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Nanobiocomposite platform based on polyaniline-iron oxide-carbon nanotubes for bacterial detection

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ABSTRACT

The nanocomposite based on polyaniline (PANI)–iron oxide nanoparticles (nFe_3O_4) and multi walled carbon-nanotubes (CNT) has been fabricated onto indium tin oxide (ITO) coated glass plate via facile electrochemical synthesis of polyaniline in presence of nFe_3O_4 (~20 nm) and CNT (20–80 nm in diameter). The results of transmission electron microscopic studies show evidence of coating of PANI and nFe_3O_4 onto the CNT. The PANI-nFe_3O_4–CNT/ITO nanoelectrode has been characterized by Fourier transform infrared spectroscopy, X-ray diffraction and scanning electron microscopy studies. The biotinylated nucleic acid probe sequence consisting of 20 bases has been immobilized onto PANI–nFe_3O_4–CNT/ITO nanoelectrode using biotin-avidin coupling. It is shown that the PANI-nFe_3O_4-CNT platform based biosensor can be used to specifically detect bacteria (*N. gonorrhoeae*) at minute concentration as low as (1×10^{-19} M) indicating high sensitivity within 45 s of hybridization time at 298 K by differential pulse voltammetry using methylene blue as electroactive indicator. This bacterial sensor has also been tested with 4 positive and 4 negative PCR amplicons of gonorrhoea affected patient samples. The results of these studies have implications towards the fabrication of a handheld device for *Neisseria gonorrhoeae* detection that may perhaps result in a decrease in the human immunodeficiency virus infections.

1. Introduction

Gonorrhoea, caused by an etiological agent, *Neisseria gonorrhoeae* (human-specific organism), is an important public health problem and is presently the second most common bacterial sexually transmitted disease. It has been estimated that about 650,000 cases of gonorrhea occur annually [1]. It is now well-recognized that gonorrhoea is a potent amplifier with five-fold amplification of the spread of human immunodeficiency virus (HIV). Therefore, accurate diagnosis and effective treatment can result in reduced HIV transmission. The diagnosis of gonococcal infection is currently conducted via a combination of clinical signs and symptoms of cervicitis/urethritis, a gram stain of urethral or cervical discharge, and the use of culture on selective media. However, over the last 20 years, the resource-rich settings have moved towards molecular assays (e.g. PCR) for diagnosing gonococcal infections. Moreover, the resource-limited settings where diagnostic testing for gonorrhoea is difficult, the affected persons are usually treated using syndrome-based algorithms for urethritis, vaginitis or pelvic inflammatory disease. There is, thus, an urgent need to develop a device that can be used to detect gonorrhoea.

Polyaniline (PANI) is presently considered to be one of the promising conducting polymers (CP) that has been found to have a wide range of applications including biosensors owing to its low cost, electrical properties and environmental stability etc. [2–6]. Carbon nanotubes (CNT), discovered by Iijima [7] have attracted much attention due to their unique structure, mechanical, electrical and thermal properties [2,8–14]. The amalgamation of CNT with conducting polymers can be an attractive route to reinforce polymer to obtain electronic properties based on morphological modification and electronic interactions between the two components. The chemical tailoring of CNT with polymeric architectures and construction of complex nanostructured composite materials in bulk has broadened applications of these materials. The properties of nanocomposites are critically dependent on dispersion of CNT in the polymer matrix and homogeneity of the material. Due to their fascinating nanoscale dimensions and high surface area, CNT can perhaps be used as a carrier to direct assembly of desired inorganic nanoparticles to obtain desired properties when used in advanced composites [15–21]. Now metal nanoparticles are playing an important

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role in fabrication of nanodevices for diagnosis of the infectious diseases because of their unique properties like large surface-to-volume ratio, high surface reaction activity, high catalytic efficiency, strong adsorption ability, particle size, electrical properties, and surface properties [22]. Due to their large specific surface area and high surface free energy, nanoparticles can play an important role towards the immobilization of biomolecules for biosensor construction. One of the major discoveries in the application of nanoparticles relates to the realization of steric stabilization that may perhaps result in the increased nanoparticle stability in the biological environment for application in biosensing. The biocompatible environment also results in enhanced long term stability of biomolecule onto the matrix leading to higher stability of the sensor. In this context, iron oxide (Fe_3O_4) nanoparticles due to good biocompatibility, low toxicity, high electron transfer capability and high adsorption ability can be used as an immobilizing matrix for application to biosensors. However, aggregation of Fe_3O_4 nanoparticles due to high surface area and magnetic dipole interaction between nanoparticles has limited their biomedical application. This problem can perhaps be overcome by dispersing Fe_3O_4 nanoparticles in a polymer composite of PANI-CNT to prepare PANI-nFe_3O_4-CNT hybrid nanobiocomposite. It has been found that conducting polymeric chains of polyaniline in the PANI–nFe_3O_4–CNT nanocomposite perhaps wrap and stabilize nFe_3O_4 onto CNT and possibly prevent nFe_3O_4 from being oxidized and CNT perhaps act as templates for the coating of nFe_3O_4 and PANI with minimum aggregation. The presence of iron oxide nanoparticles in PANI–nFe_3O_4–CNT nanobiocomposite increases the electroactive surface area of the matrix resulting in improved immobilization of DNA which may ultimately lead to improved stability and increased electron transfer kinetics between medium and the electrode.

Xuan et al. have synthesized super paramagnetic Fe_3O_4-polyaniline core/shell microspheres with blackberry-like morphology via a simple *in-situ* surface polymerization method [23]. Ding et al. have prepared cage-like and electromagnetically functionalized polyaniline composite nanostructures [24]. And the synthesis of PANI-CNT composite has been investigated by several groups [2,25–27]. The properties of PANI, CNT and nFe_3O_4 can perhaps be improved by synthesizing nanocomposites based on the nanomaterials.

We report results of the studies relating to electrochemically prepared PANI-nFe_3O_4–CNT nanocomposite as a platform for bacterial infectious disease (*N. gonorrhoeae*) detection.

2. Experimental

2.1. Chemicals and reagents

Aniline, methylene blue (MB), tris base, ethylene diamine tetra acetic acid (EDTA), potassium monohydrogen phosphate, potassium dihydrogen phosphate, N-ethyl-N′-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), nFe_3O_4 and avidin (Avi) have been procured from Sigma–Aldrich, USA. Indium-tin-oxide (ITO) coated glass plates (0.25 cm^2) have been obtained from Balzers, UK. The multiwalled carbon nanotubes (CNT, 20–80 nm diameters) have been prepared via catalytic chemical vapor deposition (CCVD) technique. All other chemicals are of analytical grade. Deionized water (resistance ~18.2 MΩ.cm) from the Millipore water purification system has been utilized for preparation of the desired aqueous solutions. All the solutions and glassware are autoclaved prior to being used.

2.2. Synthesis of artificial DNA sequences

Oligonucleotide probe sequence for detection of *N. gonorrhoeae* has been identified from the *Opa* gene (a multi-copy gene) of *N. gonorrhoeae*. All oligonucleotide sequences have been procured from Sigma–Aldrich, USA. The GenBank accession No. for oligonucleotide probe sequences (Table 1) used for immobilization in this study is as follows.

Table 1
The oligonucleotide probe sequences used for immobilization.

Oligonucleotide	Sequence
Probe DNA: Biotin (BDNA)	5′-CCGGTGCTTCATCACCTTAG-3′
Complementary target DNA (compDNA)	5′-CTAAGGTGATGAAGCACCGG-3′
One-base mismatch DNA (obmDNA)	5′-CTAAG**T**TGATGAAGCACCGG-3′
Non-complementary DNA (ncompDNA)	5′-GTATG**G**TGATCAAGCTCCCG-3′

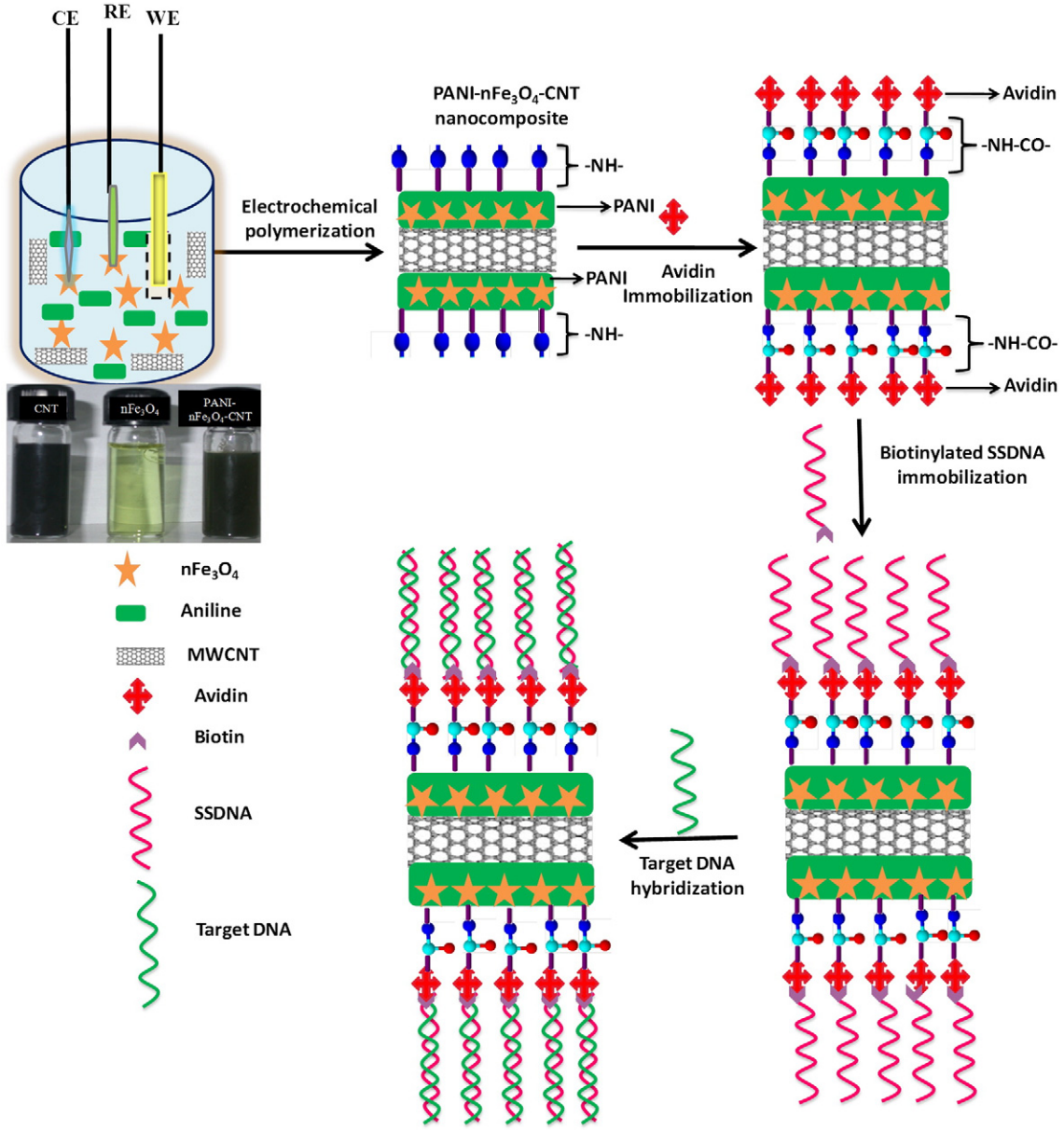
Probe: BDNA or Biotin-(GenBank Accession No: PUID 9716119 snum 2705).

2.3. Electrochemical deposition of PANI-nFe_3O_4-CNT onto ITO electrode

CNT are prepared via CCVD technique [28] followed by purification and functionalization using boiling acid treatment [2,29]. For carboxyl functionalization, CNT are refluxed with concentrated boiling nitric acid for about 12 h, and then washed with distilled water followed by rinsing with ethanol and dried at 333 K. The nanostructured Fe_3O_4 when dispersed in 1 M hydrochloric acid (HCl) turns yellow that has been used for further studies. The ITO coated glass plates are pre-cleaned with acetone, ethanol, and a copious amount of deionized water. To obtain uniformly distributed OH groups onto the ITO surface, ITO plates are immersed in a solution of H_2O_2/NH_4OH/H_2O (1:1:5, v/v) for about half an hour at 353 K for hydrolysis, after which they are rinsed thoroughly with deionized water and dried. PANI-nFe_3O_4–CNT nanocomposite is deposited onto ITO coated glass plates (0.25 cm^2) by electrochemical polymerization using three-electrode electrochemical cell containing a mixture comprising of aniline (0.1 M) in 1 M HCl, 1 mL of nFe_3O_4 dispersed in 1 M HCl and 1 mL of carboxyl functionalized CNT (1 mg/mL) in water (Potentiostat/Galvanostat, Autolab Eco Chemie, Netherlands). The polymerization is accompanied chronopotentiometrically at 50 μA for about 900 s (via intermittent washing using autoclaved Millipore water after every 300 s of polymerization) [5]. The resulting nanocomposite film is washed with the background electrolyte solution to exclude any residual monomer from the electrode. The electrode is dried under vacuum at room temperature (298 K) for about 24 h to obtain green-black PANI-nFe_3O_4–CNT/ITO nanocomposite films.

2.4. Biological tailoring of PANI-nFe_3O_4-CNT/ITO nanoelectrode with DNA using biotin–avidin interaction

Biotinylated probe DNA (BDNA) is immobilized onto electrochemically deposited PANI-nFe_3O_4–CTN/ITO nanoelectrode using biotin-avidin interaction. For this purpose, 10 μl of 1 mg/mL activated avidin (activated using 15 mM EDC and 30 mM NHS for 2 h at 298 K) is first immobilized onto PANI-nFe_3O_4–CNT/ITO electrode. The Avi-PANI-nFe_3O_4–CNT/ITO nanoelectrode is then washed and subject to 300 s incubation with 20-mer biotinylated oligonucleotide probe (BDNA, 10 μl and 1.0 μM) in a humid chamber at 298 K (Scheme 1). The incubation of BDNA solution on Avi-PANI-nFe_3O_4–CNT/ITO nanobioelectrode results in the biotin–avidin interaction due to high affinity between immobilized avidin and biotin attached to DNA. This fabricated DNA functionalized Avi-PANI-nFe_3O_4–CNT/ITO nanobioelectrode has been characterized using Fourier transform infrared spectroscopy (FT-IR, Perkin Elmer, Spectrum BX II), scanning electron microscopy (SEM, LEO 440), High resolution transmission electron microscopy (HRTEM, Model Tecnai G2 F3O STWIN, supported with field emission electron gun source), UV–visible spectroscopy (UV, Model 2200DPCV, phoenix), X-ray diffraction (XRD, JCPDS–International center diffraction data, PDF cards 3–864 and 22–1086), cyclic voltammetry (CV), differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS, Potentiostat/Galvanostat, Autolab Eco Chemie, Netherlands),. The BDNA–Avi-PANI-nFe_3O_4–CNT/ITO nanobioelectrode has been optimized for hybridization time and is subjected to incubation in the



Scheme 1. Preparation of PANI–nFe_3O_4–CNT nanocomposite and immobilization process of biotinylated DNA using biotin-avidin coupling followed by hybridization for bacterial (*N. gonorrhoeae*) detection.

concentration range (1×10^{-19} M to 1×10^{-6} M) of complementary (compDNA) target DNA for 45 s at 298 K. Further, the studies have been carried out by incubating the bioelectrode with non-complementary (ncompDNA) and one-base mismatch (obmDNA) target DNA to prove specificity of the electrode. These electrodes have also been tested with positive and negative PCR amplicons of gonorrhoea affected patient samples. Subsequently, the DPV measurements of BDNA–Avi-PANI-nFe_3O_4–CNT/ITO nanobioelectrode have been carried out in 20 μM MB, pre-treatment at +0.1 V for 10 s, at step potential of 3 mV and modulation amplitude of 50 mV, 0.05 M PBS (pH 7.0, 0.9% NaCl). The detailed mechanism of fabricating the BDNA-Avi-PANI-nFe_3O_4–CNT/ITO nanobioelectrode is depicted in Scheme 1.

3. Results and discussion

3.1. Scanning electron microscopic studies

The scanning electron microscopic (SEM) images obtained for PANI-nFe_3O_4–CNT/ITO, Avi-PANI-nFe_3O_4–CNT/ITO and BDNA-Avi-PANI-nFe_3O_4–CNT/ITO nanoelectrodes are shown in Fig. 1. A thin coating of gold is sputtered onto samples prior to SEM studies for better resolution of the desired nanostructures. PANI–nFe_3O_4–CNT nanocomposite exhibits dense nano structured morphology with interwined wire like structures. However, after DNA immobilization, the globules get attached to these nanostructures in a regular fashion. This is attributed to the conducive functional groups (amino functional groups in polyaniline, COOH groups of functionalized CNT aligned in the presence of nFe_3O_4 moieties).

3.2. High resolution transmission electron microscopic studies

The high resolution transmission electron microscopy (HRTEM) has been adopted to characterize bare CNT and a nanocomposite of CNT co-existing with nFe_3O_4 and polyaniline (Fig. 2 a, b). Characterization of bare CNT indicates nanotubes of average diameter in between 20 and 80 nm (Fig. 2 a (i)) whereas, in the nanostructured composite of CNT with PANI and nFe_3O_4, it can be seen that a coating of PANI and nFe_3O_4 onto CNT is present throughout the nanotubes

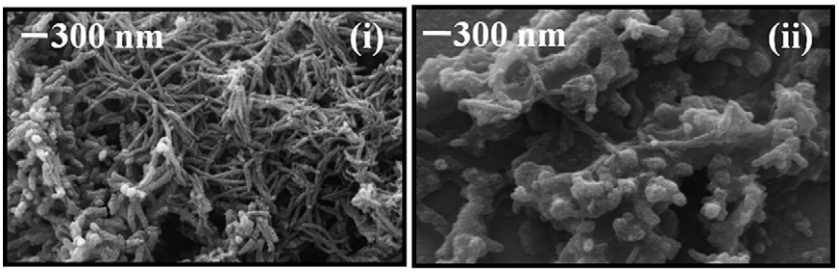
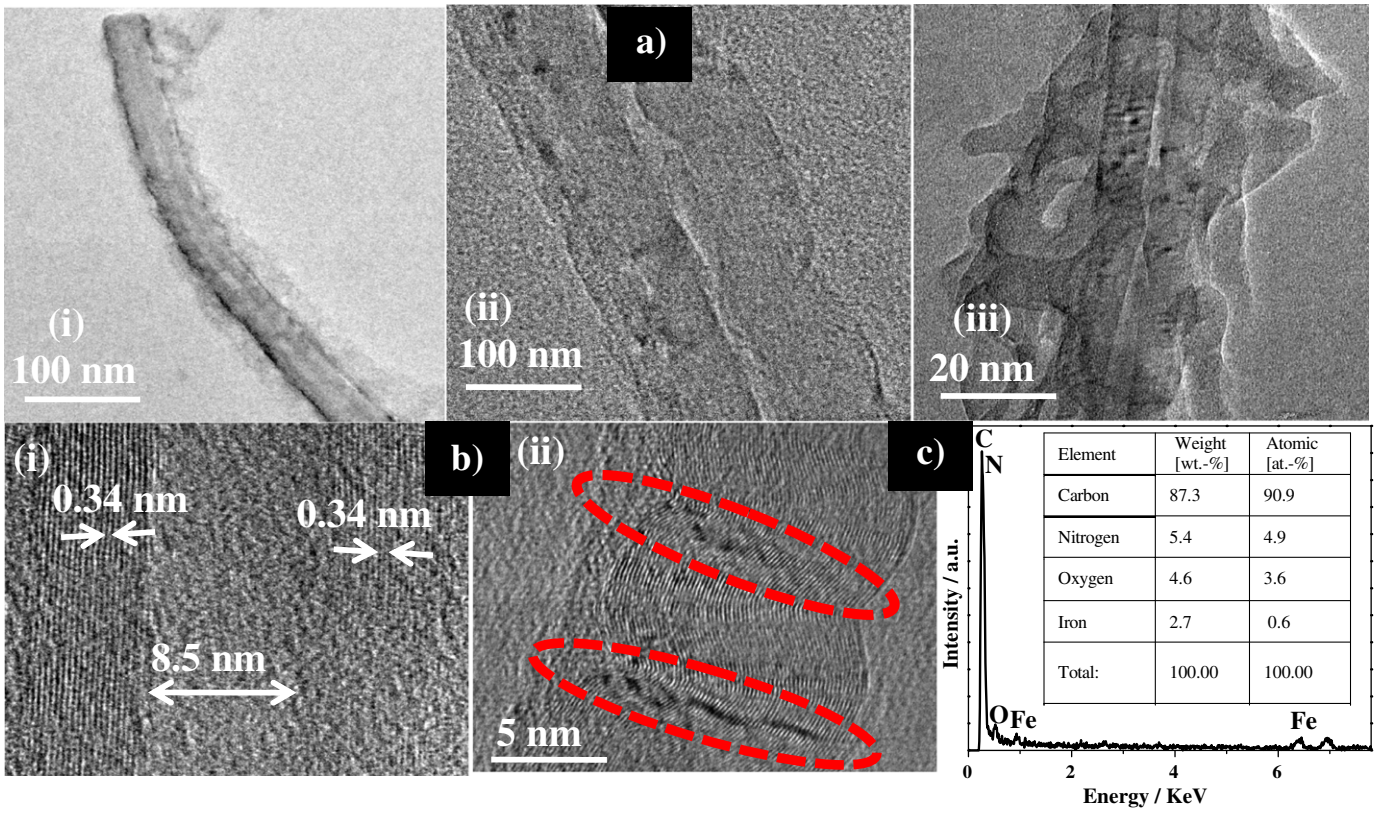


Fig. 1. SEM images of (i) PANI–nFe_3O_4–CNT/ITO nanoelectrode and (ii) BDNA-Avi-PANI–nFe_3O_4–CNT/ITO nanobioelectrode.

(Fig. 2 a (ii)). It is further observed that excess nanoparticles of Fe_3O_4 with PANI overcoat CNT along the longitudinal direction (Fig. 2 a (iii)). It can be seen (Fig. 2 a (iii)) that conducting polymeric chains of polyaniline in the PANI–nFe_3O_4–CNT nanocomposite perhaps wrap and stabilize nFe_3O_4 onto CNT and possibly prevent nFe_3O_4 from being oxidized. It appears (Fig. 2a (iii)) that CNT perhaps act as templates for coating of the nFe_3O_4 and PANI with minimum aggregation. A detailed lattice scale imaging of bare CNT and PANI-nFe_3O_4 coated CNT reveals slight structural alteration in the multi-walled structure of CNT. Fig. 2 b (i) shows multiwalled structure of a single CNT with clear distinction between the walls on both sides of the tube and the inner core-diameter of the tube about 8.5 nm. An individual wall separation of about 0.34 nm is marked between two interlayers of the tube (Fig. 2 b (i)). The coating of CNT with PANI and nFe_3O_4 results in surface modification leading to distortion of the CNT structure (Fig. 2 b (ii)). This may perhaps be attributed to embedding of PANI and nFe_3O_4 between the walls of nanotubes resulting in changes in alignment of the multiwalled structure of nanotubes. For instance, two regions of included PANI and nFe_3O_4 in CNT multiwalls can be clearly seen as indicated by dotted lines in Fig. 2b (ii). The inclusion of PANI and nFe_3O_4 (dark grey contrast in Fig. 2 b (ii)) leads to curly shape of walls of the tubes (Fig. 2 b (ii)) as compared to those of the straight and aligned walls delineated in bare nanotubes (Fig. 2 b (i)). Moreover, inclusion of PANI and nFe_3O_4 between multiwalls of the tubes results in bulging of the individual nanotubes, resulting in enhanced surface area of the nanotubes at atomic scale.

3.3. Energy dispersive X-ray spectroscopic studies

Fig. 2c displays EDX spectra of the PANI-nFe_3O_4–CNT nanocomposite. The EDX spectrum exhibits peaks at 0.24 and 0.52 keV corresponding to carbon and oxygen, respectively. The peaks seen at 0.69 and 6.41 keV are perhaps due to the presence of Fe. The weight ratios obtained from EDX of PANI-nFe_3O_4–CNT nanocomposite are: C, 87.3%; N, 5.4%; O, 4.6%; Fe, 2.7%, respectively.



Element	Weight [wt.-%]	Atomic [at.-%]
Carbon	87.3	90.9
Nitrogen	5.4	4.9
Oxygen	4.6	3.6
Iron	2.7	0.6
Total:	100.00	100.00

Fig. 2. HRTEM of (a) (i) bare CNT, (ii) nano-composite of CNT coexisting with nFe_3O_4 and PANI (iii) nano-composite of CNT coexisting with nFe_3O_4 and PANI along with its longitudinal direction (b). (i) Straight and aligned multiwall structure of a bare CNT of outer diameter about 25 nm. (ii) Curly shape of the walls of the tubes after loading of CNT with PANI and nFe_3O_4 (dark grey contrast-inclusion of nFe_3O_4). (c) The EDX spectra of PANI–nFe_3O_4–CNT nanocomposite.

3.4. Fourier transform infrared spectroscopic studies

Fourier-transform infrared (FT-IR) spectra of PANI–nFe_3O_4–CNT nanocomposite, BDNA-Avi-PANI–nFe_3O_4–CNT nanobiocomposite are shown in Fig. 3a, b. In the IR spectra of PANI–nFe_3O_4–CNT/ITO composite, the peak seen at ~810 cm^{-1} and the band between 800 and 500 cm^{-1} are assigned to aromatic C–H bending of 1, 4-disubstituted aromatic ring [30] and the vibration of C–H bond in the benzene rings, respectively (Fig. 3a) [31]. The peaks seen at 1500 and 1580 cm^{-1} can be attributed to the stretching vibration of benzoid and quinoid rings, respectively, indicating oxidation state of the PANI (emeraldine salt) [3]. The peaks seen at around 1300 and 1140 cm^{-1} are attributed to the C–N and C=N stretching of secondary aromatic amine of PANI, respectively (Fig. 3a). The peak found at around 3428 cm^{-1} is due to the N–H stretching [32]. In the FT-IR spectrum of BDNA-Avi-PANI–nFe_3O_4–CNT nanobiocomposite, vibration bands observed at 1067 and 1243 cm^{-1} are due to asymmetric stretching of P–O–C vibration and stretching vibration of P=O of the phosphoric acid group, respectively. The peaks found at 1492 and 1660 cm^{-1} are associated with carbonyl stretching vibration and C=C bonds in the purine and pyrimidine rings (Fig. 3b).

3.5. X-ray diffraction studies

Fig. 3c shows XRD spectra of the PANI–nFe_3O_4–CNT nanocomposite. The presence of diffraction peaks pertaining to PANI–nFe_3O_4–CNT nanocomposite indicates that nFe_3O_4 in the nanocomposite have a spinel structure. The reflection peaks found at $2\theta = 30.68^o$, 35.55^o correspond to the hexagonal spinel phase of nFe_3O_4. The diffraction peaks of the PANI-nFe_3O_4–CNT nanocomposite can be seen at $2\theta = 21.6^o$, 30.6^o, 35.5^o, 43.2^o, 53.4^o and 56.9^o, respectively. The average crystallite size of nFe_3O_4 in PANI–nFe_3O_4–CNT nanocomposite has been estimated to be as 20 nm using Scherrer formula [33], based on the peak at 30.68^o in its XRD spectrum. The UV–vis spectroscopy has been used to delineate the electronic states of PANI, PANI-CNT and PANI-nFe_3O_4–CNT (Supplementary data S4 and Supplementary Fig. 1).

3.6. Electrochemical studies

3.6.1. Differential pulse voltammetry (DPV)

Fig. 4a shows results of the DPV measurements carried out on the (i) BDNA-Avi-PANI–nFe_3O_4–CNT/ITO (ii) compDNA-BDNA-Avi-PANI–nFe_3O_4–CNT/ITO (iii) obmDNA-BDNA-Avi-PANI–nFe_3O_4–CNT/ITO (iv) ncompDNA-BDNA-Avi-PANI–nFe_3O_4–CNT/ITO nanoelectrodes in 20 μM MB, pretreatment at +0.1 V for 10 s, at step potential of 3 mV and modulation amplitude of 50 mV, 0.05 M PBS (pH 7.0, 0.9% NaCl). The electroactive redox indicator methylene blue (MB) has been used to investigate DNA hybridization at the electrode surface [2,5,22,32,34]. MB is known to associate with the unpaired nitrogenous guanine bases on single stranded DNA as compared to that of the double stranded DNA. The DPV curve of BDNA-Avi-PANI–nFe_3O_4–CNT/ITO nanobioelectrode indicates strong affinity of MB for free guanine bases at the BDNA-Avi-PANI–nFe_3O_4–CNT/ITO nanobioelectrode (Fig. 4 a (curve i)) [34]. A decreased peak current observed after the duplex formation indicates that the interaction between MB and guanine residues of the probe is perhaps prevented due to hybrid formation on the electrode surface. However, the decreased peak current after hybridization indicates that change in current due to intercalation is smaller compared to that from direct interaction with the guanine bases (curve ii) [5,6,23]. The observed change in MB peak current on its treatment with the obmDNA (curve iii) and ncompDNA (curve iv) indicates non-hybridization. Fig. 4b describes results of the sensing studies of BDNA-

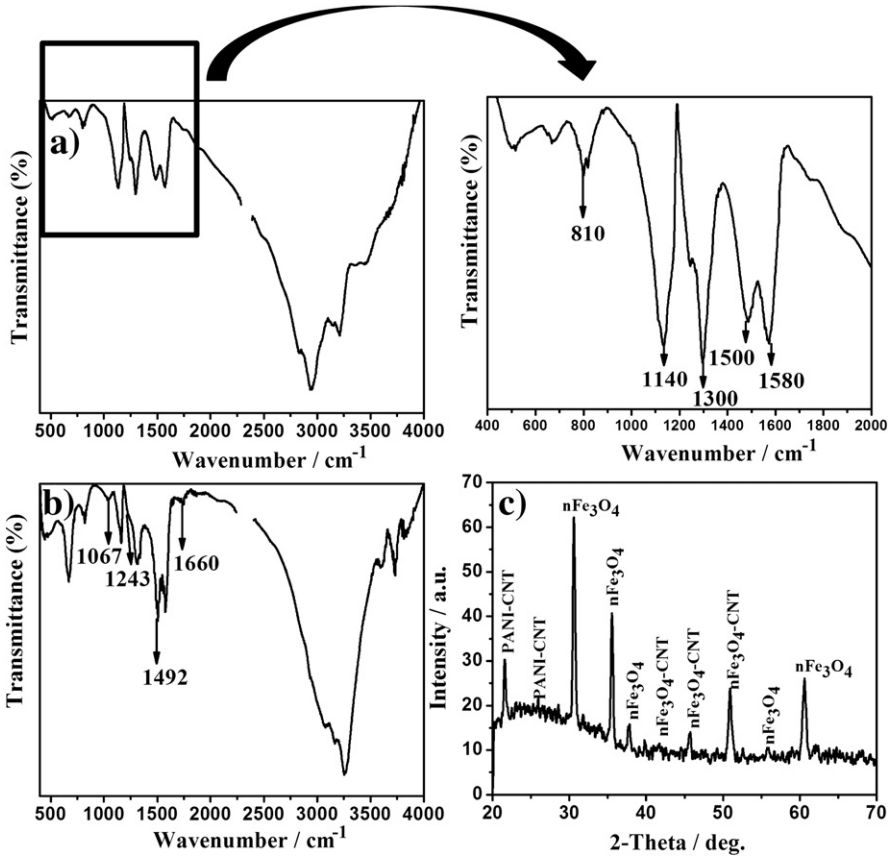


Fig. 3. FTIR spectra of (a) PANI–nFe_3O_4–CNT nanocomposite, (b) BDNA-Avi-PANI–nFe_3O_4–CNT naobiocomposite, (c) XRD spectra of PANI–nFe_3O_4–CNT nanocomposite.

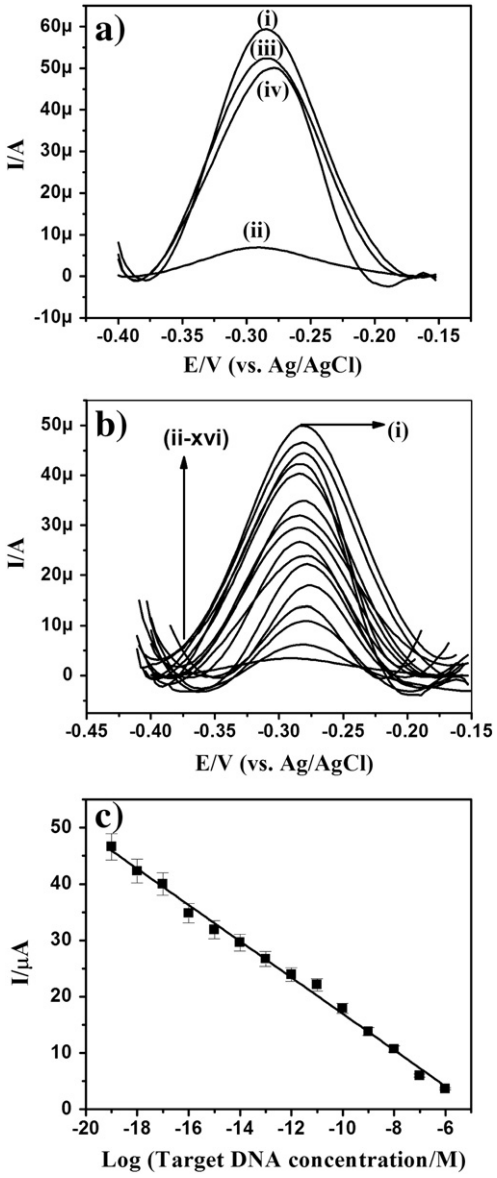


Fig. 4. (a) DPV of (i) BDNA-Avi-PANI–nFe_3O_4–CNT/ITO (ii) compDNA- BDNA-Avi-PANI–nFe_3O_4–CNT/ITO (iii) obmDNA-BDNA-Avi-PANI-nFe_3O_4–CNT/ITO (iv) ncompDNA-BDNA-Avi-PANI-nFe_3O_4–CNT/ITO at step potential of 3 mV and modulation amplitude of 50 mV, in 20 μM MB, pretreatment at +0.1 V for 10 s, 0.05 M PBS (pH 7.0, 0.9% NaCl), (b) Response studies of BDNA-Avi-PANI–nFe_3O_4–CNT/ITO (i) after hybridization with compDNA concentration 1×10^{-6} M (ii), 1×10^{-7} M (iii), 1×10^{-8} M (iv), 1×10^{-9} M (v), 1×10^{-10} M (vi), 1×10^{-11} M (vii), 1×10^{-12} M (viii), 1×10^{-13} M (ix), 1×10^{-14} M (x), 1×10^{-15} M (xi), 1×10^{-16} M (xii), 1×10^{-17} M (xiii), 1×10^{-18} M (xiv), 0.5×10^{-18} M (xv), 1×10^{-19} M (xvi) at step potential of 3 mV and modulation amplitude of 50 mV, in 20 μM MB, pre-treatment at +0.1 V for 10 s, 0.05 M PBS (pH 7.0, 0.9% NaCl), (c) The MB peak height as a function of compDNA concentration.

Avi-PANI–nFe_3O_4–CNT/ITO nanobioelectrode obtained on hybridization with different concentrations of compDNA of bacteria *N. gonorrhoeae* ranging from 1×10^{-19} M to 1×10^{-6} M. The absence of the MB peak after hybridization with the compDNA is attributed to steric inhibition of MB packing between double helix of the hybrid [35–37]. The MB peak current of BDNA-Avi-PANI–nFe_3O_4–CNT/ITO nanobioelectrode increases with decrease in the concentration of compDNA, indicating formation of the duplex at the surface (Fig. 4b). The average current of the DNA bioelectrode is linear in the compDNA concentration range of 1×10^{-19} M to 1×10^{-6} M. The detection limit of BDNA-Avi-PANI–nFe_3O_4–CNT/ITO nanobioelectrode is 1×10^{-19} M with hybridization time of 45 s. It is found that decrease in the MB peak current with respect to compDNA concentration (Fig. 4c) with value of regression co-efficient as 0.9975 follows Eq. (1)

$$\text{Current}(\mu A) = 3.2[\log(\text{compDNA concentration})] + 13.7 \quad (1)$$

The experiments have been repeated at least 15 times and the error bars are included in Fig. 4c. Reusability of the BDNA-Avi-PANI–nFe_3O_4–CNT/ITO nanobioelectrode has been estimated by dipping the used electrode in 0.5 M NaOH. The regeneration of the electrode is followed by measuring the signal at a given range of concentration (1×10^{-19} M to 1×10^{-6} M). The experiments have been repeated at this range of concentration after regenerating the electrode. It is found that the sensor is stable enough to allow for ready regeneration after washing the electrodes with 0.5 M NaOH for 30 s at room temperature and it is found that this sensor can be used at least for 15 times before significant degradation is observed. The proposed DNA sensor has good stability of about 150 days possibly due to favourable interactions between PANI backbone, nFe_3O_4 and CNT. The PANI–nFe_3O_4–CNT/ITO based DNA sensor shows higher sensitivity and linearity than that of the PANI based sensor, indicating that presence of CNT significantly enhanced the performance of this nucleic acid biosensor. The Fe_3O_4 nanoparticles in PANI–nFe_3O_4–CNT nanobiocomposite result in increased electro-active surface area of the nanobiocomposite that provides desirable environment for the loading of DNA with better conformation leading to better stability and increased electron transfer kinetics between the medium and electrode. Besides this, presence of CNT in PANI–nFe_3O_4–CNT nanocomposite results in increased conductivity and specific surface area resulting in increased amount of DNA immobilized onto the composite. A comparison of the response characteristics of the bacterial (*N. gonorrhoeae*) sensor is given in Table 2. Characterization of the nanobioelectrode has been done using CV and EIS. (Supplementary data S5 and S6, Supplementary Fig. 2 a,b).

3.6.2. Hybridization studies with patient samples

The BDNA-Avi–PANI-nFe_3O_4-CNT/ITO based bacterial sensor has been used to detect sequence specific DNA based on the DPV current change of MB before and after hybridization [36,37]. Fig. 5a shows DPV response of the fabricated bacterial DNA immobilized bioelectrode. The performance of the BDNA-Avi–PANI-nFe_3O_4-CNT/ITO nanobioelectrode has been investigated by incubating it with DNA extracted from *N. gonorrhoeae* culture isolated sample, pus sample spiked with *N. gonorrhoeae* and *N. gonorrhoeae* positive male patient sample. The details about the processing of the samples have been given as Supplementary data S1 and S2. The observed significant decrease in the DPV signal shows that the complementary DNA present in the clinical sample hybridizes with the probe DNA (Fig. 5a). As there is sample-to-sample variation in the bacterial DNA load that cannot be presently quantified perhaps results in variation of the observed signal.

3.6.3. Hybridization studies with PCR Positive and negative amplicons

Fig. 6b shows performance of the bacterial sensor for detection of *N. gonorrhoeae* positive and the negative amplicons. The details of the processing of the PCR amplicons are given as Supplementary data S3. The exposure of BDNA-Avi-PANI–nFe_3O_4–CNT/ITO nanobioelectrode to PCR positive amplicons of *N. gonorrhoeae* results in significant decrease in the peak height indicating hybridization. And the incubation of the bioelectrode with the PCR negative amplicons results in negligible change in the peak height indicating specificity of the fabricated sensor for the detection of *N. gonorrhoeae*.

4. Conclusions

Well-assembled PANI–nFe_3O_4–CNT nanocomposite has been prepared using a facile electrochemical polymerization technique. CNT attached with nFe_3O_4 constitute the cores of the novel nanocomposite and PANI acts as the “External covering” that protects the

Table 2
Response characteristics of PANI–nFe_3O_4–CNT nanocomposite based bacterial (*N. gonorrhoeae*) sensor along with those reported in literature.

S. no.	Electrode	Sensing technique	Method of immobilization	Detection limit	Detection range	References
1.	*N. gonorrhoeae* sensor (thssDNA-ZnO/ITO bioelectrode)	Differential pulse Voltammetry (DPV)	Electrostatic interaction	0.14×10^{-15} M	1×10^{-15} M to 1×10^{-6} M	Ansari et al 2009
2.	*N. gonorrhoeae* sensor (thssDNA/Au bioelectrode)	DPV	Thiol-Au bonding	1×10^{-18} M	0.5×10^{-18} M to1.0×10^{-6} M	Singh et al 2010c
3.	*N. gonorrhoeae* sensor (BDNA–Avi–Chit-MWCNT/ITO nanobioelectrode)	DPV	Biotin–avidin coupling	1×10^{-16} M	1×10^{-17} M to 1×10^{-6} M	Singh et al 2010b
4.	*N. gonorrhoeae* sensor (BDNA-Avi/Chit -Fe_3O_4/ITO nanobioelectrode)	DPV	Biotin–avidin coupling	1×10^{-15} M	1×10^{-16} M to 1×10^{-6} M	Singh et al 2011
5.	*N. gonorrhoeae* sensor (BDNA-Avi-nsPANI/ITO nanobioelectrode)	DPV	Biotin–avidin coupling	0.5×10^{-15} M	1×10^{-16} M to 1×10^{-6} M	Singh et al 2009
6.	*N. gonorrhoeae* sensor (aminoDNA-Glu-PANI-CNT/ITO nanobioelectrode)	DPV	Cross-linking (Glutaraldehyde)	1.2×10^{-17} M	1×10^{-17} M to 1×10^{-6} M	Singh et al 2010a
7.	*N. gonorrhoeae* sensor (BDNA-Avi-PANI-nFe_3O_4-CNT/ITO nanobioelectrode)	DPV	Biotin–avidin coupling	1×10^{-19} M	1×10^{-19} M to 1×10^{-6} M	Present work

nanostructures. The nanocomposite has been utilized as a platform for fabrication of ultra-sensitive bacterial (*N. gonorrhoeae*) genosensor. This genosensor can be used to specifically detect up to 1×10^{-19} M of complementary target in the wide detection range of 1×10^{-19} M to 1×10^{-6} M within 45 s of hybridization time at 298 K. This interesting nanocomposite has implications for detection of other important bacterial infectious diseases.

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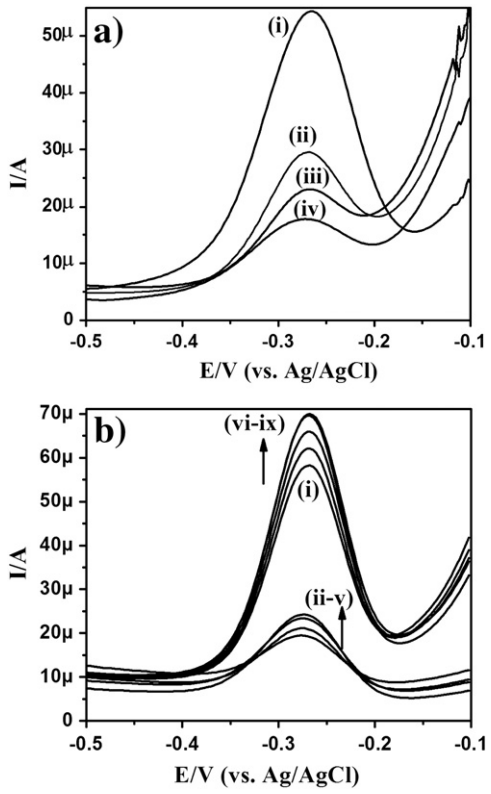

Fig. 5. a) DPV of (i) BDNA-Avi-PANI–nFe_3O_4–CNT/ITO nanobioelectrode after treatment with (ii) culture isolated sample, (iii) spiked pus sample and (iv) patient sample (b) DPV of (i) BDNA-Avi-PANI–nFe_3O_4–CNT/ITO nanobioelectrode after treatment with 4 PCR positive (ii-v) and 4 negative amplicons (vi-ix) at step potential of 3 mV and modulation amplitude of 50 mV, in 20 μM MB, pre-treatment at +0.1 V for 10 s, 0.05 M PBS (pH 7.0, 0.9% NaCl).

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.bioelechem.2012.01.005.

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