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Tin oxide nanoparticles-polymer modified single-use sensors for electrochemical monitoring of label-free DNA hybridization

Mihrican Muti[a,b], Filiz Kuralay[c,**], Arzum Erdem[a,*], Serdar Abaci[c], Tugrul Yumak[d], Ali Sinağ[d]

[a] Ege University, Faculty of Pharmacy, Analytical Chemistry Dept., Bornova, 35100 Izmir, Turkey
[b] Adnan Menderes University, Faculty of Science, Chemistry Department, 09010 Aydın, Turkey
[c] Hacettepe University, Faculty of Science, Department of Chemistry, Beytepe, 06532 Ankara, Turkey
[d] Ankara University, Faculty of Science, Department of Chemistry, Beşevler, 06100 Ankara, Turkey

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ABSTRACT

In this study, SnO_2 nanoparticles (SNPs)-poly(vinylferrocenium) (PVF^+) modified single-use graphite electrodes were developed for electrochemical monitoring of DNA hybridization. The surfaces of polymer modified and polymer–SNP modified pencil graphite electrodes (PGEs) were firstly characterized by using SEM analysis. The electrochemical behaviours of these electrodes were also investigated using the differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS) techniques. The polymer–SNP modified PGEs were then tested for the electrochemical sensing of DNA based on the changes at the guanine oxidation signals. Experimental parameters, such as; different modifications in DNA oligonucleotides, DNA probe concentrations were examined to obtain more sensitive and selective electrochemical signals for nucleic acid hybridization. After optimization studies, DNA hybridization was investigated in the case of complementary of hepatitis B virus (HBV) probe, mismatch (MM), and noncomplementary (NC) sequences.

1. Introduction

Nanobiotechnology plays an important role in the development of electrochemical biosensors for different purposes [1–3]. It combines the electronic, optical, magnetic and catalytic properties of nanomaterials with the high selectivity and high sensitivity of electrochemical methods [4]. Nanostructured metal and metal oxides have attracted great attention in recent years for biosensing due to their good compatibility with biomolecules such as enzymes, proteins, antibodies, and DNA [5]. These nanoparticles provide high electrochemically active surface area, high detection sensitivity, non-toxicity, ease of fabrication and chemical stability [6].

DNA biosensors are important topics in many fields, such as: genetics, pathology, forensic applications, pharmacogenetics and food engineering [7]. Electrochemistry of nucleic acids and particularly of oligonucleotides (ODNs) is an important field closely related to the fast growing research and development of electrochemical sensors for DNA hybridization [8]. Because of their remarkable sensitivity, compatibility, inherent miniaturization and low cost modern electrochemical DNA biosensors are extremely attractive for obtaining the sequence-specific information in a simpler, faster, and cheaper manner compared to traditional methods [9].

Electrochemical detection of DNA hybridization takes a long way with the use of nanoparticle materials [10]. To stabilize the dispersion of nanoparticles on electrode surface, polymer modification can be used successfully [11]. The immobilization of DNA using polymer film is very simple, and the adsorption, electrostatic interaction or covalent binding methods can be simultaneously applied for DNA immobilization [12].

The numbers of studies including the advantages of both nanoparticles and polymer are increasing nowadays in biosensing applications [13–24]. Cu@Au alloy nanoparticle as oligonucleotides labels for electrochemical stripping detection of DNA hybridization using conducting polypyrrole (PPy) film at the glassy carbon electrode (GCE) was carried out [17]. Ag nanowires and nanoparticles were formed by Farha Al-Said on hybrid λ-DNA/conducting polymer templates using oxidative polymerization of alkynyl-thienylpyrrole [18]. DNA biosensor based on polypyrrole and CdS nanoparticles was prepared by Travas-Sejdic et al. [19]. Gold nanoparticle/polyaniline nanotube membranes on the glassy carbon electrode (Au/nanoPAN/GCE) were fabricated for DNA hybridization [20]. Liu et al. reported polythionine/gold nanoparticles/multi-wall carbon nanotubes modified electrode for simultaneous determination of adenine and guanine in DNA [21]. DNA biosensor was developed by modified multilayer of ssDNA, cytochrome c, L-cysteine, gold nanoparticles and chi-

* Corresponding author. Tel.: +90 232 311 10 101x5131; fax: +90 232 388 5258.
** Co-corresponding author. Tel.: +90 312 297 7969; fax: +90 312 299 2163.
E-mail addresses: filizkur@hacettepe.edu.tr (F. Kuralay), arzum.erdem@ege.edu.tr (A. Erdem).

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tosan [22]. Electrochemical method for the detection of DNA-PNA hybridization using ferrocene-containing cationic polythiophene on nanogold modified electrode was performed by Fang et al. [23]. Chang et al. developed a protocol using Au nanoparticles (Au-NPs) for DNA hybridization by assembly of alternating DNA and poly(dimethyldiallylammonium chloride) (PDDA) multilayer films layer-by-layer [24].

Tin dioxide (SnO_2) is a semiconductor that presents a proper combination of chemical, electronic and optical properties that make it advantageous in several applications [6,25–31]. Due to the conductive properties of tin oxide, the SnO_2 nanoparticles were used for several electrochemical applications. SnO_2–graphene nanocomposite was used as anode materials for lithium-ion batteries by Yao et al. [28]. In the study of Ansari et al. a chitosan–tin oxide nanobiocomposite film was deposited onto the surface of indium–tin oxide glass plate for the preparation of platform to detect cholesterol [29]. Direct electrochemistry and electrocatalysis of horse radish peroxidase immobilized on sol–gel derived SnO_2/gelatin composite film was studied by Jia et al. [30]. The nanocrystalline SnO_2 electrodes were developed for detection of two proteins, cytochrome *c* and hemoglobin by using cyclic voltammetry and spectroelectrochemistry without the use of any electron-transfer promoters or mediators [31]. Thin mesoporous nanocomposite films of SnO_2-poly(diallyldimethylammonium chloride) were formed in a layer-by-layer deposition process for heme proteins methemoglobin and cytochrome $P450_{cam}$ by Milsom et al. [6].

Poly(vinylferrocenium) (PVF^+) is a redox polymer which has long been used as a fundamental conducting polymer system with the advantages of simple electrochemistry, high stability, and the ease of deposition of thin films using a variety of methods [32]. PVF^+ modified electrodes have recently been used for electrochemical monitoring of nucleic acid hybridization [33].

To the best of our knowledge, SnO_2 nanoparticles (SNP)-poly(vinylferrocenium) (PVF^+) modified single-use graphite electrodes were developed as the first time in our study as a novel electrochemical DNA sensor. Firstly characterization of the surfaces of polymer modified and polymer–SNP modified pencil graphite electrodes (PGEs) were performed by using SEM. The electrochemical behaviours of these electrodes were also investigated using the differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS) techniques. Then, polymer–SNP modified graphite electrodes were used for the electrochemical sensing of DNA by measuring the changes at the guanine oxidation signals. The effect of different modifications in DNA oligonucleotides (ODNs), and also various DNA-ODN probe concentrations were examined in order to develop more sensitive and selective electrochemical sensors. Label-free DNA hybridization was consequently investigated in the case of complementary of hepatitis B virus (HBV) probe (target), mismatch (MM), noncomplementary (NC) sequences, and also in a mixture sample (target and MM, 1:1 ratio). The features of polymer–SNP modified electrode developed for DNA hybridization were discussed and compared with those ones previously reported for other DNA hybridization electrodes in the literature.

2. Material and methods

2.1. Apparatus

All experimental measurements were carried by using AUTOLAB-PGSTAT 302 electrochemical analysis system supplied with GPES 4.9 software package (Eco Chemie, The Netherlands). Differential pulse voltammetry (DPV) was used for the electrochemical measurements. The three-electrode system was consisted of pencil graphite electrode (PGE), an Ag/AgCl/KCl reference electrode (BAS, Model RE-5B, W. Lafayette, USA) and a platinum wire as the auxiliary electrode. These electrodes were also used for the electrooxidation of PVF^+/PVF^+–SnO_2 nanoparticle performed in methylene chloride.

The potential-controlled coulometric and AC impedance studies were carried out with CH Instruments System, Model 660 B (USA). SEM images were obtained by Zeiss Evo 50 EP-SEM (USA).

2.2. Chemicals

The synthetic oligonucleotides were purchased from TIB-MOLBIOL (Berlin, Germany). Their base sequences are:

Amino linked DNA: (21 base; DNA-ODN)
5′-NH_2–$(CH_2)_6$-gAg ggT gTC TgA Agg ggg– 3′
Thiol linked DNA
5′-SH–$(CH_2)_6$–gAg ggT gTC TgA Agg ggg-3′
Bare DNA:
5′-gAg ggT gTC TgA Agg ggg-3′
HBV DNA probe (20 base):
5′-NH_2–$(CH_2)_6$–AAT ACC ACA TCA TCC ATA TA-3′
Target (20 base):
5′-TAT ATg gAT gAT gTg gTA TT-3′
Single-base mismatch (20 base; MM):
5′-TAT cTg gAT gAT gTg gTA TT-3′
Noncomplementary (23-base; NC):
5′-AAT ACC TgT ATT CCT CgC CTg TC-3′

The oligonucleotide stock solutions (500 μg/mL) were prepared with Tris–EDTA buffer solution (10 mM Tris–HCl, 1 mM EDTA, pH: 8.00; TE) and kept frozen. More diluted solutions of DNA-ODN and HBV probe were prepared in 0.5 M acetate buffer solution containing 20 mM NaCl (pH: 4.80; ABS). More diluted target/NC/MM solutions were prepared in 0.05 M phosphate solution containing 20 mM NaCl (pH: 7.4; PBS).

Methylene chloride was obtained from Riedel de Haen. Vinylferrocene was purchased from Aldrich. 2,2′-Azo-bis(2-methylpropionitrile) (AIBN) was Alfa. Tetra-n-butyl ammonium hydroxide (TBAOH), ethyl alcohol (EtOH) and methanol were purchased from Merck. Perchloric acid and benzene were obtained from BDH.

Other chemicals were in analytical reagent grade and they were supplied from Sigma and Merck. Ultrapure and deionized water was used in all solutions.

2.3. Preparation and characterization of SnO_2 nanoparticles (SNP)

Synthesis of SnO_2 nanoparticles was performed by hydrothermal method according to the earlier procedure [34]. The SNPs were also characterized in the study of Gokdai et al. [34].

2.4. Supporting electrolytes

Tetra-n-butyl ammonium perchlorate (TBAP) was used as the supporting electrolyte of the polymer solution in nonaqueous medium. It was obtained by the reaction of TBAOH (40% aqueous solution) with perchloric acid and recrystallized from the mixture of water and EtOH [35]. Buffer solutions were used as the supporting electrolyte in aqueous medium.

2.5. Chemical polymerization of vinylferrocene and preparation of poly(vinyferrocene) (PVF)/PVF–SnO_2 nanoparticle (SNP) solutions

Poly(vinyferrocene) (PVF) was prepared by the chemical polymerization of vinylferrocene with AIBN initiator [35]. 1.0 mg/mL

PVF polymer solution was prepared in methylene chloride/TBAP solvent/supporting electrolyte system. 1.0 mg/mL PVF–1.0 mg/mL SnO_2 nanoparticle solution was prepared in methylene chloride/TBAP solvent/supporting electrolyte system. The solutions were deoxygenated by bubbling pure nitrogen gas (BOS) before electrochemical experiments.

2.6. Procedure

All the experiments were done at room temperature. A new polymer electrode/polymer–SNP modified electrode was used in each electrochemical detection cycle.

2.6.1. The preparation of polymer PGE/polymer–SNP modified PGE by potential-controlled coulometry

The polymer PGE/polymer–SNP modified PGE was prepared electrochemically by electrooxidation at +0.8 V vs. Ag/AgCl reference electrode in PVF/PVF–SNP solution. The thicknesses of polymer/polymer–SNP films were controlled by the charge passed during the electroprecipitation. This charge was considered as an indication of polymeric film thickness [12]. A film thickness up to a value that corresponded to the passage of a charge of 5.0 mC during the electroprecipitation of the polymer/polymer–SNP was used in the study.

2.6.2. Microscopic characterization of unmodified PGE, polymer modified PGE and polymer–SNP modified PGE by scanning electron microscopy (SEM)

The microscopic characterization of unmodified, polymer modified and polymer–SNP modified PGEs was performed with SEM in various resolution magnitudes; 10 μm, 1 μm and 200 nm.

2.6.3. Impedance measurements

Electrochemical impedance spectroscopy (EIS) measurements were controlled at the open-circuit value; +0.4 V vs. Ag/AgCl and the frequency was varied over the range 10^5 to 10^{-1} Hz with amplitude of 10 mV in the presence of 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) mixture as a redox probe prepared in 0.1 M KCl.

2.6.4. Electrochemical measurements

Each test was repeated three times, and average values were presented in the histograms with error bars.

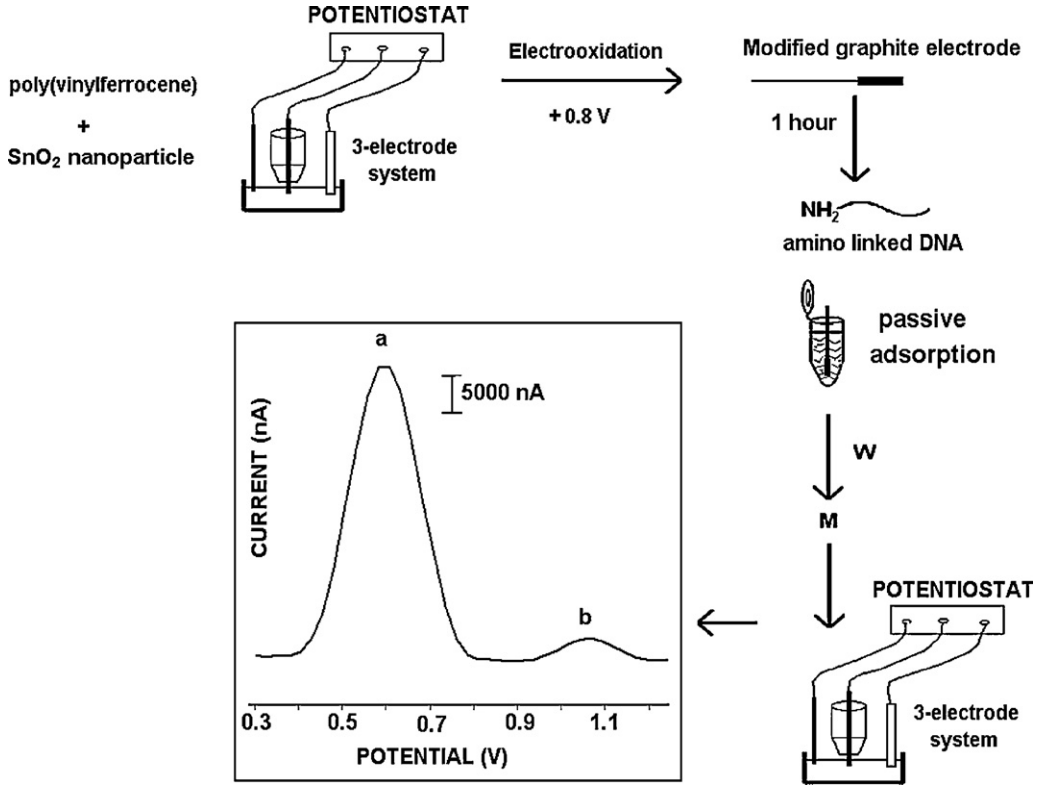
The preparation of polymer–SNP modified PGEs developed for DNA monitoring is illustrated in Scheme 1.

2.6.4.1. DNA-ODN immobilization onto the surfaces of polymer and polymer–SNP modified PGE. Modified PGEs were immersed into the vials containing 150 μg/mL of amino linked DNA-ODN solution prepared in ABS during 30 min. Each of the electrodes was then rinsed with ABS for three times to remove unbound probe from electrode surface.

2.6.4.2. Voltammetric transduction. The oxidation signals coming from polymer and DNA were monitored in a blank ABS by using polymer modified PGEs and polymer–SNP modified ones in combination with differential pulse voltammetry technique (DPV). DPV measurements were performed by scanning from −0.10 to +1.45 V at the pulse amplitude; 50 mV and scan rate; 50 mV/s.

2.6.5. Electrochemical monitoring of DNA hybridization by using polymer–SNP modified PGE

2.6.5.1. Probe immobilization onto the surface of polymer and polymer–SNP modified PGE. Modified PGEs were immersed into the vials containing 150 μg/mL of amino linked HBV probe solution



Scheme 1. Electrochemical sensing of DNA attached onto the surface of polymer–SNP by using disposable pencil graphite electrodes (PGE) followed by steps: (1) electrooxidation of poly(vinylferrocene)–SnO_2 nanoparticle mixture onto the surface of pencil graphite electrode by using potentiostat at +0.8 V with three-electrode system, (2) attachment of amino linked DNA-ODN immobilized onto the surface of polymer–SNP modified PGE by using dip-coating procedure, (3) voltammetric measurements in connection with three-electrode system, and (4) the oxidation signals of polymer (a) and guanine (b) in the same voltammetric scale; W, washing step; M, measurement.

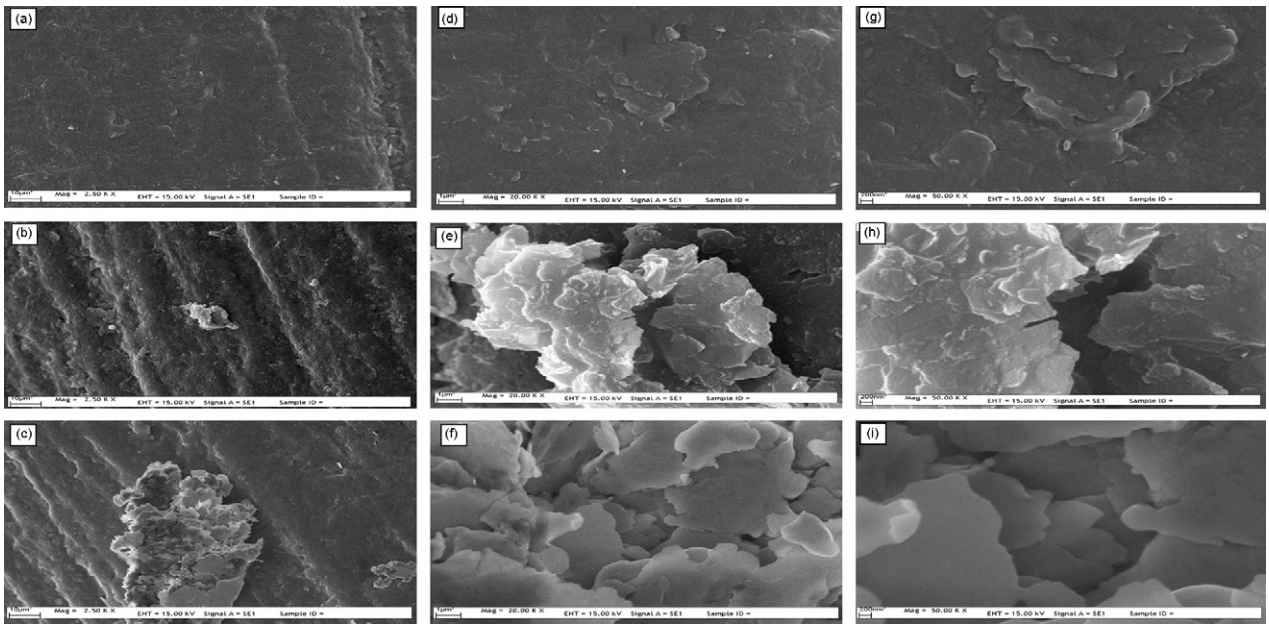


Fig. 1. SEM images of unmodified PGE (a, d, g), polymer modified PGE (b, e, h) and polymer–SNP modified PGE (c, f, j) in various magnitudes; 10 μm, 1 μm, 200 nm.

prepared in ABS during 30 min. Each of the electrodes was then rinsed with ABS for three times to remove unbound probe from electrode surface.

2.6.5.2. Hybridization at the surface of probe immobilized polymer–SNP modified PGE. Probe immobilized electrodes were immersed into the vials containing 100 μg/mL target (complementary sequences of HBV probe) prepared in PBS for hybridization process during 30 min. Each of electrodes was then rinsed with PBS for three times.

The same experimental procedure was repeated by using NC and MM sequences instead of target in order to check the selectivity of HBV DNA probe immobilized polymer–SNP modified PGE.

2.6.5.3. Voltammetric transduction. After DNA immobilization onto the surface of bare PGE/polymer modified PGE/polymer–SNP modified PGE, the differential pulse voltammetry (DPV) measurements were performed in a blank ABS to measure the guanine oxidation signal by scanning from −0.10 to +1.45 V at the pulse amplitude; 50 mV and scan rate; 50 mV/s.

3. Results and discussion

The surface morphologies of unmodified PGE, polymer modified PGE and polymer–SnO_2 nanoparticle (SNP) modified PGE were examined by using SEM analysis in magnitudes of 10 μm, 1 μm and 200 nm (shown in Fig. 1). The surface roughness of unmodified PGE was monitored successfully by SEM and shown in Fig. 1a, d and g, respectively. After the modification of polymer onto the PGE, some parts of the PGE surface was covered with polymer film (Fig. 1b). Modification of the polymer onto the electrode surface was seen clearly in lower magnitudes (Fig. 1e and h). SEM images of polymer–SNP modified PGE are given in Fig. 1c, f and j. It is clear that in the case of polymer–SNP, some parts of the PGE surface was covered with PVF^+–SNP (Fig. 1c). It is significant that different SEM images were obtained for polymer–SNP modified PGE (Fig. 1f and j) compared to polymer modified PGE (Fig. 1e and h) indicating different modifications on the PGE surface.

EIS was also used to identify and differentiate the modifications of polymer, polymer–SnO_2 bulk and polymer–SnO_2 nanoparticle modified onto the surface of pencil graphite electrode, PGE. EIS technique can provide information on the impedance changes of the electrode surface during the modification process [36]. In the Nyquist plot of impedance spectra, the diameter of the semicircle represents the charge-transfer resistance (R_{ct}) at the electrode surface [37,38]. Fig. 2a–d shows the impedance spectra of unmodified PGE, polymer modified PGE, polymer–SnO_2 bulk modified PGE and polymer–SnO_2 nanoparticle modified PGE, respectively. R_{ct} value increased (Fig. 2b to a) after the modification of PGE with polymer due to the charge limitation at the electrode surface. There were also increases at R_{ct} values after polymer–SnO_2 bulk (Fig. 2c) and polymer–SnO_2 nanoparticle (Fig. 2d) modification compared to the R_{ct} value obtained with polymer modified PGE (Fig. 2b). The R_{ct} value obtained with polymer-nanoparticle modified electrode was higher than the R_{ct} value obtained with polymer-bulk modified electrode indicating improved resistance to the charge-transfer occurred at the electrode surface. These results were in a good agreement with the results obtained by SEM analysis (shown in Fig. 1) proving the incorporation of SNPs onto polymer at PGE surface. Our results were also found in parallel to the earlier EIS results obtained with nanomaterials [3,36].

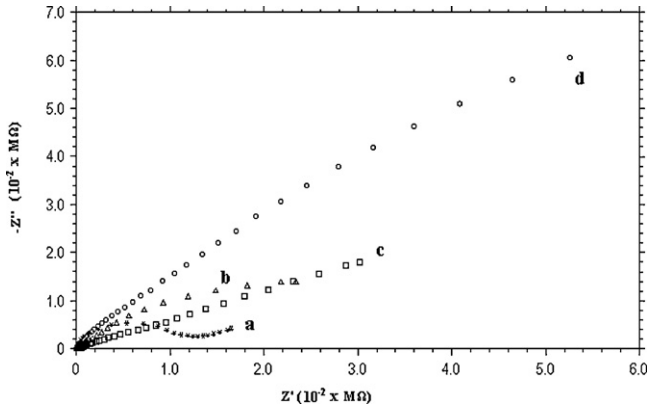


Fig. 2. Nyquist diagrams of (a) unmodified electrode, (b) polymer modified electrode, (c) polymer–SnO_2 bulk modified electrode, (d) polymer–SnO_2 nanoparticle modified electrode in 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) mixture containing 0.1 M KCl.

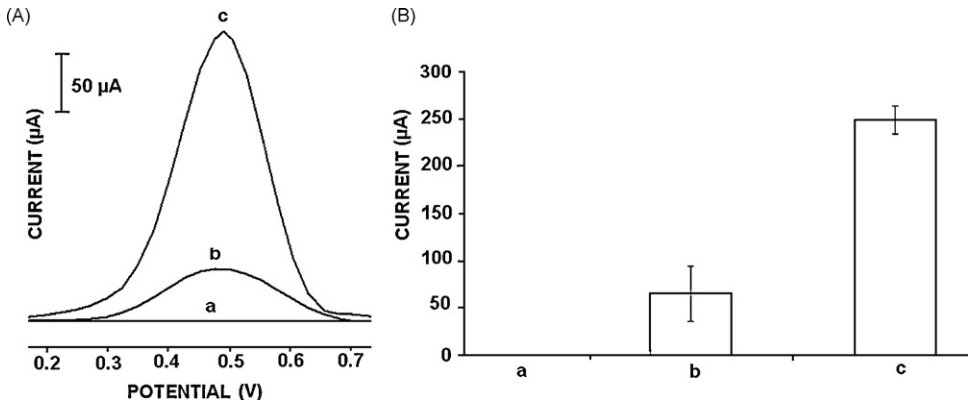


Fig. 3. DPVs and histograms of polymer oxidation signal of at bare electrode (a), polymer modified electrode (b) and polymer–SNP modified PGE (c).

Fig. 3a and b shows DPVs and histograms of the oxidation signal of PVF^+ on the surface of unmodified PGE and modified ones. The oxidation peak of PVF to PVF^+ both in the absence and presence of SnO_2 nanoparticle was observed almost at +0.48 V. After SnO_2 nanoparticle addition into the polymer matrix, the PVF^+ oxidation peak current increased about four times in comparison to the signal obtained in the absence of SnO_2 nanoparticle due to the conductive character of nanoparticle.

The effect of different ODN modifications on the response was examined in respect to their binding performance onto the positively charged polymer matrix and SnO_2 nanoparticle consisting polymer matrix. The changes at the oxidation signal of DNA electroactive base, guanine by using thiol linked, amino linked and bare ODNs were presented in Fig. S1. The maximum increase in the peak current and consequently, the maximum interaction with positively charged polymer matrix was obtained using thiol linked amino ODN. Maximum increase in the peak current and consequently, the maximum interaction with SnO_2 nanoparticle consisting positively charged polymer matrix was obtained in the presence of amino linked ODN. Thus, amino linked ODN was chosen for further experiments in order to obtain higher sensitivity and selectivity by using DNA modified polymer–SNP based electrode.

The effect of amino linked DNA-ODN concentration on the oxidation signal of guanine was studied in various ODN concentrations from 10 to 150 μg/mL (Fig. 4). The guanine oxidation signal increased when the concentration of ODN was increased to 150 μg/mL. The optimum immobilization concentration for amino linked ODN was chosen as 150 μg/mL in order to obtain the full surface coverage of polymer–SNP modified electrode. Additionally, the relative standard deviation was calculated as 13.4% for three successive determinations at 150 μg/mL DNA-ODN concentration. In parallel to the gradual increase at the guanine signal while increasing the concentrations of ODN immobilized onto the polymer–SNP modified surfaces, a decrease at polymer oxidation signal (not shown) was also obtained gradually due to amino linked DNA-ODN immobilization process, similarly to decrease at polymer signal shown earlier reports [12,32,33].

For the application of DNA immobilized polymer–SNP modified electrode, an electrochemical sensing of DNA hybridization was studied. The selectivity of hybridization between amino linked probe and NC, or MM sequences was checked. The higher oxidation signal of guanine was obtained at the hybridization of amino linked probe with its complementary target. There was no guanine oxidation signal measured in the case of hybridization between probe and NC similarly to the signal observed with HBV probe alone, however NC sequence was containing three guanine bases. Even if there was a mismatch sequence instead of target, no signal was measured indicating a full hybridization (Fig. 5). For further selectivity of HBV probe immobilized onto polymer–SNP modified electrode, the experiment was done in the mixture sample containing target and mismatch (1:1). It was found that DNA hybridization could be monitored selectively with a small increase at the guanine signal (Fig. 5e), however there are unwanted substituents in the sample; such as, MM sequence containing single-base point mutation. It

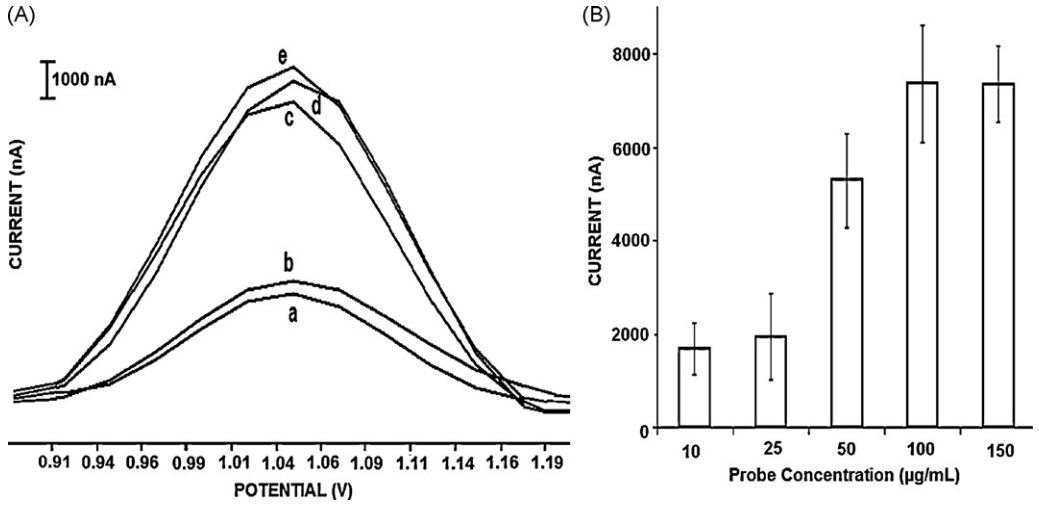


Fig. 4. (A) DPVs and (B) histograms showing the guanine oxidation signals measured by using DNA immobilized polymer–SNP modified electrode. Amino linked DNA-ODN concentrations varying from (a) 10 μg/mL, (b) 25 μg/mL, (c) 50 μg/mL, (d) 100 μg/mL and (e) 150 μg/mL.

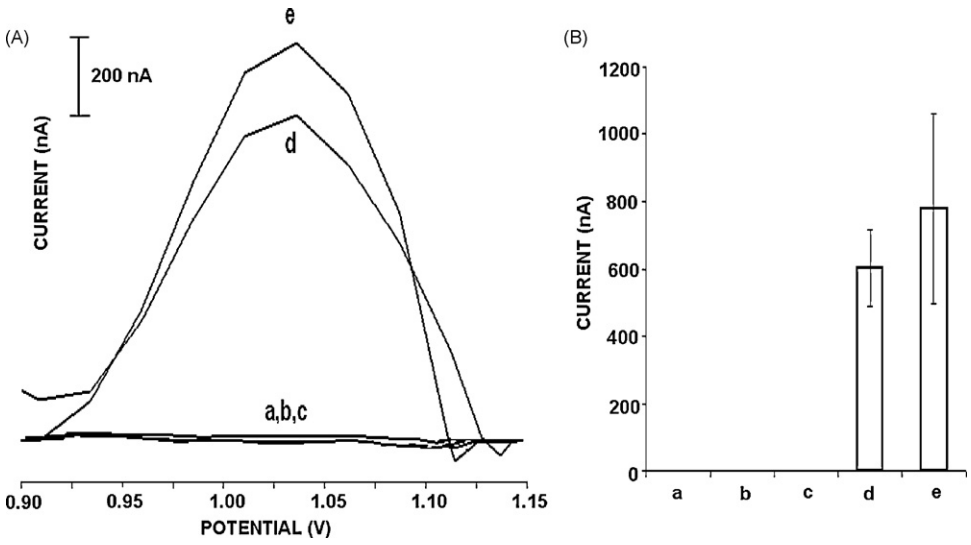


Fig. 5. (A) DPVs and (B) histograms for the guanine oxidation signals observed by using polymer–SNP modified PGEs in the presence of HBV probe alone (a), probe hybridization with 100 μg/mL NC (b), hybridization with 100 μg/mL MM (c), hybridization with 100 μg/mL target (d), hybridization in mixture samples containing target/MM in ratio (1:1) (e). 150 μg/mL amino linked HBV probe was immobilized onto polymer–SNP modified PGEs for 30 min and hybridization time was 30 min.

shows that probe immobilized polymer–SNP modified electrode has very high selectivity and specificity to its complementary DNA sequences compared to the earlier results [17,23,25].

The effect of DNA target concentration based on the oxidation signals of guanine after hybridization was also studied in various target concentration from 20 to 160 μg/mL. The oxidation peak current of guanine after hybridization increased and leveled off, when the concentration of DNA target was increased to 140 μg/mL (Fig. S2 and S3). A commonly used definition for the limit of detection is the analyte concentration giving a signal equal to the blank signal, plus three standard deviations of the blank [39]. According to this procedure, the detection limit was calculated as 1.82 μg/mL (equals to 279 nM).

4. Conclusions

Electrochemical DNA hybridization biosensor developed by using polymer–SNP modified PGE was carried out as the first time in our study. This DNA sensing approach combined with the advantages of nanotechnology and polymer technology. To the best of our knowledge, the incorporation of SNPs onto polymer was performed as the first time herein for development of electrochemical sensor monitoring DNA hybridization. With the addition of SNPs to the polymer system, the enhanced guanine oxidation signals were obtained compared to the results of earlier reports [12,21,32,33,40]. In aspects of the electrochemical results, it has been found in our study that SNP increased the electrochemical response of the polymer modified electrode resulting with the enhanced monitoring scheme for nucleic acids and DNA hybridization.

The preparation of this modified electrode was simpler, faster and cheaper in comparison to earlier studies based on polymer-nanoparticle modified electrodes [13,18,20–24] and it showed a linear response to guanine in the concentration ranges of target from 20 to 140 μg/mL with the detection limit as 1.82 μg/mL. The concentration level of short DNA sequences were found lower in our study in contrast to earlier studies in the literature [7,12,32,33]. In addition, probe immobilization onto these surfaces and hybridization process between HBV probe and its complementary were resulted in shorter time compared to earlier reports [7,12,22,32,33,36,41]. The linearity in the calibration plot obtained by a wide range of target DNA concentrations was investigated in a lower detection limit by the advantages of nanomaterials in contrast to the DL values of earlier polymer ((PVF$^+$) based DNA sensors [12,32].

DNA immobilization onto the surface of electrode was also presenting much easier process by passive adsorption resulting with much more effective discrimination of nucleic acids hybridization in a good selectivity and sensitivity. This electrode also showed a satisfactory reproducibility for DNA hybridization.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2010.07.040.

References

[1] A. Liu, Biosens. Bioelectron. 24 (2008) 167–177.
[2] A. Erdem, Talanta 74 (2007) 318–325.
[3] A. Erdem, H. Karadeniz, A. Caliskan, Electroanalysis 21 (2009) 464–471.
[4] S. Guo, S. Dong, Trends Anal. Chem. 28 (2009) 96–109.
[5] X.Q. Yang, L.H. Guo, Anal. Chim. Acta 632 (2009) 15–20.
[6] E.V. Milsom, H.A. Dash, T.A. Jenkins, C.M. Halliwell, A. Thetford, N. Bligh, W. Nogola, M. Opallo, F. Marken, J. Electroanal. Chem. 610 (2007) 28–36.
[7] S. Mathur, A. Erdem, C. Cavelius, S. Barth, J. Altmayer, Sens. Actuators B: Chem. 136 (2009) 432–437.
[8] A. Erdem, P. Papakonstantinou, H. Murphy, Anal. Chem. 78 (2006) 6656–6659.
[9] A. Erdem, in: S. Alegret, A. Merkoci (Eds.), Comprehensive Analytical Chemistry, Elsevier, 2007, pp. 403–411.
[10] J. Wang, G. Liu, R. Polksy, A. Merkoçi, Electrochem. Commun. 4 (2002) 722–726.
[11] Y. Wang, J.Y. Lee, Electrochem. Commun. 5 (2003) 292–296.
[12] F. Kuralay, A. Erdem, S. Abacı, H. Özyörük, A. Yıldız, Electroanalysis 20 (2008) 2563–2570.
[13] D. Ragupathy, A.I. Gopalan, K.P. Lee, Electrochem. Commun. 11 (2009) 397–401.
[14] Y. Du, X.L. Luo, J.J. Xu, H.Y. Chen, Bioelectrochemistry 70 (2007) 342–347.
[15] M.T. Sulak, Ö. Gökdoğan, A. Gülce, H. Gülce, Biosens. Bioelectron. 21 (2006) 1719–1726.
[16] L. Ma, R. Yuan, Y. Chai, S. Chen, J. Mol. Catal. B: Enzym. 56 (2009) 215–220.
[17] H. Cai, N. Zhu, Y. Jiang, P. He, Y. Fang, Biosens. Bioelectron. 18 (2003) 1311–1319.
[18] S.A. Farha Al-Said, R. Hassanien, J. Hannant, M.A. Galindo, S. Pruneanu, A.R. Pike, A. Houlton, B.R. Horrocks, Electrochem. Commun. 1 (2009) 550–553.
[19] J. Travas-Sejdic, H. Peng, R.P. Cooney, G.A. Bowmaker, M.B. Cannell, C. Soeller, Curr. Appl. Phys. 6 (2006) 562–566.
[20] Y. Feng, T. Yang, W. Zhang, C. Jiang, K. Jiao, Anal. Chim. Acta 616 (2008) 144–151.
[21] H. Liu, G. Wang, D. Chen, W. Zhang, C. Li, B. Fang, Sens. Actuators B: Chem. 128 (2008) 414–421.

[22] M. Li, S. Huang, P. Zhu, L. Kong, B. Peng, H. Gao, Electrochim. Acta 54 (2009) 2284–2289.
[23] B. Fang, S. Jiao, M. Li, Y. Qu, X. Jiang, Biosens. Bioelectron. 23 (2008) 1175–1179.
[24] Z. Chang, M. Chen, H. Fan, K. Zhao, S. Zhuang, P. He, Y. Fang, Electrochim. Acta 53 (2008) 2939–2945.
[25] C.A. Ibarguen, A. Mosquera, R. Parra, M.S. Castro, J.E. Rodriguez-Paez, Mater. Chem. Phys. 101 (2007) 433–440.
[26] T. Wang, Z. Ma, F. Xu, Z. Jiang, Electrochem. Commun. 5 (2003) 599–602.
[27] W. Chen, D. Ny, S. Chen, J. Power Sources 195 (2010) 412–418.
[28] J. Yao, X. Shen, B. Wang, H. Liu, G. Wang, Electrochem. Commun. 11 (2009) 1849–1852.
[29] A.A. Ansari, A. Kaushik, R.S. Pratima, B.D. Malhotra, Electroanalysis 21 (2009) 965–972.
[30] N. Jia, Q. Zhou, L. Liu, M. Yan, Z. Jiang, J. Electroanal. Chem. 580 (2005) 213–221.
[31] E. Topoglidis, Y. Astuti, F. Duriaux, M. Grtzel, J.R. Durrant, Langmuir 19 (2003) 6894–6900.
[32] F. Kuralay, A. Erdem, S. Abacı, H. Özyörük, A. Yıldız, Electrochem. Commun. 11 (2009) 1242–1246.
[33] F. Kuralay, A. Erdem, S. Abacı, H. Özyörük, A. Yıldız, Anal. Chim. Acta 643 (2009) 83–89.
[34] Z. Gokdai, A. Sınağ, T. Yumak, Biomass Bioenergy 34 (2010) 402–410.
[35] F. Kuralay, H. Özyörük, A. Yıldız, Sens. Actuators B: Chem. 109 (2005) 194–199.
[36] P. Du, H. Li, Z. Mei, S. Liu, Bioelectrochemistry 75 (2009) 37–43.
[37] E. Barsoukov, J.R. MacDonald, Impedance Spectroscopy Theory, Experiment, and Applications, Wiley-Interscience, USA, 2005.
[38] M.E. Ghica, R. Pauliukaite, O. Fatibello-Filho, C.M.A. Brett, Sens. Actuators B: Chem. 142 (2009) 308–315.
[39] R.L. McCreery, in: A.J. Bard, M. Dekker (Eds.), Electroanalytical Chemistry, New York, 1990, pp. 221.
[40] H. Karadeniz, A. Erdem, A. Caliskan, Electroanalysis 20 (2008) 1932–1938.
[41] I.O. K'Owino, S.K. Mwilu, O.A. Sadik, Anal. Biochem. 369 (2007) 8–17.