Chapter 3 Polypyrrole Nanotubes-Polyaniline Composite for DNA Detection Using Methylene Blue as Hybridization Indicator

3.1 Introduction

Nanostructured CPs (NCP) have attracted much attention in biosensors [211], electronics [212] and energy storage devices [213], because of their unique physicochemical characteristics such as high catalytic, optical and electronic properties that could not be achieved by their bulk counterparts. Recently, NCP are used to enhance the detection sensitivity by creating number of active sites in a controlled manner for immobilizing biomolecules.

PPy is one of the most intensively investigated CP for biosensor applications owing to its stability, conductivity, and biocompatibility [214,215]. Another versatile CP is polyaniline is attracted special attention on the account of homogenous unique redox properties, high electrical conductivity and strong adherence to electrode surface [216-218]. During the preparation procedure for the electrochemical DNA biosensor, the immobilization techniques of the ssDNA probe on the transducer surface concerns several parameters of the sensor such as the stability of the probe-modified surfaces and efficiency of DNA hybridization, because the restricted use of biosensors is due to the difficulties related to the electrochemical behavior of immobilized biomolecules, which dominates its reactivity and electrochemical activity [219,220]. Different immobilization techniques such as physical adsorption [221,9], electrochemical adsorption [222], electrochemical entrapment [223], biotin-avidin coupling [224,225] and covalent immobilization [226] have been utilized.
Compared to these techniques, covalent and biotin-avidin immobilization approaches have been proven to be the effective and sensitive methods for the fabrication of DNA hybridization sensors. But these techniques require labor intensive, expensive chemicals, time consuming and costly coupling reagents to bind the biomolecules with the modified electrode. In order to overcome this disadvantage, the covalent coupling of gluteraldehyde (GA) is used for biomolecules immobilization. Since, GA allows working with label-free targets to minimize background signal and to attain the discrimination of mismatches in the DNA hybridization. Also the covalent linking of biomolecules using linkage reagents such as glutaraldehyde on a transducer is an efficient method of immobilization to result more sensitivity and selectivity.

Methylene blue (MB) is a phenothiazine dye that is commonly used to monitor the hybridization reaction [227,228]. In general there are at least three different interaction modes between MB and DNA. MB can interact with anionic phosphate of DNA by electrostatic binding, the electrostatic and/or intercalation with the major or minor grooves of ds-DNA helix, and preferential binding between MB and guanine bases. The PANi-Fe₃O₄ nanocomposite [229], PANi thin film coated electrode [7] and amine functionalized gold surface [230] have utilized gluteraldehyde (GA) as linker for a effective and easy immobilization of ssDNA on the electrochemically modified electrodes. Currently, many reports are available for the electrochemical detection of DNA hybridization based on different substrate using tedious techniques. However, they have generally used expensive nanomaterials such as MWCNT [231], Au [232], Ag [233] etc., and coupling reagents (EDC/NHS) [234] for DNA immobilization. Furthermore, the construction of nanostructured CP is another issue currently limiting their applications in biosensor.
Normally, hard-template and soft-template approaches are widely employed in the synthesis of CP nanostructures. However, simple, efficient, controlled and large-scale method for preparation of nanostructured CPs are still lacking.

In this chapter, PPy nanotubes were prepared in high yield without consideration of conventional templates and surfactants but by the use of FeCl₃-methyl orange (FeCl₃-MO) reactive self-degraded template. We have fabricated nanotubes of the PPy-PANi by a simple chemical method on the electrode surface. It may be noted that there is no report on the efficiency of covalent attachment of ssDNA on the nanotubical structure is available using gluteraldehyde linker. The PPy nanotubes structure increases the detection selectivity by enhancing the conductivity of the PPy-PANi nanocomposite and providing large active sites for DNA immobilization by gluteraldehyde crosslinker. This results indicate that this approach for label-free electrochemical DNA target detection with the lower detection limit of 50 fM.

3.2 Experimental

3.2.1 Materials

Pyrrrole and aniline monomers were procured from SRL, India. These monomers were distilled under reduced pressure prior to use and stored under inert nitrogen atmosphere. Sodium-saline citrate (SSC) buffer, gluteraldehyde (GA), ammonium peroxydisulfate (APS) and ferric chloride (FeCl₃·3H₂O) were obtained from Sigma-Aldrich and sodium dodecyl sulfate (SDS), methyl orange, methylene blue (MB) were received from Himedia, India. All other chemicals were used without any further purification.
5’-amine modified short chain 15 mer synthetic oligonucleotides were supplied by MWG biotech, Ebersberg, Germany. The sequences of the probe and targets are as follows:

- Capture probe DNA: 5’-NH₂-(CH₂)₆-AGT ACA GTC ATC GCG-3’
- Complementary target DNA: 5’-CGC GAT GAC TGT ACT-3’
- Non-complementary target DNA: 5’-TAT CTA CGT ACA TGT-3’
- Single base mismatch (underlined) target DNA: 5’-CGC GAT G₇C TGT ACT-3’
- Double base mismatch (underlined) target DNA: 5’-CGC CAT G₇C TGT ACT-3’

3.2.2 Instrumentation

Electrochemical measurements were performed using a CHI 6005D & 1022A electrochemical workstation (CH Instruments, Austin, USA). The electrochemical cell of 10 mL with a three electrode system containing gold working electrode (0.03 cm²), Ag/AgCl (3M KCl) reference electrode and platinum wire auxiliary electrode, respectively. The electrolytic cell was deaerated with high-purity nitrogen to remove the oxygen from the solution during all the measurements. Prior to the DNA immobilization, gold electrode was polished with alumina slurry, rinsed with double distilled (DD) water, and sonicated using ethanol to remove the bound particulates. CV was performed in the potential window - 0.2 and 0.7 V at a scan rate 50 mV/s in PBS (pH 7.0) containing 1.0 mM [Fe(CN)₆]³⁻/⁴⁻. EIS was performed in buffer containing 1mM [Fe(CN)₆]³⁻/⁴⁻ in the frequency range 100 KHz - 1 Hz. The impedance data are obtained in Nyquist plots and charge transfer resistance (R_CT Ω
cm$^{-2}$) values using Randel’s equivalent circuit, $R_s$ ($Q_{dl}$ ($R_{CT}W$)) by simulation of Zsimwin software. DPV was performed in the potential region from 0.0 to -0.50 V, amplitude 0.05 V, step potential 0.07 V, and scan rate 0.02 V/s. Conductivity of the samples were measured by four-probe method using Keithley (USA) nanovoltmeter after pressing the samples into 1 cm dia, 1.5 mm thick pellets under 4 ton pressure. Thermogravimetric analysis (TGA) experiments were performed with a TA instrument model SDT Q600 via heating under air at 20 °C/min. SEM images were obtained using a JSM-6390 (JEOL, Tokyo, Japan) at an accelerating voltage 20 kV. The FT-IR measurements were carried out using a nexus-670 instrument.

### 3.2.3 Synthesis of PPy-PANi Composite Film

The PPy nanotubes were prepared according to earlier reported procedure [235]. In brief, 0.5 g FeCl$_3$ and 5 mM methyl orange solution (60 mL) was stirred for 30 minutes. Then 210 µL pyrrole monomer was slowly added into the above solution in 10 minutes and maintained for 36 hours at -5°C. The black precipitate was filtered, purified with DD water, ethanol and dried for 12 hours at 45°C. The PPy nanotubes (20 % weight) were then dispersed in 1M HCl (45 mL), ultrasonicated 1 hour and transferred into an ice bath. Subsequently, aniline monomer (80 % weight) and oxidizing agents 0.4 g APS in 5 mL 1 M HCl were added to form PPy-PANi nanocomposite. The conventional PPy-PANi was also prepared in the same manner but without the addition of methyl orange (MO) into the reaction mixture.

### 3.2.4 DNA Probe Immobilization and Hybridization

The PPy-PANi modified electrode was kept in 1 % GA for 4 hours at room temperature [7]. The GA treated PPy-PANi film was washed with DD water and then 5’-NH$_2$-ssDNA (10 µL of 1µM) were immobilized onto PPy-PANi film and
incubated for about 10 hours at 5°C, then washed with 0.1% SDS PBS (pH 7.0) to remove unbound probe DNA (Figure 3.1). Hybridization reaction was performed by immersing the probe DNA modified electrode in a hybridization solution (4×SSC buffer) containing different concentrations of target DNA for 40 minutes at room temperature. After that, the electrode was rinsed with a 0.1% SDS PBS to remove the non-hybridized target DNA. Then the hybridized electrode was placed in the methylene blue (50 µM) in PBS (pH 7.0) for 15 minutes and washed with PBS to remove the physically adsorbed molecules.

Figure 3.1 Fabrication procedure of this electrochemical DNA biosensor. (A) Bare Au, (B) A + PPy-PANi nanotubes, (C) B + GA, (D) C + ssDNA, (E) D + non-complementary and (F) D + complementary target.
Chapter 3

3.3 Results and Discussion

3.3.1 Surface Characterization of the PPy-PANi Composite

The SEM (Figure 3.2 A) shows the cylindrical morphology of the PPy nanotubes with diameter 150 nm and after the coating of PANi, the diameter appeared to be 500 nm (Figure 3.2 B). This confirms the deposition of PANi on the PPy nanotubes. This assumption was also supported by the literature [235].

Figure 3.2 SEM images of (A) PPy, (B) PPy-PANi and (C) PPy-PANi-GA-ssDNA on gold coated silicon chip.
The immobilization of ssDNA shrinks and deforms the structure of the composite and destabilized the nanotubical structure and hence resulted in decreased conductivity, as confirmed by CV and FT-IR (Figure 3.2 C). Further, the conventional bulky PPy-PANi composite have been prepared under identical condition (without addition of MO) for comparative study with nanostructured PPy-PANi nanocomposite. The surface morphology of the prepared bulky PPy-PANi nanocomposite is shown in Figure 3.3 (A and B) which shows the granular morphology observed.

![Figure 3.3 SEM images of conventional (A) PPy and (B) PPy-PANi on gold coated silicon chip.](image)

The FT-IR spectra of PPy, PANi and PPy-PANi composite (with KBr powder pellets) were recorded (Figure 3.4). The main characteristic peaks of PPy show various vibrational frequencies at 1545 and 1440 cm\(^{-1}\) (pyrrole ring), 1300 cm\(^{-1}\) (\(=\)C-H in plane deformation), 1150 and 970 cm\(^{-1}\) (C-N) stretching of the pyrrole ring. The peak represent at 1577 cm\(^{-1}\) (C-C stretching), 1488 cm\(^{-1}\) (C=C) stretching,
1300 cm\(^{-1}\) (C-N stretching) and 1101 cm\(^{-1}\) (N=Q=N) of PANi respectively. These results are in agreement with earlier reports [235-237].

Figure 3.4 FT-IR spectra of (a) PPy, (b) PANi and (c) PPy-PANi recorded as KBr pellets.

The PPy-PANi (Figure 3.4 (curve c) and Figure 3.5 (curve b)) showed similar FT-IR pattern in terms of the peak intensities and position ascertaining the effective interaction between the PPy and PANi (1569, 1496, 1300, 1142 and 804 cm\(^{-1}\)). After the DNA immobilization (Figure 3.5 (curve c)) on GA treated PPy-PANi composite film the new vibration bands from the GA and DNA corresponding to C=O group of aldehyde, primary, secondary alcohol of GA 1739, 1054 and 1134 cm\(^{-1}\) are obtained.
respectively. Further, 1276 cm$^{-1}$ (cytosine), 1513 cm$^{-1}$ (adenine), 1739 cm$^{-1}$ (G-C base pair) and 820 cm$^{-1}$ (O-P-O) vibrational frequencies in DNA are also noticed [238-242]. These stretching frequencies confirmed the effective immobilization of ssDNA on the GA activated PPy-PANi film.

Figure 3.5 FT-IR spectra of (a) PPy, (b) PPy-PANi and (c) PPy-PANi-GA-ssDNA on gold coated silicon chip.

Figure 3.6 shows the Raman spectra of PPy (curve a) and PPy-PANi (curve b). The main characteristic peak of PPy shows broad peak at 1200-1600 cm$^{-1}$ (N-H and C=C stretching in pyrrole ring) [243]. The PPy-PANi (curve b) shows similar the
Raman spectra of PPy with few additional peaks. These findings show that polyaniline uniformly coated on the PPy nanotubes [244].

![Figure 3.6 Raman spectra of (a) PPy and (b) PPy-PANi.](image)

Thermogravimetric curves for nanostructured PPy-PANi (curve a) and conventional bulky PPy-PANi (curve b) composites are given in Figure 3.7. Both the PPy-PANi composite show three stages of thermal transition leading to weight losses. First thermal transition is related to removal of dopants [245]. The second thermal transition corresponds to the loss of low molecular weight oligomers or side products (hydroquinone or quinine) and the final transition is due to the degradation of backbone units of PPy-PANi. The TGA profile suggested that the decomposition of the materials started at 251°C and 227°C for nanostructured PPy-PANi and bulky...
Figure 3.7 TGA curves for (a) nanostructured PPy-PANi and (b) conventional bulky PPy-PANi.

PPy-PANi, respectively, with an initial loss of moisture around 100°C. The weight-loss of nanostructured PPy-PANi (~35%) and bulky PPy-PANi (~45%) has been observed in the transition temperature between 250°C and 450°C due to the loss of the dopant in the polymer composite. These result confirmed that the nanotubical structure of PPy-PANi composite has better thermal stability than conventional bulky PPy-PANi composite. Furthermore, the nanotubical structure of PPy-PANi shows a compact structure with increased thermal stability.
3.3.2 Electrochemical Characterization of PPy-PANi Film in 1M HCl

The CV behaviour of PPy-PANi nanocomposite has been characterized in 1M HCl. Two pairs of redox peaks due to the conversion of insulating leucoemeraldine to conducting protonated emeraldine and then conducting emeraldine to insulating pernigraniline in the potential region of -0.2 to 0.8V is observed for PPy-PANi composite modified Au electrode surface (Figure 3.8).

Figure 3.8 Cyclic voltammogram of nanostructured PPy-PANi composite modified Au electrode for continuous 15 cycles at a scan rate of 50 mV/s in the potential range between -0.2 to 0.8 V in 1M HCl.

It may be noted that the stability of the nanostructured PPy-PANi composite film for 15 continuous cycles showed only 6 % loss (Figure 3.8) from its initial value, whereas the conventional PPy-PANi almost 20 % loss (Figure 3.9) after 15 cycles. It is clearly indicated that the nanostructured PPy-PANi composite film showed a good
reversible oxidation wave with greater stability compared to that of conventional bulky PPy-PANi composite. Further, the linear dependence of the oxidation peak current on the scan rate indicated that the oxidation reaction was controlled by the charge transfer rather than by diffusion step (Figure 3.10). In addition, the four probe conductivity measurement suggested that the superior conductivity for nanotubical structure of the PPy-PANi (0.7237 S/cm) composite when compared to the conductivity value of conventional bulky PPy-PANi (1.592 ×10^{-3} S/cm) composite. The nanostructure of PPy-PANi composite is nearly 472 times higher conductivity than the conventional PPy-PANi composite.

Figure 3.9 Cyclic voltammogram of conventional bulky PPy-PANi composite modified Au electrode for continuous 15 cycles at a scan rate of 50 mV/s in the potential range between -0.2 to 0.8 V in 1M HCl.
Figure 3.10 Cyclic voltammogram of PPy-PANi nanotubes modified Au electrode and cycled in the potential range between -0.2 and 0.8 V in 1M HCl at a scan rate between 10 and 100 mV/s (in 10 mV/s increment steps) Inset: shows the peak current vs. square root of the scan rate.

3.3.3 Electrochemical Characterization of PPy-PANi Nanotubes in Phosphate Buffer Solution

Figure 3.11 shows the results of the electrochemical characterization of PPy-PANi (curve a), PPy-PANi-GA (curve b) and PPy-PANi-ssDNA (curve c) in PB (pH 7.0) solution. The broad peak at about 0.272 V (curve a) arose due to the merger of the peaks responsible for the oxidation of aniline to the first radical cation (phenazine...
rings) and then the oxidation of the radical cation to the radical dication of the PPy-PANi modified electrode [246]. It can be seen that there is a considerable decrease in the intensity of these redox peaks after gluteraldehyde activation, confirming the binding of aldehyde moieties onto the PPy-PANi surface (curve b). In addition, the observed potential shift and enhancement in redox peaks after DNA immobilization is attributed to the binding of ssDNA probes onto the gluteraldehyde activated PPy-PANi surface (curve c) [247].

Figure 3.11 Cyclic voltammogram of (a) PPy-PANi, (b) PPy-PANi-GA and (c) PPy-PANi-GA-ssDNA modified Au electrode in the potential range between -0.2 to 0.8 V at a scan rate of 30 mV/s in 0.1 M PB (pH 7.0) solution.
3.3.4 CV and EIS Characterization of PPy-PANi Modified Electrode in PB (pH 7.0) Solution Containing 1mM [Fe(CN)₆]³⁻/⁴⁻

Figure 3.12 shows the results of CV behaviour of the modified electrodes in the presence of the 1mM [Fe(CN)₆]³⁻/⁴⁻ in 0.1 M PBS (pH 7.0) at a scan rate 50 mV/s.

Figure 3.12 CV behaviour of the modified Au electrode measured in presence of 1mM [Fe(CN)₆]³⁻/⁴⁻ in 0.1 M PBS (pH 7.0) at a scan rate of 50 mV/s, in the potential range between -0.2 to 0.7 V. Curves a: bare Au, curve b: PPy-PANi nanotubes, curve c: b + GA, curve d: c + ssDNA and curve e: d + complementary DNA.

Deposition of the PPy-PANi onto the Au electrode surface decrease the reversibility and small change in peak current of [Fe (CN)₆]³⁻/⁴⁻ (Iₚa: 9.0 µA & ΔEₚ (Eₚa-Eₚc): 120
mV (curve b)) compared to the unmodified bare Au electrode ($I_{pa} : 8.40 \mu A$ and $\Delta E_p : 80 \text{ mV (curve a)}) due to the barrier properties of the PPy-PANi film. It can be seen that a considerable decrease in redox peak current after the activation of GA onto the PPy-PANi film ($I_{pa} : 8.0 \mu A$ and $\Delta E_p : 260 \text{ mV (curve c)})}, Table 3.1, confirming the effective attachment of GA on PPy-PANi film. After the immobilization of ssDNA on PPy-PANi-GA, the reversibility of the $[\text{Fe(CN)}_6]^{3/-4}$ redox reaction is further decreased ($I_{pa} : 5.5 \mu A$ and $\Delta E_p : 270 \text{ mV (curve d)}) due to electrostatic repulsion between the negatively charged ssDNA and $[\text{Fe(CN)}_6]^{3/-4}$. In addition, the ssDNA modified surface hybridized with fully complementary target DNA increases two times the negative charge density and decreases the reversibility of the $[\text{Fe(CN)}_6]^{3/-4}$ redox reaction ($I_{pa} : 5.3 \mu A$ and $\Delta E_p : 460 \text{ mV (curve e)})}.

Table 3.1 Cyclic voltammetry parameters of the modified Au electrodes in PB (pH 7.0) solution containing 1mM $[\text{Fe(CN)}_6]^{3/-4}$.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$E_{pa}$ (V)</th>
<th>$E_{pe}$ (V)</th>
<th>$\Delta E_p$ (V)</th>
<th>$I_{pa}$ (A)</th>
<th>$I_{pe}$ (A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bare Au</td>
<td>0.24</td>
<td>0.16</td>
<td>80</td>
<td>$8.40 \times 10^{-6}$</td>
<td>$8.66 \times 10^{-6}$</td>
</tr>
<tr>
<td>PPy-PANi</td>
<td>0.26</td>
<td>0.14</td>
<td>120</td>
<td>$9.04 \times 10^{-6}$</td>
<td>$8.48 \times 10^{-6}$</td>
</tr>
<tr>
<td>PPy-PANi-GA</td>
<td>0.29</td>
<td>0.03</td>
<td>260</td>
<td>$8.40 \times 10^{-6}$</td>
<td>$7.67 \times 10^{-6}$</td>
</tr>
<tr>
<td>PPy-PANi-ssDNA</td>
<td>0.30</td>
<td>0.03</td>
<td>270</td>
<td>$5.50 \times 10^{-6}$</td>
<td>$5.41 \times 10^{-6}$</td>
</tr>
<tr>
<td>PPy-PANi-dsDNA (com)</td>
<td>0.43</td>
<td>-0.03</td>
<td>460</td>
<td>$5.38 \times 10^{-6}$</td>
<td>$6.28 \times 10^{-6}$</td>
</tr>
</tbody>
</table>
Further, the charge transport properties of the composite film modified electrodes were characterized by monitoring charge transfer resistance ($R_{CT}$) at the electrode/electrolyte interface. Figure 3.13 shows the EIS behaviour of the modified Au electrode in presence of 1mM [Fe(CN)$_6$]$^{3/-4-}$ in PBS at frequency 100 kHz - 1Hz.

Figure 3.13 EIS behaviour of the modified Au electrode measured by impedance in the frequency 100 kHz to 1 Hz, DC potential: 230 mV, AC potential ± 5 mV in presence of [Fe(CN)$_6$]$^{3/-4-}$ in PBS (pH 7.0) Curve a : Bare Au, curve b : PPy-PANi , curve c : b + GA, curve d : c + ssDNA and curve e : d + complementary DNA.

The $R_{CT}$ value of the bare GC (curve a), PPy-PANi (curve b) and PPy-PANi-GA (curve c) electrodes were 1078, 2618 and 6677 $\Omega$ cm$^2$, respectively. The $R_{CT}$ value of PPy-PANi-GA is higher than that of PPy-PANi and bare GC electrode due to the activation of non conducting GA on PPy-PANi surface. Further, the immobilization
of ssDNA on PPy-PANi-GA surface the $R_{CT}$ value (12690 $\Omega$ cm$^{-2}$, (curve d)) is nearly 2 fold higher than the PPy-PANi-GA (6677 $\Omega$ cm$^{-2}$ (curve c)) modified surface due to the negatively charged ssDNA repel the negatively charged $[\text{Fe(CN)}_6]^{3/-4-}$ at the electrode/electrolyte interface [248,249]. Similarly, hybridization with complementary target DNA increases two times the negatively charge density and enhancing the $R_{CT}$ value to 15770 $\Omega$ cm$^{-2}$ (Table 3.2). These results clearly indicate the controlled immobilization of ssDNA on high active sites of the PPy-PANi nanotube surface and it provides efficient coiling of target DNA to improve the hybridization efficiency.

Tabel 3.2 Electrochemical impedance spectroscopy parameters of the modified Au electrodes in PB (pH 7.0) solution containing 1mM $[\text{Fe(CN)}_6]^{3/-4-}$.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>EIS parameters</th>
<th></th>
<th></th>
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<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Chi Square ($(\chi^2)$)</td>
<td>$R_e/\Omega$</td>
<td>$Q_{dl}/F$ cm$^{-2}$</td>
<td>n</td>
<td>$R_{CT}/\Omega$</td>
<td>Warburg</td>
</tr>
<tr>
<td>Bare Au</td>
<td>9.97 x 10$^{-4}$</td>
<td>154</td>
<td>2.83 x 10$^{-6}$</td>
<td>0.85</td>
<td>1078</td>
<td>1.25 x 10$^{-4}$</td>
</tr>
<tr>
<td>PPy-PANi</td>
<td>1.33 x 10$^{-3}$</td>
<td>159</td>
<td>2.25 x 10$^{-6}$</td>
<td>0.87</td>
<td>2618</td>
<td>1.44 x 10$^{-4}$</td>
</tr>
<tr>
<td>PPy-PANi-GA</td>
<td>1.05 x 10$^{-3}$</td>
<td>155</td>
<td>1.75 x 10$^{-6}$</td>
<td>0.88</td>
<td>6677</td>
<td>1.46 x 10$^{-4}$</td>
</tr>
<tr>
<td>PPy-PANi-ssDNA</td>
<td>7.75 x 10$^{-4}$</td>
<td>156</td>
<td>1.65 x 10$^{-6}$</td>
<td>0.91</td>
<td>12690</td>
<td>1.33 x 10$^{-4}$</td>
</tr>
<tr>
<td>PPy-PANi-dsDNA (com)</td>
<td>8.16 x 10$^{-4}$</td>
<td>158</td>
<td>1.57 x 10$^{-6}$</td>
<td>0.91</td>
<td>15770</td>
<td>1.29 x 10$^{-4}$</td>
</tr>
</tbody>
</table>
3.3.5 Differential Pulse Voltammetric DNA Hybridization Detection using Methylene Blue

Figure 3.14 shows DPV reduction signal of the MB responses for different modified electrodes immobilized with ssDNA probe. The experiment has been made as follows. The surface of the modified electrodes were washed with SDS, incubating in MB (50 µM) solution for 15 minutes without applying any potential and finally washed with PB (pH 7.0) solution to remove the non specific adsorbed MB [250]. The accumulated MB normalized reduction peak current ($\Delta I_{pc}$) values observed after the hybridization of the complementary (curve b), non-complementary (curve c), single base mismatched (SMM) (curve d) and double base mismatched (DMM) (curve e) target sequences are 3.4, 0.2, 2.1 and 1.1 µA, respectively.

It is clear that the sensor discriminates the hybridized and unhybridized surfaces by indicating 95 % reduction peak current ($\Delta I_{pc}$) changes between ssDNA and dsDNA due to the intercalative binding take place between the duplex of the dsDNA and small molecules of the MB. The peak current with solely ssDNA immobilized electrode observed was 2.6 µA while the non-complementary target DNA (2.8 µA) which indicated the nonspecific adsorption was negligible. Similarly, after the ssDNA modified electrode was hybridized with single (62 %) and double (32 %) base mismatched target sequences, the decrease of peak current values of MB were smaller than that obtained from the hybridization with the complementary target sequence due to less number of dsDNA formed on the ssDNA modified electrode surface. Thus MB could be used as good electrochemical indicator to discriminate the ssDNA, dsDNA and mismatches by our easy fabrication strategy. This observation was in agreement with the results of the investigation carried out by Hejazi et al. [250]
for peptide nucleic acid (PNA) assembled on Au surface. These data clearly indicate that the PPy-PANi nanotubical structures enhances the conductivity and acts as a good platform for the sensitive and selective discrimination of the completely complementary, non-complementary, single and double base mismatched target DNA sequences in presence of the intercalated MB. These experiments were done at $1 \times 10^{-9}$ M target concentration.

Figure 3.14 DPV of intercalated MB reduction peak current at (a) PPy-PANi-GA-ssDNA, (b) PPy-PANi-GA-dsDNA (com), (c) PPy-PANi-GA-dsDNA (non-com), (d) PPy-PANi-GA-dsDNA (SMM) and (e) PPy-PANi-GA-dsDNA (DMM). Inset: Corresponding bar diagram of normalized change in $I_{pc}$. 

83
Figure 3.15 shows the effect of varying the complementary target DNA concentration on the DPV responses of the PPy-PANi-GA-ssDNA. Inset Figure 3.15 shows the variation of the $I_{pc}$ obtained after target hybridization at each concentration with log concentration of DNA. For these experiments, the hybridized surface was dehybridized by rinsing with hot (90°C) ultrapure water for 3 minutes followed by rapid cooling in an ice bath and re-hybridized with the different target DNA concentration.

Figure 3.15 DPV detection of different target concentration using the PPy-PANi-GA-ssDNA (a) 0, (b) $1.0 \times 10^{-13}$, (c) $1.0 \times 10^{-12}$, (d) $1.0 \times 10^{-11}$, (e) $1.0 \times 10^{-10}$ and (f) $1.0 \times 10^{-09}$ M. Inset shows the resulting logarithmic calibration plot.
The sensor showed a linear range between $1 \times 10^{-13}$ and $1 \times 10^{-09}$ M with a regression coefficient 0.9959, the lower detection limit noticed was $5.0 \times 10^{-14}$ M which is lower than the previous reported values such as AuNP/MWCNT modified gold electrode ($4.3 \times 10^{-13}$ M) [231], AuNP/HDT modified gold electrode ($1.0 \times 10^{-12}$ M) [232] and AgNP/MWCNT modified glassy carbon electrode ($6.4 \times 10^{-13}$ M) [233]. Besides, these sensors required costly nanomaterials like CNT, AgNP and AuNP whereas our sensor fabrication is very simple and cost effective than the reported results.

The higher sensitivity indicates the lower target DNA concentration of $1 \times 10^{-09}$ M is sufficient to saturate the ssDNA immobilized on the PPy-PANi-GA-ssDNA modified electrode. In addition, high surface area, good conductivity and excellent porosity exhibited a measurable current even for low concentration of DNA. In order to evaluate the stability of the biosensor, repeated hybridization and dehybridization process were made using $1 \times 10^{-09}$ M target concentration on the same PPy-PANi-GA-ssDNA surface and the DPV studies were also made. The results showed a constant $I_{pc}$ for the five consecutive measurements with relative standard deviation of 3.5 %. The stability of PPy-PANi-GA-ssDNA investigated after 7 days storage at 4°C and further used to hybridize with the target ssDNA sequence, 98.6 % of the initial sensitivity remained (Figure 3.16).
Figure 3.16 DPV response of PPy-PANi-GA-ssDNA modified electrode (a and c) and hybridized with complementary target DNA (b and d) measured in PB (pH 7.0) solution.

To ascertain the reproducibility of the results, three different GC electrodes were modified with the PPy-PANi-GA-ssDNA and their response towards the hybridization of $1 \times 10^9$ M target DNA was tested (Figure 3.17). The DPV peak current obtained in the measurement of three independent electrodes (5.9, 5.4 and 5.3 µA) showed a relative standard deviation of 5.81 %, confirming that the results are reproducible. The above results showed that the present modified electrode is very much stable and reproducible towards the recognition of target DNA. These results indicate that the combination of biopolymer (PPy-PANi) can be a good platform to construct the biosensor with increased sensitivity and lower detection limit.
Further, our sensor fabrication is very simple and cost effective than the PPy/MWCNT/AuNP and MWCNT/AgNP reported sensors [251, 252].

Figure 3.17 DPV responses of PPy-PANi-GA-ssDNA modified three independent electrodes hybridized with complementary target DNA, measured in PB (pH 7.0) solution.

3.4 Conclusion

The proposed electrochemical sensor has been fabricated using the of PPy-PANi nanotubes prepared by simple chemical method and drop casted on the Au transducer, which showed good stability as indicated by electrochemical measurements and also confirmed by SEM analysis. The fabricated PPy-PANi-GA-ssDNA electrode exhibited a better electrochemical performance (472 times greater conductivity and detection limit of 50 fM) in label-free electrochemical DNA
hybridization detection, which is comparable with the literature reports. This is further evidenced by the 95% of difference in MB reduction peak current between ssDNA and complementary dsDNA. The stability measurements suggest that the loss of activity after five consecutive measurements is noticed. This is due to the fact that the ssDNA on the PPy-PANi-GA film might undergo conformational changes before and after hybridization. In order to improve the stability and reproducibility, we have changed the probe ssDNA immobilization method. Because the method used to immobilize probe ssDNA on a transducer surface play an important role to obtain higher sensitivity, reproducibility and stability. For this purpose, we have used simple and efficient gold-thiol (Au-S) interaction for anchoring the probe ssDNA onto PPy-PANi-AuNP nanocomposite film modified gold electrode have been investigated in chapter 4.