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An electrochemical DNA biosensor based on Ni doped ZnO thin film for meningitis detection

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ABSTRACT

Nickel doped zinc oxide (Ni-ZnO) thin film based matrix is used for fabricating highly stable and sensitive DNA biosensor for detecting a life-threatening disease, Meningitis. The present DNA biosensor has been fabricated after the immobilization of 23mer oligonucleotide sequence of DNA over the surface of Ni-ZnO/ITO electrode via. electrostatic interaction. Scanning electron microscopy (SEM) studies show the formation of nanostructured Ni doped ZnO thin film surface morphology which facilitates higher loading of single stranded thiolated DNA (ss th-DNA) molecules over the Ni doped ZnO matrix. The ss th-DNA/Ni-ZnO/ITO bioelectrode response studies were done using differential pulsed voltammetry (DPV) in the methylene blue (MB) mediated buffer. Linear response over wide DNA concentration (5 ng/μl–200 ng/μl) was obtained with a high sensitivity of 49.95 μA/decade. The present biosensor is found to exhibit very low detection limit (5 ng/μl) with a quick hybridization time of only 30 s. Electrochemical impedance spectroscopy (EIS) has also been used for studying the sensing response of ss th-DNA/Ni-ZnO/ITO bioelectrode.

1. Introduction

Neisseria meningitides is often referred to as meningococcus, which is having thirteen serogroups based capsular polysaccharides with Gram-negative diplococcus [1], is the causative agent of life threatening disease called Meningitis. Though meningitis can be caused by three types of infections such as bacterial, viral or fungal [2], but among all, bacterial meningitis is the most severe one as almost 90–95% deaths in meningitis cases are due to bacterial infection. Hence, correct detection is very essential for the meningitis infection. As its symptoms including neck stiffness, fever, vomiting, headache etc. resembles with other common diseases. This disease is generally confirmed by inspection of cerebrospinal fluids (CSF) by latex agglutination test, Gram staining, CT scanning and X-ray, PCR, biochemical test and immunological test [3–17]. However, they are non-confirmatory, expensive and time-consuming. So, worldwide research has been going on towards the development of cost-effective, non-invasive, sensitive and accurate detection for meningitis.

For this purpose, biosensors have been greatly emerged over the past few decades through the invention of modern technologies and the introduction of new methodologies. Within this broad biosensor field, comes nucleic acid (DNA) biosensors which are in great demand due to their accurate detection of DNA targets sequences related to pathogenic or genetic diseases. In molecular diagnostics, pharmaceutical, gene analysis, tissue matching and forensic applications there is a growing demand of DNA biosensors for detecting sequence specific DNA [18–24]. Biosensors based on variety of techniques have been developed for DNA detection which includes fluorescence [25], piezoelectric [26], surface plasmon resonance spectroscopy [27], quartz crystal microbalance [28], electrochemical-luminescence [29] and electrochemical methods [30]. Among them electrochemical method for DNA detection has become very important due to their simplicity, better reliability, high sensitivity and good selectivity [31,32].

Also, impedimetric technique for DNA detection is very popular because of its simple and easily comprehensible manner. Electrochemical impedance spectroscopy (EIS) is generally used for analyzing the capacitive and resistive properties of the electrode-electrolyte interface when subjected to a small amplitude sinusoidal excitation signal [33,34]. A wide range of alternating current (AC) frequencies are used for scanning the impedance of the system. The impedance spectra thus obtained is interpreted with the help of equivalent circuit models. It has also been used extensively as a tool for investigating electrode kinetics but however the biosensors based on this technique generally suffers from low sensitivity.

The essential factor regarding fabricating DNA biosensor is immobilization of good amount of probe DNA layer which can offer fast hybridization with the target oligonucleotides DNA sequences [35]. Therefore, by controlling the properties of the recognition layer, enhanced DNA biosensor characteristics can be achieved [36]. Recently, several novel

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and highly efficient materials for DNA biosensors fabrication have been reported in literature for biological, medical and electronic device applications, however there is still scope to attain all the improved biosensing characteristics [37].

For the fabrication of DNA biosensors material such as graphene [38], CNTs [39], metal nanoparticles [40,41] thin film of metal oxides such as zinc oxide, manganese oxide, nickel oxide, zirconium oxide, titanium oxide, iridium oxide, tungsten oxide, copper oxide, iron oxide etc. are the potential matrices for immobilization of several proteins and biomolecules because of high biocompatibility, high electrical conductivity, non-toxicity, large surface area, chemical stability, ease of preparation, electrochemical activity and excellent adhesion to the substrate [42]. Zinc oxide (ZnO) having high isoelectric point (IEP) (~9.5) is one of the most popular metal oxide being utilized as a matrix for biosensing work [43]. In literature, a lot of work has been reported on ZnO based DNA biosensors [44–46]. However, there is still scope for further developments. With the introduction of a suitable dopant into ZnO, modification and enhancement of the biosensing properties may be achieved. Ni is preferred as a dopant in ZnO due to similar ionic sizes of Ni^{2+} ions (0.72 Å) with Zn^{2+} (0.74 Å) ions which makes facile incorporation of Ni into ZnO without altering ZnO morphology and secondly Ni doped ZnO is expected to give improved electrochemical behaviour and hence an efficient matrix for DNA biosensor. So, there is an urgent need to harness such a promising matrix (Ni doped ZnO) for the biosensor fabrication. However, till date to the best of our knowledge no work has been reported in literature on Ni doped ZnO based meningitis DNA biosensor. The present work focuses on the realization of DNA biosensor using Ni doped ZnO matrix for meningitis detection.

2. Experimental

2.1. Chemicals and reagents

N. meningitides oligonucleotides, Triton X-100, nickel acetate tetrahydrate ($(CH_3COO)_2Ni\cdot 4H_2O$, 99.0%), zinc acetate dihydrate ($C_4H_{10}O_6Zn\cdot 2H_2O$, 99.0%) were purchased from Sigma-Aldrich (USA). Ammonia solution (min. 25% GR) was purchased from Merck India Ltd., India. Sodium phosphate dibasic dihydrate and monobasic anhydrous, ethylene diaminetetra acetic acid (EDTA) and Tris buffer were procured from Sisco chemicals, India. No further purification of the chemicals was done before use. 50 mM phosphate buffer saline (PBS) (pH 7.0, 0.5 M NaCl) was prepared by proportionate mixing of the monobasic and dibasic sodium phosphate solutions and then adding 0.5 M NaCl to it. De-ionized (DI) water (resistivity ~ 18.2 MΩ-cm) was used for all the aqueous solution preparation. All oligonucleotides solutions for varying concentrations were prepared in Tris-EDTA (TE) buffer (10 mM Tris, pH 8.0, 1 mM EDTA). The following are the DNA sequences used in the present work:

Probe DNA	5′-HS-GATACGAATGTGCAGCTGACACG-3′
Complementary target DNA	3′-CTATGCTTACACGTCGACTGTGC-5′
Non-complementary target DNA	3′-CTTAGCAAACTGGTGCACACACG-5′

2.2. Preparation of Ni doped ZnO (Ni-ZnO/ITO) electrode

Preparation of the zinc acetate solution (0.455 M) in DI water was done at room temperature. Dropwise addition of ammonia solution (25%) with constant stirring was done for the formation of milky white precipitates (pH = 8–9) and then allowed to stir for 4–5 h at room temperature. DI water was used for washing the precipitates so as to reach neutral pH = 7.0 and thereafter addition of 1 M dilute HNO_3 was done for obtaining transparent solution (pH = 1). To this transparent solution, nickel acetate (6 wt%) was added with constant stirring for 1 h at 80 °C. The concentration for nickel acetate (6 wt%) has already been optimized in our earlier work for obtaining the best redox behaviour [47]. Hence, in the present work, the optimized value of nickel acetate (6 wt%) is being used. Triton-X was also added to the resultant solution for achieving uniform coating. With the help of spin coating, the prepared solution was spin coated over ITO coated glass platform. Repetition of the spin coating followed by pyrolysing at 300 °C for 10 min was done for obtaining the desired thickness. At 300 °C, the prepared films were annealed for 3 h in air for achieving desired crystallinity [45].

2.3. Fabrication of ss th-DNA/Ni-ZnO/ITO bioelectrode

Ni-ZnO/ITO electrode was first washed with DI water before the immobilization of probe single stranded DNA (ss-DNA) and subsequently dried in air at room temperature. Thereafter, immobilization of 10 μl of DNA probe solution (ss-DNA, 5 ng/10 μl) was done over the fabricated Ni-ZnO/ITO electrode and subsequently kept for 3 h at 25 °C in humid chamber. The immobilization of DNA onto Ni-ZnO/ITO occurs via. electrostatic interaction between positively charged Ni-ZnO (IEP ~ 9.5) and negatively charged DNA (IEP ~ 4.2) at the physiological pH ~ 7.0. Since, the negative charge density for the thiol molecule is more in comparison to the negatively charged phosphate backbone of the DNA, hence, at pH ~ 7.0, the negatively charged thiol group is more attracted to the positively charged Ni-ZnO thin film rather than the phosphate group of DNA. The whole DNA sensing mechanism is shown in Scheme 1. In order to remove the unbound probe from the bioelectrode, several washings with TE buffer were done followed by drying in air. For DNA hybridization reaction to occur, incubation of the prepared ss-DNA/Ni-ZnO/ITO bioelectrode was done with desired concentrations of non-complementary and complementary target solutions for 30 s at room temperature. After hybridization, before using the bioelectrode, several washings of the hybridized electrode were done for removing unbound ss-DNA.

2.4. Experimental characterizations

The X-ray diffraction [Bruker D8 Discover] was done for studying the structural property for Ni doped ZnO thin film. The electrode's surface morphology was studied with the help of scanning electron microscopy (SEM) [Quanta FEI 200]. Potentiostat/galvanostat [Gamry Inc. 600] along with the three-electrode system was used for carrying out the electrochemical measurements in 0.05 M $[Fe(CN)_6]^{3-/4-}$ mediated phosphate buffer saline (PBS) solution (50 mM, pH 7.0, 0.5 M NaCl) having Ag/AgCl as the reference electrode. All DPV measurements were carried out in PBS (50 mM, pH 7.0, 0.5 M NaCl) mediated with 20 μM methylene blue (MB).

3. Results and discussion

3.1. XRD studies

X-ray diffraction (XRD) spectra for the Ni doped ZnO thin film deposited on ITO coated glass substrate is shown in Fig. 1. The Ni doped ZnO crystallites are found to possess hexagonal wurtzite structure (space group *P*63*mc*). As shown in Fig. 1, all the XRD peaks can be assigned as hexagonal phase of zinc oxide as reported in JCPDS card No. 36-1451 and good sharpness of the peaks reveal highly crystallized structure. The lattice parameters as calculated are found to be a = b = 3.222 Å and c = 5.231 Å which are slightly different from the reported unstressed single crystal parameters (a = b = 3.249 Å and c = 5.206 Å) which can be attributed to the stress developed in the film due to formation of defects [41]. With regard to Ni doping, no additional peaks corresponding to Ni or NiO were observed, indicating Ni doping has no change in ZnO structure. The presence of several other ZnO diffraction planes as shown in Fig. 1 reveals polycrystalline nature of the film with the c-axis i.e. (002) peak as the most prominent axis of orientation for the crystallites. The average crystallite size (D) has been calculated