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An electrochemical genosensor for *Salmonella typhi* on gold nanoparticles-mercaptosilane modified screen printed electrode

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ABSTRACT

In this work, we fabricated a system of integrated self-assembled layer of organosilane 3-mercaptopropyltrimethoxy silane (MPTS) on the screen printed electrode (SPE) and electrochemically deposited gold nanoparticle for *Salmonella typhi* detection employing Vi gene as a molecular marker. Thiolated DNA probe was immobilized on a gold nanoparticle (AuNP) modified SPE for DNA hybridization assay using methylene blue as redox (electroactive) hybridization indicator, and signal was monitored by differential pulse voltammetry (DPV) method. The modified SPE was characterized by cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and atomic force microscopy (AFM) method. The DNA biosensor showed excellent performances with high sensitivity and good selectivity. The current response was linear with the target sequence concentrations ranging from 1.0×10^{-11} to 0.5×10^{-8} M and the detection limit was found to be 50 (±2.1) pM. The DNA biosensor showed good discrimination ability to the one-base, two-base and three-base mismatched sequences. The fabricated genosensor could also be regenerated easily and reused for three to four times for further hybridization studies.

1. Introduction

Molecular-based (DNA) electrochemical biosensor has widely reported in recent years due to the advantages such as high sensitivity, specificity, simplicity, low cost, and easy to miniaturize (Drummond et al., 2003; Farjami et al., 2012; Ferapontova, 2011; Hao et al., 2011; Henry et al., 2010; Jin et al., 2007; Lai et al., 2006; Liu et al., 2008). In electrochemical DNA biosensor, the stability and amount of the immobilized single stranded DNA (ss-DNA) probe on the electrode surface as well as its accessibility toward the target DNA played an important role in the performance of the biosensor (Cederquist et al., 2008; Liu et al., 2005). With the advent of the nanoscience, various kinds of the nanoparticles are utilized for the DNA probe immobilization due to their unique character such as high surface area, biocompatibility and strong adsorption ability. Gold nanoparticles (AuNPs) have elegantly been used to immobilize the thiolated DNA probe on the electrode surface, due to their unique properties such as retaining biological activity, efficient conducting interfaces with electrochemical signal transduction and for amplifying the electrical response (Elsholz et al., 2009; Li et al., 2010; Paredes et al., 2010). The self-assembly of AuNPs and silica core shell nanoparticles are reported for diverse applications (Cheung et al., 2003; Ming et al., 2002). The organosilane binds to the nanoparticle surface and thus forms the interface between core and shell. Moreover, gold has strong binding with thiol group containing molecules such as mercaptosuccinic acid, mercaptopropionic acid and cysteamine, which may be considered due to the formation of strong thiol-gold bond (Bain and Whitesides, 1989; Giersig and Mulvaney, 1993; Gittins and Caruso, 2001; Ulman, 1996). The formation of self-assembly on the electrode surface sometimes blocks the electrical conductivity between nanoparticles and electrode surface which is not favorable for the fabrication of biosensor. In this scenario, the development of simple and conductive electrode surface (nanoparticle modified) is highly desirable for the fabrication of biosensor. The organosilane 3-mercaptopropyltrimethoxy silane (MPTS) has offered self-assembled monolayer on gold and proven to act efficiently between the electrode surface and sol–gel derived films (Cheung et al., 2003; Wang et al., 1998).

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Here, we utilized this integrated self-assembled polymeric network layer of MPTS on the screen printed electrode (SPE) and electrochemical deposition of gold nanoparticles for the immobilization of thiol-modified DNA probe for the detection of Vi gene of *Salmonella typhi*. The genus *Salmonella* consists of bacilli that infect the intestines of the large number of vertebrate species including human beings, leading to typhoid or enteric fever, gastroenteritis, septicemia. Most *Salmonellosis* infections originate by ingestion of contaminated food and products such as poultry, meat and fresh products have also been a vehicle for infection. These diseases can be potentially fatal if timely treatment is not provided (CDC, 2006; USDA, 2010). The Salmonella species are responsible for 93.8 million cases of gastroenteritis and 155,000 deaths each year, worldwide both developing and developed countries (Macjowicz et al., 2010). The Center for Disease Control and Prevention (CDC) has listed *Salmonella* as a category B bioterrorism agent. The most important member of the genus is *Salmonella typhi*, which is the causative organism for enteric fever or typhoid. *Salmonella* are Gram negative rods, and possesses following antigens on the basis they are classified and identified: (i) flagellar antigen H, (ii) somatic antigen O, and (iii) a surface antigen Vi. The flagella antigen (H) is the heat labile protein, somatic antigen (O) is a phospholipid–protein–polysaccharide complex which forms an integral part of the cell wall, and Vi antigen (virulence) it is a capsular polysaccharide it acts as a virulence factor by inhibiting phagocytosis (Dieckmann et al., 2008; Lin et al., 2007; Sharma and Qadri, 2004). The significance of Vi capsular polysaccharide detection is it differentiates *S. typhi* from *S. typhimurium*. More than 98% *S. typhi* isolates from blood of the typhoid patient shows expression of Vi antigen, it correlates the virulence factor of *S. typhi* (Tran et al., 2010; Sharma and Qadri, 2004). Therefore, rapid detection and identification of this pathogen is extremely important to maintain public health safety and security.

A number of methods have been developed for the detection of *Salmonella* including conventional culture method, immunoassay methods including enzyme-linked immunosorbent assay (ELISA), immunochromatographic (ICT) (Kumar et al., 2008; Preechakasedkit et al., 2012), amperometric immunosensors (Afonso et al., 2013; Rao et al., 2005), metalloimunoassays (Dungchai et al., 2008), capacitive immunosensor (Jyang et al., 2009), dot blot immunoassays (Chaicumpa et al., 1995) which mainly rely on specific microbiological and biochemical identifications. There is numerous ELISA plate based assay commercially available such as *Salmonella* ELISA (BIO ART SA), TRANSIA PLATE *Salmonella* Gold (BioControl), and RIDASCREEN® *Salmonella* ELISA (R-Biopharm AG), Vidas Salmonella (Biomerieux SA). Lateral flow immunoassays typically use sandwich ELISA are also commercially available such as DuPont lateral flow system, Singlepath Salmonella (Merck), Reveal Salmonella lateral flow (Neogen). All commercially available products based on ELISA assay provides the results in ~48 h which include the enrichment of sample (~18–24 h) and sequential incubation of bio reagents for ~15 h. Besides, this label free electrochemical impedance spectroscopy method, surface plasmon resonance method (Nandakumar et al., 2008; Zhang et al., 2012), nucleic acid sequences real time Polymerase Chain Reaction (PCR) has also been used for *Salmonella* detection (Malorny et al., 2004; Verdoy et al., 2012). Several PCR based method is commercially available such as BAX system (DuPont Qualicon, USA), Micro SEQ *Salmonella* (Applied Biosystems, CA, USA) where specific gene detected by system. In the case of PCR based detection required total analysis time is ~18 h including enrichment step.

There is a need for new technology that offers rapid, precise, sensitive, simple and preferably for onsite detection. The detection of specific base sequences is becoming important in several areas such as clinical diagnostics, food safety, and environmental research. The application of molecular based biosensor technologies showed excellent performance with high sensitivity and good selectivity. In this direction, electrochemical DNA hybridization offers remarkable attention due to its specificity, selectivity, and sensitivity which is suitable for miniaturization as it has compatibility with micro-fabrication technology. Nanomaterials have also accelerated the performance of electrochemical application by improving bio-compatibility, enhancing electron transfer rate due to this enhanced signal can be achieved. Various methods have been reported for the detection of *Salmonella* from food and environmental samples (Diaz-Serrano et al., 2011; Farabullini et al., 2007; Ikebukuro et al., 2002; Weber et al., 2011). Electrochemical DNA (E-DNA) sensor also reported for *Salmonella typhirium* detection using DNA probe which forms a stem-loop configuration that holds the redox tag in proximity to the electrode surface after hybridization the redox tag moves away from the electrode surface and decrease in current was observed as a signal (Lai et al., 2006; Lubin et al., 2009). In this work, we have taken advantage of Vi gene detection as in many cases high number of false-positive and false-negative Widal test results limit its clinical usefulness (Bhan et al., 2005). We utilized the self-assembled layer of MPTS sol–gel matrix porosic in nature on SPE and electrochemical deposition of AuNPs that offers a good approach for the immobilization of thiol-modified DNA probe for *S. typhi* detection. This study also offers a facile and efficient route for the gold nanoparticles modification on the electrode surface and further for application in the fabrication of electrochemical genosensor. We used DNA probe immobilized electrode for hybridization assay with target sequence and methylene blue as an electrochemical indicator. The signal generated is monitored by differential pulse voltammogram (DPV) method.

2. Materials and methods

2.1. Chemicals and reagents

(3-Mercaptopropyl) trimethoxysilane (MPTS), 6-mercapto-1-hexanol, 97% (MCH) methylene blue, Gold (III) chloride trihydrate ($HAuCl_4{\cdot}3H_2O$), potassium ferrocyanide ($K_4Fe(CN)_6{\cdot}3H_2O$), potassium ferricyanide $K_3Fe(CN)_6$ were obtained from Sigma–Aldrich, Milwaukee, USA and all other chemicals used in the present studies were of molecular biology grade. All the reagents were prepared in MilliQ water, and the solutions and glasswares were autoclaved prior to being used. The DNA probes for *S. typhi* detection were as follows:

- Immobilized probe HS–$(CH_2)_6$– 5′ GCA TAT CGG TAT TCT GGC GGC 3′
- Complementary target (Tr) 1: 5′ GCC GCC AGA ATA CCG ATA TGC 3′
- Mismatch Tr-1-M-(1 base): 5′ GCC GCC TGA ATA CCG ATA TGC 3′
- Mismatch Tr-1-M-(2 base): 5′ GCC GCC TTA ATA CCG ATA TGC 3′
- Mismatch Tr-1-M-(3 base): 5′ GCC GCC TTT ATA CCG ATA TGC 3′

2.2. Apparatus

The electrochemical measurements were carried out on a PGSTAT 302 from Autolab with GPES software (EcoChemie, The Netherlands). A conventional three-electrode system comprising screen printed electrode ($\Phi = 3$ mm) as working electrode after modification with MPTS and AuNP denoted as MPTS/Au NP/DNA, platinum wire as auxiliary electrode and Ag/AgCl electrode as a reference electrode. Electrochemical impedance spectroscopy (EIS), differential pulse voltammetry (DPV) and cyclic voltammetry (CV) were performed in 10 mM phosphate buffer (pH 7.4).

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2.3. Fabrication of screen-printed electrodes (SPE)

A semi-automatic screen-printing machine was used for the fabrication of SPEs as reported elsewhere (Sharma et al., 2010). For this purpose, initially a layer of silver ink (conductive ink) was screen-printed on the alumina substrate and subsequently applied the homemade carbon ink (polystyrene:graphite powder ratio is 40%:60%) and dried. Finally, a layer of dielectric ink was screen printed over the electrode to expose the area of working electrode and dried at 80° C for 2 h. The size of the electrodes was 37 mm × 5 mm.

2.4. Electrode modification with MPTS and AuNPs electrodeposition

The ~50.0 mM MPTS sol was prepared by mixing MPTS with double distilled water at a 0.5:100 ratio and 600 μL of 0.1 M HCl, the mixture was sonicated for 9.0 min followed by stirring for 45.0 min until a clear homogeneous solution obtained. Then from this homogeneous MPTS sol 4 μL was dropped on the SPE and kept for 4.0–6.0 h to dry at room temperature (25° C). This ~50 mM MPTS was optimum to expose the thiol groups (—SH) to bind the AuNPs. The high concentrations of MPTS on the electrode surface lead to the formation of multilayer of MPTS, which in turn to prevent the deposition of AuNPs. The MPTS concentration plays a crucial role for the exposure of thiol-groups. On the MPTS modified SPE, controlled electrodeposition of AuNP was performed in 1 mM $HAuCl_4$ containing 0.5 M H_2SO_4 with potentiostaic method at −0.2 V for 200 s. After the AuNPs electrodeposition on the MPTS modified SPE, gently washed with distilled water and dried at room temperature.

2.5. Immobilization of DNA probe on AuNPs modified SPE

After the electrochemical deposition of AuNPs, 2.0 μL of thiolated ss-DNA (10 μM) in 10 mM phosphate buffer (pH 7.4) was dropped on modified SPE. This electrode was incubated at 30 °C for 1 h. Then the DNA modified electrode was thoroughly rinsed with 10 mM phosphate buffer (pH 7.4) followed by distilled water to remove the weakly adsorbed DNA probe and dried. Thiolated ss-DNA probe modified electrode was further immersed in 1.0 mM 6-mercapto-hexanol (MCH) for 1 h to block the uncovered electrode surface. Finally, the modified electrode was rinsed thoroughly with 10 mM phosphate buffer (pH 7.4) and double distilled water, respectively. To study the behavior of MB with ss-DNA, electrode was immersed in 20 μM methylene blue (MB) for 5 min and is characterized by DPV in 10 mM phosphate buffer (pH 7.4). The probe ss-DNA modified electrode is denoted as ss-DNA/AuNPs-MPTS/SPE in the text.

2.6. DNA hybridization and detection using methylene blue

The DNA hybridization was performed by pipetting 2 μL of different concentrations of target DNA (Tr-1) and mismatch (Tr-1-M) onto ss-DNA/AuNPs-MPTS/SPE for 1 h at 37 °C (Scheme 1). After hybridization, the electrodes were washed with phosphate buffer carefully to remove the unbound DNA. In order to detect DNA hybridization, the electrode was incubated in 20 μM methylene blue (MB) for 5 min. After accumulation of MB, the electrode was washed with 10 mM phosphate buffer thoroughly. Differential pulse voltammetry (DPV) was performed in 10 mM phosphate buffer (pH 7.4).

2.7. Regeneration of the modified DNA hybridized electrode

The SPE obtained after DNA hybridization were regenerated by rinsing the electrode surfaces with hot distilled water (90° C) for 5 min followed by rapid cooling in an ice bath. The reusability of the SPE was monitored by repetitive hybridization with target ss-DNA.

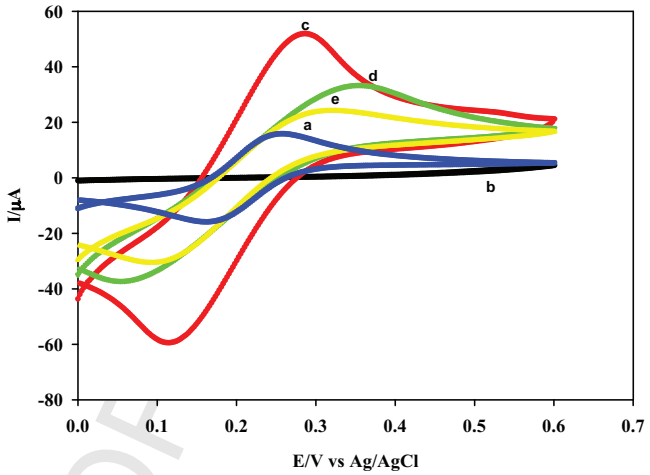

Fig. 1. Cyclic voltammograms in 5 mM $Fe(CN)_6^{3-/4-}$ containing 0.1 M KCl at 10 mV/s of different modified SPE: (a) un-modified SPE, (b) MPTS modified SPE, (c) AuNPs-MPTS/SPE, (d) ss-DNA/AuNPs-MPTS/SPE, and (e) ds-DNA/AuNPs-MPTS/SPE.

3. Results and discussion

3.1. Electrochemical characterization of the modified SPE after DNA hybridization

The electrochemical behavior of MPTS/AuNPs modified SPE was characterized by cyclic voltammogram (CV) and electrochemical impedance spectroscopy (EIS). In the present DNA biosensor, the effect of each modification on SPE such as MPTS self assembly, AuNPs electrodeposition, ss-DNA immobilization and hybridization (ds-DNA) were characterized by CV in 5 mM $Fe(CN)_6^{3-/4-}$ in phosphate buffer (Fig. 1). Before modification, CV of the SPE was recorded and found well-defined redox peak current of $Fe(CN)_6^{3-/4-}$ (Fig. 1, curve a). After, the modification of SPE with MPTS sol no peak was observed. It reveals that the MPTS polymeric network hindered the electron transfer at the surface of the SPE for diffusion of ferricyanide toward the electrode surface (Fig. 1, curve b). Here, the concentration of MPTS plays an important role for the exposure of thiol-groups on the electrode surface for further anchoring of the gold nanoparticles (Cheung et al., 2003; Ming et al., 2002). After the electrochemical deposition of AuNPs on MPTS modified (AuNPs-MPTS/SPE) electrode, the peak current increased more and peak potential (ΔEp) became ~150 mV (Fig. 1, curve c).

The result indicates that AuNPs has deposited on the MPTS modified electrode that anchored with exposed thiol-groups leads to enhancing the electroactive surface area, and is responsible for an increase in the peak current. The AuNPs provide an excellent electron conducting pathways to the surface of MPTS and coupling between the MPTS surface and underlying SPE. As a result high exchange electron densities occurred and decrease in charge transfer resistance and increase in peak current is observed (Barfidokht et al., 2013; Shein et al., 2009). The average value of the electroactive surface area was calculated according to the Randles–Sevick equation (Bard and Faulkner, 2000):

$$Ip = 2.69 \times 105\, AD^{1/2} n^{3/2} v^{1/2} C \quad (1)$$

where n is the number of electrons participating in the redox reaction, A is the area of the electrode (cm^2), D is the diffusion coefficient of the molecule in solution (6.7×10^{-6} cm^2 s^{-1}), C is the concentration of the probe molecule in the bulk solution (mol cm^{-3}), and v is the scan rate (V s^{-1}). The electroactive surface area for unmodified

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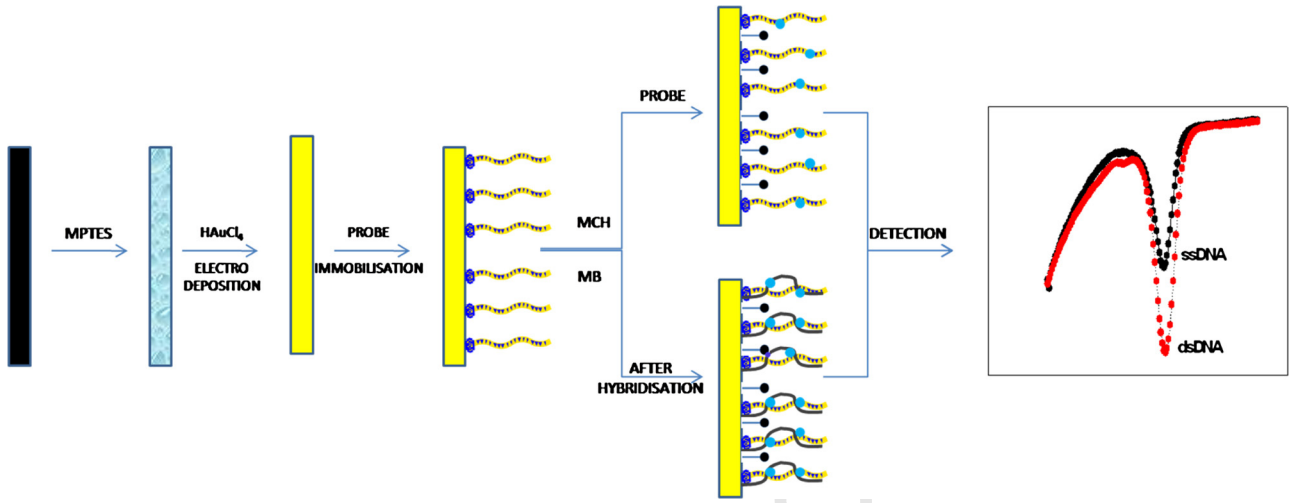

Scheme 1. The schematic representation of the MPTS-AuNPs modified SPE platform for the genosensor.

SPE and Au NPs modified electrode were $(2.1 \pm 0.3) \times 10^{-4}$ cm^2 and $(7.7 \pm 0.2) \times 10^{-3}$ cm^2, respectively.

After, immobilization of the thiolated ss-DNA probe ss-DNA/AuNPs-MPTS/SPE, peak current decreases and ΔEp value increases (Fig. 1, curve d) due to the electrostatic repulsion between ferricyanide and ss-DNA probe on the electrode surface. It indicates that ss-DNA probe was immobilized on the electrode surface. After hybridization with the complementary target the peak current further decreases and ΔEp value increases due to the similar reasons as that of in the case of ss-DNA probe immobilization on the ds-DNA/AuNPs-MPTS/SPE (Fig. 1, curve e).

EIS was employed to study the surfaces of the modified SPE in 1.0 mM $Fe(CN)_6^{3-/4-}$ containing 0.1 M KCl solution and Nyquist plots were plotted with a frequency range of 0.01 Hz to 10 kHz. The amplitude of the applied potential was set at 0.2 V. In the Nyquist plot of impedance spectra, the semicircle portion at higher frequencies corresponds to the electron-transfer-limited process and the linear portion seen at lower frequencies may be ascribed to the diffusion. An increase in the diameter of the semicircle reflects the increase in the electron transfer resistance (R_{ct}). As shown in Fig. S1, the R_{ct} value of the AuNPs modified SPE is ~200 Ω (Fig. S1, curve a). It is almost a straight line, which is a characteristic of a mass diffusional limiting electron-transfer process. After the immobilization of thiolated DNA probe, the value of R_{ct} increased, and the EIS showed a large interfacial resistance compared to AuNPs modified SPE (Fig. S1, curve b). The increase in R_{ct} value is due to the immobilization of negatively charged probes on the electrode surface that electrostatically repels the negatively charged redox probe $Fe(CN)_6^{3-/4-}$ and, as a result, inhibits interfacial charge transfer. The hybridization with target DNA induced a larger R_{ct} value (Fig. S1, curve c) which further corroborates that more negatively charged DNA hybridizes on the probe immobilized SPE lead to more repulsion of the negatively charged redox probe. These results were consistent with the fact that the DNA sensor was fabricated as expected and in corroboration with the CV results EIS also showed similar behavior.

Supplementary figure related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jbiotec.2014.08.002.

3.2. AFM image of electrode after modification of electrode

Fig. 2(a) showed AFM image of Au NPs electrodeposition on SPE, the topographical image clearly shows the presence of gold nanoparticles on the SPE and it covers the whole surface of the SPE and their *Z*-scale height is ~30 nm and size of Au NPs is ~20–40 nm. The deposition of AuNPs indicates the interaction between thiol groups of MPTS that are freely available after modification of the SPE. After that DNA probe immobilized on the SPE, AFM image clearly shows a change in morphology of the SPE and its *Z*-scale height is ~75 nm (Fig. 2(b)). It shows that on the surface DNA probe is immobilized due to which change in morphology and increase in height was observed as compared to Fig. 2(a). Further, on hybridization with target DNA change in morphology observed as well as its *Z*-scale height also increased to ~260 nm Fig. 2(c). It confirms qualitatively that the proper hybridization occurred on the electrode surface, and it could be visualized by increasing their *Z*-scale height.

3.3. Optimization of the experimental conditions

The hybridization temperature and time were optimized to obtain high sensitivity and reproducibility of the biosensor. The DNA probe modified electrode was hybridized with target nucleic acid at different temperatures such as 25, 37, 45 and 55° C and different times such as 20, 30, 45, and 60 min and DPV were monitored (Fig. S2). The increase in the current response at 25° C was less. While, at 37° C, an increase in the current response was more as compared to 25° C. The hybridization time was optimized 60 min, after that the current response started to get saturated. Further on increasing the hybridization temperature to 45 and 55° C the increase in the current response was observed, but it decreased after 20 min. The hybridization temperature is kept below the theoretical melting temperature (T_m) of the probes (Mao et al., 2008). T_m of the probe and target DNA is 52° C and 58° C, respectively. Above the T_m of the probes, it may get denatured/break the hybridized DNA. Thus, the optimal hybridization temperature was chosen as 37° C and hybridization time is 60 min for further experiments.

Supplementary figure related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jbiotec.2014.08.002.

3.4. Analytical performance of the DNA biosensor

The performance of the DNA biosensor was monitored by hybridization of ss-DNA/AuNPs-MPTS/SPE with different concentrations of target ss-DNA sequence. Fig. 3(A) showed the DPV reductive signals of MB at the ss-DNA/AuNPs-MPTS/SPE (DNA probe modified electrode) and after hybridization with different concentrations of target ss-DNA sequence. MB is an electrochemically active dye it has reversible redox characteristics. It gives well-defined voltammetric response, the reduction potential of

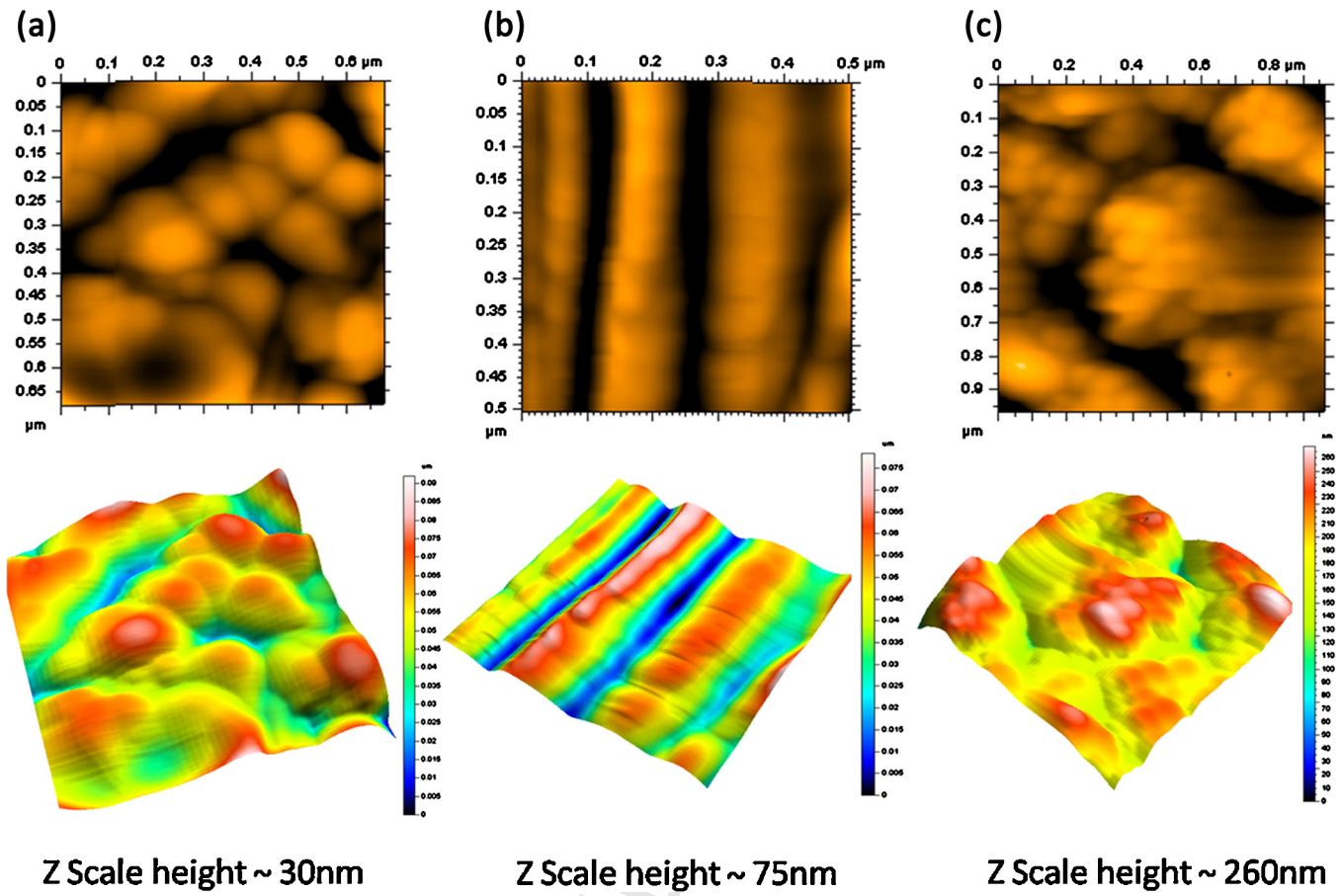

Fig. 2. Topography AFM image of AuNPs-MPTS/SPE (image a), ss-DNA/Au NPs/MPTS/SPE (image b), ds-DNA/AuNPs/MPTS/SPE (image c) and their respective 3D image with scale height.

Table 1
Q6 Comparison of analytical performances with other DNA biosensor for *salmonella* detection.

Reported biosensor	Detection technique	Detection range (M)	Analysis time[a]	Detection limit	Reference
Electrochemical DNA sensor	Osteryoung square wave voltammetry	$5.7–8.0 \times 10^{-7}$ M	–	550.0 nM	Diaz-Serrano et al. (2011)
Amperometric DNA sensor	Amperometric	$5.0 \times 10^{-8}–1.0 \times 10^{-5}$ M	–	–	Ikebukuro et al. (2002)
Electrochemical impedance DNA sensor	Impedance	–	–	1.0 nM	Weber et al. (2011)
Electrochemical genosensor	Differential pulse voltammetry		–	5.0 nM	Farabullini et al. (2007)
Surface plasmon resonance (SPR) DNA biosensor	SPR	$5.0 \times 10^{-9}–1000 \times 10^{-9}$ M	–	0.5 nM	Lubin et al. (2009)
Label free immunosensor	Impedance spectroscopy	–	6.0 min	500 CFU/mL	Nandakumar et al. (2008)
Magneto-immunoassay using gold nanoparticles	Differential pulse voltammetry	–	1.3 h	143 cells/mL	Alfonso et al. (2013)
Immunochromatographic strip test	Using gold nanoparticle color development	–	15.0 min	1.14×10^5 CFU/mL	Preechakasedkit et al. (2012)
Micro SEQ	Real time PCR	–	2.0 h	–	Applied Biosystem, CA, USA
BAX System	PCR assays	–	24 h	–	Du Pont, USA
Electrochemical genosensor on MPTS modified SPE	Differential pulse voltammetry	$1.0 \times 10^{-10}–0.5 \times 10^{-8}$ M	~2.0 min	0.05 nM	Present method

[a] In all methods for analysis time sample preparation and electrode preparation time has not included. In present method analysis time is less than 2.0 min after electrode incubated with target sample.

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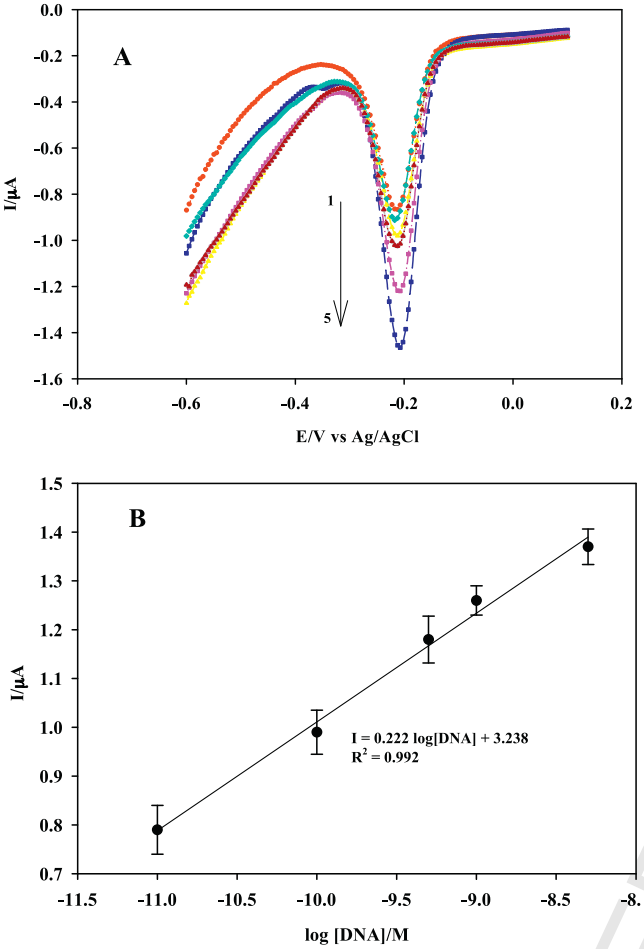

Fig. 3. (A) Differential pulse voltammograms (DPV) of methylene blue on ss-DNA probe-modified SPE and after hybridization with different concentrations of target sequences. Concentrations of target sequences from 1 to 5 are 1.0×10^{-11} (cyan), 1.0×10^{-10} (yellow), 0.5×10^{-9} (brown), 1.0×10^{-9} (pink), 0.5×10^{-8} (blue) and probe curve represents red color. (B) Calibration curve of the sensor response to target sequence, plot of difference of peak current from DPV before and after hybridization versus logarithm of target sequence concentration.

intercalated MB was observed at ~−0.2 V. It interacts in a different way with ss-DNA and ds-DNA such as intercalation between the base pairs, insertion into the minor or major groove (Henry et al., 2010; Kelley et al., 1999; Rohs et al., 2000). The redox active MB intercalated into the ds-DNA base stacks, the electrocatalytic signals obtained from MB carries electron transport from ds-DNA to the electrode surface. It indicates that more ds-DNA was adsorbed on the modified electrode surface more MB accumulates on the DNA, resulting in the increase in reduction of peak current. The calibration plot was plotted between difference of peak current before and after hybridization and with their logarithmic value of target sequence concentrations. It was found linear from 1.0×10^{-11} to 0.5×10^{-8} M ($n = 3$) (Fig. 3(B)) and linear regression equation was calculated as

$$I = 0.222 \log[DNA] + 3.238 \quad (2)$$

where log [DNA] is the target sequence concentration. The detection limit was 50 (±2.1) pM. With the addition of AuNPs, an increase in surface area that helped to increase the loading of DNA amount and improved the sensitivity of the genosensor. The sensitivity and analytical performances of the present study were compared in Table 1 with other reported methods and commercial method for

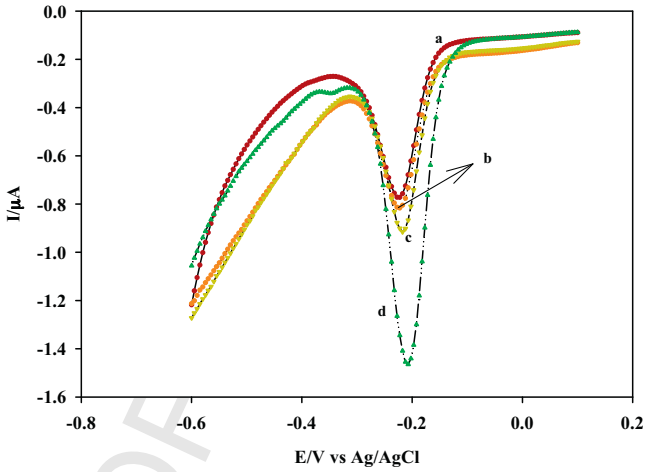

Fig. 4. DPV signal of methylene blue at the different SPE: (a) ss-DNA/AuNPs/MPTS/SPE, (b) ss-DNA/AuNPs/MPTS/SPE hybridized with two base mismatch sequences, (c) ss-DNA/Au NPs/MPTS/SPE hybridized with three base mismatch sequences, and (d) ss-DNA/Au NPs/MPTS/SPE hybridized with complementary sequences.

the detection of *Salmonella*. The present strategy demonstrates a feasible and rapid molecular detection approach compared to other methods.

3.5. Specificity of the DNA biosensor

The specificity of the biosensor was evaluated by analyzing entirely complementary target sequence, one-base, two-base and three-base mismatched oligonucleotide sequences under the same optimized hybridization condition and other electrochemical condition parameters. Fig. 4 presents the DPV signal at the different electrodes. A well-defined DPV signal was obtained in case of complementary sequence (Fig. 4, curve d). The DPV current signals for two-base and three-base mismatched sequences were significantly weaker than that of complementary sequences and even for three-base mismatched sequence showed less response (Fig. 4, curves b and c). It indicates that the hybridization was not achieved with mismatch sequences. In case of mismatch the DPV signal obtained from MB which accumulated on the ds-DNA base stacks that block the electron transfer due to the improper hybridization. The results demonstrated that this DNA biosensor showed high specificity for the hybridization detection.

3.6. DNA biosensor response in serum

In order to see the matrix effect of human blood serum, the performance of ss-DNA/AuNPs-MPTS/SPE has been studied in diluted 1:10 serum with phosphate buffer (10 mM, pH 7.4). Fig. S3 shows that DPV response monitored in only buffered solution, then with diluted serum sample. We found that the response is almost equal in both matrix with slight variation in peak potential (toward higher potential) is observed it may be due to the matrix effect (serum sample). The serum sample spiked with 10 nM complementary target and hybridized as previously mentioned. The small difference in DPV peak potential and peak current is obtained due to the same reason of matrix effect (serum proteins and other species). This study shows that DNA biosensor is selective enough to measure the complementary target sequences directly in blood serum samples.

Supplementary figure related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jbiotec.2014.08.002.

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3.7. Regeneration of the DNA biosensor

Reusability of the DNA biosensor is necessary for continuous monitoring of the target sample. Although we have used SPE even then we have studied the regeneration of the DNA biosensor. DNA biosensor was regenerated by dipping the used SPE in hot water (90 °C) for 5 min, followed by rapid cooling in an ice bath for 5 min. It was observed that the DNA biosensor could be regenerated three to four times with about 15–20% loss of the original signal. The cyclic voltammogram showed that after regeneration of the SPE response similar to the case of ss-DNA (Fig. S4, curve a). Fig. S4, curve b shows voltammogram after hybridization of same regenerated electrode. The signal attenuation seems to be attributed due to the loss of AuNPs and thiolated probes on the SPE.

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4. Conclusion

We have demonstrated an efficient DNA electrochemical biosensor for rapid detection of Vi gene for *S. typhi*. We fabricated DNA biosensor by immobilizing thiol labeled DNA probe on MPTS and AuNPs modified SPE. The surface of the modified electrode is characterized by different methods including, AFM, CV, EIS. The DNA hybridization was monitored by DPV measurements using methylene blue as an electrochemical indicator. The linearity was found from 1.0×10^{-11} to 0.5×10^{-8} M and the detection limit was 50 (±2.1) pM. Hybridization temperature and time were optimized at 37° C for 60 min, respectively. Furthermore, the specificity of the DNA biosensor was also studied and it showed good specificity and it could easily discriminate between complementary targets and mismatch target sequences. The reusability of the DNA biosensor was also checked and was found that it could be easily reused for three to four times. It can be concluded at this juncture that the proposed DNA biosensor has a lower detection limit and wider linear range for the target ss-DNA sequence assay. In the future, we will be focused for Salmonella detection in real samples and utilized this platform for other pathogens.

Author contributions

Sanjay Upadhyay designed research and wrote the manuscript; Ritu Das performed the experiments; Mukesh Kumar Sharma analyzed the data; B.K. Bhattacharya and Iti Garg design the oligonucleotides; V. Venkatesh performed the AFM experiment and analyzed the image data; and Vepa K. Rao reviewed the work.

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