Chapter 5 Polypyrrole-Poly(3,4-ethylenedioxythiophene)-Silver Nanoparticles (PPy-PEDOT-AgNP) Nanocomposite for Label Free Electrochemical DNA Hybridization Sensing

5.1 Introduction

Wide-scale sequence specific detection require the development of easy to use, fast, miniaturized analytical devices and there is a high demand for faster, cheaper nucleic acid assay that would full-fill the need of modern diagnostics and biomedical research. The sequence-specific detection of DNA targets (for genetic or pathogenic diseases) is receiving consideration attention in molecular diagnostics [287-293]. Many methods for hybridization detection have been developed including electrochemical [40,294], optical [295,296] and acoustic [297] and so on. Compared with other techniques the advantages of electrochemical techniques are low cost, small size and miniaturization. However, the main disadvantages are their low sensitivity thus improving their sensitivity has become one of the main issues in the development of electrochemical DNA sensors. In recen years, the development of nanomaterials for ultra-sensitive detection for biological species has received great attention because of their unique optical, electronic and chemical properties. In the direction of fabrication of highly sensitive and selective transducers materials like nanogold [58-60], quantum dots [61,62], carbon nanotubes [298,65], graphene [299,70] and conducting polymers [72,73] have been used to prepared the nanostructures for DNA sensing. Among them, conducting polymers are well-known functional materials for biosensing applications due to their unique properties. Particularly, the nanosturcted conducting polymers such as nanowire, nanotubes,
micro-structured films currently demonstrated to improve the sensitivity of the sensors. The nanostructured CPs could greatly improved diffusion since they have much greater exposed surface area as well as much penetration depth compared to their bulk counterparts.

Generally, two different immobilization techniques of biomolecules are followed for the polymer surfaces. First method involves the non-covalent doping of DNA by physical adsorption. But in this method, the capture probes are less accessible for hybridization as they are imperfectly oriented in the polymer matrix. In the second method involves the covalent binding of modified (-NH₂, -COOH, -SH etc.) DNA on the functional groups substituted CP matrix using coupling reagents such as 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and N-hydroxy succinimide (EDC/NHS) [248]. Unfortunately, the later one involves complex chemistry for the functionalization of the native monomer. Polymer nanocomposite materials have attracted due to their synergetic effect and hybrid properties derived from several compounds. The major advantages of this biosensor are: (i) we can avoid functionalization steps on the native monomer. (ii) The noble metal nanoparticles (Au & Ag) provide a stable platform for the immobilization of biomolecules that retain their bioactivities for the fabrication of biosensors. (iii) This CP-metal nanocomposite has good conductivity and excellent porosity leading to recordable currents even for low concentration of DNA. Recently, CP metal nanocomposites such as MWCNT-PPy-AuNP, PANi-AuNP and PEDOT-AuNP are used for DNA sensing applications [231,279,301]. However, in these work, researchers have generally used complex nanomaterials preparation and DNA immobilization techniques. For example, Spain et al. [301] recently developed DNA sensor based on PEDOT-AuNP composite
prepared by vapour phase polymerization technique. In addition, in this work horseradish peroxidase (HRP) enzyme tagged probe DNA was used to improve the sensitivity and selectivity. This enzyme tagged method has shortcoming arising from limited tagging efficiency and complex multistep analysis.

Recently, Ag nanoparticles are extensively used in DNA hybridization sensing for amplification of hybridization owing to their catalytic properties and also exhibit the highest electrical and thermal conductivity among all the metals. Also, the Ag nanoparticles demonstrate many advantages when compared to Au nanoparticles, such as higher extinction coefficients, sharper extinction bands and higher field enhancement. Many techniques including chemical reduction in aqueous solution with or without stabilizing agent, thermal decomposition in organic solvents, chemical, photochemical and microwave irradiation have been employed in the synthesis of Ag nanoparticles. However, these methods are obviously involving complicated with high energy consuming and sophisticated techniques.

In the present work, Ag nanoparticles functionalized PPy-PEDOT nanotubes are prepared by simple method without using any expensive capping or reducing agent and the time required for synthesis is around 2 hours only. Further, the Ag nanoparticles have been uniformly deposited along the walls of the nanotubes. Subsequently, the HS-ssDNA are immobilized onto the Ag nanoparticles [233,302] and used for the label-free DNA hybridization sensing in phosphate buffer for the first time. The sensor behaviour is characterized by cyclic voltammetry and impedance studies. The morphological change of the modified electrode surfaces are examined by SEM, Raman and FT-IR techniques.
5.2 Experimental

5.2.1 Materials

Pyrrole monomer and silver nitrate (AgNO₃) were procured from SRL, India. The pyrrole monomer was distilled under reduced pressure prior to use and stored under nitrogen. EDOT, sodium-saline citrate (SSC) buffer, 6-mercaptohexanol (MCH) and ferric chloride (FeCl₃) were used as received from Sigma-Aldrich and sodium dodecyl sulfate (SDS), methyl orange (MO) were purchased from Himedia. All other reagents were and used without further any purification.

Thiolated short chain 27 mer synthetic oligonucleotides were procured from MWG biotech, Ebersberg, Germany with HPLC purification. Their base sequences are as below:

- Capture probe DNA: 5’-HS-(CH₂)₆- CGA T CTG TTT TAT GTA GGG TTA GGT CA-3’ (I)
- Complementary target to I : 5’-TG ACC TAA CCC TAC ATA AAA CAG-3’(II)
- Non complementary target to I: 5’-TAC CATTCT CAT CTC TGA AAA CTT CCG-3’ (III)
- Single base mismatch (underlined) target to I: TGA CCT AAC CCC ACA TAA AAC AG-3’(IV)
- Double base mismatch (underlined) target to I: TGA CCT CAC CCC ACA TAA AAC AG-3’(V)

5.2.2 Instrumentation

The CV and EIS measurements were carried out on a CHI6005D electrochemical workstation (CH Instruments, Austin, USA), which was in
connection with a glassy carbon (GC) working electrode (0.07 cm²), a Ag/AgCl (3M KCl) reference electrode and a platinum wire auxiliary electrode was used. All the solutions were deaerated with inert nitrogen gas during experiments. Prior to the sensor fabrication, the working electrode surface was polished with 1, 0.5, 0.05 µm alumina slurry, rinsed with DD water and cleaned ultrasonically using ethanol. After cleaning, the polished GC electrode was subjected to the following modifications. CVs were recorded between a potential window -0.2 and 0.7 V at a scan rate 50 mVs⁻¹ and EIS measurements were taken at amplitude 5 mV over the dc potential of 200 mV. SEM was carried out using a TESCAN (VEDA3) at an accelerating voltage 15 kV, X-ray diffraction patterns of the samples were recorded using a Bruker AXS D8 advanced diffractometer with Cu Kα radiation (λ = 1.5406 Å), Raman spectroscopy was recorded on STR spectrometer (Japan) with 514.5 nm laser excitation and FT-IR spectra were recorded using nexus-670 Spectrometer from Thermo Nicolet.

5.2.3 Synthesis of PPy-PEDOT-AgNP Nanocomposite

5.2.3.1 Chemical Synthesis of Polypyrrole-Poly(3,4-ethylenedioxythiophene) (PPy-PEDOT) Nanotubes

Preparation of PPy nanotubes was performed as described in chapter 3 section 3.2.3. The average size of the PPy nanotube is approximately 170 nm as seen in SEM (Figure 5.1 A). The PPy nanotubes (50 % weight) were dissolved in 1M HCl (45 mL), ultrasonicated 1 hour and transferred into an ice bath. To this, EDOT monomer (50 % weight) was added and oxidized using appropriate amount of FeCl₃ to form PPy-PEDOT nanotubes. Thus the PEDOT coated PPy nanotubes diameter increased to 350 nm as depicted in SEM, Figure 5.1 B.
5.2.3.2 Functionalization of Silver Nanoparticles (AgNP) on the PPy-PEDOT Composite Film

Synthesis of PPy-PEDOT-AgNP composite was prepared according to the simple procedure reported by Zhang [303]. Freshly synthesized PPy-PEDOT nanotubes (10 mg) were added to 20 mL vial containing 10 mL of an aqueous 0.01 M AgNO₃ solution. After 2 hour the PPy-PEDOT-AgNP composite was centrifuged, washed with de-ionized water, ethanol and dried under vacuum oven for 12 hours at 40°C. The PPy-PEDOT nanotubes react with AgNO₃, yielding Ag spheres uniformly distributed along the walls of the nanotubes (Figure 5.1 C).

Figure 5.1 Typical SEM images of (A) PPy, (B) PPy-PEDOT and (C) PPy-PEDOT-AgNP.
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It may be noted that the Ag nanoparticles are well dispersed on the walls of the nanotubes without using any capping or reducing agents and the nanoparticles are well separated from each other providing enough space between each HS-ssDNA immobilized on the polymer film. This behaviour is very essential to immobilize the capture probe with enough space for efficient coiling of target DNA to improve the hybridization efficiency.

Figure 5.2 Schematic illustration of HS-ssDNA covalently immobilized onto the PPy PEDOT-AgNP nanocomposite by the Ag-thiol interaction at room temperature.
5.2.3.3. Immobilization of DNA Probe and Hybridization

The immobilization of thiolated probe DNA onto the PPy-PEDOT-AgNP composite electrode was performed with 10 μL of 1 μM capture probe for 4 hours at room temperature (Figure 5.2). Then the modified surface was washed with 1 wt% SDS in PB (pH 7.0) solution to eliminate the non-specifically adsorbed probe DNA molecule on the electrode surface. Subsequently, the ssDNA modified electrode subjected to MCH treatment of 1mM solution for 1 hour to further eliminate the non-specifically adsorbed DNA molecules and to hold a good orientation of probe DNA for its good recognition ability. Then the hybridization experiments were performed by immersing the PPy-PEDOT-Ag-S-ssDNA surface into the solution containing 1.0 × 10^{-11} M target DNA in 4 × SSC buffer for 1.5 hour at room temperature and washed with the PB (pH 7.0) solution to remove the unreacted target DNA.

5.3. Results and Discussion

5.3.1 FT-IR, Raman, XRD and UV-Vis Characterizations of PPy-PEDOT-AgNP Nanocomposite

The FT-IR spectra of PPy, PEDOT, PPy-PEDOT and PPy-PEDOT-AgNP composites are presented in Figure 5.3. The main characteristic peaks of PPy were assigned as follows: 3429 cm^{-1} (N-H stretching), 1524 cm^{-1} (C=C and C-H stretching), 1440 cm^{-1} (N-H stretching) and 1280 cm^{-1} (C-H & N-H deformation) in the pyrrole ring. In the FT-IR spectra of PEDOT, peaks seen at 1543 and 1311 cm^{-1} (C=C and C-C in the thiophene ring), 905 cm^{-1} (C-S bond), 668 cm^{-1} (C-S-C stretching) in the thiophene ring and 905 cm^{-1} (ethylenedioxy ring deformation) were in agreement with the literature reports [304,305]. After coating of PEDOT over PPy
nanotubes, a strong interaction between PPy and PEDOT is observed in terms of difference in the peak intensity and position (Figure 5.3, curve c) (1543, 1328, 1176, 1021, 918 and 670 cm$^{-1}$). Deposition of Ag nanoparticles on the PPy-PEDOT composite observed shift in the peak intensity and position at 905 cm$^{-1}$ (ethylenedioxy ring deformation), 670 cm$^{-1}$ (C-S-C stretching) and 1553 and 1301 cm$^{-1}$ (C=C and C-C in the thiophene ring) (Figure 5.3, curve d) indicating the efficient dispersion of the Ag nanoparticles onto the PPy-PEDOT composite and their interaction with the functional groups of the polymer composite.

Figure 5.3 FT-IR spectra of (a) PPy, (b) PEDOT, (c) PPy-PEDOT and (d) PPy-PEDOT-AgNP in KBr medium.
The Raman spectra of PPy, PPy-PEDOT and PPy-PEDOT-AgNP and PPy-PEDOT-AgNP-S-ssDNA modified substrates are presented in Figure 5.4.

Figure 5.4 Raman spectra of (a) PPy, (b) PPy-PEDOT, (c) PPy-PEDOT-AgNP and (d) PPy-PEDOT-AgNP-S-ssDNA.

The main characteristic peak of PPy shows broad peak at 1200-1600 cm\(^{-1}\) (N-H and C=C stretching in pyrrole ring). The peaks represented at 1513 (C\(_{\alpha'}\) = C\(_{\beta'}\) stretching), 1430 (C\(_{\gamma}\)=C\(_{\beta}\) stretching), 1125 (C\(_{\alpha}-C_{\alpha'}\) stretching), 990 (C\(_{\beta}-C_{\text{alkyl}}\) stretching) and 706 cm\(^{-1}\) (C\(_{\alpha}-S-C_{\alpha'}\) ring deformation) of PEDOT. These results are identical with earlier reports [243,244]. The PPy-PEDOT (Figure 5.4, curve b) shows different Raman
pattern in terms of the peak intensities and position ascertaining the effective interaction between the PPy and PEDOT (1241, 1415, 1521 and 3231 cm$^{-1}$). Similar peak patterns are observed for the Ag nanoparticles modified PPy-PEDOT (curve c), which indicates the incorporation of the Ag nanoparticles is not affecting the polymer structure. After DNA immobilization on the PPy-PEDOT-AgNP nanocomposite (curve d), new peaks appeared at 661 cm$^{-1}$ (thyamine breathing), 1338 cm$^{-1}$ (adenine stretching) and 1492 cm$^{-1}$ (symmetric stretching of adenine and quinine) frequencies respectively, confirming the presence of DNA on the polymer nanocomposite [178].

Figure 5.5 shows the XRD pattern of PPy (curve a), PPy-PEDOT (curve b) and PPy-PEDOT-AgNP (curve c). As expected for both PPy and PPy-PEDOT, the patterns do not yield any characteristics peaks except the low angle peak at ~ 25° indicating the amorphous nature of the polymeric materials. The PPy-PEDOT-AgNP nanocomposite shows the diffraction features appearing at 2θ as 32.3°, 38.2°, 44.3°, 64.5° and 77.4° that correspond to the (100), (111), (200), (220) and (311) planes of the Ag respectively. This observation demonstrates that the (111) plane with face-centered cubic lattice of silver are more predominant. The average particle size of nanoparticles was estimated based on Scherrer correlation of particle diameter (D) with peak width for Bragg diffraction from ideal single domain crystallites $L=0.9\lambda K_{\alpha1}/B_{(20)} \cos \theta_{\text{max}}$. The estimated average diameter of the silver nanoparticles on PPy-PEDOT nanotubes was ~ 28 ± 5 nm.

The UV-Vis spectra of PPy-PEDOT and PPy-PEDOT-AgNP nanocomposites are reported in Figure 5.6. The UV-Vis spectrum of PPy-PEDOT exhibits absorption peaks at 385 nm and 560 nm due to the $\pi-\pi^*$ electronic transitions of the aromatic
Figure 5.5 XRD pattern of (a) PPy, (b) PPy-PEDOT and (c) PPy-PEDOT-AgNP.

Figure 5.6 UV-Visible spectra of (a) PPy-PEDOT and (b) PPy-PEDOT-AgNP in N-methyl-2-pyrrolidone (NMP).
rings (Figure 5.6, curve a). When the silver nanoparticles are attached onto PPy-PEDOT nanocomposite, a strong peak around 416 nm arises (Figure 5.6, curve b), which represents the silver nanospheres surface plasmon [306].

5.3.2 Electrochemical Behaviour of PPy-PEDOT-AgNP Composite in 1M HCl

Cyclic voltammetry is used to study the PPy, PEDOT and PPy-PEDOT-AgNP composite films behaviour in 1M HCl (Figure 5.7 and 5.8). The CV exhibited a pair of redox peak corresponding to the doping/dedoping in the PPy and PEDOT film between the potential windows - 0.4 to 0.9 V (Figure 5.7 A & B).

Figure 5.7 Cyclic voltammogram responses of (A) PPy, (B) PPy-PEDOT and (C) PPy-PEDOT-AgNP modified GC electrode measured in 1M HCl in the potential window - 0.4 to 0.9 V at a scan rate (10-50 mV/s); Inset shows the square root of scan rate vs. current graph.
It is evident that the increase in peak currents with the scan rates, keeping potential constant suggest the occurrence of surface confined and reversible diffusionless redox transitions within the PPy and PPy-PEDOT film (Inset of Figure 5.7 A and B). The CV measurement of the PPy-PEDOT-AgNP modified surface in 1M HCl exhibits very sharp redox current with a narrow voltage range at ~ 0 V vs. Ag/AgCl reference electrode as shown in Figures 5.7 C and 5.8 (curve c). The oxidation potential of Ag nanoparticles is 0.02 V, corresponding to \( \text{Ag} + \text{Cl}^- \rightarrow \text{AgCl} \) [307]. These results clearly indicate the efficient interaction of Ag nanoparticles with polymer active groups as well as good electroactivity.

![Cyclic voltammograms comparison](image)

**Figure 5.8** Comparative cyclic voltammograms of (a) PPy, (b) PPy-PEDOT and (c) PPy-PEDOT-AgNP modified GC electrode in 1M HCl at a scan rate 50 mV/s.
5.3.3 Electrochemical Study of PPy-PEDOT-AgNP Nanocomposite Modified Electrode in PBS Containing 1mM \([\text{Fe(CN)}_6]^{3-/4-}\)

Figure 5.9 depicts the CV behaviour of the GC electrode at different stages of modifications made in presence of the 1mM \([\text{Fe(CN)}_6]^{3-/4-}\) in PBS at a scan rate 50 mV/s. Deposition of PPy and PPy-PEDOT onto the GC electrode surface decrease the reversibility while apparant increase in peak current of \([\text{Fe(CN)}_6]^{3-/4-}\) (\(I_{pa}: 28.8 \text{ and } 35.0 \mu\text{A} \& \Delta E_p (E_{pa}-E_{pc}) : 208 \text{ and } 113 \text{ mV}\), is noticed compared to the unmodified GC electrode (\(I_{pa}: 21.3 \mu\text{A} \text{ and } \Delta E_p : 70 \text{ mV}\) (Table 5.1).

Figure 5.9 CV behaviour of modified GC electrode measured in presence of 1mM \([\text{Fe(CN)}_6]^{3-/4-}\) in PB (pH 7.0) solution at a scan rate 50 mV/s in the potential range - 0.2 to 0.6 V. Curves a: Bare GC, curve b: PPy nanotubes, curve c: PPy-PEDOT nanotubes, curve d: C+ AgNP, curve e: d + ssDNA, curve f: e + complementary DNA, curve g: e + non-complementary.
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Tabel 5.1 Cyclic voltammetry parameters of the modified GC electrodes in PB (pH 7.0) solution containing 1mM \([\text{Fe(CN)}_6]^{3/4-}\).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Cyclic voltammetry parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(E_{pa}) (V)</td>
</tr>
<tr>
<td>Bare GC</td>
<td>0.228</td>
</tr>
<tr>
<td>PPy</td>
<td>0.282</td>
</tr>
<tr>
<td>PPy-PEDOT</td>
<td>0.242</td>
</tr>
<tr>
<td>PPy-PEDOT-Ag</td>
<td>0.234</td>
</tr>
<tr>
<td>PPy-PEDOT-AgNP-S-ssDNA</td>
<td>0.265</td>
</tr>
<tr>
<td>PPy-PEDOT-AgNP-S-dsDNA (com)</td>
<td>0.289</td>
</tr>
<tr>
<td>PPy-PEDOT-AgNP-S-dsDNA (non-com)</td>
<td>0.263</td>
</tr>
</tbody>
</table>

On the other hand, dispersion of the Ag nanoparticles onto the PPy-PEDOT film enhances the reversible process (\(I_{pa}: 47.3 \mu A\) and \(\Delta E_p: 90\) mV), Table 5.1, due to the more efficient electron transfer of the Ag nanoparticles.

Further, the charge transport properties of the composite film modified electrodes were characterized by electrochemical impedance spectroscopy (EIS). In EIS, the semicircle diameter could represent the electron-transfer resistance, \(R_{CT}\), which dominate the electron transfer kinetics of the redox probe at the electrode interface/electrolyte interface. Therefore, the data are generally interpreted using the Nyquist plot in Figure 5.10. The double layer capacitance (\(Q_{dl} \ F \ cm^{-2}\)) and charge transfer resistance (\(R_{CT} \ \Omega \ cm^{-2}\)) were found using Randles equivalent circuit. The fitting values of parameters are given in Table 5.2.
Frequency dependent impedance $Z(\omega)$ and $Q_{CPE}$ are related by equation (5.1)

$$Z(\omega) = \frac{1}{Q_{CPE} (j\omega)^n}$$

(5.1)

$\omega = 2\pi f$ is the angular frequency, $f$ is the frequency, $n$ is coefficient directly related to the degree of the surface roughness and inhomogeneity of the electrode surface which varies between 0 and 1.

Figure 5.10 EIS behaviour of modified GC electrode measured by impedance in the frequency region from 100 KHz to 1Hz at the DC potential 0.2 V and AC potential ± 5 mV in presence of 1mM [Fe(CN)$_6$]$_{3/4}^-$ in PB (pH 7.0) solution. Curves a: Bare GC, curve b: PPy nanotubes, curve c: PPy-PEDOT nanotubes, curve d: C+ AgNP, curve e: d + ssDNA, curve f: e + complementary DNA, curve g: e + non-complementary.
In this work ‘n’ varies from 0.6 to 0.9 confirming the presence of rough surface and the inextricability of the $Q_{CPE}$ from the equivalent circuit. The near constant ‘n’ values for the PPy (0.6), PPy-PEDOT (0.7) modified and unmodified GC surface (0.9) clearly indicates the porous polymer structures. Since the PPy-PEDOT nanotubes is drop casted on the electrode and the dropcasting is completely covering the electrode resulting in the constant $Q_{CPE}$ exponent. Figure 5.1 A & B show the SEM images of the nanotubical structure of PPy and PPy-PEDOT composite. It is to be noted that the PEDOT coated PPy nanotubes have the thickness approximately
350 nm and surface roughness is also observed as in Figure 5.1 B. The efficiency of simulating the circuit parameters is validated by observing constant $\chi^2 = 7.88 \pm 4 \times 10^{-4}$ and this very small value suggests that this model fit the experimental data well.

As compared with bare GC (314 $\Omega$ cm$^{-2}$) the PPy coated electrode $R_{CT}$ value (282 $\Omega$ cm$^{-2}$) was lower due to nanotube structure of PPy. In the PPy-PEDOT modified surface $R_{CT}$ value decreased to 136 $\Omega$ cm$^{-2}$ and the PPy-PEDOT-AgNP deposited surface $R_{CT}$ value further decreased to 31 $\Omega$ cm$^{-2}$. Similarly, the observed $Q_{CPE}$ values for the PPy, PPy-PEDOT and PPy-PEDOT-AgNP surfaces were $4.12 \times 10^{-5}$, $6.63 \times 10^{-5}$ and $1.26 \times 10^{-7}$ F cm$^{-2}$, respectively indicating the high catalytic activity of Ag nanoparticles present on the PPy-PEDOT surface.

**5.3.4 DNA Hybridization Detection at PPy-PEDOT-AgNP Nanocomposite**

Effect of immobilization of the thiolated DNA capture probe on the PPy-PEDOT-AgNP nanocomposite measured in presence of 1mM [Fe (CN)$_6$]$^{3/-4}$ is shown in Figures 5.9 and 5.10 (curve e). The reversibility of the [Fe(CN)$_6$]$^{3/-4}$ redox reaction is decreased ( $I_{pa}$ : 12.4 $\mu$A and $\Delta E_p$ : is 131 mV (curve e)) for the PPy-PEDOT-AgNP-S-ssDNA. The PPy-PEDOT-AgNP electrode witnessed a small $R_{CT}$ value of about (31 $\Omega$ cm$^{-2}$) (curve d). The $R_{CT}$ was increased to be about 2248 $\Omega$ cm$^{-2}$ for the PPy-PEDOT-AgNP-S-ssDNA surface (curve e). The $R_{CT}$ value was further increased to 6181 $\Omega$ cm$^{-2}$ after hybridized with complementary target DNA (Figure 5.10, curve f, Table 5.2). The increase of $R_{CT}$ after probe DNA immobilization and hybridization could be well ascribed to the repulsion of redox probe from approaching electrode surface by negatively charged phosphate skeletons of DNA. The results of impedance experiments were in good agreement with that of cyclic voltammetry experiments.
On the other hand, the interaction of the non-complementary probe has insignificant effect on both the CV and impedance behaviour with that of PPy-PANi-AgNP-SS-sDNA (curve g). That is, the PPy-PEDOT-AgNP surface discriminates the ssDNA and dsDNA effectively and selectively, Figures 5.9 and 5.10. Two factors contributing to the increased diffusion restriction of $[\text{Fe(CN)}_6]^{3/4-}$ and the enhanced $R_{CT}$ for the PPy-PEDOT-AgNP surface are: (i) the polymer PPy-PEDOT composite film blocks electron transfer kinetics at the electrode surface and reduces the interfacial capacitance ($Q_{\text{CPE}}$, $1.26 \times 10^{-7}$ F cm$^2$) thus decreased reversibility of $[\text{Fe(CN)}_6]^{3/4-}$) (ii) the thiol capped capture DNAs immobilized directly onto the Ag nanoparticles in the polymer matrix also enhances the negative charge density at the electrode/electrolyte interface. That is, the micro environment of the AgNP in the PPy-PEDOT-AgNP matrix are well dispersed and separated from each other to provide enough space between each HS-ssDNA immobilized on them. This enhances the target coiling and increases the hybridization efficiency indicated by the $\Delta R_{CT}$ 3933 $\Omega$ cm$^2$.

Figure 5.11 shows the thiol reductive voltammetry for the PPy-PEDOT-AgNP and PPy-PANi-AgNP-S-ssDNA surfaces in 0.5 M KOH solution at a scan rate 100 mV/s, (Figure 5.11), in which two distinctive reduction peaks at (P1) - 408 and (P2) - 972 mV/s (Figure 5.11, curve b) suggesting that the higher potential desorption peak corresponds to desorption of ssDNA and MCH and the strands may be separated by nm scale [276]. In contrast, the PPy-PEDOT-AgNP (curve a) surface did not show any characteristic peak. Therefore, the presence of PPy-PEDOT in the polymer matrix induces a well separated HS-ssDNA immobilization on the PPy-PEDOT-AgNP
matrix. In order to confirm this further, the surface coverage was calculated from the equation (5.2).

\[ \Gamma_0 = \frac{Q}{nFA} \quad (5.2) \]

Where \( Q \) is the charge consumed for the reduction of thiolated DNA, \( n \) is number of electron (\( n = 1 \)), \( F \) is Faraday constant 96,485 C, and \( A \) is electrode area 0.07 cm\(^2\). The estimated number of HS-ssDNA for the PPy-PEDOT-Ag-S-ssDNA surface is \( 66.734 \times 10^{12} \) molecules/cm\(^2\). For this purpose, the total area from the peaks P1 and P2 are considered for the \( \Gamma_0 \) calculation. Theoretical calculations and earlier reports suggest that the efficiency of DNA hybridization with the surface-bound probe is maximized when the probe densities are in the order of \( 10^{12}-10^{13} \) molecules/cm\(^2\) [308].

![Cyclic voltammetric responses](image)

Figure 5.11 Cyclic voltammetric responses of (a) PPy-PEDOT-AgNP and (b) PPy-PEDOT-AgNP- S-ssDNA in 0.5 M KOH at a scan rate of 100 mV/s.
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The impedance behaviour of the PPy-PEDOT-AgNP-S-ssDNA after hybridization with the completely complementary, non-complementary, single and double base mismatched targets is shown in Figure 5.12.

Figure 5.12 EIS behaviour of (a) PPy-PEDOT-AgNP-S-ssDNA, (b) PPy-PEDOT-AgNP-dsDNA (com), (c) PPy-PEDOT-AgNP-dsDNA (non-com), (d) PPy-PEDOT-AgNP-dsDNA (SMM) and (e) PPy-PEDOT-AgNP-dsDNA (DMM) in presence of 1mM [Fe(CN)₆]³⁻/⁴⁻ in PB (pH 7.0) solution. Inset: Corresponding bar diagram of normalized change in $R_{CT}$.
The selectivity of this assay was investigated by using the DNA probe to hybridize with different DNA sequences as shown in Figure 5.12. The ΔR_{CT} values observed after the hybridization of the complementary (curve b), non-complementary (curve c), single base mismatched (curve d) and double base mismatched (curve e) target sequence with the capture DNA (curve a) are 3933, 207, 2911 and 1740 Ω cm^{-2} respectively. The observed 95 % of impedance change between the HS-ssDNA and HS-dsDNA show obviously the discrimination. On the other hand, single and double base mismatches in the target sequences reduce the ΔR_{CT} by 26 and 56 % owing to less number of dsDNAs formed on the surface. These data clearly confirm that the combination of the PPy-PEDOT and Ag nanoparticles facilitates the conductivity and acts as a good platform for the sensitive and selective discrimination of different target sequences. This work was done at 1×10^{-11} M target concentration. Figure 5.12 shows the Nyquist diagrams of varying target concentration hybridized with PPy-PEDOT-AgNP-S-ssDNA. Inset of Figure 5.12 shows the calibration curve of DNA calculated from the variation of ΔR_{CT} before and after target hybridization at each concentration with log of concentration of DNA. For the regeneration experiments, the hybridized electrode was dehybridized by immersing hot (90 °C) ultrapure water for 2 minutes followed by rapid cooling in an ice bath and re-hybridized with the different target DNA concentration. This sensor exhibited a linear correlation to the logarithm of the target DNA concentration range between 1.0 x10^{-14} and 1.0 x10^{-11} M with a regression coefficient 0.9902. The detection limit of the proposed biosensor has been calculated by using the formula 3σ/b, where σ is the standard deviation of the blank and b is the slope of the calibration curves and is found as 5.4 ± 0.3 × 10^{-15} M, which is nearly 3 fold lower than that of, PANi-AuNP, MWCNT-PPy-AuNP and
MWCNT-AgNP composites [279,231,233]. The higher sensitivity indicates that the lower target DNA concentration of $1.0 \times 10^{-11} \text{ M}$ is found sufficient to saturate the ssDNA immobilized on the PPy-PEDOT-Ag modified electrode.

Figure 5.13 EIS detection of different target concentration using the PPy-PEDOT-AgNP-S-ssDNA. (a) 0, (b) $1.0 \times 10^{-14}$, (c) $2.0 \times 10^{-14}$, (d) $4.0 \times 10^{-14}$, (e) $8.0 \times 10^{-14}$, (f) $1.0 \times 10^{-13}$, (g) $4.0 \times 10^{-13}$, (h) $8.0 \times 10^{-13}$, (i) $1.0 \times 10^{-12}$, (j) $5.0 \times 10^{-12}$ and (k) $1.0 \times 10^{-11} \text{ M}$. Inset: Variation of $\Delta R_{CT}$ with log ($C_{\text{target DNA}}$).
Further, the controlled (distance between two AgNP spot) deposition of the AgNP on PPy-PEDOT matrix may help to immobilize the capture DNAs which are well separated from each other to maximize the target coiling efficiency. Besides sensitivity and reusability are also an extremely important features for the newly developed biosensor applications such as clinical diagnosis.

The reproducibility of three different biosensors constructed in the same manner, for $4.0 \times 10^{13}$ M target DNA, showed the response of $\Delta R_{CT}$ values (2517, 2750 and 2560 $\Omega$ cm$^2$) with a relative standard deviation (RSD) value of 4.75% (Figure 5.14 A). This result demonstrated the reliability in the sensor construction procedure.

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**Figure 5.14** (A) EIS response of PPy-PEDOT-AgNP-S-ssDNA modified three independent electrodes hybridized with complementary target DNA. (B) EIS response of PPy-PEDOT-AgNP-S-ssDNA modified electrode (a and c) and hybridized with complementary target DNA (b and d)
The stability of PPy-PEDOT-AgNP-S-ssDNA modified surface was investigated after 7 days storage at 5°C and was further used to hybridize with the target ssDNA sequences (1.0 × 10^{-13} M) and observed 91.5% of the initial sensitivity (Figure 5.15 B). This indicates that the combination PPy-PEDOT bipolymer and Ag nanoparticles increases the conductivity and the detection limit. The main advantage is that the PPy-PEDOT-AgNP nanocomposite preparation, electrode modification and finally DNA immobilization employed in the present study is very simple when compared to the reported tedious procedures for nanomaterials preparation and electrode modification.

5.4 Conclusion

We have demonstrated a simple, convenient electrochemical technique to fabricate PPy-PEDOT-AgNP nanocomposite films modified GC electrode for DNA sensor. The microdispersed Ag nanoparticles in the PPy-PEDOT nanotubes provides good platform for anchoring the thiolated HS-ssDNA with controlled manner. The hybridization efficiency is enhanced by 2.7 times due to this platform accommodates the optimum probe DNA density (66.734 × 10^{-13} molecules/cm^2) and the microdispersed Ag nanoparticles in the PPy-PEDOT nanostructure on which thiol capped DNAs are immobilized and well separated from each other to provide higher target coiling efficiency. This is further evidenced by the detection limit obtained for PPy-PEDOT-AgNP-S-ssDNA (5.4 ± 0.3 × 10^{-15} M) modified surface is nearly 2 and 1 fold higher when compared to the PPy-PANi-AuNP-S-ssDNA (1.2 × 10^{-13} M) and PPy-PANi-GA-ssDNA (5.0 × 10^{-14} M) modified surfaces, respectively. Further, the PPy-PEDOT-AgNP-S-ssDNA modified surface shows a better stability and reproducibility when compared to PPy-PANi-AuNP-S-ssDNA and PPy-PANi-GA-
ssDNA modified surfaces. In term of biosensors applications, electrochemical polymerization is widely used because of several advantages: (i) it is performed at ambient temperature, (ii) the polymer film formed is confined to the electrode and the thickness can be controlled in the nanometer to micrometer range and (iii) the electrochemical polymerization process may only take few minutes. Hence, we have planned to prepare a electrochemical biosensor using gold nanoparticles functionalized PEDOT thin film for high sensitive label-free DNA detection in Chapter 6