



# Detection of DNA by using bio-conducting polymer–Nile blue composite electrode; Nile blue as an indicator

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## ABSTRACT

The amplified electrochemical sensing of DNA was accomplished by electrodeposited PEDOT on the electrode surface and incorporation of Nile blue (NB) as redox active intercalator into DNA. Herein, above modified electrode called as PEDOT/DNA/NB composite electrode. PEDOT/DNA/NB composite electrode exhibited well defined redox peak at  $-0.35$  V (Ag/AgCl) corresponding to NB. The composite electrode surface coverage ( $\Gamma$ ) and  $\Delta E_p$  were compared with PEDOT/NB and DNA/NB modified electrode. Atomic Force microscopy (AFM), and cyclic voltammetry (CV) were used to characterize the PEDOT/DNA/NB composite electrode. The composite electrode was exhibited as surface confined redox process in neutral pH. The composite electrode was found to be pH dependent. The composite electrode exhibited catalytic property towards reduction of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). The composite electrode was utilized to amperometric study and its response towards H<sub>2</sub>O<sub>2</sub> detection was less than 6 s and the detection limit was 0.1  $\mu$ M. Moreover, we tested PEDOT/DNA/NB composite electrode to electrocatalytic reduction of cytochrome *c* (Cyt *c*).

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## 1. Introduction

The electrostatic and topographic property of biological macromolecules such as DNA complexes can be exploited for the templated generation and assembly of supramolecular aggregates of organic and inorganic building blocks [1,2]. The power of DNA as a molecular template is enhanced by our ability to synthesize virtually any DNA sequence by automated methods, and to amplify any DNA sequence from microscopic to macroscopic quantities by means of polymerase chain reaction (PCR). Therefore, DNA is particularly suitable to serve as a construction component in nano sciences [3]. The use of DNA as template for spontaneous assemblies of cationic cyanine dye [4], fullerene derivatives [5], CdS semiconductor nanoparticles [6], silver nanowires [7], and gold nanowires [8–10] to form supramolecular structures has been extensively reported. In all cases, the negatively charged phosphate backbone of the DNA double helix has been employed to interact electrostatically with inversely charged species presented in solution. Because metal nanoparticles are always wrapped by charged organic layer, the metal nanowires formed by such metal nanoparticles for electronic interconnections of nanometer-scale electronics devices should be resistive [7]. The conductive polymers may provide another option for this problem.

Conductive polymers (CPs) have been extensively studied because of their highly electrical conductivity and mechanical flexibility, their ability to be electrochemically switched between electronically insulating and conducting states. It has been well established that

disorder is one of the limiting factors in electron/hole transport in doped conducting polymers [11] and the incorporation of the DNA and probe oligonucleotide into a conducting polymer is attractive because of electrochemical and an electronic properties of conjugated polymers are a sensitive function of their environment, so that hybridization of the incorporated probes would be expected to perturb the electrochemical response of the polymer [12–21]. Also, DNA has a unique secondary structure where a stack of  $\pi$  electrons in the base pair promoted the possibility of DNA to form a “molecular conduit” [22]. Wrapping of a conducting polymer on the DNA surface might be useful to connect the molecular wires [23] and these hybrids might play an important role to bridge the human–machine interface.

Among the conducting polymers, CPs, poly(3,4-ethylenedioxythiophene), or PEDOT, has been reported to exhibit good stability after incorporation of the biological material and to get enhanced electrical signal [24–25]. Since, PEDOT modified conducting polymer films have a high regularity of the polymeric chain due to the lack of  $\alpha$ – $\beta$  linkages between the monomers, a high stability of the p-doped state and a high conductivity [24–26].

Recently, Fang et al. [27] used ferrocene functionalised polythiophene as transducer for label free DNA detection. Shinkai and co workers [28] prepared PEDOT/DNA/terracationic porphyrin (TMpyP) composite and concluded that DNA is useful as a scaffold to arrange redox active couples in one dimensional matrix. Mouffouk and Higgins [29] reported selective electrochemical response to hybridization based on oligonucleotide-functionalised PEDOT coated micro electrodes. Moreover, Goto et al. [30] prepared PEDOT polymer by using DNA as a liquid crystal electrolyte and the prepared polymer exhibited optically active.

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Nile blue A (NB), one of phenoxazine dyes, is a well-known electroactive molecule. It has been used as a mediator for electron transfer with a two-electron redox conversion to modify solid electrodes and used for electrocatalytic applications [31–33]. The incorporation of phenothiazine [34] and phenoxazine dyes [15,35] into DNA modified electrode have been reported. Recently, Hu et al. [35] studied the interaction between Nile blue and immobilized single- or double-stranded DNA and its application in electrochemical recognition and calculated binding constants. But, they have not been used electrode to electrocatalytic application.

Cytochrome *c* (Cyt *c*) plays a major role in electron transport in biochemical transformations. It is a water-soluble heme protein that exists in the cytosol between the inner and outer membranes of mitochondria. Under physiological conditions, it transfers electrons between cytochrome *c* reductase and cytochrome *c* oxidase, which are both embedded in the mitochondrial membrane. Reversible electron transfer between electrode and redox proteins immobilized in films provides a basis for constructing biosensors, biomedical devices, and enzymatic bioreactors [36]. Many reports have described the electrochemistry of cytochrome *c* in terms of modifier electrode and modifier-protein interactions [37–40]. Many promoters, such as some small organic compounds [41–42], small peptides [43] and conductive polymers [44] have been found to promote the direct electrochemistry of cytochrome *c* on the electrode surface. Various dyes associated with electrode surfaces have been proven to align cytochrome *c* and to facilitate interfacial electron transfer [45–46].

In the present work, we report that the amplified electrochemical sensing of DNA is accomplished by electrodeposited PEDOT on the electrode surfaces and incorporation of Nile blue (NB) as redox active intercalator into DNA. This modified composite electrode is utilized towards catalytic reduction of hydrogen peroxide. To the best of our knowledge, there is no literature available on elsewhere.

## 2. Experimental

### 2.1. Reagents and solutions

EDOT, DNA, and NB were purchased from Aldrich. All reagents were of analytical grade and used without any further purification. Solutions were prepared with doubly-distilled water. High purity nitrogen was used for deaeration. The buffer and sample solutions were purged with highly purified nitrogen for at least 10 min prior to the experiments. Nitrogen atmosphere was maintained over the solutions during the experiments to prevent the reentry of atmospheric oxygen.

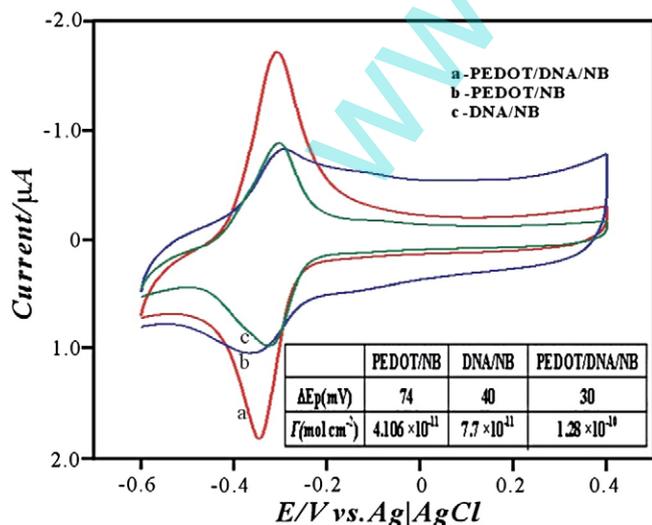


Fig. 1. CVs of (a) PEDOT/DNA/NB composite (b) PEDOT//NB (c) DNA/NB electrode. Electrolyte: 0.2 M PBS solution, pH:7; scan rate: 50 mV/s.

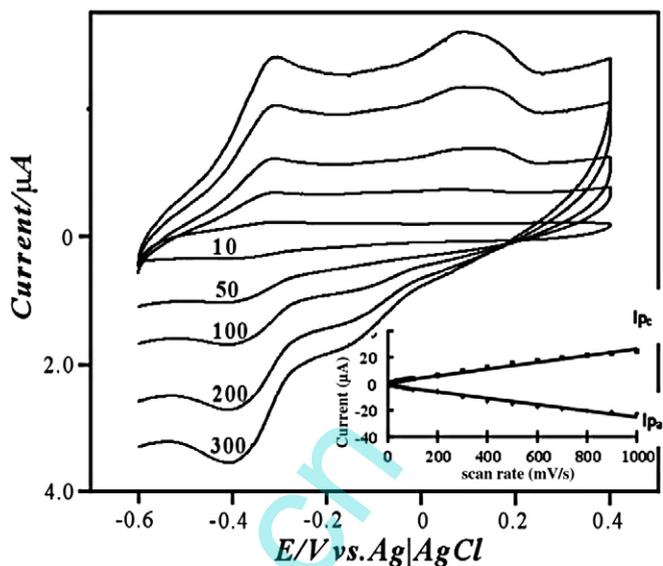


Fig. 2. CVs of PEDOT/DNA/NB composite electrode at different scan rates: scan rate: (a) 0.01 (b) 0.05 (c) 0.1 (d) 0.2 (e) 0.3 (V/s); Inset: scan rate vs  $I_{pa}/I_{pc}$ . Electrolyte: 0.2 M PBS solution, pH 7.

### 2.2. Apparatus

Electrochemical experiments were performed with CH Instruments (Model CHI-400) using CHI-750 potentiostat. Glassy carbon electrode (geometric area 0.07 cm<sup>2</sup>) obtained from BAS served as the working electrode. Pt wire was used as counter electrode and Ag/AgCl with the saturated KCl solution used as reference electrode. All the potentials given in this paper were referred Ag/AgCl (saturated KCl solution).

### 2.3. Preparation of PEDOT/DNA/NB composite electrode

Prior to modification, glassy carbon electrode (GCE) was polished with 0.05  $\mu$ m alumina on Buehler felt pads and then ultrasonically cleaned for about a minute in water. Finally, the electrode was washed thoroughly with double distilled water and used. After being cleaned, the polished GC surface was subjected to electrochemical deposition of poly(3,4-ethylenedioxythiophene) (PEDOT) film (three cycles within the potential range  $-0.5$  to  $1.1$  V) from the solution containing 0.01 M EDOT, and 0.1 M LiClO<sub>4</sub> potentiodynamically. The 20  $\mu$ l of 2 mg DNA solution was spread over the PEDOT modified electrode and dried to get the film

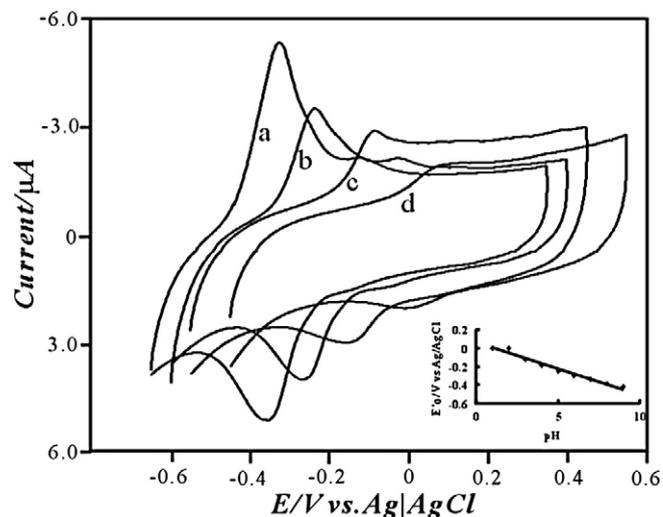


Fig. 3. CVs of different pH (a) 7 (b) 5 (c) 3 (d) 1. Scan rate: 50 mV/s. Inset: pH vs  $E^{0'}$ .

by hot air oven for 30 min. Then PEDOT/DNA modified electrode was dipped into PBS solution containing 1 mM NB for 10 min. This electrode called as PEDOT/DNA/NB composite electrode. PEDOT/DNA/NB composite electrode was thoroughly washed further with extra pure water and stored in the PBS for further studies.

### 3. Results and discussions

#### 3.1. Electrochemical behavior of PEDOT/DNA/NB composite electrode

The PEDOT/DNA/NB composite electrode shows a well-defined reversible voltammogram corresponding to the redox peak of NB at  $-0.35$  V (Ag/AgCl) (Fig. 1a). The observed redox peak has good agreement with previously reported NB modified electrode [31–

33,35]. The peak-to-peak separation ( $\Delta E_p$ ) was typically small (30 mV), although not zero, as expected for a reversible ideal case; the ratio of anodic and cathodic peak current  $I_{pa}/I_{pc}$  was close to unity. On the other hand, surface coverage of PEDOT/NB and DNA/NB were  $4.106 \times 10^{-11}$  mol  $\text{cm}^{-2}$  and  $7.7 \times 10^{-11}$  mol  $\text{cm}^{-2}$  respectively. The  $\Delta E_p$  of PEDOT/NB and DNA/NB electrodes were 74 and 40, respectively. This higher  $\Delta E_p$  and lower surface coverage ( $\Gamma$ ) in PEDOT/NB and DNA/NB compared with PEDOT/DNA/NB composite electrode might be due to the absence of electrostatic binding with NB in PEDOT/NB electrode and lower surface coverage in DNA/NB might be due to less conductivity. Higher surface coverage and lesser  $\Delta E_p$  of PEDOT/DNA/NB composite electrode is due to strong electrostatic binding of NB on the backbone of DNA that electrostatically interacted with PEDOT conducting polymer. The formal

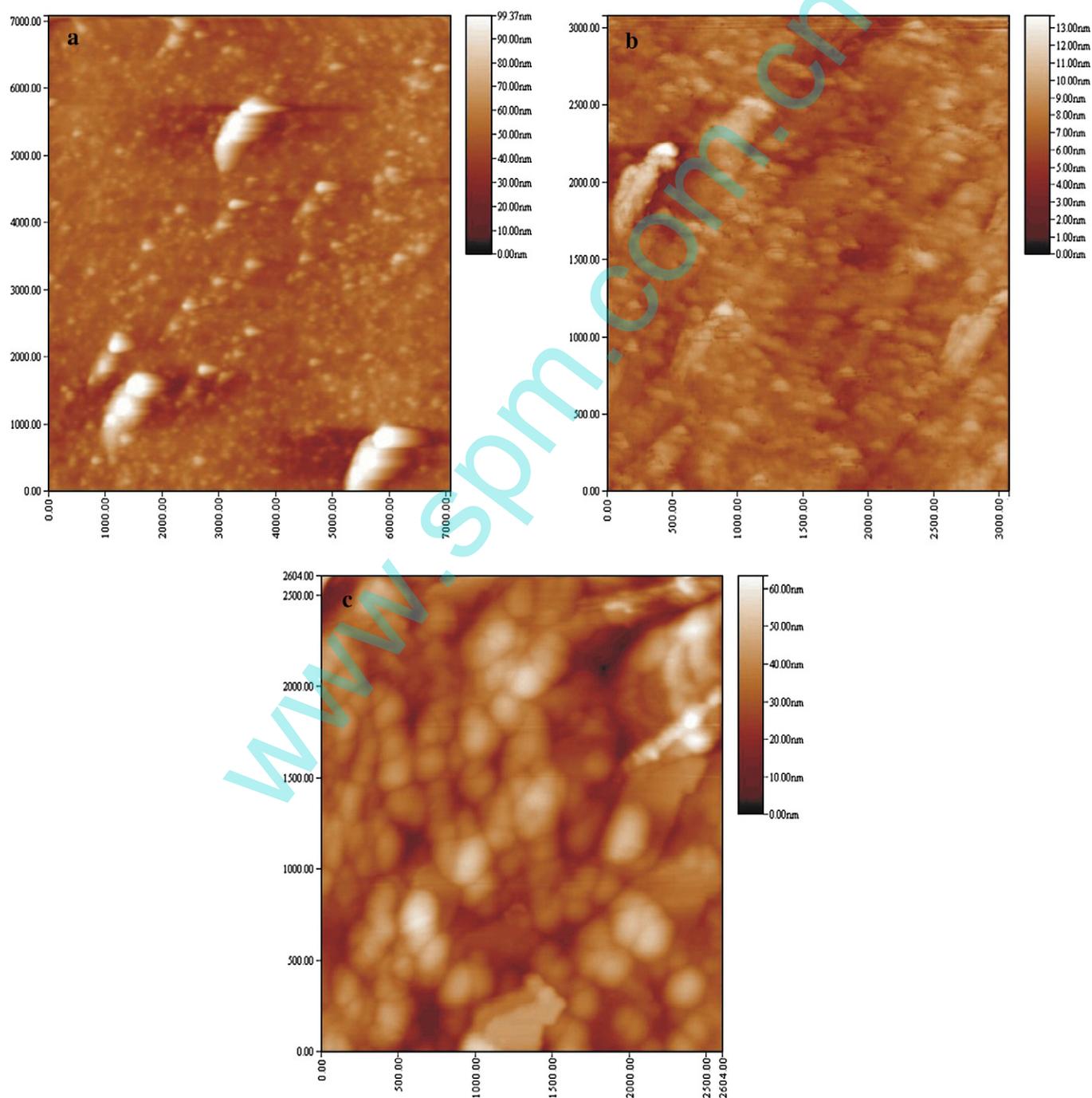


Fig. 4. Tapping mode AFM image of (a) DNA/NB ITO glass electrode (b) PEDOT/NB and (c) PEDOT/DNA/NB composite film coated in ITO glass.

potential of composite electrode shifted by 30 mV in the negative direction compared to DNA/NB electrode. Such behavior is characteristic of electrostatic interaction of NB with adsorbed ssDNA [47]. Here, similar type of characteristic property is expected PEDOT/DNA/NB composite electrode.

As shown, the anodic and cathodic peaks were rather broad and the magnitude of the peak current was significantly lower than that observed on the PEDOT/DNA/NB composite electrode. The enhanced peak current of NB in composite electrode is due to the presence of DNA and PEDOT and gives good stability. Since the anodic and cathodic peak currents gradually decrease during the subsequent sweeps, possibly due to the leaching of NB from the PEDOT and DNA surface. But composite electrode provides good electrode stability. Because, due to high conductivity PEDOT act as good matrix [24,25,48,49].

### 3.2. Electrochemical characterization of PEDOT/DNA/NB composite electrode

Fig. 2 shows the cyclic voltammograms of PEDOT/DNA/NB composite electrode in a deaerated PBS solution at different scan rates. A redox couple with well-defined peak appeared. The ratio of anodic to cathodic peak currents was nearly unity and a plot of {inset of Fig. 2 (-0.34 V)} peak current as a function of scan rate was linear as expected for surface confined species [50] and the charge transfer was fast in the coating [51]. The separation of peak potentials,  $\Delta E_p$  was 55 mV.  $\Delta E_p$  was close to  $2.303/RT$  (or 59/n mV at 25 °C) [50].

We have estimated, the apparent surface coverage,  $\Gamma$  by using Eq. (1)

$$\Gamma = Q/nFA_e. \quad (1)$$

Where  $Q$  was charged from the area under the PEDOT/DNA/NB composite electrode oxidation peak corrected for the base line (at the scan rate of  $10 \text{ mV s}^{-1}$ );  $A$  area of the electrode:  $0.07 \text{ cm}^2$ ,  $F$ : Faraday constant. In the present case, the calculated surface coverage ( $\Gamma$ ) was  $1.28 \times 10^{-10} \text{ mol cm}^{-2}$  for assuming a two electron process.

Fig. 3 displays the pH-dependent voltammetric response of PEDOT/DNA/NB composite electrode. In order to ascertain this, the voltammetric responses of PEDOT/DNA/NB electrode were obtained in the solutions of different pH varying from 1.0 to 9. As can be seen in Fig. 3, the formal potential of the redox couples were pH dependent and it negatively shifted by increasing the solution pH. The pH dependence suggests that the electroactive sites on the PEDOT/DNA/NB composite electrode behave as true surface active groups

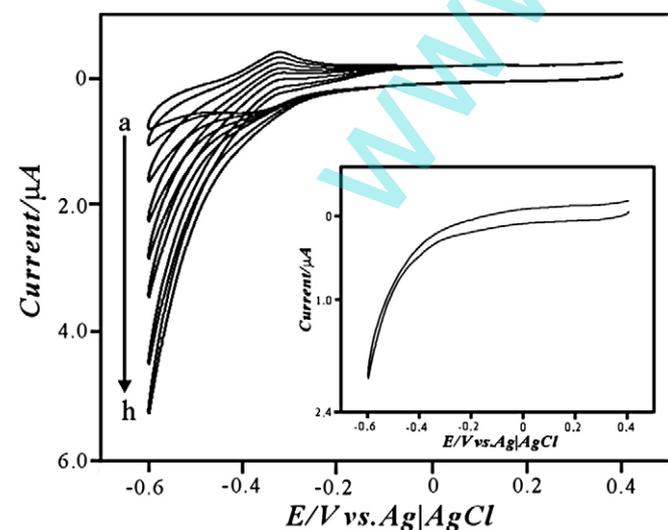


Fig. 5. PEDOT/DNA/NB composite electrode response towards different mM of  $[\text{H}_2\text{O}_2]$  (a) 0 (b) 0.02 (c) 0.04 (d) 0.06 (e) 0.08 (f) 0.1 (g) 0.12 (h) 0.14; scan rate:  $50 \text{ mV/s}$ . Inset: bare GC electrode in the presence of  $0.14 \text{ mM}$  of  $\text{H}_2\text{O}_2$  solution.

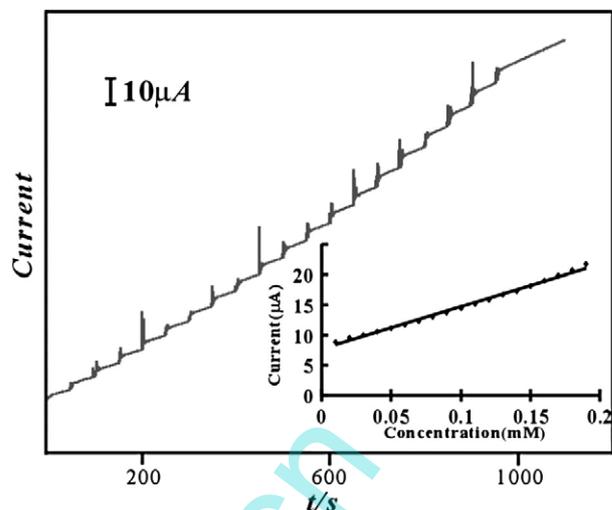


Fig. 6. Amperometric response to  $\text{H}_2\text{O}_2$  at PEDOT/DNA/NB composite electrode. Applied potential  $-0.35 \text{ V}$ . Rotation rate:  $750 \text{ rpm}$ .

influenced by specific solution conditions and not shielded within the electrode interior. The plot of  $E_{1/2}$  vs pH yields straight line with a slope of  $57 \text{ mV}$  per unit change in solution pH as shown in the inset of Fig. 3 which was very close to the anticipated Nernstian value of  $59 \text{ mV}$  for processes in which equal numbers of electrons and protons were involved in the electrode reactions.

### 3.3. Characterization of surface morphology using AFM

Fig. 4 shows the Tapping mode AFM images of PEDOT/NB, DNA/NB and PEDOT/DNA/NB electrode. PEDOT/DNA/NB (Fig. 4c) electrode shows that NB uniformly distributed on the surface of PEDOT–DNA film and thickness of film decreased compared to DNA/NB electrode. Since, PEDOT–DNA film provides facile matrix to immobilize dye molecules. Fig. 4a and b show AFM images of DNA/NB and PEDOT/NB electrode, respectively and observed that NB was randomly distributed and exhibited rough surface.

### 3.4. Electrocatalytic reduction of $\text{H}_2\text{O}_2$

Electroanalytical application of the PEDOT/DNA/NB composite electrode was studied for the analysis of  $\text{H}_2\text{O}_2$ . The electrochemical responses to the reduction of  $\text{H}_2\text{O}_2$  was shown in Fig. 5, where the current response was recorded at the bare (inset of Fig. 5) and PEDOT/DNA/NB composite electrode in the absence and presence of  $\text{H}_2\text{O}_2$ . As one can see at the bare electrode (curve b) the reduction of  $\text{H}_2\text{O}_2$  requires a higher potential and also gives a very poor response. In contrast, at the modified electrode with the addition of  $\text{H}_2\text{O}_2$  to the solution, change in the cyclic voltammogram occurs with an increase in cathodic current and a concomitant decrease in the anodic current (curves d and e). The catalytic reduction occurs at a much lesser potential of  $-0.37 \text{ V}$  at the PEDOT/DNA/NB composite electrode. A significant improvement in the magnitude of the cathodic current at a lower potential reflects the high electrocatalytic activity of the PEDOT/DNA/NB composite electrode.

### 3.5. Amperometry

Quantitative determination of  $\text{H}_2\text{O}_2$  was also carried out amperometrically in a stirred solution of PBS solution maintained at pH 7.0. Fig. 6 depicts the typical amperometric response of the PEDOT/DNA/NB composite electrode recorded at a applied potential of  $-0.35 \text{ V}$  for successive addition of  $1 \text{ mM}$   $\text{H}_2\text{O}_2$  solution. A rapid and well-defined response was observed for each addition of  $\text{H}_2\text{O}_2$ . The sensor responds quickly to the concentration of  $\text{H}_2\text{O}_2$ , a steady state current being

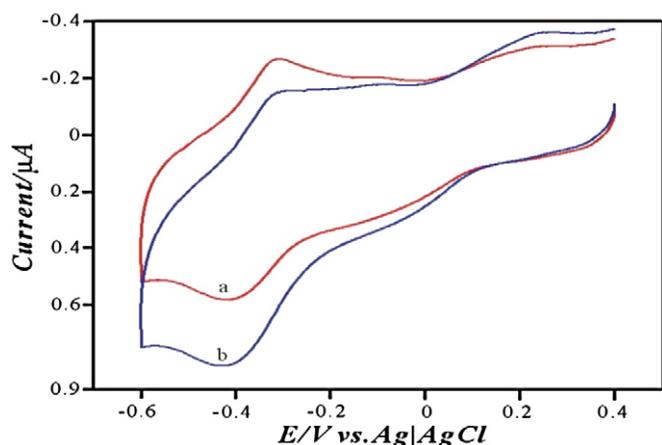


Fig. 7. PEDOT/DNA/NB composite electrode response to (a) pH 7 PBS solution (b) 0.1 mM Cyt *c* solution. Scan rate: 50 mV/s.

reached within less than 6 s. Under optimum conditions, the modified electrode showed a linear response to  $H_2O_2$  in the concentration range of 6  $\mu M$  to 0.2 mM and detection limit ( $S/N=3$ ) was found to be 0.1  $\mu M$  and sensitivity of 10  $\mu A/mM$  for  $H_2O_2$  at the composite electrode. The results indicate that the modified electrode maintained a good sensitivity and had a stable amperometric response under hydrodynamic condition.

### 3.6. Electrocatalytic reduction of Cyt *c* at PEDOT/DNA/NB composite electrode

Fig. 7 demonstrates the electrochemical behavior of Cyt *c* at PEDOT/DNA/NB composite electrode. When Cyt *c* was added to the PBS, an increase in the reduction peak current of NB and a corresponding decrease in the oxidation peak current was observed. Such behavior of electrode indicates mediated electrocatalytic reduction reaction. The heterogeneous electron transfer between Cyt *c* and conventional electrodes is very slow owing to its extended three-dimensional structure, inaccessibility of the electroactive center, adsorptive denaturation, unfavorable orientations and conformational equilibria of protein at electrodes [52]. As well known, Cyt *c* in pH 7.0 buffers shows no response at bare GC electrode, thus, the NB in PEDOT/DNA/NB composite electrode might have great effect on the kinetics of the electrode reaction for Cyt *c*. It shows that the NB can electrocatalyze the reduction of Cyt *c* ( $Fe^{III}$ ) in neutral media through the electron transfer reaction at the heterogeneous boundary layer NB and Cyt *c*. Similar electrocatalytic reduction of cytochrome *c* was carried out using methylene blue modified electrode [45].

## 4. Conclusion

PEDOT/DNA/NB composite electrode was prepared by dip coating method. PEDOT/DNA/NB composite electrode exhibited well defined redox peak at  $-0.35$  V (Ag/AgCl) corresponding to NB. The composite electrode was compared with PEDOT/NB and DNA/NB modified electrode and an enhanced peak current and reduced  $\Delta E_p$  were found. The composite electrode was exhibited surface confined redox process in neutral pH. The composite electrode was found pH dependent. The composite electrode exhibited catalytic property towards reduction of hydrogen peroxide. The composite electrode was utilized to amperometric study and its response towards  $H_2O_2$  detection was less than 6 s and the detection limit was 0.1  $\mu M$ . Moreover, we have utilized PEDOT/DNA/NB composite electrode to electrocatalytic reduction of Cyt *c*.

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