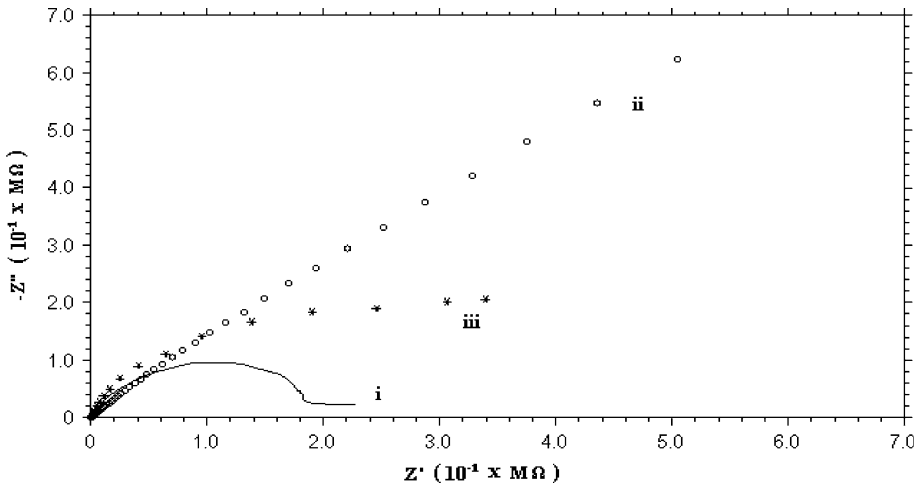

Fig. 9 Impedance spectra of (*i*) polymer modified Au electrode, (*ii*) dsDNA immobilized polymer modified Au electrode after immersing into 1000 μg mL^{-1} dsDNA solution, (*iii*) dsDNA immobilized polymer modified Au electrode after interaction with 50 μg mL^{-1} MC in PBS containing 0.1 M $NaClO_4$. Other conditions are same in Figs. 4 and 7

Acknowledgments This study was supported by Turkish Scientific and Technological Council (TUBITAK) (Project no: 106T065). A.E. expresses her gratitudes to the Turkish Academy of Sciences (TUBA) as an Associate member for their support.

References

1. Kafka J, Panke O, Abendroth B, Lisdat F (2008) Electrochim Acta 53:7467
2. Peng H, Zhang L, Soeller C, Travas-Sejdic J (2009) Biomater 30:2132
3. Wang J (2002) Chim Acta 469:63
4. Erdem A (2007) Talanta 74:318
5. Gooding JJ (2002) Electroanal 14:1149
6. Lucarelli F, Marrazza G, Turner APF, Mascini M (2004) Biosens Bioelectron 19:515
7. Singh R, Prasad R, Sumana G, Arora K, Sood S, Gupta RK, Malhotra BD (2009) Biosens Bioelectron 24:2232
8. Wang J (2005) In: Palecek E, Scheller F, Wang J (eds) Electrochemistry of nucleic acids and proteins: towards electrochemical sensors for genomics and proteomics. Elsevier, Amsterdam
9. Fan C, Song H, Hu X, Li G, Zhu J, Xu X, Zhu D (1999) Anal Biochem 271:1
10. Wang J, Kawde AN, Erdem A, Salazar M (2001) Analyst 126: 2020
11. Nakayama M, Ihara T, Nakano K, Maeda M (2002) Talanta 56:857
12. Erdem A, Papakonstantinou P, Murphy H (2006) Anal Chem 78:6656
13. Karadeniz H, Erdem A, Caliskan A, Pereira CM, Pereira EM, Ribeiro JA (2007) Electrochem Commun 9:2167
14. Keighley SD, Estrela P, Li P, Migliorato P (2008) Biosens Bioelectron 24:906
15. Karadeniz H, Erdem A, Kuralay F, Jelen F (2009) Talanta 78:187
16. Wang J (2000) Nucleic Acids Res 28:3011
17. Silva TAR, Ferreira LF, Souza LM, Goulart LR, Madurro JM, Brito-Madurro AG (2009) Mater Sci Eng C 29:539
18. Kuralay F, Özyörük H, Yıldız A (2006) Sens Actuators B 114:500
19. Chang Z, Chen M, Fan H, Zhao K, Zhuang S, He P, Fang Y (2008) Electrochim Acta 53:2939
20. Arora K, Prabhakar N, Chand S, Malhotra BD (2007) Sens Actuators B 126:655
21. Garnier F, Bouabdallaoui B, Srivastava P, Mandrand B, Chaix C (2007) Sens Actuators B 123:13
22. Zhu N, Chang Z, He P, Fang Y (2006) Electrochim Acta 51:3758
23. Nie G, Zhang Y, Guo Q, Zhang S (2009) Sens Actuators B 139:592
24. Korri-Youssoufi H, Makrouf B (2001) Syn Met 119:265
25. Li N, Guo L, Jiang J, Yang X (2004) Biophys Chem 111:259
26. Li CZ, Liu Y, Luong JHT (2005) Anal Chem 77:478
27. Wang J, Ozsoz M, Cai X, Rivas G, Shiraishi H, Grant DH, Chicharro M, Fernandes J, Palecek E (1998) Bioelectrochem Bioenerg 45:33
28. Song M, Zhang R, Wang X (2006) Mater Lett 60:2143
29. Karadeniz H, Alparslan L, Erdem A, Karasulu E (2007) J Pharm Biomed Anal 45:322
30. Kuralay F, Erdem A, Abacı S, Özyörük H, Yıldız A (2008) Electroanalysis 20:2563
31. Kuralay F, Erdem A, Abacı S, Özyörük H, Yıldız A (2009) Anal Chim Acta 643:83
32. Kuralay F, Erdem A, Abacı S, Özyörük H, Yıldız A (2009) Electrochem Commun 11:1242
33. Zhao YD, Pang DW, Hu S, Wang ZL, Cheng JK, Qi YP, Dai HP, Mao BW, Tian ZQ, Lou J, Lin ZH (1999) Anal Chim Acta 388:93
34. Kuralay F, Özyörük H, Yıldız A (2005) Sens Actuators B 109:194
35. Shimei J, Yue W (1999) Spectrochim Acta Part A 55:1025
36. Arora K, Prabhakar N, Chand S, Malhotra BD (2007) Biosens Bioelectron 23:613
37. Degefa TH, Kwak J (2008) J Electroanal Chem 612:37
38. Sanchez-Pomales G, Santiago-Rodriguez L, Rivera-Velez NE, Cabrera CR (2007) J Electroanal Chem 611:80
39. Prabhakar N, Singh H, Malhotra BD (2008) Electrochem Commun 10:821
40. Kerman K, Morita Y, Takamura Y, Tamiya E (2003) Electrochem Commun 5:887
41. Chen SM, Wang CH (2007) Bioelectrochem 70:452
42. Ozkan D, Karadeniz H, Erdem A, Mascini M, Ozsoz M (2004) J Pharm Biomed Anal 35:905
43. Perez P, Teijeiro C, Marin D (1999) Chem Biol Interact 117:65
44. Saoudi B, Despas C, Chehimi M, Jammul N, Delemar M, Bessiere J, Walcarius A (2000) Sens Actuators B 62:35
45. Wu J, Zhao Y, Li X, Liu H, Shen G, Yu R (2005) Sens Actuators B 104:43
46. Li J, Liu G, Liu Y, Liu S, Shouzhuo Y (2005) Anal Biochem 346:107
47. Liu Y, Hu N (2007) Biosens Bioelectron 23:661
48. Kumar RS, Arunachalam S (2007) Polyhedron 26:3255