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DNA biosensor for detection of *Neisseria gonorrhoeae* causing sexually transmitted disease

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ABSTRACT

A sequence-specific electrochemical sexually transmitted disease (STD) sensor based on self-assembled monolayer of thiolated DNA probe specific to target *opa* gene for detection of Gonorrhoea, a sexually transmitted disease has been fabricated. 6-Mercapto-1-hexanol (MCH) has been used as a blocking agent to facilitate oligos "stand" up at the surface, a configuration favoring subsequent DNA hybridization and to repel non-specific adsorption of undesired DNA. The results of differential pulse voltammetric studies of this STD sensor reveal low detection limit (1.0×10^{-18} M) and a wide dynamic range (from 1.0×10^{-6} M to 0.5×10^{-18} M) arising due to the stable hybridization using methylene blue as an electro-active DNA hybridization indicator. The experimental results with genomic DNA, clinical patient sample of *Neisseria gonorrhoeae*, culture of non-*N. gonorrhoeae Neisseria* species (NgNS) and gram negative bacteria indicate that the fabricated sensor is specific to this STD.

1. Introduction

Gonorrhoea is a sexually transmitted disease (STD) caused by a Gram-negative diplococcus *Neisseria gonorrhoeae* (Gerbase et al., 1998). Gonorrhoea presents as lower genital tract infection, pelvic inflammatory disease (PID) and related sequelae in women (infertility and ectopic pregnancy); urethritis and epididymitis in men; and proctitis, pharyngitis, conjunctivitis and disseminated gonococcal infection in both sexes (World Health Organization, 1995; City of Philadelphia department of public health division of disease control gonococcal infection). It can also be transmitted from a mother's genital tract to the newborn during birth to cause ophthalmia neonatorum and systemic neonatal infection. Many techniques such as microscopy, culture of urethral exudates, endocervical specimens (Usategui et al., 1982) and PCR techniques can be used to detect gonorrhoea. Culture is presently considered to be the test of choice. However, it is quite laborious, time-consuming (14–72 h), and can be used for detection of living cells only (Nam et al., 2004). Some of the other disadvantages include fastidiousness of the organism, which makes successful growth less likely if isolation media, specimen transport, and laboratory techniques are not optimized. In addition, multiple steps are necessary for processing specimens, and the quality assurance needs to be addressed at each step. PCR operation needs well-trained laboratory personnel and is expensive. Also, both the commercial and in-house developed PCRs have been shown to yield variable sensitivities and specificities depending upon the gene targeted and the population genetics of the area under consideration. The currently available diagnostic techniques like microscopy, culture and PCR have specificity of about 95–100%, 100% and 88.9–99.5%, respectively with detection time ranging from 30 min to 72 h (Alam et al., 2002; Ho et al., 1992; Tabrizi et al., 2005; Herring et al., 2006; Koumans et al., 1998). Hence, detection of gonorrhoea is highly complicated due to the non-availability of a reliable method of screening the population. It has been reported that 10% of the infected males and as many as 50–80% of females are asymptomatic. The human immunodeficiency virus (HIV) cases are known to rapidly multiply. Numerous epidemiological studies suggest that there is approximately two- to fivefold greater risk of acquiring HIV in the presence of gonorrhoea. Early and appropriate diagnosis and treatment of gonorrhoea is an integral part of any quality, comprehensive HIV

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prevention strategy (Korenromp et al., 2005; Nusbaum et al., 2004; Hawkes and Santhya, 2002; Pais, 1996; Dallabetta, 1994). There is thus an urgent need for the development of a cost-effective, easy to perform, rapid screening test to diagnose this important STD (Alam et al., 2002; Ho et al., 1992; Davies et al., 1998; Buchanan et al., 1973).

Self assembled monolayers (SAMs) are well-ordered, homogeneous, simple, and versatile for immobilization of the desired biomolecules. SAMs have recently been used as substrates for bioanalyte immobilization (Martins et al., 2003; Barrias et al., 2005; Onoe et al., 2004) and for the fabrication of high performance biosensors with shorter response time, reduced non-Faradaic background current and improved reproducibility etc. (Yi et al., 2000). In this context, thiolated DNA SAMs on gold substrates have attained much interest during recent past as these matrices have been demonstrated for more ordered DNA surface confinement with predominantly well-aligned vertical conformation to the electrode surface (Steel et al., 1998; Thiel et al., 1997). Since DNA strands are highly negatively charged, multivalent cations can be electrostatically trapped within the DNA film. Moreover, Au-thiolates are known to be quite stable as the heat of adsorption ranges in tens of kcal/mol. DNA immobilization at the surface plays an important role in the performance of a DNA sensor. (Herne and Tarlov (1997) have shown a classical interfacial design by employing a mixed SAM of thiolated oligonucleotides (SH-DNA), and a dilution molecule, MCH at a gold surface. In this design, MCH not only helps DNA oligos "stand" up at the surface, a configuration favoring subsequent DNA hybridization, but also repels non-specific adsorption of undesired DNA molecules (Steel et al., 2000). SH-DNA/MCH-based electrochemical DNA sensors are able to sensitively detect DNA targets with high sequence specificity in pure DNA samples (Petrovykh et al., 2004). MCH has been used previously as an Au passivating species on 2D surfaces and large nanostructures. It displaces the non-covalent and non-specific adsorption of thiolated DNA, improving hybridization to the complementary strands (Liu et al., 2003). Park et al. (2004) have reported that the conformation of thiol-linked oligonucleotides on an Au surface can be controlled by treatment with MCH. MCH displaces non-covalent base adsorption to the surface, changing the oligo conformation on the Au surface. Many studies on influence of MCH concentration and reaction time on hybridization ability of Au–DNA have recently been reported, suggesting a reduced nonspecific adsorption of the oligo to the Au surface (Park et al., 2004). In this context, Lee et al. (2006) demonstrated that the chemisorption of mercaptoundecanol on HS-ssDNA-modified Au surface leads to the reorientation of the oligomers into a more upright position thus increasing response (Lee et al., 2006). DNA chips based on surface-confined DNA probe arrays have recently been reported to play an important role in the development of DNA sequencing and gene mapping technologies for pharmaceutical, clinical and forensic applications (Shumaker-Parry et al., 2004). Electrochemical biosensors offer many advantages over the existing devices based on optical schemes, because electrochemical transduction provides rapid, simple and low-cost point-of-care detection of specific nucleic acid sequences (Mikkelsen, 1996; Palecek and Fojta, 2001; Wang, 1999, 2000). Hence, an electrochemical DNA hybridization biosensor with low detection limit and high sensitivity is highly desired for specific DNA sequence detection (Drummond et al., 2003; Wang, 2002; Kavanagh and Leech, 2006; Chang et al., 2008).

In this manuscript, we report results of the studies relating to the fabrication of a highly sensitive DNA hybridization sensor based on the self assembled monolayer of thiolated probe DNA specific to target *opa* gene, multicopy gene present in *N. gonorrhoeae* onto gold electrode.

2. Experimental

2.1. Chemicals and reagents

Oligonucleotide probes, Tris–Base, ethylene diamine tetra acetic acid (EDTA), potassium monohydrogen phosphate, potassium dihydrogen phosphate, methylene blue (MB) have been procured from Sigma–Aldrich, USA. 6-Mercapto-1-hexanol has been purchased from Fluka, UK. All the solutions and glassware are autoclaved prior to being used. All solutions (molecular biology grade) are prepared in deionized water (Milli Q 10 TS). A 5′-thiol modified 20bases oligonucleotide specifically targeting the *opa* gene (a multi copy gene) of *N. gonorrhoeae* (thssDNA) is used as the probe. Culture of *N. gonorrhoeae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Neisseria sicca* and the clinical samples (urethral and endocervical swabs) of patients have been obtained from clinical microbiology laboratory, All India Institute of Medical Sciences (AIIMS), New Delhi and Safdarjung Hospital, New Delhi, India. The sequences of oligonucleotide probes used in this study are as follows: Probe: thssDNA DNA or Thiolated- (genbank accession no: PUID 9716119 snum 2705).

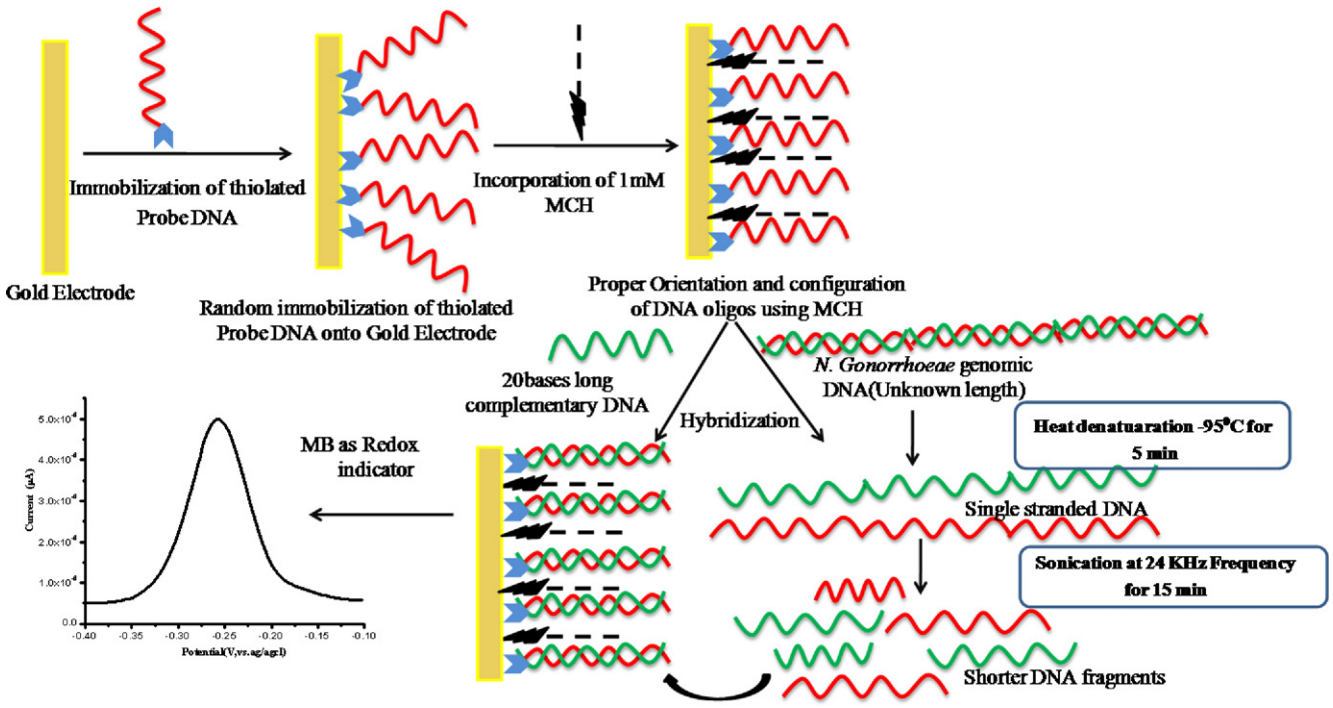
Probe DNA: 5′-SH-CCGGTGCTTCATCACCTTAG-3′
Complementary target DNA: 5′-CTAAGGTGATGAAGCACCGG-3′
One-base mismatch DNA: 5′-CTAAGTTGATGAAGCACCGG-3′

2.2. Formation and modification of DNA self-assembled monolayer (SAM) onto gold (Au) electrode (thssDNA–Au)

Prior to SAM formation, Au plates ($0.25\,cm^2$) are cleaned with acetone, ethanol, and with copious amount of de-ionized water. Further, Au plates are treated with 'Piranha'-etch solution (H_2SO_4/H_2O_2; 7:3) followed by rinsing with deionized water, and then with ethanol. Thiolated probe DNA is suspended in tris-EDTA (TE) buffer of pH 8.0 and 20 μl of thiolated probe DNA immobilized onto gold surface by utilizing SH–Au bond. After 2 h, the solution is rinsed with TE buffer and the electrode is dried under a flow of nitrogen. The electrode is then immersed in 1 mM solution of MCH for 2 h to remove non-specific DNA binding from the surface and to cover any regions of pristine gold (Martins et al., 2003), followed by thorough rinsing in deionized water and dried again under nitrogen. The mechanism of the thiolated probe DNA immobilization onto Au electrode and hybridization with target DNA for the detection of *N. gonorrhoeae* is depicted in Scheme 1. Electrochemical characterization has been performed using a three-electrode system in phosphate buffer saline electrolyte (PBS) (0.05 M, pH 7.0, 0.9% NaCl) containing 5 mM $[Fe(CN)_6]^{3-/4-}$ using platinum as counter electrode and Ag/AgCl as reference electrode. The fabricated nucleic acid functionalized gold electrode has been characterized using Fourier transform infrared spectroscopy (FT-IR, Perkin Elmer, Spectrum BX II), scanning electron microscopy (SEM, LEO-440), cyclic voltammetry (CV), differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS) (Potentiostat/Galvanostat, Autolab Eco Chemie, Netherlands) techniques.

2.3. Hybridization studies of thssDNA–Au electrode

The hybridization detection studies have been performed onto thssDNA/Au electrode by incubating with complementary and one-base mismatched DNA sequence. The target droplet is air-dried and cooled to room temperature. It is then rinsed with TE buffer for 10 s to remove any unbound DNA. Prior to characterization, thssDNA/Au electrode is stored at 4 °C under nitrogen gas. The thssDNA–Au electrode has been optimized for hybridization time and is subject to incubation in wide dynamic range from 1.0×10^{-6} M to 0.5×10^{-18} M of complementary target DNA within response time



Scheme 1. Schematic representation of procedure used to modify the electrode with thiolated DNA oligomers and MCH blocking agent. MCH prevents nucleotide adsorption, resulting in a more radial configuration of the DNA and subsequent DPV experiments with MB as redox indicator.

of 60 s at 25 °C. DPV measurements of thssDNA–Au electrode have been carried out after 20 μM MB pretreatment at +0.1 V for 10 s, in 0.05 M phosphate buffer pH 7.0 containing 0.9% NaCl.

2.4. DNA isolation from culture

DNA has been isolated from a panel of strains comprising of control strains of *N. gonorrhoeae* (WHO C), *E. coli*, *K. pneumoniae*, *S. aureus*, and a clinical strain of *N. sicca* for specificity studies. For this 100 μl sterile MilliQ water is taken in a 1 ml eppendorf. A suspension of colonies is made and then vortexed. The suspension is boiled for about 10 min and is centrifuged at 8000 rpm for about 5 min. To this, equal volume (100 μl) of 24:1 (v/v) chloroform: isoamylalcohol is added followed by centrifuging at 12,000 rpm for about 10 min. The aqueous layer containing the DNA is carefully pipetted out into another eppendorf. This extracted DNA is kept at −20 °C prior to being used.

2.5. Extraction of DNA from urethral and endocervical swab patient samples

Endocervical and urethral swabs from patients have been obtained from the STD Clinics, Dermatology OPD of All India Institute of Medical Sciences (AIIMS), New Delhi and Safdarjung Hospital, New Delhi, India. The swabs have been collected in triplicate for microscopy, culture and DNA extraction, respectively. Endocervical/urethral swabs have been plated on two selective media, (one with and one without inhibitors). The culture plates are then incubated at 36 °C in an atmosphere of 5% CO_2 for 24–48 h following which they are inspected for appearance of *N. gonorrhoeae* colonies. Suspected colonies are subjected to Gram stain, oxidase, superoxol test and rapid carbohydrate utilization test (RCUT) for confirmation. DNA has been extracted by using QIAamp DNA mini kit from *N. gonorrhoeae* culture, pus sample spiked with *N. gonorrhoeae* and patient sample.

2.6. Genomic DNA sample preparation

N. gonorrhoeae genomic DNA solution (0.2 μg/mL) in TE buffer was denatured by heating in a water bath (95 °C) for 5 min and was immediately chilled in ice to obtain denatured single-stranded DNA. Same aliquot of genomic DNA solution was subject to sonication (15 min at 120 V, 2 A and 24 kHz frequency using ultrasonic cleaner, Vibronics Pvt. Ltd., Mumbai, India) to break the long DNA strands into smaller fragments of the DNA. This genomic DNA was utilized for hybridization studies using the thssDNA–Au bioelectrode.

3. Results and discussion

3.1. SEM studies

The morphological studies onto Au and thssDNA–Au electrodes have been performed using scanning electron microscope and the results are shown in Fig. 1. A thin coating of gold is applied on the desired samples prior to SEM studies for better resolution. The SEM micrograph of Au electrode indicates smooth and homogenous morphology of Au electrode (Fig. 1(i)). However, after immobilization of thssDNA on Au electrode, the morphology porous with appearance of shiny bright/light streaks assigned to the accumulation of static charge onto surface of thssDNA–Au electrode. This is attributed to the immobilization of fibrous thiolated DNA molecules (Fig. 1(ii)) present onto gold electrode. Contact Angle Measurements have also been done to confirm the DNA immobilization (Supplementary Information Fig. 1).

3.2. Electrochemical characterization of DNA/Au electrode

Au and thssDNA/Au electrode have been electrochemically characterized using CV in PBS (0.05 M, pH 7.0, 0.9% NaCl) containing 5 mM $[Fe(CN)_6]^{3-/4-}$. After immobilization with thssDNA (Fig. 2(a)), the peak current has been found to decrease indicat-

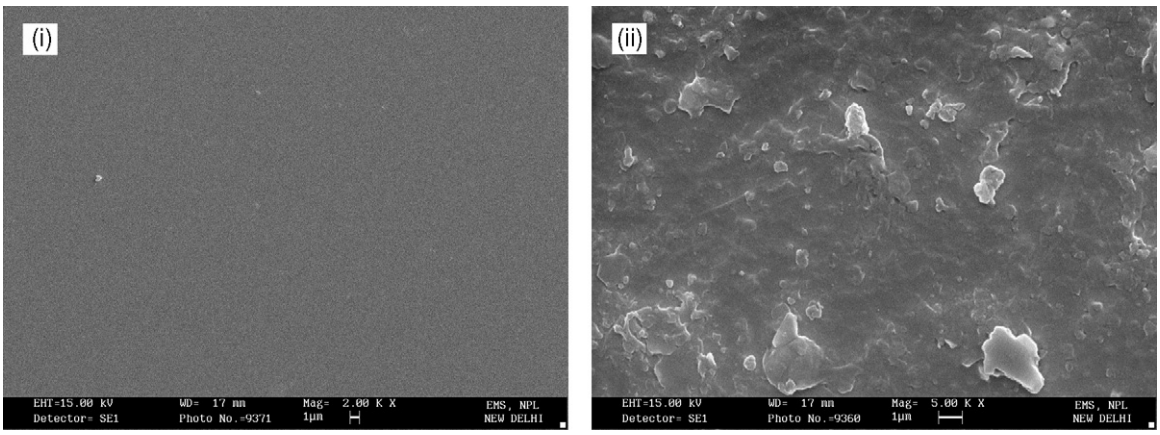


Fig. 1. SEM image of (i) pristine gold and (ii) thssDNA–Au electrode at 10 K×.

ing immobilization of DNA. The decrease in peak current indicates the thiolated ssDNA SAM formation and has been attributed to the insulating nature of ssDNA and due to the presence of large number of negative phosphate groups which hinder the diffusion of negatively charged redox molecule toward surface. The shift in peak potentials of the $[Fe(CN)_6]^{3-/4-}$ redox-reaction at the thssDNA probe immobilized electrode is due to repulsive electrostatic interactions between negatively charged DNA and ferricyanide ions that perhaps impede the movement of anions, ferricyanide ions, to reach the DNA immobilized Au surface. Therefore, these results indicate that thiolated probe ssDNA molecules were immobilized onto the Au substrate.

Fig. 2(b) shows the Nyquist plot of electrochemical impedance spectroscopy (EIS) measurements carried out as a function of frequency in the presence of PBS (50 mM, pH 7.0, 0.9% NaCl) containing 5 mM $[Fe(CN)_6]^{3-/4-}$. The impedance spectra include a semicircle portion at higher frequencies relating to the electron transfer-limited process and a linear part at lower frequencies corresponding to diffusion. The R_{ct} for the pristine Au electrode is $1.42232 \times 10^{-3}\ \Omega$. The increase in diameter of the semicircle reflects increase in the interfacial charge-transfer resistance (R_{ct}). The charge transfer resistance (R_{ct}) of the thssDNA–Au electrode is found to be $2.48573 \times 10^{-3}\ \Omega$ indicating immobilization of the thssDNA onto Au electrode.

3.3. Surface concentration

We have investigated the electron-transfer properties of MB at both thssDNA and dsDNA modified electrodes. Peak current (I_p) of MB is found to be linearly proportional to scan rates at both the thssDNA and dsDNA modified electrodes (Fig. 3), Fig. 3(a) and (b) having regression coefficient (R) of 0.997 and 0.9842, respectively, suggesting that the MB electrochemistry is a surface confined process and that MB is strongly adsorbed on DNA strands at the gold electrode (Kelley et al., 1997). The cyclic voltammeric studies have been carried out as a function of scan rate (1–20 mV/s) onto thssDNA–DNA bioelectrode to determine the total surface concentration. It is observed that the anodic peak potential E_{pa} varies linearly with logarithm of ν and the trend of the variation in the peak potential with scan rate follows Eq. (1):

$$E_{pa} = 0.01296 \log \nu + 0.25282; \quad R = 0.99; \quad SD = 0.001 \tag{1}$$

According to Laviron's theory (Laviron, 1979), the slope represents

$$\frac{RT}{\alpha nF} = 0.01296 \tag{2}$$

where α is the transfer coefficient. The value of $RT/\alpha nF$ has been used to calculate the total surface concentration of ionic species

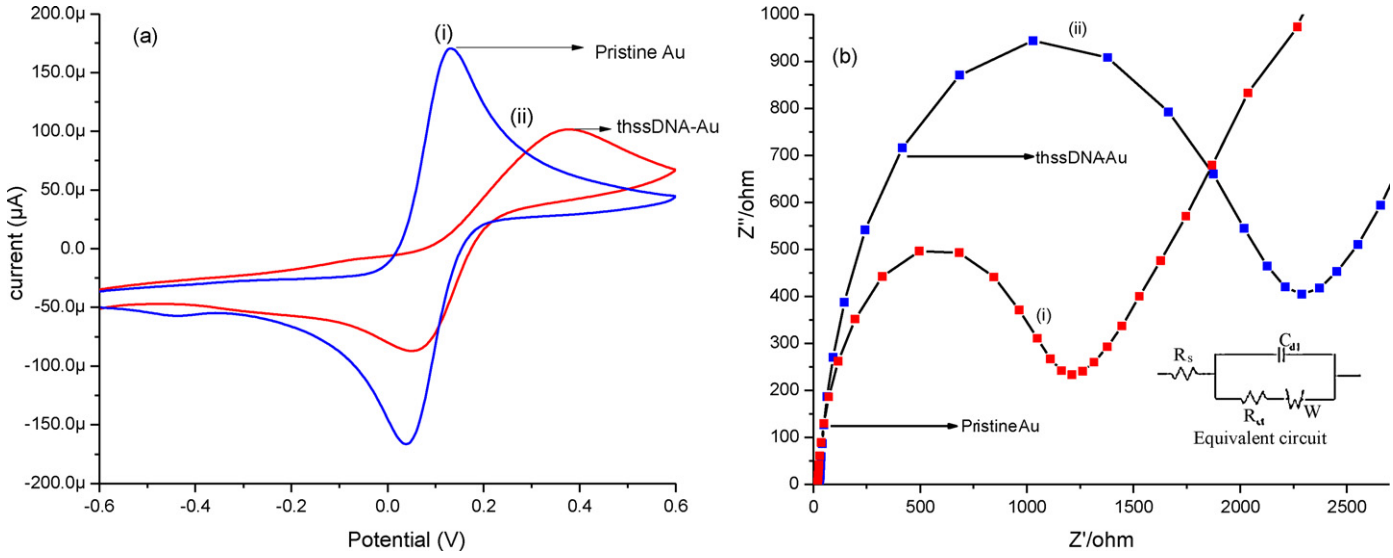


Fig. 2. Immobilization studies: (a) CVs of (i) pristine gold and (ii) thssDNA–Au electrode at 50 mV/S scan rate and (b) Electrochemical impedance spectra of (i) pristine gold and (ii) thssDNA–Au electrode in 0.05 M phosphate buffer of pH 7.0 containing 0.9% NaCl, containing 5 mM $[Fe(CN)_6]^{3-/4-}$ respectively.

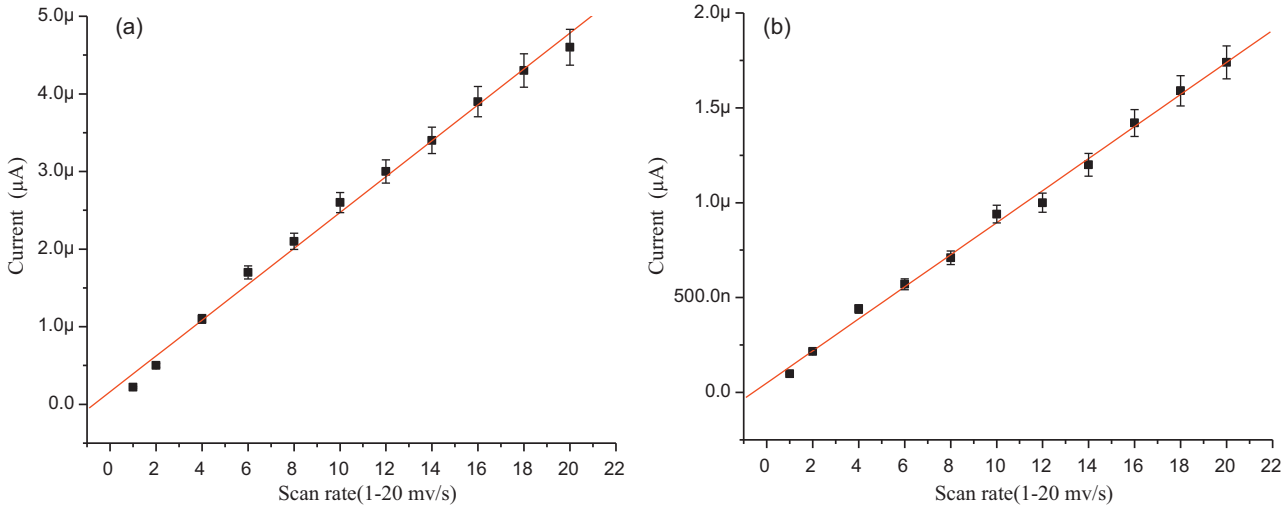


Fig. 3. The plots of oxidation peak currents vs scan rate: Influence of scan rates from 1 to 20 mV/s on (a) thssDNA–Au electrode and (b) dsDNA–Au electrode in presence of MB containing 0.05 M phosphate buffer of pH 7.0 containing 0.9% NaCl. (no. of samples $n = 5$)

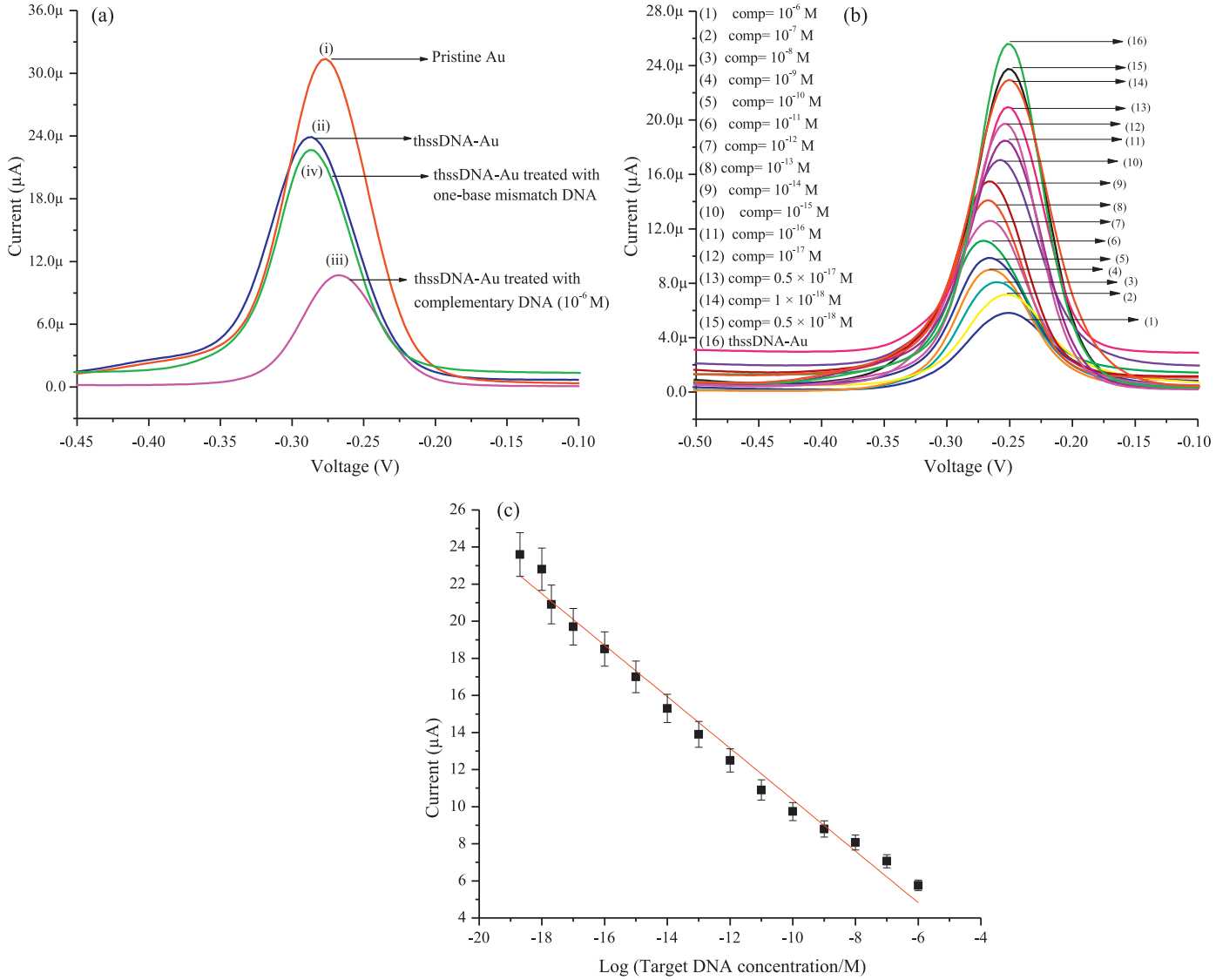


Fig. 4. (a) DPVs of (i) pristine gold in MB (ii) thssDNA–Au electrode, (iii) thssDNA–Au electrode after hybridization with one base mismatch target DNA (iv) thssDNA–Au electrode after hybridization with complementary target DNA; (b) DPV of thssDNA–Au electrode after hybridization with complementary target concentration 1×10^{-6} to 0.5×10^{-18} M, at pulse height of 50 mV and pulse width of 70 ms, after 20 μM MB, pretreatment at +0.1 V for 10 s in 0.05 M phosphate buffer of pH 7.0 containing 0.9% NaCl and (c) linear relationship between the peak current and the log [target DNA concentration].

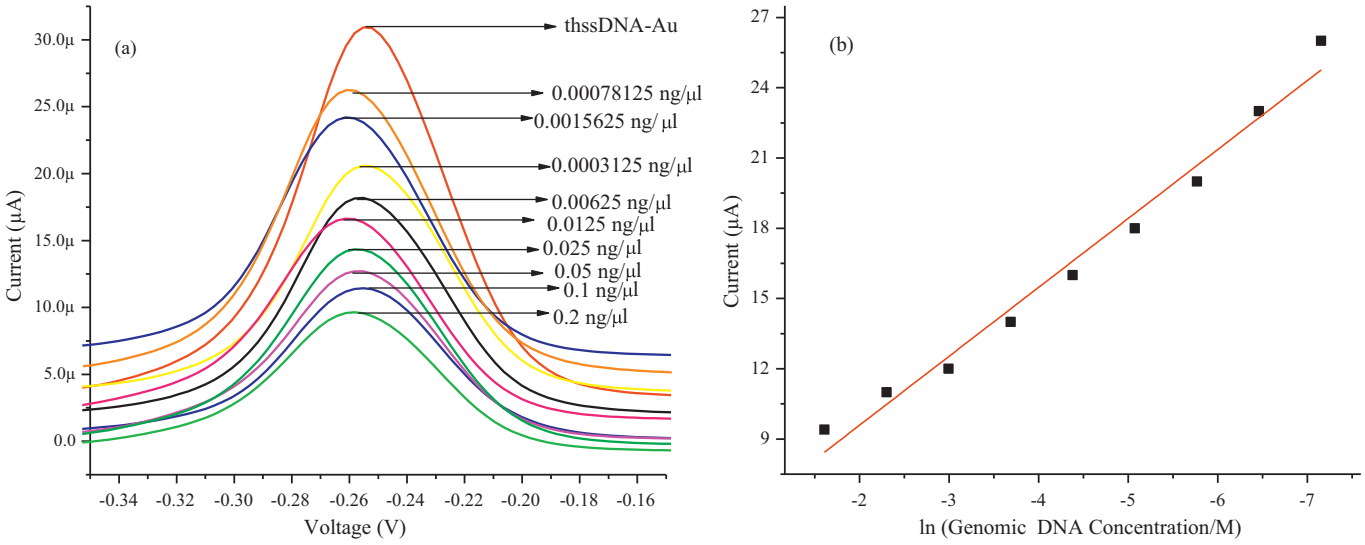


Fig. 5. (a) DPVs of thssDNA–Au bioelectrodes after hybridization with *Neisseria gonorrhoeae* genomic DNA (0.00078–0.2 ng/μL) in 20 μM MB 20 μM MB, 0.05 M phosphate buffer of pH 7.0 containing 0.9% NaCl. (b) Linear relationship between the peak current and ln [genomic DNA concentration].

(Γ) onto thssDNA–Au bioelectrode using Eq. (3):

$$I_p = n^2F^2\Gamma\nu A(4RT)^{-1} \quad (3)$$

where I_p/ν can be calculated from the slope of I_p vs. ν plot.

The total surface concentration of ionic species onto thssDNA–Au and dsDNA–Au bioelectrode is found to be 3.87×10^{-6}, 13.5×10^{-5} mol cm^{-2}, respectively indicating better immobilization and hybridization of DNA onto Au electrode revealing thssDNA–DNA is a promising matrix for fabrication of DNA biosensor.

3.4. Monitoring methylene blue oxidation

Fig. 4 shows results of electrochemical hybridization response studies of MCH/thssDNA/Au with complementary target and one-base mismatch synthetic oligomers in PBS, (0.05 M, and pH 7.0, 0.9% NaCl) in presence of 20 μM methylene blue, a hybridization redox indicator. Generally three kinds of binding modes including electrostatic, groove and intercalative binding take place between MB and DNA. The binding properties depend on the both the MB and the experimental conditions, such as the ionic strength, the concentration of MB and DNA, the buffer pH etc. The DPV of MB on a pristine gold electrode is shown in Fig. 4(a) (i). Fig. 4(a)(ii–iv) shows response of DNA/Au electrode investigated with complementary DNA and one-base mismatch. The observed decrease (Curve (ii)) in peak current with respect to pristine gold indicates that thssDNA is successfully immobilized on Au electrode. After incubation and treatment with perfect complementary DNA, DPV of MB on dsDNA–Au electrode, the peak current is observed to decrease indicating hybridization arising due to the steric inhibition of MB intercalation between double helix of the DNA. The relatively higher MB signal with virgin thssDNA probe onto Au electrode may be due to the strong affinity of MB for the exposed guanine bases resulting in the MB accumulation occurs at this surface. The

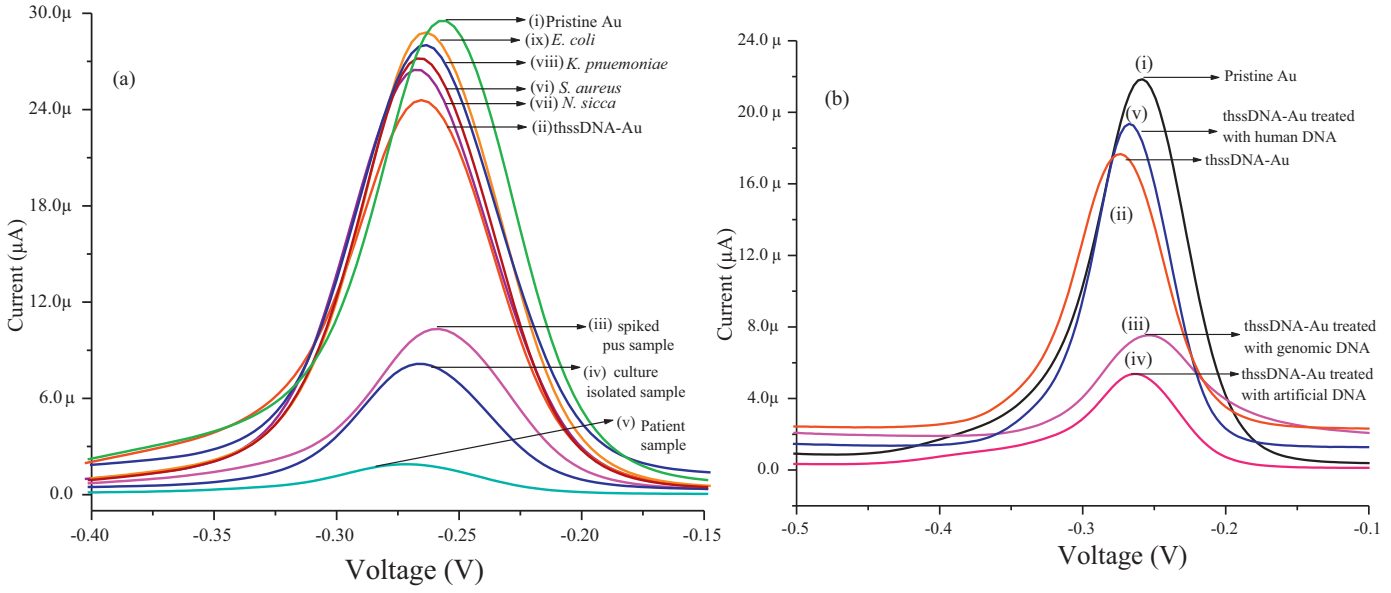


Fig. 6. DPVs of thssDNA–Au electrode to (a) detect presence of complementary target sequence in *Neisseria gonorrhoeae* culture and non-complementary target sequence in other *Neisseria* species and other GNBs and (b) DPV of thssDNA–Au electrode using Human DNA; at pulse height of 50 mV, pulse width of 70 ms in 20 μM MB, 0.05 M phosphate buffer of pH 7.0 containing 0.9% NaCl.

decreased peak current observed after duplex formation indicates interaction between the MB and guanine residues of the probe was prevented by hybrid formation on the electrode surface. However, the decreased peak current after hybridization indicates that the change in current due to intercalation is small compared to that from direct interaction with the guanine bases (Singh et al., 2009; Zari et al. 2009; Erdem et al., 2001; Ansari et al., 2009; Prabhakar et al., 2008; Tani et al., 2001; Henry et al., 2009). However, after treatment with the single base mismatch, negligible change in the peak potential is observed indicating specific interaction of the probe DNA. Further, difference of the electrochemical response of MB on thssDNA–Au and dsDNA–Au electrode indicates that the distinguishing capability of MB indicator to ssDNA or dsDNA is quite high on the surface of Au electrode (Arora et al., 2007). Table 2 shows the percentage (%) reduction in the current (I_{pa}) for thssDNA–Au and complementary and on-base mismatch treated electrodes. A significant percentage (%) decrease in current (I_{pa}) to 67% is observed after thssDNA–Au electrode hybridized with complementary DNA indicates the successful hybridization.

Fig. 4(b) shows results of DPV studies carried out as a function of target analyte concentrations ranging from 1×10^{-6} M to 0.5×10^{-18} M. The MB peak height increases with decrease in the complementary target concentration from 1×10^{-6} M to 0.5×10^{-18} M, revealing 1×10^{-18} M as the detection limit and 0.5×10^{-18} M as the saturating concentration of the electrode. The peak current of the STD sensor exhibits (Fig. 4(c)) a linear correlation to the log [target concentration] from 1×10^{-6} M to 0.5×10^{-18} M and obeys Eq. (4).

$$\text{MB oxidation current } I_{\mu A} = -0.6 \times \log(\text{target DNA concentration}) - 3.5 \quad (4)$$

with regression coefficient 0.983. The experiments have been repeated at least 10 times.

In order to avoid the non-specific alignment and further improve the sensitivity, an appropriate blocking reagent; MCH has been used to block the uncovered surface region to facilitate favorable conformation and vertical alignment of the covalently linked probe oligonucleotide sequence prior to the hybridization. It displaces the noncovalent and nonspecific adsorption of thiolated DNA, improving hybridization to complementary strands resulting in the improved sensitivity. Compared with other DNA detection methods, the electrochemical method shows high sensitivity, wide linear range and it can be used to discriminate a single-base difference in the DNA target with high specificity. Table 1 shows characteristics of the thssDNA–Au bioelectrode including those reported in the literature.

3.5. Response studies of thssDNA–Au bioelectrode using N. gonorrhoeae genomic DNA

Fig. 5(a) shows DPVs of thssDNA–Au bioelectrode on hybridization with heat-denatured sonicated *N. gonorrhoeae* genomic DNA (15 min, 24 kHz frequency). With decreasing *N. gonorrhoeae* genomic DNA concentration (0.00078–0.2 ng/μL), MB oxidation current ($I_{\mu A}$) increases, indicating decreased number of DNA duplexes formed at the thssDNA–Au bioelectrode surface. This can be attributed to the denaturation of genomic DNA arising due to breakage of hydrogen bonds between paired nitrogenous bases and the breakage of long DNA strands to smaller fragments brought about by sonication. Fig. 5(b) shows that the peak current of the STD sensor exhibits a linear correlation to the ln [genomic DNA concentration] from 0.00078 ng/μL to 0.2 ng/μL and obeys Eq. (5).

$$\text{MB oxidation current } I_{\mu A} = -2.94 \times \ln(\text{genomic DNA concentration}) + 3.7 \quad (5)$$

Table 1
Response characteristics of thssDNA–Au bioelectrode along with those reported in literature.

S. no.	Electrode	Method of immobilization	Electrochemical technique	Detection limit	Detection range	Hybridization time	Reference
1	DNA immobilization onto modified Au QCM and hybridization	Covalent immobilization	Frequency change	10^{-15} M	–	–	Liu et al., 2003
2	STD sensor (BdNG-avidin-nsPANI/ITO electrode)	Biotin-avidin coupling	Cyclic voltammetry, differential pulse voltammetry using methylene blue as indicator	0.5×10^{-15} M	1×10^{-6}–1×10^{-16} M	60 s	Singh et al., 2009
3	HS-ssDNA onto the screen-printed gold electrode (SPGE)	Covalent immobilization	Differential pulse voltammetry using methylene blue as indicator	2 pg ml^{-1}	0–5 ng ml^{-1}	5 min	Zari et al., 2009
4	ssDNA-modified Carbon Paste Electrode	Physical attachment	Voltammetric and spectrophotometric methods, DPV using MB and $[Ru(bpy)_3]^{2+}$	–	–	5 min	Erdem et al., 2001
5	STD sensor (th-ssDNA-ZnO/ITO bioelectrode)	Electrostatic interaction	Cyclic voltammetry, Differential pulse voltammetry using Methylene Blue as indicator	0.000704 fmol	0.000524 fmol–0.524 nmol	60 s	Ansari et al., 2009
6	Polypyrrole-polyvinyl sulphonate film electro-chemically deposited onto indium-tin-oxide (ITO) glass for *M. tuberculosis* in serum	Covalent immobilization	Square wave voltammetry using guanine oxidation, Methylene blue, ruthenium complex	0.1 fmol, 0.1 attomol, and 1.0 pmol,	0.1–80.0 fmol, 0.1–12.0 attomole	60 s	Prabhakar et al., 2008
7	STD sensor (aDNA-Glu-PANI/CNT/ITO bioelectrode)	Cross-linking (Glutaraldehyde)	Cyclic voltammetry, differential pulse voltammetry using methylene blue as indicator	1.2×10^{-17} M	1×10^{-6}–1×10^{-17} M	60 s	Singh et al., 2010
8	PANI coated Pt disc electrode for *Escherichia coli*	Biotin–avidin coupling	Differential pulse voltammetry using methylene blue as indicator	0.001 fmol	0.00025–0.125 fmol	60 s	Arora et al., 2007
9	STD sensor (thssDNA/Au bioelectrode)	Au–SH specific bonding	Cyclic voltammetry, differential pulse voltammetry using methylene blue as indicator	1.0×10^{-18} M	1×10^{-6}–0.5×10^{-18} M	60 s	Present work

Table 2
Percentage (%) decrease in current of thssDNA–Au electrode, complementary and one-base mismatch treated thssDNA–Au electrode.

Electrode	Current ($I_{pa)}$ of MB (μA)	% decrease in I_{pa}
Pristine Au	31	0
thssDNA–Au	23	25
thssDNA–Au treated with one-base mismatch DNA	22	29
thssDNA–Au treated withcomplementary DNA	10	67

with regression co-efficient 0.979. Hence, 0.2 ng/μL may be taken as the saturating concentration of the thssDNA–Au bioelectrode surface. No DNA duplex is formed for an *N. gonorrhoeae* genomic DNA concentration of 0.00078 ng/μL indicating it as the detection limit. It can be concluded that thssDNA–Au bioelectrode can be used to detect the presence of complementary sequences in *N. gonorrhoeae* genomic DNA. Reusability of the thssDNA–Au bioelectrode has been estimated by dipping a used electrode in 0.1 M NaOH. It is found that 5 min is sufficient to regenerate single-stranded probe molecules immobilized at the thssDNA–Au surface. These genoelectrodes lose about 20% of MB oxidation current after the tenth use (data not shown). It has been found that 600 copies of DNA can be detected using the fabricated Gonorrhoea sensor. As *opa* is a multiple copy gene with 11–13 copies being present in a single genome resulting in increased sensitivity. The designed probe is capable of binding to different *opa* types which may be present in the genome.

3.6. Specificity of the DNA sensor

The performance of this electrode has been investigated by checking thssDNA–Au electrode hybridization with. DNA extracted from *N. gonorrhoeae* culture, pus sample spiked with *N. gonorrhoeae* and *N. gonorrhoeae* positive male patient. The specificity of the bioelectrode has been studied by incubating the probe electrode with DNA extracted from other non-*N. gonorrhoeae Neisseria* species (NgNs) as well as other gram negative bacteria. Fig. 6 shows DPV response of the STD sensor to DNA extracted from *N. gonorrhoeae* culture, pus sample spiked with *N. gonorrhoeae*, patient sample and culture of *E. coli*, *N. sicca*, *S. aureus*, *K. pneumoniae*. The observed significant decrease in the DPV signal shows that the bioelectrode (thssDNA–Au) is highly specific for the *N. gonorrhoeae*. However, no significant decrease in the signal is obtained in presence of *E. coli*, *N. sicca*, *S. aureus*, *K. pneumoniae* DNA indicating the specificity of probe DNA for *N. gonorrhoeae* detection. The studies have also been performed using DNA extracted from human blood which was used as internal control. The signal of human DNA treated thssDNA–Au electrode is similar to that of the single stranded thiolated probe DNA immobilized gold electrode indicating non-hybridization. The high sequence-specificity of this sensor is further confirmed by the non-hybridization of the human DNA.

4. Conclusions

The thssDNA–Au electrode has been fabricated to detect gonorrhoea by immobilizing the probe DNA specific to *N. gonorrhoeae* onto gold electrode. The sensing characteristics have been studied with the complementary and single base mismatch target DNA. The detection limit for this biosensing electrode with complementary DNA target is found to be 1.0×10^{-18} M within hybridization time of 60 s. This thssDNA–Au electrode can be used to detect the complementary sequence in genomic DNA following sonication. Besides this, the performance of the thssDNA–Au electrode has been checked using DNA extracted from the urethral swab of a confirmed positive male patient and the results indicate that the electrode is specific to *N. gonorrhoeae* and it can be used to distinguish the *N. gonorrhoeae* from other *Neisseria* species and other gram negative bacteria. Efforts should be made to further optimize sonications experiments on genomic DNA followed by evaluation of the bioelectrode on a large number of clinical samples. It can then also be used for detection of other sexually transmitted infections.

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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.jbiotec.2010.09.935.

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