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Nanostructured ZnO-based biosensor: DNA immobilization and hybridization

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ABSTRACT

An electrochemical DNA biosensor was successfully fabricated by using (3-aminopropyl) triethoxysilane (APTES) with zinc oxide (ZnO) nanorods synthesized using microwave-assisted chemical bath deposition method on thermally oxidized SiO_2 thin films. The structural quality and morphology of the ZnO nanorods were determined by employing scanning electron microscopy (SEM) and X-ray diffraction (XRD), which show a hexagonal wurtzite structure with a preferred orientation along the (101) direction. The surface of the SiO_2 thin films was chemically modified with ZnO. Label-free detection DNA immobilization and hybridization were performed using potassium hexacyanoferrate with cyclic voltammetry (CV) measurements. The capacitance, permittivity, and conductivity profiles of the fabricated sensor clearly indicate DNA immobilization and hybridization. Results show that the capacitance values of bare, ZnO- modified surface immobilization, and target DNA hybridization were 46×10^{-12} F, 47×10^{-8} F, 27 μF, and 17 μF, respectively, at 1 Hz. The permittivity measurement increased from 3.94×10^3 to 251×10^3 and 165×10^3 at the frequency range of approximately 200 to 1 Hz for bare and DNA immobilization and hybridization, respectively. The measured conductivity values for the bare, ZnO, immobilized, and hybridization device were 2.4×10^{-9}, 10×10^{-8}, 1.6×10^{-7}, and 1.3×10^{-7} S cm^{-1}, respectively.

1. Introduction

The development of a new generation of DNA sensors recently gained substantial recognition in the research on gene analysis, detection of genetic disorders, tissue matching, and forensic applications [1].

DNA sensors are label-free, highly sensitive, specific, simple, and low-cost; hence, DNA sensors can be widely used in the determination of genetic variations [2], forensic applications [3], and food analysis [4,5].

Various techniques were developed to detect DNA, such as electrochemical sensing [6], surface plasmon resonance (SPR) [7], fluorescence [8], and chromatography combined with mass spectrometry [9].

The semiconductor zinc oxide (ZnO), a representative of groups II-VI, gained particular interest because its novel properties and characteristics. A direct band gap (3.4 eV) of ZnO crystallizes within a hexagonal wurtzite-type structure with lattice parameters a = 0.325 nm and c = 0.521 nm. ZnO is intensively studied because of its unique properties and versatile applications in transparent electronics, ultraviolet light sensors [10–12], piezoelectric devices, chemical sensors and spintronics (ZnO doped with magnetic transition metals) [13,14]. Low-dimensional ZnO nanostructures generated significant interest because of their enhanced physiochemical properties [15–19] and the convenience fabricating these nanostructures into various morphologies, such as nanowires [15,20], nanoflakes [16], nanorods [17], nanobelts [18], nanorings [19], nanocables [21], nanotubes [22,23], nanocolumns [24], nanocombs [25], and nanoneedles [26]. Various methods were used for the deposition of ZnO thin films, such as chemical vapor deposition (CVD) [27], electrodeposition [28], sol-gel spin coating [16], physical vapor deposition (PVD) [29], spray pyrolysis [30], radio frequency sputtering [31], and ink-jet printing [32].

The current study focuses on biosensor technology based on multifunctional ZnO nanorods for biological, biochemical and chemical applications. The nanostructures were fabricated using chemical bath deposition (CBD). Before probe DNA immobilization and target DNA hybridization detection, the surface of SiO_2 thin films was modified with ZnO because of its chemical composition, superior conductivity, and substantial attachment surfaces [33]. A novel, label-free, and sensitive DNA sensor was developed using an interdigitated electrode (IDE) with ZnO-modified SiO_2 thin films. The current study explains the

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preparation of ZnO nanorods using microwave-assisted CBD to produce nano-biosensors generate new compounds with high sensitivity.

2. Materials and methods

2.1. Chemical materials

All chemicals were used without further purification and were purchased from commercial sources. The utilized chemicals are as follows: zinc chloride ($ZnCl_2$), polyvinyl alcohol [$CH_2CH(OH)$]n (PVA), Zn nitrate hexahydrate $Zn(NO_3)_2{\cdot}6H_2O$, and hexamethyl enetetramine ($C_6H_{12}N_4$).

2.2. Preparation of ZnO solution

The ZnO nanorods were prepared using CBD method. Before allowing the epitaxial growth of ZnO nanorods, the substrates were cleaned (RCA cleaning processing to remove organic residues from silicon wafer). The experimental setup and the growth mechanism used to deposit ZnO nanorods include 0.1 mol/L aqueous solution of ($ZnCl_2$) vigorously stirred at 70 °C for 10 min. 1.5 g aqueous solution of PVA was stirred at 80 °C for 30 min. These solutions were mixed together via high-speed stirring and placed on a hot plate at 70 °C for 2 h. The solution was transferred to a microwave oven for 15 min at 80 °C to facilitate the complexation of Zn ions with PVA. Ammonia solution was added to the mixture until a pH of 8 was reached, and the nanocomposite PVA–$Zn(OH)_2$ solution was synthesized. The as-obtained nanocomposite PVA–$Zn(OH)_2$ solution was spin-coated over a SiO_2 substrate, thereby serving as seed layer, and annealed at 210 °C for 1 h, resulting in the decomposition of Zn $(OH)_2$ to ZnO. The temperature was increased to 380 °C for 2 h. After the annealing process, the substrate was inserted vertically in a beaker containing 0.1 mol/L of Zn $(NO_3)_2{\cdot}6H_2O$ and an equal molar concentration of ($C_6H_{12}N_4$) dissolved in deionized water (DI) water. The beaker was placed on a hot plate for 2 h at 85 °C. The grown nanorods all over the substrate were washed with hot ethanol to remove the remaining salt.

2.3. Modification of SiO_2 thin films with ZnO

A p-type silicon (100) wafer was ultrasonically cleaned with acetone and isopropanol and immersed in a buffered oxide etch (BOE) solution to remove the native oxide. The p-type silicon was rinsed with DI water. After oxidation, the silicon oxide (SiO_2) layer with a thickness of roughly 50 nm and high purity aluminum (99.99%) were deposited on the backside of Si using a thermal evaporator.

The selectivity of the DNA biosensor was studied using a ZnO/APTES/SiO_2/Si/Al electrode. The SiO_2 surface was functionalized with APTES solution, which was prepared by mixing 2% APTES with 93% of ethanol and 5% of DI water. To modify the surface of SiO_2 with APTES, 10 µL of prepared APTES solution was deposited on the SiO_2 surface and incubated for 2 h. The surface was washed three times and dried, 10 µL of ZnO was dropped on the surface at 150 °C for 20 min using a hot plate. This procedure was repeated three times to obtain a ZnO layer on the SiO_2 surface. After the procedure, the electrode is ready for further characterization.

2.4. Probe DNA immobilization on modified ZnO

The utilization of ZnO nanostructures in the enzyme immobilization in electrochemical biosensors gained interest. Various research on the methods of ZnO synthesis and related features, such as biosensor performance and biosensor construction, e.g., modified electrodes and enzyme immobilization, were published [34]. Probe DNA was purchased from BASE Pte Ltd. (Malaysia). The probe DNA sequences were 5′-CTG ATA GTA GAT TTG TGA CCG TAGAAA-C6. The probe DNA was dropped to the ZnO-modified SiO_2 electrode for immobilization and was incubated for 2 h. After 2 h, the electrode was carefully rinsed using DI water to remove any un-bonded DNA probe and dried at room temperature. The probe-modified device, which is denoted as DNA/ZnO/APTES/SiO_2/Si/Al, was ready for electrochemical measurements.

2.5. DNA of hybridization

The transduction of the hybridization of DNA at a DNA-modified recognition interface is commonly achieved electrochemically, optically, or by using mass-sensitive devices [10]. Electrochemical transduction is highly sensitive, independent from solution turbidity, compatible with micro fabrication, low cost, requires low power, and has a simple instrumentation, which is compatible with small portable devices. Electrochemistry helps control DNA hybridization and denaturing processes [35,36] and offers novel approaches to transduction of hybridization. The oligonucleotides used in the current study were purchased from First BASE Pte Ltd. (Malaysia). Hybridization with complementary DNA sequences was 5′-CTA CGG TCA TCA CAA ATC TAC TAT CAG-3′. 5 µL of the target DNA (10 µmol/L) was dropped to the ZnO electrode and incubated for 4 h to allow hybridization. The ZnO electrode was washed successively with phosphate-buffered saline (PBS) (pH 6.8, 50 mmol/L NaCl) solution and DI water. The ZnO electrode was dried under air blow to remove any non-hybridized DNA and dried at room temperature. 10 µL of 0.5 µM methylene blue was dropped to the ZnO electrode and incubated for 3 min. The ZnO electrode was washed with DI water to remove any excess methylene blue. Finally, the ZnO electrode is ready for electrical measurements.

2.6. Electrochemical measurements

The changes in the capacitance of the interface are induced by the DNA hybridization events with a single-stranded target DNA on a probe platform. To improve the performance of the DNA sensor, the probe layer must be fabricated using a well-defined surface chemistry, which prevents non-specific binding, as well as other side reactions, to induce high selectivity for a specific target DNA. As a result, various DNA sensors were used on electrodes modified with various platforms, such as self-assembled monolayers (SAMs), mixed SAMs, conducting polymer films, various nanomaterials, such as gold nanoparticles (AuNPs), and ZnO nanostructures. The design of the probe layer depends on whether the sensor is faradaic or non-faradaic. The changes in electrical properties at the DNA probe layer are usually extracted using a best fitting model. Each circuit element obtained by fitting impedance responses to an electrical circuit can be utilized to analyze the type and amount of target DNA, as well as its conformational changes [37,38]. Electrochemical measurements were performed using a dielectric analyzer, as shown in Fig. 1. The tests were conducted by using Ag/AgCl as a reference electrode and ZnO-modified electrode as a working electrode. Al acts as a back gate. The responses of DNA immobilization and hybridization were investigated in 10 µM potassium hexacyanoferrate III, and $K_3Fe(CN)_6$ aqueous solution containing 0.1 M KCl as an electrolyte.

2.7. Characterizations

The morphological surface of the ZnO thin films were investigated by scanning electron microscopy (SEM) using Jeol JSM-6460 LV microscope operating at 10 kV. Energy dispersive X-ray spectrometer (EDX) attached to SEM was used to determine the elemental chemical composition. The structure evolution of the as-prepared ZnO nanorods was examined by high-resolution X-ray diffraction (HR-XRD) using PANnalytical X'Pert Pro MRD diffractometer equipped with Cu-Kα-radiation (λ = 0.15418 nm) operating at 40 kV and 30 mA. DNA immobilization and hybridization were tested using a nova control dielectric analyzer (Germany).

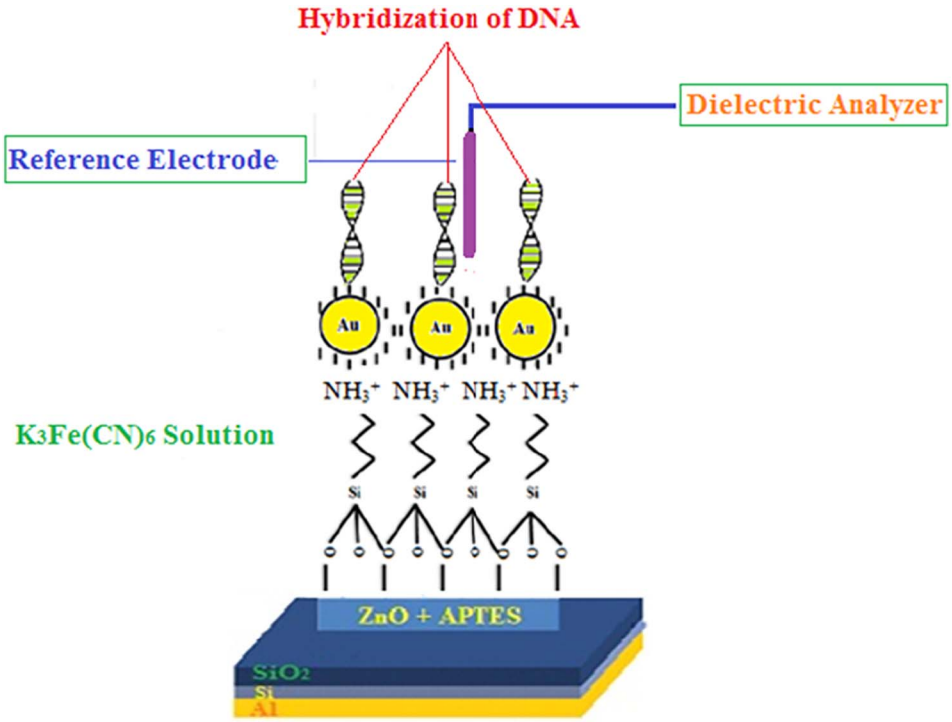


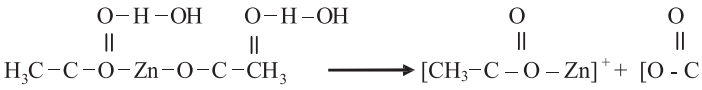
Fig. 1. Scheme of the probe DNA-ZnO/APTES/Au electrode.

3. Results and discussion

3.1. Reaction mechanism

The CBD method is based on the formation of a solid phase from a solution and involves two steps, namely, nucleation and particle growth. During nucleation, clusters of molecules undergo rapid decomposition and particles combine to form a film with a certain thickness by heterogeneous reactions at the substrate surface.

a) Zinc-diacetae-dihydrate is converted to zinc-acetate ion (cation), acetate ion, photon, and hydroxy anion.



$$H_3C-C(=O)-O-Zn-O-C(=O)-CH_3 \longrightarrow [CH_3-C(=O)-O-Zn]^+ + [O\text{-}C(=O)-CH] + 2\,H^+ + 2\,OH^-$$

b) The product of the above reaction is converted into acetic acid and zinc hydroxide. When this solution was heated, the ionic product exceeds the solubility product and precipitation occurs on the substrate and in the solution to form ZnO nuclei

$$[CH_3 - C - O - Zn]^+ + 2\,H^+ + 2\,OH^- \rightarrow CH_3COOH + Zn(OH)_2$$

c) ZnO is produced by removing water molecules. This process is described below.

$$Zn(OH)_2 \rightarrow ZnO + H_2O$$

3.2. Characterization of surface morphology

Fig. 2 shows the SEM images of as-deposited the ZnO film show that the ZnO thin films completely covers the SiO_2 substrate, and no pinholes or cracks can be observed. The formed ZnO exhibits a nanorod-like morphology with a typical diameter of 40–50 nm.

3.3. Crystal quality of the ZnO nanorods

The XRD pattern of the ZnO thin films is illustrated in Fig. 3. All the observed diffraction peaks completely match with the standard JCPDS card No. 01-079-0205, and are consistent with the results in the literature [39]. The diffraction peak (101) shows the highest intensity, indicating a preferred orientation along the (101) direction (ZnO nanorods grow preferentially along the (101) crystallographic plane).

All the diffraction peaks are sharp and narrow, thus indicating high crystallinity. Table 1 shows the XRD data of ZnO from the JCPDS card compared with the measured XRD results.

3.4. Capacitance measurement

Fig. 4 shows the changes of dielectric properties (here capacitance) before and after immobilization and target DNA hybridization at different frequencies. The measurements were investigated at a frequency range of 1 Hz to 1 MHz. Before DNA target detection, the dielectric properties of the ZnO-modified SiO_2 thin films were measured, which will serve as baseline (reference) for further measurements. The capacitance was measured when the DNA-probe was immobilized to the ZnO-modified SiO_2 thin films, and hybridization was tested on the same sample. The results show that the capacitance values of bare, ZnO-modified surface immobilization and target DNA hybridization were 46×10^{-12} F, 47×10^{-8} F, 27 μF, and 17 μF, respectively, at 1 Hz. The capacitance value of the ZnO-modified surface is higher than that of the bare device. Thus, the immobilization and hybridization of DNA were successfully detected, as indicated by the highest capacitance values of 27 μF and 17 μF, respectively, on the modified ZnO electrode. The capacitance value between the ZnO-modified surface and DNA immobilization and hybridization indicates a successful DNA detection using the biosensor fabricated using the ZnO thin films. Reyes et al. [40] reported a ZnO-nanostructure-based quartz crystal microbalance

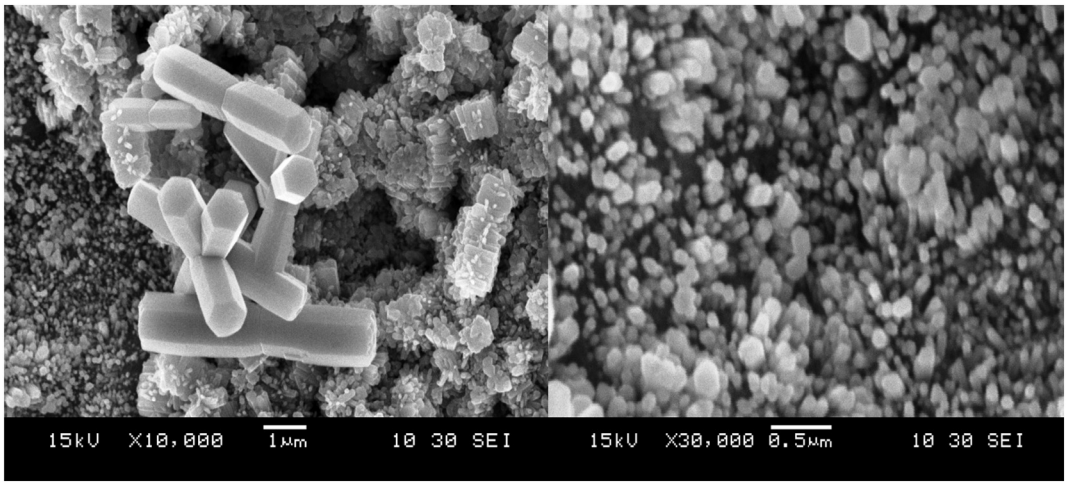


Fig. 2. SEM images of ZnO nanorods grown on ZnO-PVA seed layer.

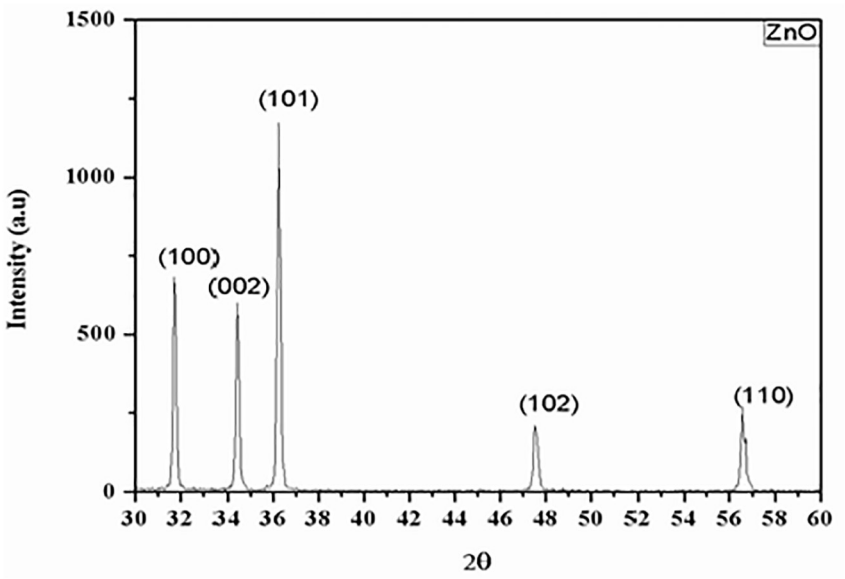


Fig. 3. X-ray diffraction pattern of ZnO nanorods grown on ZnO-PVA seed layer.

Table 1
Structural and microstructural parameters of ZnO nanorods as obtained from XRD analysis.

hkl	2θ (degree)
	Observed
100	31.737
002	34.379
101	36.215
102	47.484
110	56.536

(nano-QCM) device for biosensing applications. ZnO nanotips are directly grown on the sensing area of a conventional QCM by metalorganic chemical vapor deposition (MOCVD). The selective immobilization and hybridization of DNA oligonucleotide molecules are confirmed by the fluorescence microscopy of the nano-QCM sensing areas. Siddiquee et al. [41] studied the sensitivity of an electrochemical DNA biosensor, which was examined using a ssDNA/ZnO/CHIT/AuE probe to hybridize the different concentrations of the target DNA related to a T. harzianum gene. Das et al. [42] synthesized hexagonal ZnO nanocrystalline films that were electrochemically deposited onto ITO coated glass substrates from an aqueous electrolyte containing $ZnCl_2$ and KCl; these nanocrystalline films were utilized to fabricate DNA bio electrodes to detect M. tuberculosis. The DNA biosensor was successfully fabricated using ZnO nanostructures modified with SiO_2 thin films in an electrolyte solution and exhibited a good performance in capacitance.

3.5. Permittivity measurement

The results of the permittivity measurements are shown in Fig. 5. Similar outcomes were observed where the largest changes in permittivity were observed with probe DNA immobilization and target DNA hybridization. However, the permittivity measurement increased from 3.94×10^3 to 251×10^3 and 165×10^3 at a frequency range of approximately 200 to 1 Hz for bare and DNA immobilization and hybridization, respectively. The capacitance profile was significantly high in a frequency of roughly 1 Hz and decreased as frequency increased. The obtained results indicated that permittivity measurement was sensitive at a low frequency. The results obtained from both measurements (capacitance and permittivity) showed that the capacitance and permittivity values of the ZnO-modified electrode considerably changed after probe DNA immobilization and hybridization detection. M. M. Ahmed [43] reported the synthesis of a biosensor using silicon oxide for biomedical applications and its effectiveness in detecting target DNA hybridization. An electrochemical DNA biosensor was successfully

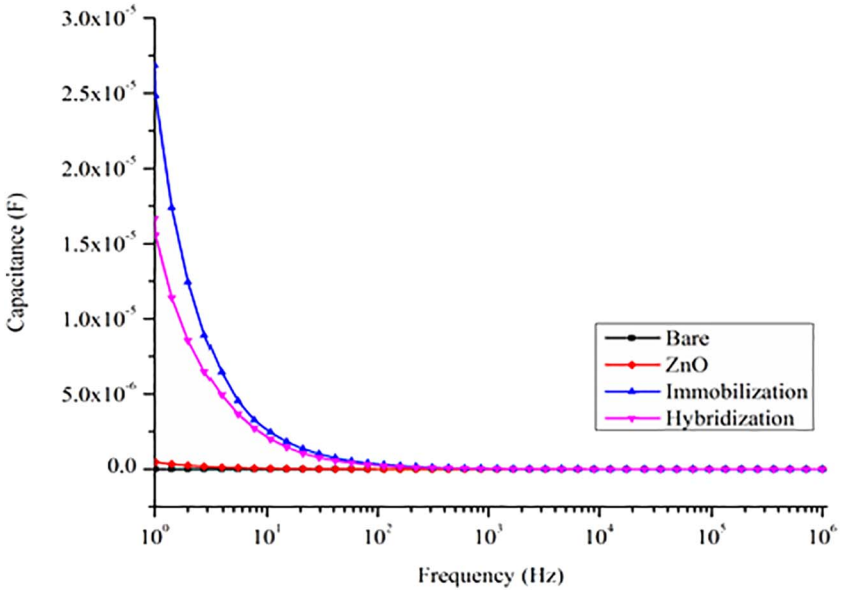


Fig. 4. The capacitance versus frequency of ZnO-modified SiO_2 thin films for DNA immobilization and hybridization detection.

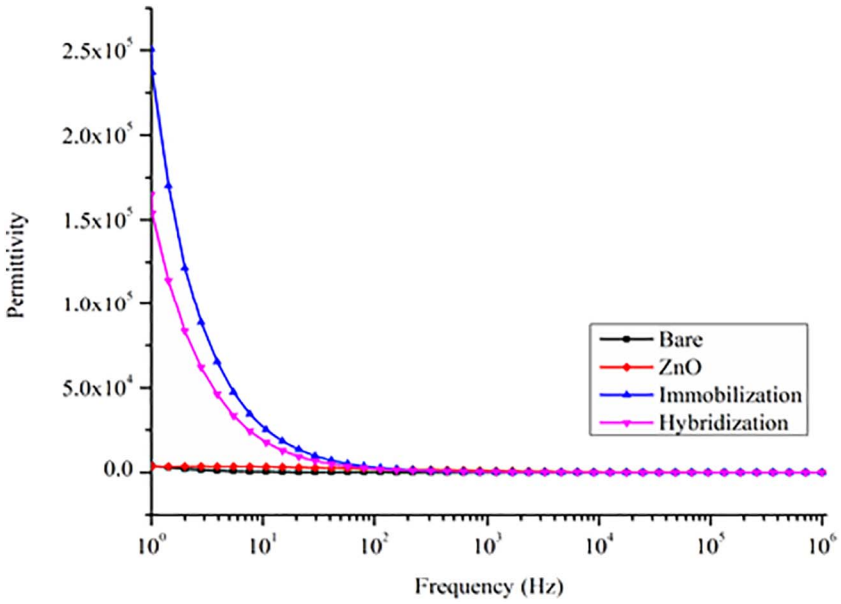


Fig. 5. The permittivity versus frequency of ZnO-modified SiO_2 thin films for DNA immobilization and hybridization detection.

fabricated by using APTES as a linker molecule combined with gold nanoparticles (GNPs) on a thermally oxidized SiO_2 thin film. The GNP-modified SiO_2 thin films were electrically characterized by measuring capacitance, permittivity, and conductivity using a low-cost dielectric analyzer. The capacitance, permittivity, and conductivity profiles of the fabricated sensor clearly differentiated DNA immobilization and hybridization. P. E. Canavar [44] developed single-walled carbon nanotube (CNT) and chitosan composite (chitosan ∗ CNT)-based sensors as DNA biosensors, which were applied for the electrochemical investigation of the interaction between anticancer drug mitomycin C (MC) and DNA.

3.6. Conductivity measurement

Conductivity measurements were also carried out to further investigate the effect of probe DNA immobilization and target DNA hybridization on the ZnO-modified SiO_2 thin films. The measured conductivity values for bare, ZnO, immobilized and hybridization device were 2.4×10^{-9}, 10×10^{-8}, 1.6×10^{-7}, and 1.3×10^{-7} S cm^{-1}, respectively. Fig. 6 shows that DNA probe was immobilized and hybridized to ZnO-modified SiO_2 thin films, and the values of conductivity of the bare electrode increased; thus, the resistivity of the device decreased. This reduction may be attributed to the strong interaction between potassium hexacyanoferrate III, $K_3Fe(CN)_6$ and the unpaired guanine base in the probe DNA [45].

The differences in the conductivity values between ZnO-modified surface and probe DNA immobilization and target DNA hybridization indicate that electron transfer occurred during the immobilization and hybridization of DNA. Therefore, the conductivity measurement enhanced the reaction of DNA immobilization and hybridization on the ZnO-modified surface, which indicated the characteristic of the electrolyte solution.

4. Conclusion

GNPs modified with ZnO nanorods, which were altered SiO_2 thin films in the electrolyte solution with IDE electrodes, was successfully completed for DNA detection. The ZnO nanorods were synthesized

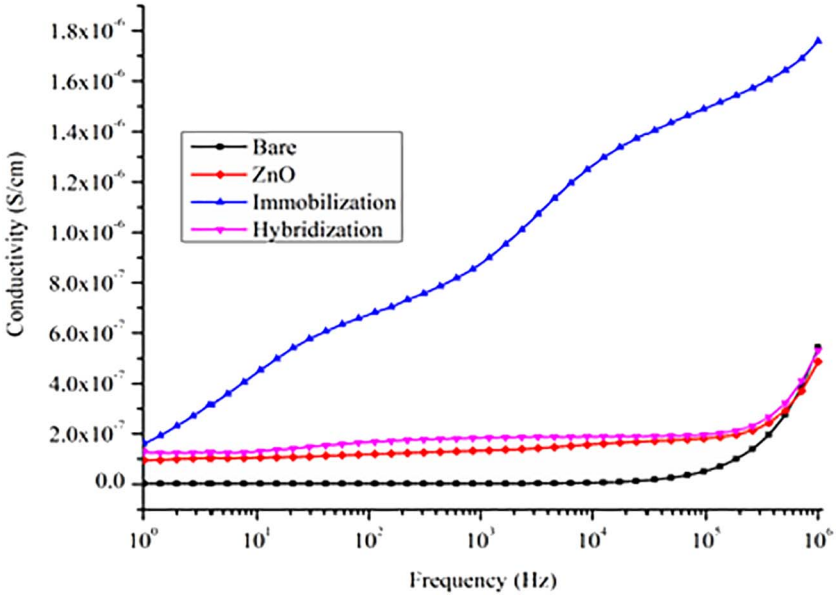


Fig. 6. The conductivity versus frequency of ZnO-modified SiO_2 thin films for DNA immobilization and hybridization detection.

through a low-cost and easily performable microwave-assisted CBD technique. The characterization of ZnO using SEM, XRD and EDX indicated the good quality of the nanostructured hexagonal wurzite ZnO, as well as the doped GNPs. The doped GNPs on the surface of the thin films provided an attachment surface for probe DNA and significantly increased the sensitivity of the device. The fabricated sensor successfully differentiated the detection of complementary and non-complementary targets based on the properties of dielectric and capacitance, permittivity and conductivity measurements in a label-free approach. The results indicate that the fabricated sensor can be applied in low-cost biodiagnostics, forensic testing, food analysis, and environmental monitoring. DNA immobilization and hybridization was detected in ZnO-modified SiO_2 thin films by controlling the surface chemistry with a $K_3Fe(CN)_6$ solution.

Conflict of interest

The authors have declared no conflict of interest.

Acknowledgements

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