

# *Omp85* genosensor for detection of human brain bacterial meningitis

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**Abstract** The 5'-thiolated DNA probe based on specific virulence gene, *Omp85*, was immobilized onto a screen-printed gold electrode followed by hybridization with 6–100 ng/6  $\mu$ l ( $5.9 \times 10^5$ – $9.3 \times 10^6$  c.f.u.) of *Neisseria meningitidis* single stranded genomic DNA (ssG-DNA) for 10 min at 25 °C from the cerebrospinal fluid (CSF) of a meningitis patient. The *Omp85* genosensor can detect as little as 6 ng ssG-DNA in 6  $\mu$ l CSF of a human brain meningitis patient in 30 min including a response time of 1 min by cyclic voltammetry, differential pulse voltammetry (DPV) and electrochemical impedance. The sensitivity of the genosensor electrode was  $2.6(\mu\text{A}/\text{cm}^2)/\text{ng}$  using DPV with regression coefficient ( $R^2$ ) 0.954. The genosensor was characterized using Fourier transform infrared spectroscopy and atomic force microscopy. *Omp85* genosensor was stable for 12 months at 4 °C with 12 % loss in DPV current.

**Keywords** Bacterial meningitis · Genosensor · *Neisseria meningitidis* · *Omp85* genosensor

## Introduction

Meningitis is a severe inflammation in the meninges (outer membrane covering) of brain and spinal cord of human. *Neisseria meningitidis* is the main responsible pathogen causing human brain bacterial meningitis (Espinosa et al. 2001; Dash et al. 2012) that can lead to damage of the brain or even death of the patient. Meningitis is generally detected from patient CSF through microscopy (Dunbar et al. 1998), biochemical assays (Negi et al. 2010), latex agglutination test (Surinder et al. 2007), PCR (Kumar et al. 2011; Dash et al. 2012a), real-time PCR (RT PCR) (Staquet et al. 2007), microarray (Mahajan et al. 2009; Sethi et al. 2010; Patnaik et al. 2012) and biosensors (Patel et al. 2009, 2010). The present available methods are though cumbersome and have one or more limitations. Therefore, genosensors are gaining preference for diagnosis of the meningitis. Thiolated DNA has always been a better choice for genosensors because of their easy fabrication, economic value and ability to function at wide range of temperature, pH and solvents (Lin et al. 2011). Methylene Blue (MB) is widely used as redox indicator for electrochemical genosensors (Kumar et al. 2009, 2012).

*Omp85* is a virulence gene that codes for a conserved outer membrane protein of *N. meningitidis*. *Omp85* can

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be targeted for the development of a vaccine (Fitzpatrick and McInerney 2005). Omp85 also assists in effective insertion of lipids and integral proteins into the outer membrane of *N. meningitidis* (Voulhoux et al. 2003). A single stranded DNA probe was designed targeting *Omp85* gene of *N. meningitidis* for specific hybridization on a screen-printed gold electrode.

## Materials and methods

### Chemicals and patient sample

The 5'-thiolated ssDNA probe (5'-CGTTTCCCAAG ACAACCTGT-3'), based on our NCBI GenBank accession number HQ712171 (Dash et al. 2010), was synthesized by Innovision Medichem. SPGE (gold: counter and working electrode, silver: reference electrode) was procured from DropSens and modified at our Institute. All other chemicals used were of analytical grade. CSF from patients with suspected meningitis were obtained from National Centre for Disease Control (NCDC), Delhi.

### Isolation of DNA from patient CSF

Patient CSF (0.5 ml) containing *N. meningitidis* was centrifuged at  $16,000\times g$  for 1 min. The upper viscous CSF was discarded and the pellet was suspended in 0.5 ml of TE (10 mM Tris/HCl, 1 mM EDTA) buffer, pH 8. The bacterial suspension was heated (for cell lysis) at 95 °C for 5 min and centrifuged at  $16,000\times g$  for 1 min. The amount of the genomic DNA (G-DNA) was quantified from the supernatant. In a second set of experiment, the *N. meningitidis* suspension was diluted serially and each dilution was plated in triplicate on Mueller–Hinton agar and grown at 37 °C for 18 h in CO<sub>2</sub> rich environment. The experiment was repeated three times and the average c.f.u. of *N. meningitidis* corresponding to G-DNA was calculated for different dilutions. The supernatant containing G-DNA was denatured for 5 min at 95 °C to make single stranded DNA (ssDNA) of 6 ng/6 µl ( $5.9 \times 10^5$  c.f.u.), 12 ng/6 µl ( $1.2 \times 10^6$  c.f.u.), 25 ng/6 µl ( $2.4 \times 10^6$  c.f.u.), 50 ng/6 µl ( $4.6 \times 10^6$  c.f.u.) and 100 ng/6 µl ( $9.3 \times 10^6$  c.f.u.) for hybridization with the immobilized probe on modified SPGE. *N. meningitidis*, after infection in human CSF, doubles every 30 min.

### Fabrication of genosensor

The working gold surface (12.6 mm<sup>2</sup>) was activated by treating with H<sub>2</sub>SO<sub>4</sub>/H<sub>2</sub>O<sub>2</sub> (1:1, v/v) for 5 min and then washed with autoclaved Milli Q water followed by absolute ethanol subsequently with water and finally dried at room temperature 25 °C. The ssDNA-SH/Au was constructed by immobilizing 5 µl of 5'-thiolated *Omp85* DNA probe (1 µM) onto SPGE for 24 h at 25 °C and the unbound DNA probe was removed by washing with PBS (50 mM phosphate buffer, 0.9 % NaCl), pH 7 and dried at room temperature for 3–4 h. The ssG-DNA 6–100 ng/6 µl from a patient CSF was hybridized at 25 °C for 10 min (optimized) with ssDNA-SH/Au. After hybridization, the electrode was washed 4–5 times with TE buffer, pH 8 to remove unhybridized *N. meningitidis* ssG-DNA and again washed with PBS, pH 7 before electrochemical study. The immobilization of ssDNA probe and hybridization with ssG-DNA was detected by CV, DPV and EI using FRA2 µAUTOLAB type iii, Metrohm. The overall experiment was repeated three times and the average values were taken for plotting graph between concentration of ssG-DNA and the oxidation peak current (I<sub>p</sub>). The schematic fabrication of the genosensor is shown in Scheme 1.

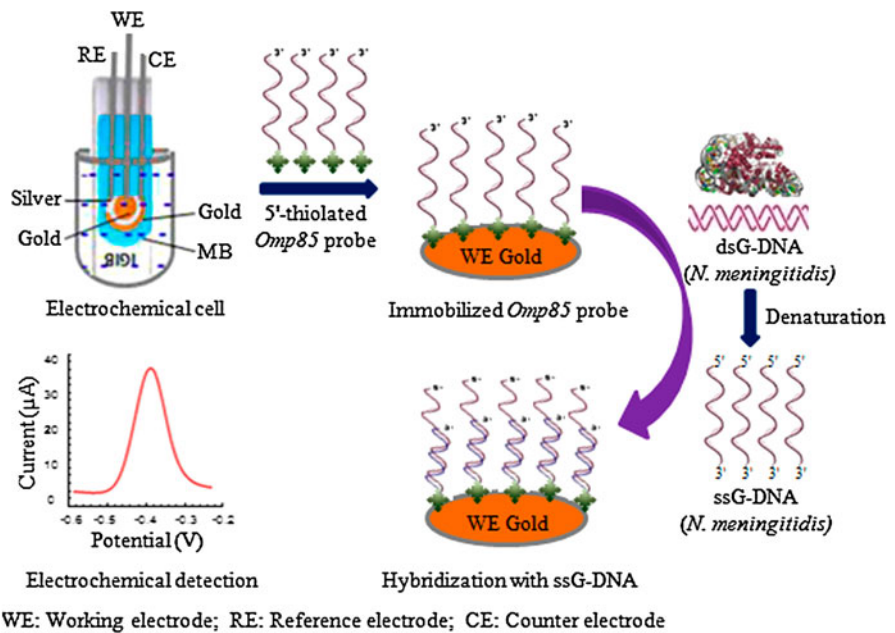
The surface topography of both ssDNA-SH/Au and dsDNA-SH/Au electrodes (after hybridization) were characterized using FTIR in a frequency range of 400–4,000/cm and AFM in non-contact mode in a surface area range of 0.25 µm<sup>2</sup> (5500SPM, Agilent Tech).

## Results and discussion

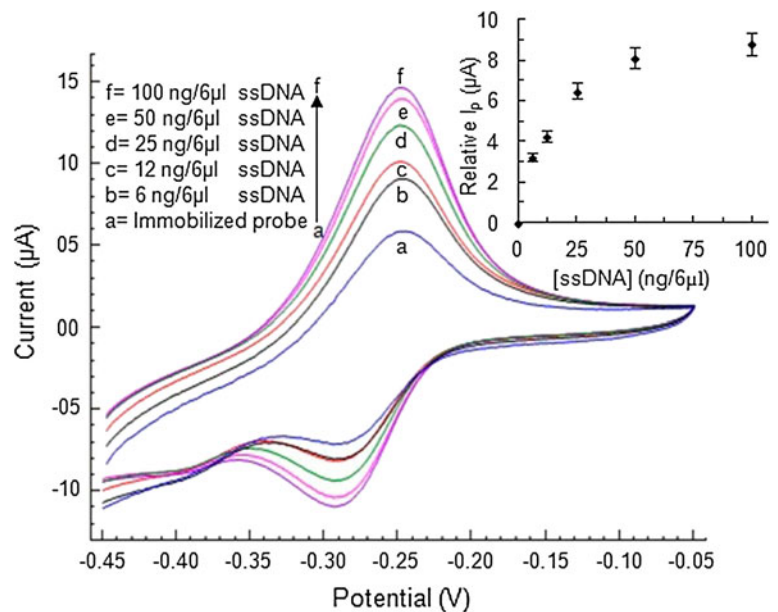
### CV studies

CV of ssDNA-SH/Au and dsDNA-SH/Au is shown in Fig. 1. The CV current of ssDNA-SH/Au (5.89 µA, curve a) was lower than that of bare gold (not shown in Fig. 1), due to higher rate of electron exchange between gold and Methylene Blue (MB). Whereas, strong association of MB with the unpaired nitrogenous bases (guanine) of ssDNA-SH/Au decreases the rate of electron exchange and hence decreases the current output. The oxidation current of dsDNA-SH/Au was 9.11 µA (curve b), 10.16 µA (curve c), 12.37 µA (curve d), 14.00 µA (curve e) and 14.69 µA (curve f) after hybridization with 6 ng/6 µl, 12 ng/6 µl, 25 ng/

**Scheme 1** Schematic representation of immobilization of 5'-thiol labeled *Omp85* ssDNA probe on SPGE and hybridization with ssG-DNA of *N. meningitidis* from CSF of the meningitis patient



**Fig. 1** Cyclic voltammetry of (a) ssDNA-SH/Au and (b–f) hybridization with 6, 12, 25, 50 and 100 ng/6 µl of *N. meningitidis* ssG-DNA at 50 mV/s using 1 mM MB in 50 mM PBS, pH 7. The inset shows linear increase of the relative peak current  $I_p$  up to 50 ng/6 µl of ssG-DNA of *N. meningitidis* used for hybridization following equation  $I (\mu A) = 0.109 (\mu A/ng) \times [ssDNA] (ng) + 2.983 (\mu A)$ . 1 c.f.u. of *N. meningitidis* corresponds to  $1.0 \times 10^{-5}$  ng of ssG-DNA



6 µl, 50 ng/6 µl and 100 ng/6 µl of ssG-DNA of *N. meningitidis*, respectively (Fig. 1). The oxidation peak current increased along with an increase in ssG-DNA was due to the presence of extra unhybridized guanine bases of G-DNA that interacted with MB molecules and resulted in increased current output. The hyperbolic curve between relative peak current (with respect to probe)  $I_p$  and concentration of ssG-DNA (Fig. 1 inset) shows that, the oxidation current

increased up to 50 ng/6 µl of ssG-DNA. On further increasing the concentration of hybridizing DNA, the peak current does not increased further because all the immobilized ssDNA probe of gold surface (12.6 mm<sup>2</sup>) get saturated at 50 ng/6 µl and does not available for further hybridization with ssG-DNA. The genosensor can detect as low as 6 ng/6 µl ssG-DNA in CSF and the sensitivity of the electrode was 0.909 (µA/cm<sup>2</sup>)/ng with  $R^2$  0.94.

## DPV studies

The DPV current of ssDNA-SH/Au (35.78  $\mu\text{A}$ , curve a) was lower than that of bare gold (not shown in Fig. 2). The current of dsDNA-SH/Au increased along with an increase in concentration of ssG-DNA of *N. meningitidis*: 42.28  $\mu\text{A}$  (6 ng/6  $\mu\text{l}$ , curve b), 47.32  $\mu\text{A}$  (12 ng/6  $\mu\text{l}$ , curve c), 50.24  $\mu\text{A}$  (25 ng/6  $\mu\text{l}$ , curve d), 57.19  $\mu\text{A}$  (50 ng/6  $\mu\text{l}$ , curve e) and 62.29  $\mu\text{A}$  (100 ng/6  $\mu\text{l}$ , curve f) (Fig. 2). The trend of variation showed by the current was similar to that of CV analysis. The saturation in relative peak current (with respect to probe)  $I_p$  was observed above 50 ng/6  $\mu\text{l}$  of ssG-DNA from patients CSF (Fig. 2 inset). The sensitivity of the genosensor electrode was 2.597( $\mu\text{A}/\text{cm}^2$ )/ng with  $R^2$  0.954.

## EI studies

The Nyquist plot of ssDNA-SH/Au and hybridization with different concentrations of ssG-DNA from the patient CSF is shown in Fig. 3. The semicircular region represents parallel combination of resistance and capacitance corresponds to the electron exchange inhibitory process, whereas, the linear region denotes for the diffusion of electrons. The applied potential of the circuit was + 0.180 V ( $\pm 0.01$ ) in a frequency range of  $10^{-2}$ – $10^5$  Hz.

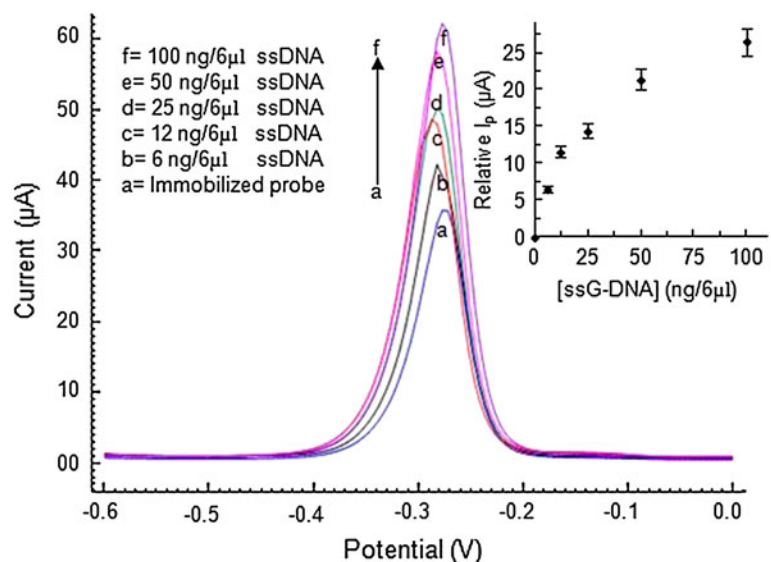
The electrochemical impedometric charge transfer resistance ( $R_{ct}$ ) of ssDNA-SH/Au obtained from the

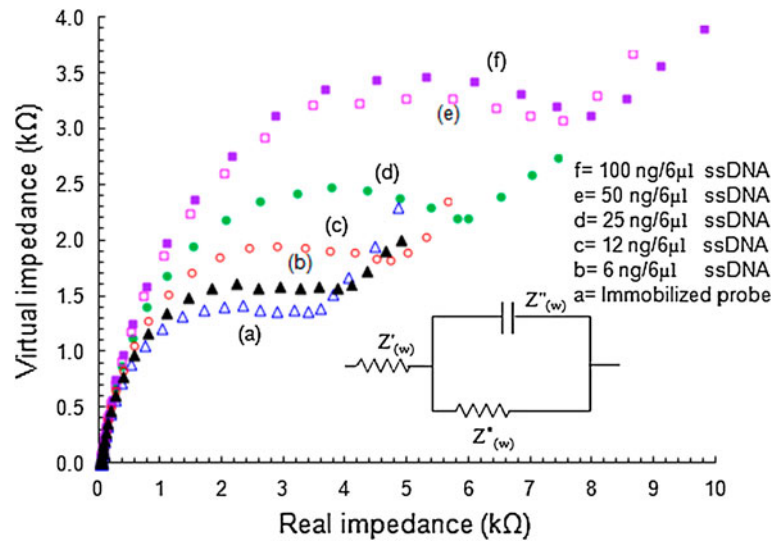
Nyquist plot was  $3.39 \times 10^3 \Omega$  (curve a). The  $R_{ct}$  value of dsDNA-SH/Au with 6 ng/6  $\mu\text{l}$  ( $5.9 \times 10^5$  c.f.u.), 12 ng/6  $\mu\text{l}$  ( $1.2 \times 10^6$  c.f.u.), 25 ng/6  $\mu\text{l}$  ( $2.4 \times 10^6$  c.f.u.), 50 ng/6  $\mu\text{l}$  ( $4.6 \times 10^6$  c.f.u.) and 100 ng/6  $\mu\text{l}$  ( $9.3 \times 10^6$  c.f.u.) of *N. meningitidis* ssG-DNA were  $3.85 \times 10^3 \Omega$  (curve b),  $4.71 \times 10^3 \Omega$  (curve c),  $5.98 \times 10^3 \Omega$  (curve d),  $7.50 \times 10^3 \Omega$  (curve e) and  $7.96 \times 10^3 \Omega$  (curve f), respectively (Fig. 3). Due to the faster electron exchange kinetic with  $[\text{Fe}(\text{CN})_6]^{3-/4-}$ , the  $R_{ct}$  value of bare gold (not shown in Fig. 3) was lower than that of ssDNA-SH/Au electrode whereas, the electronegative phosphate groups of DNA probe prevented the interaction of  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  ions with gold. The extra deoxyribose backbone of unhybridized DNA increased repulsion with  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  ions resulting in an increased  $R_{ct}$  value of the dsDNA-SH/Au electrode.

## Characterization of sensor (FTIR and AFM)

The hybridization of ssG-DNA with immobilized probe on gold electrode was confirmed by FTIR spectra. The immobilized probe showed peaks at 1,421.53, 1,532.63, 1,638.66 and 1,701.45/cm and after hybridization peaks at 1,435.98, 1,543.29, 1,644.44 and 1,724.13/cm correspond to cytosine (in-plane vibration of cytosine), adenine (C7=N vibration), thiamine (C2=O stretching) and guanine (C=O stretching), respectively suggest the presence of all four nucleotides (Marty et al. 2009). The peaks at

**Fig. 2** Differential pulse voltammetry of (a) ssDNA-SH/Au and (b–f) hybridization with 6, 12, 25, 50 and 100 ng/6  $\mu\text{l}$  of *N. meningitidis* ssG-DNA using 1 mM MB in 50 mM PBS, pH 7. The inset shows linear increase of the relative peak current  $I_p$  value above 50 ng/6  $\mu\text{l}$  of ssG-DNA of *N. meningitidis* used for hybridization following the equation  $I$  ( $\mu\text{A}$ ) = 0.312 ( $\mu\text{A}/\text{ng}$ )  $\times$  [ssDNA] (ng) + 6.233 ( $\mu\text{A}$ )





**Fig. 3** Nyquist plot for (a) ssDNA-SH/Au and (b–f) hybridization with 6,12, 25, 50 and 100 ng/6 μl of *N. meningitidis* ssG-DNA using 5 mM  $K_3Fe(CN)_6$  in 50 mM PBS, pH 7. Applied potential: +0.180 (±0.01) V. Frequency range:  $10^{-2}$ – $10^5$  Hz. The inset shows the circuit diagram for the equation  $Z^*_w(\Omega) = Z'_w(\Omega) + j \cdot Z''_w(\Omega)$ , the angular frequency  $w = 2$

$< \pi > f$ , where  $f$  is the frequency. The  $Z'$  (real part of  $Z^*$ ) mostly gives the resistive part of the circuit, whereas, the  $Z''$  (the imaginary part of  $Z^*$ ) provides information related with reactive (working capacitance) behavior of the circuit

1,022.42 and 1,287.94/cm of immobilized probe DNA and the peaks at 1,014.31 and 1,304.11 correspond to symmetric and asymmetric  $PO_2^-$  stretches of phosphodiester deoxyribose backbone, respectively (Malins et al. 2005). The decrease in transmittance, higher resolve of peaks at the region of 1,400–1,800/cm and broadening of the peaks at the region of 3,000–3,500/cm in case of dsDNA-SH/Au as compared to ssDNA-SH/Au, confirms hybridization of ssG-DNA with the immobilized probe.

The AFM image of the bare gold showed an average roughness (Ra) of 3.92 nm (image not shown) which was increased to 12.00 nm for ssDNA-SH/Au electrode. After hybridization of the immobilized probe with ssG-DNA of *N. meningitidis*, the surface roughness of dsDNA-SH/Au was further increased to 24.37 nm. The change in the morphology and increase in roughness confirms immobilization of probe and hybridization with ssG-DNA of *N. meningitidis* (Bowen et al. 2000).

**Table 1** Comparison of ssDNA-SH/Au electrode based genosensor with earlier reported meningitis sensors

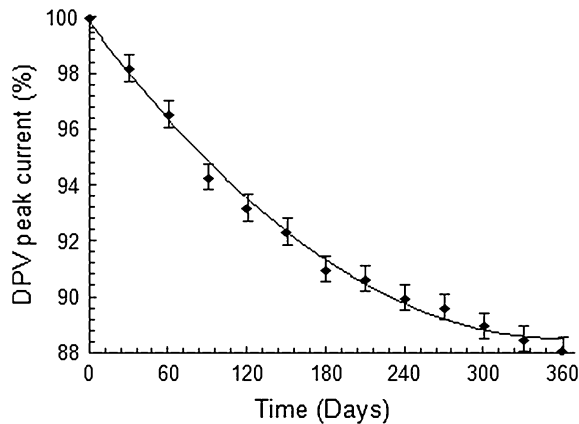
S. No.	Detection method <sup>a</sup>	Target DNA	Lowest detection limit (ng) ssDNA	Detection time	Sensitivity ( $\mu A/cm^2$ )/ng	References
1	CV	Synthesized 23 mer ssDNA	70	–	115.8	Patel et al. 2009 ( <i>CtrA</i> DNA sensor)
2	CV	PCR (532 bp) Isolated G-DNA	50	24 h 20 h	0.0056 0.0115	Patel et al. 2010 ( <i>CtrA</i> DNA sensor <sup>b</sup> )
3	CV	Meningitis patient DNA in CSF	06	30 min	0.9092	<i>Omp85</i> genosensor
4	DPV	Meningitis patient DNA in CSF	06	30 min	2.5967	<i>Omp85</i> genosensor

DPV differential pulse voltammetry

<sup>a</sup> CV cyclic voltammetry

<sup>b</sup> Lowest detection limit 100 ng reported for dsG-DNA





**Fig. 4** Differential pulse voltammetry (% peak current) of ssDNA-SH/Au electrode at different days with a regular interval of 30 days for 12 months on storage at 4 °C. Each value is the average of three readings at same conditions

#### Specificity and stability of the sensor

Table 1 presents the comparison between *Omp85* genosensor and *ctrA* (capsule transporter A) gene-based sensors reported earlier for detection of meningitis. The sensitivity of the sensor using PCR product (532 bp), which is lower when compared to genomic DNA, may be due to the presence of some nucleotides used in PCR reaction and left in the PCR product which then interfere in the hybridization causing a decrease in sensitivity. *Omp85* genosensor is better than earlier reported sensor due to higher sensitivity, low detection limit and low detection time. To check the cross-reactivity, the genosensor was hybridized with 25 ng/6 µl of ssG-DNA from *Escherichia coli*, *Streptococcus pyogenes*, *Salmonella typhi*, *Streptococcus pneumoniae* and CSF without *N. meningitidis* as a control. The electrochemical studies showed no change in  $I_p$  (DPV current) and found almost same as the probe, which confirms the specificity of the genosensor only to *N. meningitidis*. The *Omp85* genosensor can be modified for detection of multiple patient samples by connecting multiple modified SPGE to the instrument.

The stability of the ssDNA-SH/Au electrode was determined using DPV at regular intervals of 30 days (Fig. 4). The ssDNA-SH/Au electrode was stable at 4 °C for 12 months with approx. 12 % loss in ssDNA-SH/Au current. The ssDNA-SH/Au can be reused 20 times by washing with 1 mM HCl for

2 min followed by PBS, pH 7 to remove the hybridized ssG-DNA.

#### Conclusions

The *Omp85* genosensor can detect as low as 6 ng ssG-DNA/6 µl in CSF in 30 min including the response time of 1 min for the confirmation of the disease. The sensitivity of the genosensor electrode was 2.597 (µA/cm<sup>2</sup>)/ng. The fabricated electrochemical genosensor is highly specific due to specific virulent *Omp85* gene based DNA probe. The *Omp85* genosensor has an advantage of quick detection of life-threatening meningitis from the CSF during an outbreak of the disease to save life of several patients.

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