

Zirconia grafted carbon nanotubes based biosensor for M.Tuberculosis detection

Maumita Das,^{1,2} Chetna Dhand,^{1,2} G. Sumana,¹ A. K. Srivastava,¹ N. Vijayan,¹ R. Nagarajan,² and B. D. Malhotra^{1,a)}

¹Department of Science and Technology Centre on Biomolecular Electronics, National Physical Laboratory, CSIR, Dr. K. S. Krishnan Marg, New Delhi 110012, India

²Department of Chemistry, University of Delhi, Delhi 110007, India

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Zirconia (ZrO₂) grafted multiwalled carbon nanotubes (CNTs) (crystallite size of ZrO₂ ~ 28.63 nm), obtained via isothermal hydrolysis of zirconium oxychloride in presence of CNT, have been electrophoretically deposited onto indium-tin-oxide (ITO) coated glass plate. High resolution electron microscopic investigations reveal assemblage of the ZrO₂ nanostructure inside and around CNT cavities. Electrochemical impedance spectroscopic studies indicate ~3.5 fold enhancement in charge transfer behaviour of NanoZrO₂-CNT/ITO electrode compared to that of NanoZrO₂/ITO electrode. Considering the synergy between biocompatible ZrO₂ and electrochemically superior CNT, this nanobiocomposite has been explored to develop an impedimetric nucleic acid biosensor for *M. Tuberculosis* detection.

Considering the advantages of many interesting phenomena occurring at nanoscale, significant efforts have been directed towards the development of micro- and nano-structured smart composite materials to explore their application in various imperative fields including biomolecular electronics. In this context, carbon nanotubes (CNTs) and its composites are currently at the vanguard of advanced materials research with prevalent applications due to their unique one-dimensional structure and remarkable electrical and mechanical properties.^{1,2} CNTs have been employed as a promising template for fabricating CNT-based metal oxide nanotubes and nanorods (e.g., Fe₃O₄, TiO₂, ZnO, SnO₂), that are likely to play an important role towards the development of next generation magnetic, optical, and electronic devices.^{3,4} There is a widespread interest towards the fabrication of one-dimensional nanoscale composite materials by filling or coating CNTs with different metals, non-metals, metal oxides, etc.

Keeping in view the application of CNT-metal oxide composite in various emerging fields, there is an augmented interest in exploring the synergy of electroactive CNTs and biocompatible metal oxides. Among the metal oxides, zirconia (ZrO₂) is a leading candidate due to its biocompatibility, chemical inertness, and affinity of groups containing oxygen that can be advantageous for application to electronic devices. Dai *et al.* have worked on integration of ZrO₂ and single-walled carbon nanotubes (SWCNTs) by forming ZrO₂ thin-films on top of individual SWCNTs and used as gate dielectrics for nanotube field-effect transistors.⁵ Liu *et al.* have synthesized ZrO₂-CNT composite and have utilized it for the detection of ethanol using chemiluminescence. CNT transistors integrated with ZrO₂ gate oxide are emerging as

promising candidates for the development of advanced computers.⁶

We report a simple and controllable electrophoretic deposition (EPD) of ZrO₂ and CNT (NanoZrO₂-CNT) nanocomposite films onto an indium-tin-oxide (ITO) coated glass substrate. This NanoZrO₂-CNT/ITO electrode has been utilized for immobilization of single stranded probe DNA (ssDNA) specific for *Mycobacterium tuberculosis* to reveal its application to biosensor for nucleic acid detection.

Zirconium oxychloride (ZrOCl₂·8H₂O), Ferrocene, toluene, magnesium nitrate [Mg(NO₃)₂·6H₂O], acetonitrile, oligonucleotide probe sequence specific to *Mycobacterium tuberculosis*, complementary target, one-base mismatch, and non-complementary DNA sequences have been procured from Sigma–Aldrich. All the solutions and glassware are autoclaved prior to being used, and desired reagents (molecular biology grade) have been prepared in de-ionized water (Milli Q 10 TS). Pre-cleaned ITO glass plates have been used for deposition of CNT-ZrO₂ nanocomposite. The sequences of DNA probes used for the electrochemical DNA hybridization detection are as follows:

- Probe: 5'-GGT CTT CGT GGC CGG CGT TCA-3'
- Complementary target: 5'-TGA ACG CCG GCC ACG AAG ACC-3'
- One-base mismatch: 5'-TGA-ACG-CCG-ACC-ACG-AAG-ACC-3'
- Non-complementary: 5'-ATG-TCT-CAA-GCC-AGC-TGC-TG-3'

CNTs (90%) are synthesized by catalytic chemical vapor deposition in presence of ferrocene in toluene as catalyst.⁷ Solid ZrOCl₂·8H₂O (0.2 mol L⁻¹) and 30 mg of pristine CNT in 100 mL are ultrasonicated for 30 min. A black suspension with homogeneously dispersed CNT is obtained. The stable aqueous suspension is then refluxed in a thermostatic water bath at 100 °C, ensuring isothermal hydrolysis of ZrOCl₂.

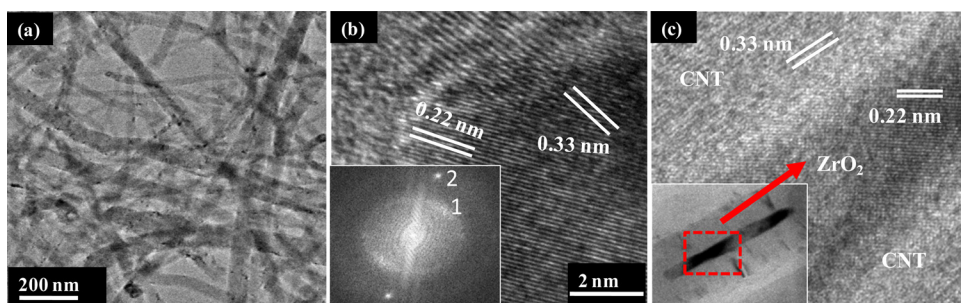


FIG. 1. (Color online) HR-TEM of NanoZrO₂-CNT composite (a) at low magnification, (b) showing entrapment of ZrO₂ nanoparticles between two CNT [inset shows fast fourier transform of ZrO₂ nanoparticles], (c) exhibiting filling up of bore diameter of CNT by ZrO₂ [inset exhibits the region where ZrO₂ is inside the CNT cavity].

After approximately 72 h, the black suspension turns grey. This solution is then filtered, dried, and grey precipitate is collected. Thus obtained ZrO₂-CNT nanocomposite (10 mg) is dissolved in 10 mL of acetonitrile and is used as stock solution.

The colloidal suspension for EPD is prepared by mixing 150 μL of NanoZrO₂-CNT stock solution (1 mg mL⁻¹) in 10 mL of acetonitrile. Grain-by-grain deposition of colloidal particles during cathodophoresis at an optimum DC potential of 80 V for 60 s results in formation of uniform, homogeneous, and thin composite film (200 nm) consisting of NanoZrO₂-CNT.

Nucleic acid functionalized NanoZrO₂-CNT/ITO electrode is fabricated by *in situ* electrophoretic entrapment of 21-mer ssDNA (10 μL) specific to 16s rRNA spacer region of *Mycobacterium tuberculosis* (ssDNA-NanoZrO₂-CNT/ITO).¹² The affinity of ZrO₂ towards the groups containing oxygen has been utilized for immobilization of DNA that facilitates strong binding without any cross-linker.⁸

EPD of NanoZrO₂-CNT/ITO and ssDNA-NanoZrO₂-CNT/ITO electrodes is carried out with a DC battery (BioRad, model 200/2.0). ssDNA-NanoZrO₂-CNT/ITO electrode is characterized using XRD (x-ray diffractometer, Bruker AXS, D8 Advance, Germany), Fourier transform infrared spectroscopy (PerkinElmer, Spectrum BX II), scanning electron microscopy (SEM, LEO 40), and high-resolution transmission electron microscopy (HR-TEM, Tecnaii-G2 F30 STWIN with field emission gun electron source). Electrochemical analysis has been conducted using Autolab Potentiostat/Galvanostat (Eco Chemie, Netherlands) using a three-electrode system with ITO as working, platinum wire as auxiliary, and Ag/AgCl as reference electrode in phosphate buffer saline (PBS) of pH 7.0 containing 5 mM [Fe(CN)₆]^{3-/4-} as redox probe.

The XRD pattern of NanoZrO₂-CNT composite shows a strongest diffraction peak at 23.2°, medium intensity peaks at 40.9° and 42.8°, and a less intensive peak at 58.5°, which can be well indexed as (110), (1 $\bar{1}$ 1), (015), and (232), respec-

tively, with interplaner spacing of 0.22 nm which is further supported by HR-TEM. The lattice parameters obtained from corresponding peaks of ZrO₂ in NanoZrO₂-CNT composite, using Difrac Plus, EVA software, indicate that the structure of nanocomposite corroborates to somewhat deviated monoclinic arrangement. The deviation from ideal crystal structure of ZrO₂ (Ref. 9) (ZrO₂ monoclinic structure) may be acknowledged to the stress generated at atomic scale during ultrathin sheathing of ZrO₂ onto side-wall of CNT and its filling inside the CNT core. The broadening in reflection planes in the XRD pattern indicates nanocrystalline nature of the particles with average crystallite size of 28.63 nm. The lattice parameters and the cell volume obtained are given in Table-SI.¹²

FT-IR spectrum of NanoZrO₂-CNT/ITO electrode exhibits characteristic peaks at 549 and 644 cm⁻¹ arising due to symmetric stretching of Zr-O-Zr bonds, indicating presence of ZrO₂ on the ITO surface. The band observed at 1642 cm⁻¹ is assigned to Zr-O-C vibrations pointing towards composite formation between CNT and ZrO₂. Peaks seen at 1289, 1528, and 1730 cm⁻¹ for ssDNA-NanoZrO₂-CNT/ITO bioelectrode [Fig. 1(c)] are due to P-O stretching of phosphate backbone and C-O stretching vibrations of purine and pyrimidine rings of DNA, respectively. The broad peak seen at 3712 cm⁻¹ is due to N-H stretching vibrations indicating immobilization of DNA onto NanoZrO₂-CNT/ITO surface.¹²

The HR-TEM investigations delineate surface morphology of NanoZrO₂-CNT composite. Figure 1(a) shows low magnification images of NanoZrO₂-CNT. The atomic scale image [Figs. 1(b) and 1(c)] elucidates the dispersion of ZrO₂ in and around CNT. Interplaner spacing of ZrO₂ ($d_{1\bar{1}1} = 0.22\text{nm}$, deviated monoclinic crystal structure) and CNTs (0.33 nm) are indicated in Figure 1(b). Fast Fourier transform (FFT) exhibits two important planes [marked as 1 and 2, inset Fig. 1(b)] of ZrO₂ nanoparticles with interplaner spacing of 0.38 and 0.21 nm, respectively. Figure 1(c) shows filling up of the bore diameter of CNTs by ZrO₂ nanostructures [inset in Fig. 1(c)]. The interplaner spacing of 0.22 nm

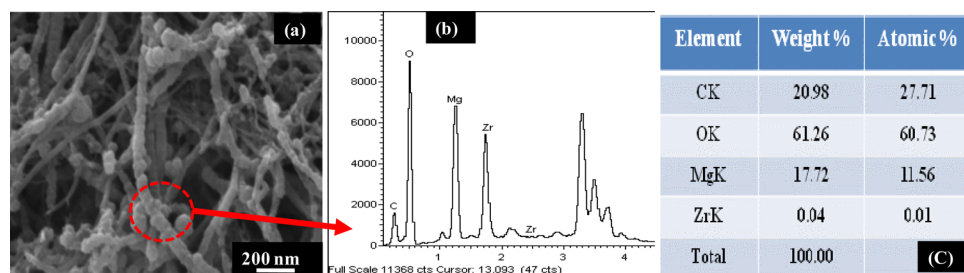


FIG. 2. (Color online) (a) SEM image of NanoZrO₂-CNT composite shows wrapping of CNT sidewalls by ZrO₂ nanoparticles, (b) EDS pattern with (c) weight % of the elements of NanoZrO₂-CNT film.

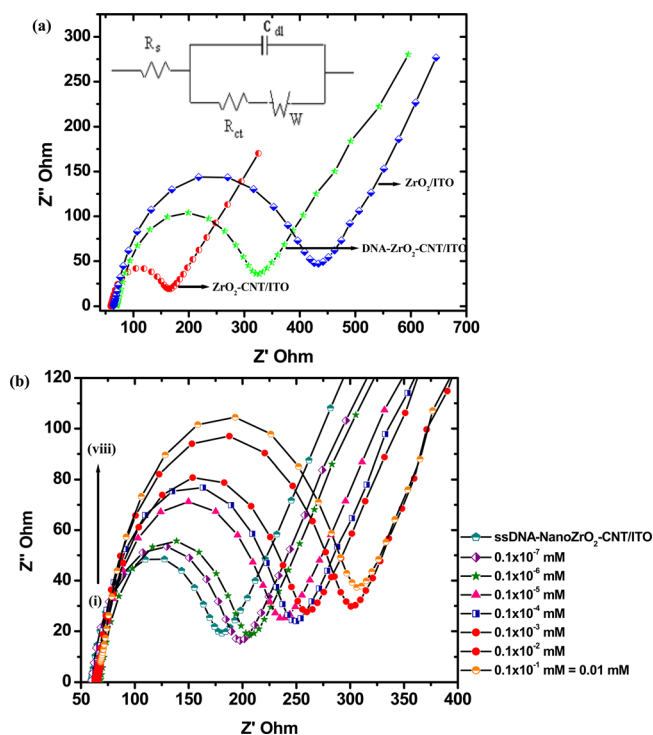


FIG. 3. (Color online) (a) Nyquist diagram of (1) NanoZrO₂/ITO, (2) NanoZrO₂-CNT/ITO, and (3) DNA-NanoZrO₂-CNT/ITO electrodes. (b) DNA hybridization of ssDNA-NanoZrO₂-CNT/ITO bioelectrode in PBS using [Fe(CN)₆]^{3-/4-} as a redox marker, at pH 7.0.

pertaining to ZrO₂ and 0.33 nm corresponding to CNT is marked on the micrograph [Fig. 1(c)]. It appears that there is good bonding between ZrO₂ and CNTs at their interface. The coating of ZrO₂ is clearly evidenced from SEM [Fig. 2(a)]. A corresponding energy dispersive spectroscopic analysis (EDS) pattern [Fig. 2(b)] illustrates the presence of zirconium in the composite [Fig. 2(c)].

Electrochemical impedance spectroscopy (EIS) has been employed to investigate the hindrance provided by the electrode material to transfer of charge from the solution to the electrode that can be correlated with the modification of surface.^{10,11} Figure 3(a) shows Nyquist plots obtained for (1) NanoZrO₂/ITO, (2) NanoZrO₂-CNT/ITO, and (3) DNA-NanoZrO₂-CNT/ITO electrodes, in frequency range of 0.01–10⁵ Hz. The charge transfer resistance (R_{CT}) is found to decrease from 365.65 to 108.59 Ω after incorporation of CNTs, as it provides conducting channel and facilitates easier electron transfer from the medium to electrode due to increased diffusion of redox species [Fe³⁺/Fe⁴⁺] onto NanoZrO₂-CNT/ITO film. The observed increase in the R_{CT} value to 254.61 Ω after DNA immobilization can be attributed to electrostatic repulsion between the negatively charged phosphate backbone of DNA and the redox couple ([Fe(CN)₆]^{3-/4-}) present in the solution.

DNA hybridization has been monitored by monitoring the change in R_{CT} after the incubation of ssDNA-NanoZrO₂-CNT/ITO bioelectrode for about 20 min in a solution as a function of complementary DNA concentration. It has been found that there is a negligible change in the R_{CT} value beyond 1×10^{-2} mM or 0.01 mM concentration. And, hence,

0.01 mM concentration of probe DNA has been used for all experiments. The impedance spectra of bioelectrode have been recorded in PBS of pH 7.0 containing 5 mM [Fe(CN)₆]^{3-/4-} solution [Fig. 3(b)]. It is found that there is a continuous increase in the R_{CT} with increasing complementary target DNA concentration. This may be attributed to the increased number of double helix formed on ssDNA-NanoZrO₂-CNT/ITO electrode surface after hybridization that retards interfacial electron transfer kinetics due to increased negative charge. The ssDNA-NanoZrO₂-CNT/ITO bioelectrode can selectively detect target DNA concentrations ranging from 1×10^{-2} to 1×10^{-8} mM with improved detection limit of 0.01 nM. The R_{CT} increases linearly with logarithm of target DNA concentrations,

$$R_{CT} = 20.80 \log C + 289.65, \quad (1)$$

with regression coefficient (R) is 0.995.¹²

The electrophoretic deposition technique has been employed to fabricate NanoZrO₂-CNT composite onto ITO coated glass plate. The HR-TEM and SEM of nanocomposite elucidate that ZrO₂ is present in and around the nanotubes resulting in deviated monoclinic crystalline structure having crystallite size of 28.63 nm. The enhanced unit cell volume of ZrO₂ provides increased surface area and addition of CNTs resulting in enhanced electron transport. The impedimetric genosensor (ssDNA-NanoZrO₂-CNT/ITO) has been fabricated by *in-situ* entrapment of 21-mer DNA probe specific to *Mycobacterium tuberculosis*. The findings of these studies have implications towards the fabrication of nanobiochips and ultrasensitive biomolecular electronic systems that are likely to revolutionize the field of genotronics.

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- ¹²See supplementary material at <http://dx.doi.org/10.1063/1.3645618> for the schematic of electrophoretic fabrication of NanoZrO₂-CNT composite film onto ITO coated glass substrate. The XRD, FT-IR, and electrochemical response studies of NanoZrO₂-CNT/ITO and DNA-NanoZrO₂-CNT/ITO electrodes have also been investigated.