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Label and Indicator Free Electrochemical Nanobiosensing of DNA Hybridization Based On MnO_2 Nanomaterial Modified GCPE

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Abstract

A label and indicator free electrochemical DNA hybridization detection based on manganese (IV) oxide nanomaterial modified glassy carbon paste composite electrode (MnO_2-Nm/GCPE) was developed. 22 mer oligonucleotides representing E. coli bacteria were used as model case. As far as it is known, this study is the first study where MnO_2-Nms were incorporated into GCPE and used for genosensor transducer. The extent of hybridization was determined by using differential pulse voltammetric signals of guanine oxidation. By incorporating MnO_2-NM into the electrode structure, more sensitive guanine signal was obtained compared to plain GCPE. After the optimization of experimental parameters, the detecion limit was calculated as 1.31 x 10^{-9}M.

Keywords: GCPE, Genosensor, MnO_2 nanomaterials

Introduction

The detection of DNA hybridization related to specific sequences offers sensing and diagnosis of viruses, pathogenic microorganisms, mutations and human genetic diseases.[1] Clinical analysis of pathogenic or inherited diseases requires sensitive and selective detection for effective treatment.[2] Genosensor technologies are rapidly developed as an alternative to conventional methods by offering low cost, rapid, sensitive and selective detection.[3-5] Various approaches have been developed in this area. Among these approaches nanomaterial modified electrochemical genosensors show great development.[6-8]

For the design of an electrochemical genosensor, immobilization of a DNA probe onto nanomaterial modified electrode surfaces has received considerable attention. Since nanomaterials possess unique physical, chemical and optical properties, they become promising matrices for sensing applications.[9-10] In addition; nanomaterials provide increased surface area for DNA immobilization to obtain improved sensitiviy and selectivity.[11-12]

In recent years vaious nanoparticles such as carbon nanotubes,[13] gold nanoparticles,[14] SiO_2 nanoparticles,[15] Fe_2O_3 nanoparticles,[16] have been widely used in biosensing applications. Manganese dioxides are a kind of inorganic materials that have been used in catalysis reactions and in electrodes

for battery applications.[17] Recently, several kinds of manganese (IV) oxide nanomaterials (MnO_2-Nms) were synthesized and used in several biosensor applications.[18-19]

Herein we describe a label free electrochemical genosensing of specific DNA hybridization by using MnO_2-Nms modified glassy carbon paste electrodes (GCPE). GCPE is a kind of composite carbon electrode which shows better electrochemical performance compared to carbon paste electrodes. GCPE can easily be formed just by mixing the proper amount of glassy carbon micro particles with mineral oil.[20] Since its composite nature, it is very easy to renew and modify this electrode and for this reason, GCPE has been extensively used in biosensor applications.[20-24] Also as far as we know there is one work that uses plain GCPE (without any nanomaterial inside) as DNA genosensor transducer.[25] On the other hand, considering the usage of MnO_2-Nms in the genosensor structure again one study has been found which glassy carbon electrode was used as a genosensor transducer and MnO_2-Nms were immobilized onto the electrode surface via suitable membranes.[18] In the present study, we combine MnO_2-Nms with a composite structure. By this way, more practical genosensor was obtained because the modification was done just by mixing proper amount of nanomaterial with paste structure. As nanomaterials have electrocatalytic properties, more effective interactions are expected in our work.

E. coli oligonucleotides were used as model case. Differential pulse voltammetry (DPV) of guanine oxidation signals before and after hybridization at MnO_2-Nms/GCPE surfaces were evaluated for direct analysis. After the optimization of experimental working conditions, analytical characteristics were examined and limit of detection (LOD) value was calculated.

Experimental

Apparatus

The oxidation signal of guanine was investigated by using DPV with AUTOLAB PGSTAT 12 electrochemical analysis system and GPES software package (ECO CHEMIE Instruments B.V.,The Netherlands). Sigma 3-16 pk was used for centrifugation. Bandelin sonorex was used for sonication. TEM images were recorded using JEOL-JEM 2100. MnO_2-Nms modified GCPE was used as working electrode. Ag/AgCl and platinum electrodes were used as reference and auxiliary electrode, respectively.

Chemicals

Glacial acetic acid, HCl, NaOH, NaCl, KH_2PO_4 and $KMnO_4$ were purchased from MERCK KGaA (Darmstadt, Germany, http://www.merck.de). Trisodium citrate dihydrate and manganase(II) acetate, were purchased from SIGMA (Milwaukee,USA, http://www.sigmaaldrich.com). All of other

chemicals were of analytical grade. Ultrapure distilled water was used in all solutions. All experiments were performed at room temperature.

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

Capture probes and target sequences in the form of lyophilized powder, were purchased from Ella Biotech GmbH (Martinsried, Germany, http://www.ellabiotech.com) with purification by HPLC. The base sequences of the oligonucleotides, were shown in Table 1.

Table 1 Olgonucleotide sequences

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

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

Methods

The hybridization detections were performed by monitoring DPV signals of guanine oxidation. The procedure of the study consisted of the following steps; MnO_2-Nms fabrication, MnO_2-Nms modified GCPE preparation, probe immobilization, hybridization, washing and electrochemical transduction.

Fabrication of MnO_2-Nms

MnO_2-Nms were prepared based on the following reaction:[27]

$$2KMnO_4 + 3Mn(Ac)_2 + 2H_2O \rightarrow 5MnO_2 + 2KAc + 4HAc$$

0.10 M potassium permanganate solution (40 mL) was added to 4 mL of 1.5M manganese(II) acetate solution under ultrasonicating at 25 ^{0}C. After the completion of the reaction, the brown solution was centrifuged (about 10,000 rpm). The deposition (MnO_2-Nms) was washed with water repeatedly until the washing solution was neutral. Finally, the prepared MnO_2-Nms were dispersed to 20 mg mL^{-1} in water by ultrasonication and stored at 4 ^{0}C for use.[26]

Preparation of MnO_2-Nms/GCPE

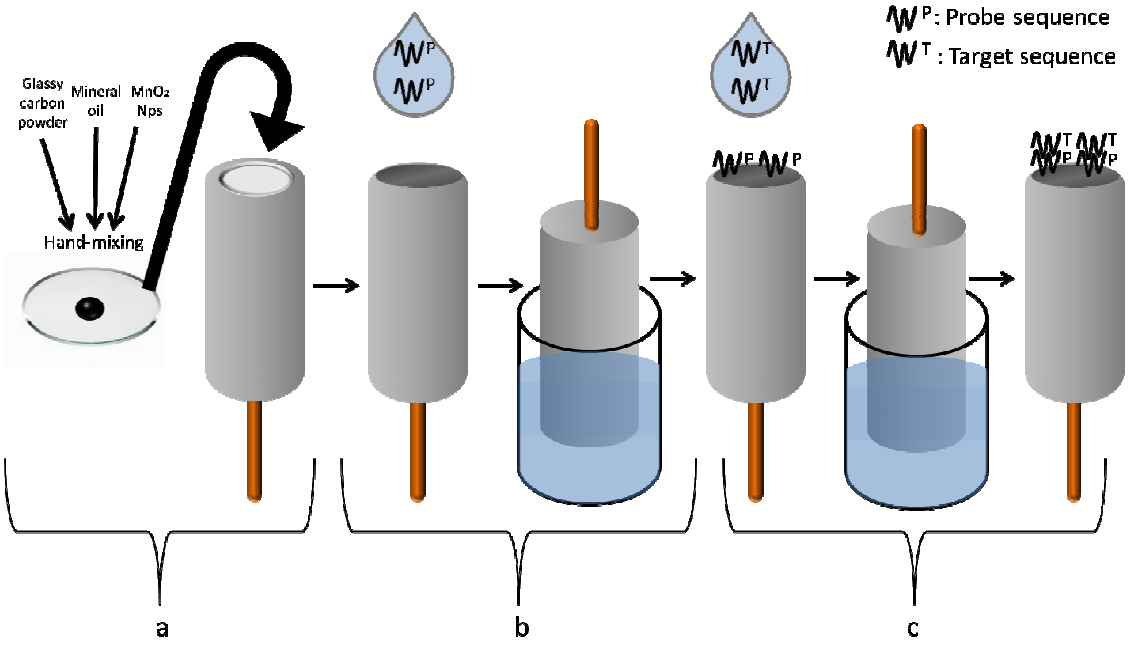
Glassy carbon paste was prepared in the usual way by hand-mixing glassy carbon powder and mineral oil in a 80:20 mass ratio. Then, 4 µL (20 mg mL^{-1}) of prepared MnO_2-Nms was added to glassy carbon paste and was mixed again. The resulting paste was then packed firmly into electrode cavity. The surface was polished on a weighing paper to a smoothed finish and rinsed carefully with doubly distilled water before use. (Scheme 1a).

Probe Immobilization

The immobilization of the ssDNA probe on the electrode surface was carried out as follows: 0.5M 50 µL acetate buffer solution (pH:4.8) containing $2.25x10^{-6}$M probe sequence was dropped on the surface of MnO_2-Nms/GCPE for 60 min at room temperature. The role of using lower pH value was to prevent probe sequence hybridization on itself. Then, the electrode was washed three times with acetate buffer (0.5M pH:4.8) to remove any unimmobilized probe sequence. (Scheme 1b)

Hybridization and Washing

The hybridization was performed at room temperature by 50 µL hybridization buffer solution (2XSSC) containing $1.5x10^{-6}$M target sequence was dropped on the surface of probe immobilized MnO_2-Nms/GCPE for 30 min at room temperature. The electrode was rinsed three times with washing buffer solution including 2XSSC to prevent the unspecific binding. (Scheme 1c) The desgined genosensor's selectivity was tested by using a 20 mer non complementary target sequence representing antrax bacteria.



Scheme 1 Schematic diagram of the DNA biosensor fabrication a) Fabrication of MnO_2-Nms, b) Probe immobilization and c) Hybridization with target sequence

Electrochemical transduction

The oxidation signal of guanine was measured by using DPV in potential range between +0.75 V-+1.3V (for guanine oxidation) at a rate of 20 mV s^{-1} with amplitude of 10 mV.

All results reported in this paper represent the means of at least three measurements, and the error bars represent the corresponding standard deviation. Everytime a fresh electrode surface was provided due to ease which the nature of composite GCPE allows.

Results and Discussion

A label free voltammetric nanogenosensor system has been developed for direct detection of DNA hybridization. MnO_2-Nms modified GCPE's were prepared as sensor surfaces. DNA hybridization detection was performed after interaction between target sequences at probe modified MnO_2-Nms/GCPE surfaces based on guanine oxidation signals.

Characterization of Prepared MnO_2-Nms

The synthesized nanostructures were examined by TEM technique. Figure 1 demonstrates TEM images of prepared MnO_2-Nms. From the Figure, an agglomeration can be seen instead of well dispersed nanomaterials. On the other hand, this situation in correspondence with similar studies in the literature containing MnO_2-Nms in the structure.[27-28] The appearance of the image was attributed to the preparation procedure.

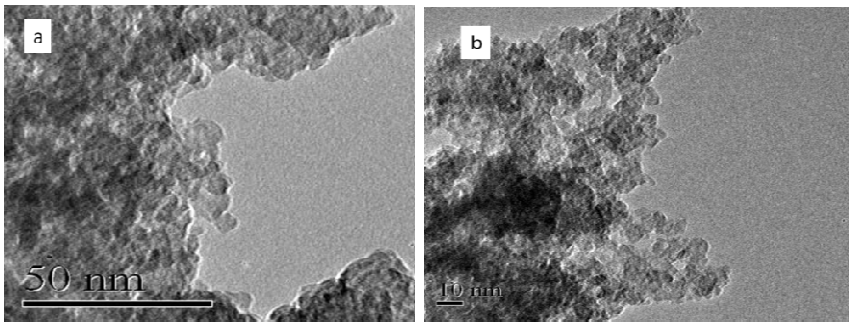


Fig. 1 TEM images of MnO_2-Nms with a)50 nm b)10 nm scale.

The Effect of MnO_2-Nms on the Probe Signal

Figure 2 shows the effect of MnO_2-Nms on guanine signal response enhancement. $7.5x10^{-7}$M of probe sequences were immobilized at plain and MnO_2-Nms modified GCPE surfaces. The oxidation peak potentials of guanine were found at around 1.0 V.[29] A significant increase in guanine signal at nanoparticles modified GCPE is observed (Fig 2a) compared to plain GCPEs (Fig. 2b). As can be seen from Figure, using MnO_2-Nms provide around 80.00 %of current enhancement in designed biosensor sensitivity.

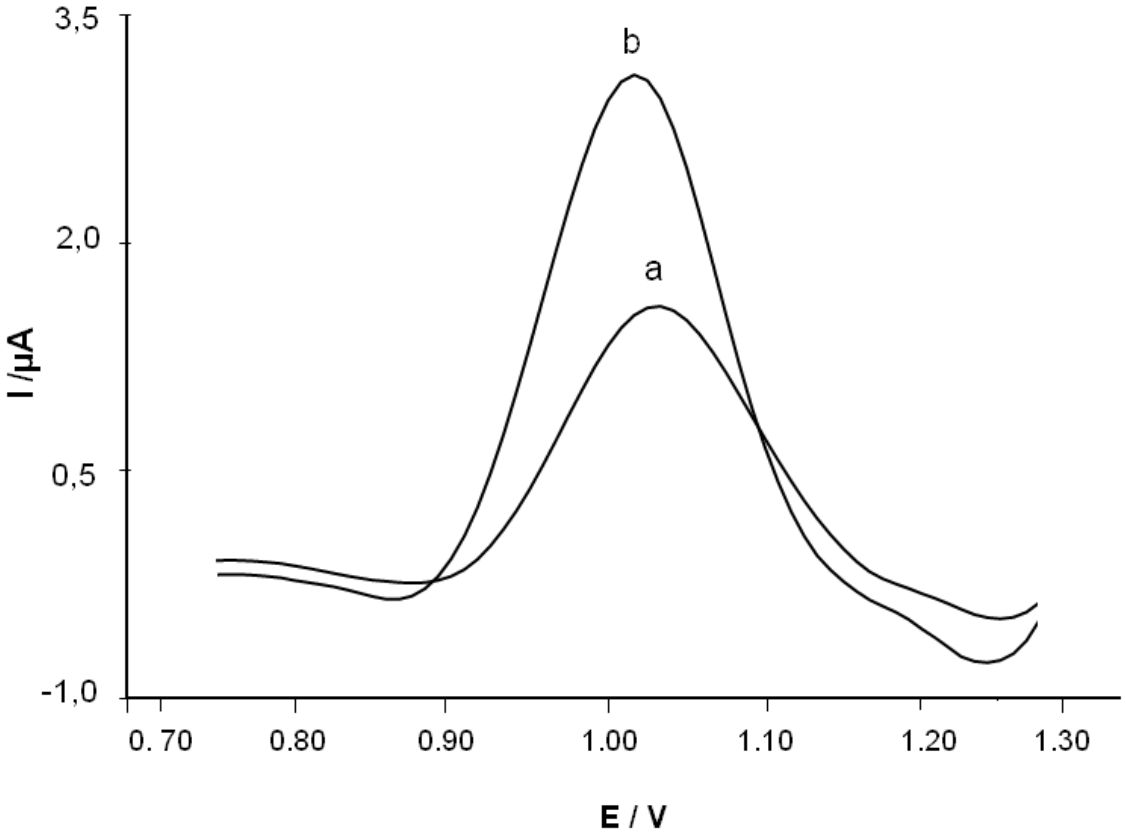


Fig. 2 DPVs of guanine oxidation at $7.5x10^{-7}$M probe modified a) plain GCPE b) 2 µL MnO_2-Nms modified composite GCPE. DPV parameters were as follows: 0.75-1.30 V; step potential: 7 mV; modulation amplitude: 50 mV; modulation time: 0.05 s; interval time: 0.5 s.

Optimization of the Developed Probe

The experimental conditions like MnO_2-Nms amount, probe and target concentration, content of hybridization buffer solution, probe immobilization and hybridization times were optimized to maximize the selectivity of the designed genosensor. The selectivity of the sensor was accomplished in terms of guanine oxidation signal before (a) and after hybridization with complementary (b) and non-complementary (c) targets at probe modified MnO_2-Nms/GCPE as shown in Figure 3.

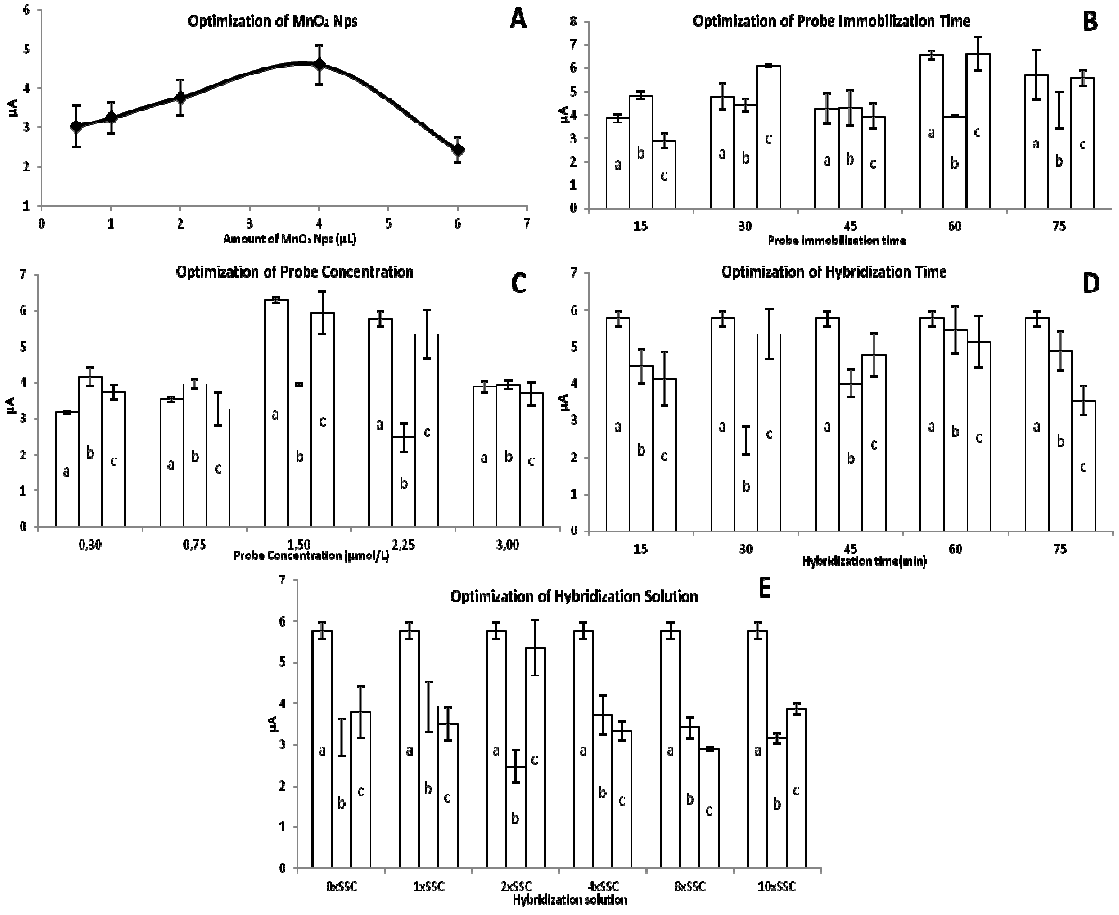


Fig. 3 Optimization of working conditions: A) MnO_2-Nms amount, B) probe immobilization time, C) probe concentration, D) hybridization time, E) hybridization solution. For the bars a)probe, b)hybridization with complemantary target, c) hybridization with non-complemantary target.

For MnO_2-Nms amount, 0.5, 1.0, 2.0, 4.0 and 6.0 µL MnO_2-Nms were tested by following the probe's guanine base current value (Fig 3 A). Since best current value was obtained with 4.0 µL MnO_2-Nms, further experiments were conducted by using this value. 15, 30, 45, 60 and 75 min were tried as probe immobilization times (Fig 3 B) under the working conditions of 4.0 µL MnO_2-Nms, 1.5µM probe concentration, 1.5µM target concentration, 30 min. target hybridization time and 2x SSC hybridization buffer (0.3M NaCl and 30mM sodium citrate). The current decrease between probe

(Fig 3 B a) and complementary target (Fig 3 B b) hybridization and between noncomplementary target (Fig 3 B c) and probe (Fig 3 B a) hybridization were followed. As can be seen from the Figure 3B, best results were observed at 60 min. For this reason, this value was utilized for further experiments. For the optimization of probe concentration 0.3, 0.75, 1.5, 2.25 and 3.0 μM probe amounts were immobilized onto the electrode surface (4.0 μL MnO_2-Nms, 60 min. probe immobilization time, 30 min. target hybridization time, 1.5μM target concentration, SSC hybridization solution). The similar current decrease as explained for Figure 3B was followed. As can clearly be seen from the Figure, optimum probe concentration was chosen as 2.25 μM, since more significant decrease was obtained when this amount was used. 15, 30, 45, 60 and 75 min. were applied as target hybridization time to the system (4.0 μL MnO_2-Nms, 60 min. probe immobilization time, 2.25μM probe concentration, 1.5μM target concentration, SSC hybridization solution). 30 min. was chosen as optimum target hybridization time considering obtained results (Fig 3D). Last working parameter that was optimized is the concentration of hybridization solution (Fig 3E). For this purpose, the concentration of hybridization solution was changed as 0XSSC, 1XSSC, 2XSSC, 4XSSC, 8XSSC and 10XSSC (4.0 μL MnO_2-Nms, 60.0 min. probe immobilization time, 2.25μM probe concentration, 30 min. target hybridization time, 1.5μM target concentration) Since the best result was obtained with 2XSSC, this solution was utilized for further studies.

In most of the obtained results above and under all optimum working conditions, (Fig. 3), guanine oxidation signals obtained before hybridization were higher than the ones obtained after hybridization with complementary target sequence due to double strand formation. The redox active groups of guanines were only partially available for oxidation and the peak current observed from hybrid modified electrodes were significantly decreased.[29] For some cases like Fig 3B and Fig. 3C, expected decrease can not be seen at some bars due to insufficient probe concentration (Fig. 3C) or shortage of time for completion of double strand DNA formation (Fig 3B). The same situation was observed when higher probe concentration is concerned. At this point, since probe can form double layer on itself, it is not possible to form double strand with target DNA and only the guanine signals on the double layered probe is observed. For this reason, a slight increase can be seen in the signals (Fig. 3C last bar)

The detection capability of developed genosensor under optimized conditions were then tested by using DPV before (a) and after hybridization with complementary (b) and non-complementary (c) target sequences (Figure 4).

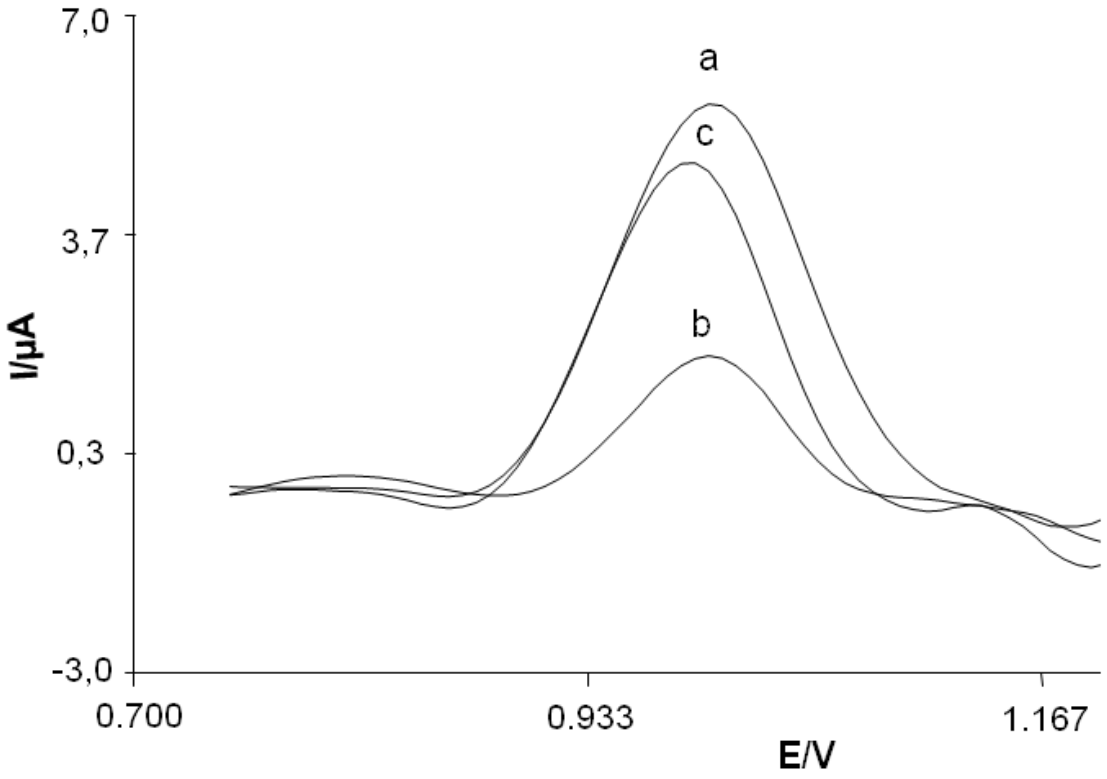


Fig. 4 DPV of guanine oxidation peak at probe sequence modified MnO_2-Nms/GCPE (a) and after hybridization with complementary sequence (b), and non-complementary sequence (c). DPV parameters were as follows: 0.75-1.2 V; step potential: 7 mV; modulation amplitude: 50 mV; modulation time: 0.05 s; interval time: 0.5 s

As can clearly be seen from the Figure, DPV signal decreases dramatically when complementary sequence was immobilized onto the probe (Figure 4b). The selectivity of the probe can also be observed when the current decrease of hybridization of complementary sequence (Figure 4b) and noncomplementary sequence (Figure 4c) was compared.

After the completion of optimization of experimental conditions, analytical characteristics were examined. As can be seen from Figure 5, ΔI (ΔI=I(probe)-I(hybridization with complemantary target)) was linear with respect to logarithmic value of complemantary target sequence over the range from $3.75x10^{-8}$ (0.25 mg /L) to $1.5x10^{-6}$M (10 mg /L). The regression equation was obtained as y = 1.2663x – 0.5655 (x was the logarithmic value of complemantary target sequence, nM; y was ΔI, μA; n=3) and a regression correlation coefficient (R^2) of the calibration line was 0.9963. The detection limit (LOD) was was also calculated based on S/N=3 and found as $1.31x10^{-9}$M (8.7 μg / L). The relative standard deviation RSD was 2.62% for five successive renewals (n=5).

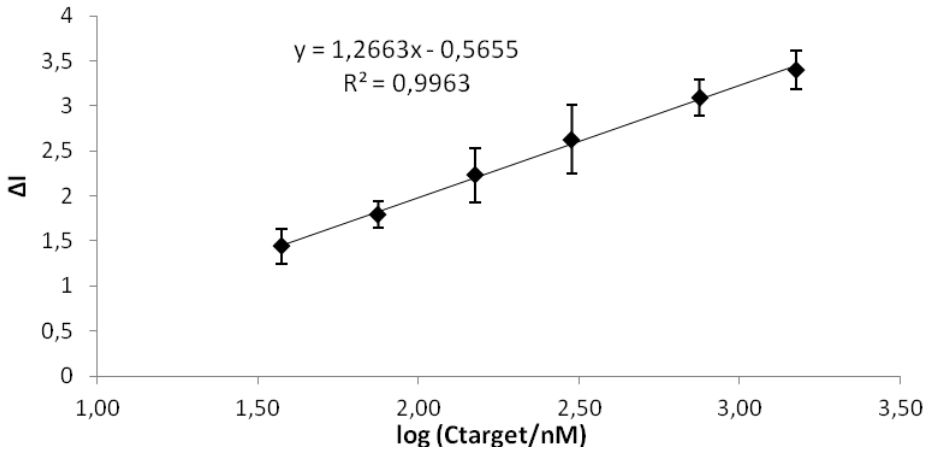


Fig. 5 Calibration curve of ΔI (I_{probe}-$I_{hybridization\ with\ complementary\ target}$) current values after hybridization with different concentration of target sequence. The potential range is between 0.75-1.3 V

The comparison of performance of developed system with various types of carbon electrode based genosensors has been made and presented at Table 2. Although it is possible to reach more sensitive LOD values with indicators, developed system's LOD value is comparable to the work with methylene blue as indicator [32]. Considering other carbon electrode based label-free genosensors in the Table, MnO_2-Nm/GCPE genosensor has better sensitivity (lower LOD) with acceptable linear range.

Table 1: The comparison of performance of developed system with various types of carbon electrode based genosensors

Electrode	Analyte or Sequence	LOD	Linear range	References
(CNTPE) modified with	salmonsperm dsDNA.	1.8 X 10^{-7} M	1 x10-7 to 1.1_10-4 mol L_{-1},	M. Mohamadi et al.[30]
poly(Py-*co*-PAA) film /GCE	probe NH2-GAT GAG TAT TGA TGC CGA-3_,	0.98 nM. .	2×10^{-9} to 2×10^{-7} M.	Peng et al.[31]
ZrO_2 /CPE; methylene blue as indicator	An oligonucleotide probe with a terminal 5′-phosphate group	($\leq2\times10^{-10}$ M	2.25×10^{-10} to 2.25×10^{-7} M	Zhuo et al.[32]
Heated CPE	Double-stranded calf thymus DNA, single-stranded calf thymus DNA	0.5 mg/L	1-8 mg/L	Wang et al.[33]

SWCNTcoated carbon fiber microelectrodes	Dopamine and Seratonin	250 nM	15–865 µM	Swamy et al.[34]

CNTPE: Carbon nanotube paste electrode; GCE: Glassy carbon electrode; ZrO_2 : zirconia; SWCNT: Single-walled carbon nanotube; Py: pyrrole monomer; PAA: 3-pyrrolylacrylic acid

Conclusions

In this work a label free nanogenosensor for the detection of E. coli bacteria was designed. MnO_2-Nms modified composite electrode (GCPE) was used as a transducer for the first time. The selectivity of the genosensor was tested by using the DPV oxidation peaks of guanine signals between E. coli and antrax sequence. The linear range for developed genosensor was found between $3.75x10^{-8}$M and $1.5x10^{-6}$M with the LOD value of $1.31x10^{-9}$M. This shows that the designed genosensor is capable to perform sensitive and direct detection and also applicable for other bacteria sequences. As a result practical, a label free sensitive and robust genosensor was obtained by combining GCPE composite nature with MnO_2-Nms.

Acknowledgements

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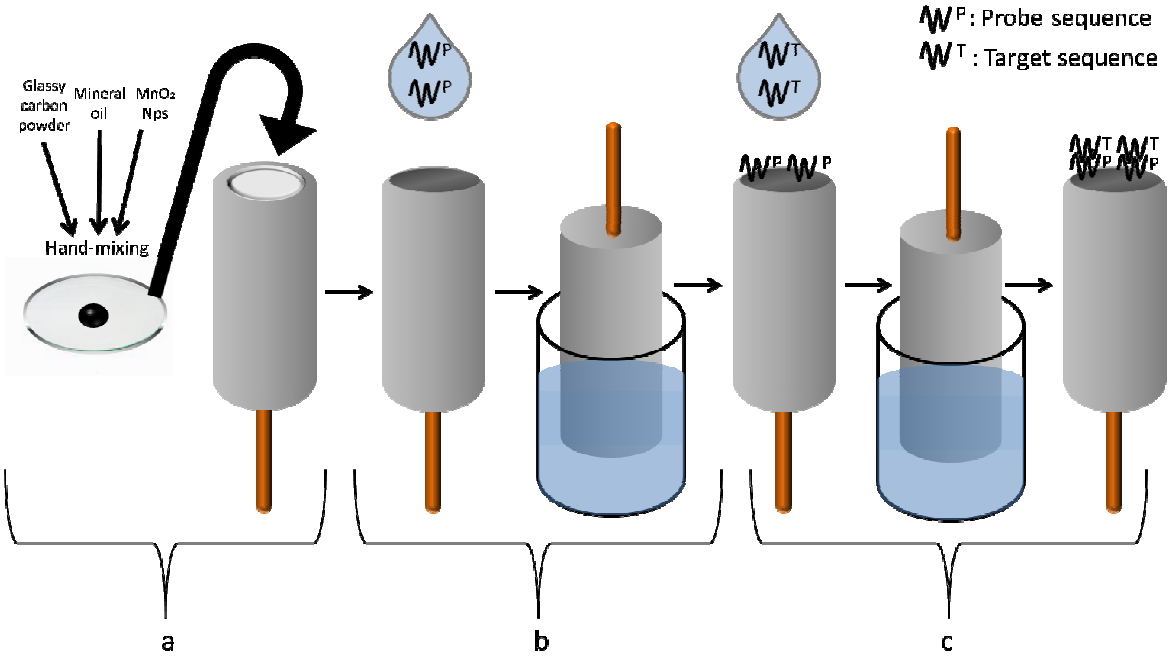

A label and indicator free electrochemical DNA hybridization detection based on MnO_2-Nps/GCPE was developed. Compared to plain GCPE, very robust and sensitive genosensor was obtained.