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# DNA hybridization electrochemical biosensor using a functionalized polythiophene

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ABSTRACT

A simple and label-free electrochemical sensor for recognition of the DNA sensor event was prepared by electrochemical polymerization of 4-hydroxyphenyl thiophene-3-carboxylate. Poly(4-hydroxyphenyl thiophene-3-carboxylate) (PHPT) was synthesized electrochemically onto glassy carbon electrode and characterized by cyclic voltammetry, FTIR and AFM measurements. An ODN-probe was physisorbed onto PHPT film and tested on hybridization with complementary ODN segments. A biological recognition can be monitored by comparison with electrochemical signal (cyclic voltammogram) of single and double strand state oligonucleotide. The oxidation current of double strand state oligonucleotide is lower than that of single strand, that is corresponding to the decrease of electroactivity of PHPT with the increase of stiffness of polymer structure. Physisorbed ODN-probe and its hybridization were observed morphologically onto ITO electrodes using AFM. The sensitivity of the electrochemical sensor is 0.02 μA/nmol, detection limit is 1.49 nmol and it has good selectivity.



## 1. Introduction

There is a great interest in the development of easy-to-use DNA sensors. Detection of specific DNA sequences is of great importance in numerous applications, such as medical research and clinical diagnosis. Although fluorescent label or surface plasmon resonance was widely used as hybridization transduction systems, these methods may be lengthy and costly [1,2]. Recently, electrochemical gene sensors have attracted increasing attention because of their simplicity, high selectivity and sensitivity. Nanoparticles [3,4], metal complexes [5], enzymes [6] and organic redox indicators [7] have been used for the electrochemical detection of DNA hybridization.

The intrinsically conducting polymers are a relatively new class of polymeric materials and are sensitive to changes in the polymeric chain environment [8–10]. Therefore, they may be used as active substrates that can sensitively report a biological recognition event, such as DNA hybridization [11]. Immobilization of oligonucleotide (ODN) probes onto conducting polymer would be expected to provide a simple and direct way to detect hybridization events, which perturb the electrochemical response of the polymer. Conducting polymers deposited on electrodes provide effective sensors where they provide an interface for entrapment or attachment of DNA probes, and also act as an electronic transducer of obtained signal [12].

The performance of electrochemical biosensors generally depends on the physic-chemical properties of the electrode materials, as well as the sensing element immobilized over the electrode surface [13]. Most of the reported electrochemical biosensors must apply a high positive charge potential on the working electrode, which minimizes the interference effects from reducing species [14].

Among conducting polymers, many functionalized thiophenes have been synthesized in the past due to their favorable properties [15,16]. Bauerle and Emge introduced nucleobase functionalized polythiophenyl derivatives. They observed changes of absorption maximum and the decrease of electrochemical activities as well due to the hydrogen bond formation with complementary [17].

This paper shows the procedure used for the novel synthesis of thiophenyl monomer and electropolymerization, the specificity of the immobilization and ability of the probe ODN physisorbed onto the PHPT compound to be hybridized. The ODN/PHPT film has been characterized using cyclic voltammetry, FTIR spectroscopy and atomic force microscopy (AFM). The advantageous features of this biosensor include a low price, ease preparation, high sensitivity and good selectivity.

## 2. Experimental

### 2.1. Materials

3-Thiophenecarboxylic acid, thionyl, hydrokinon, diethylether, sodiumbicarbonate, anhydrous magnesium sulfate and acetonitrile (99.8%, anhydrous) were purchased from Aldrich. Phosphate buffer

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(pH 7.4) was obtained from Sigma. An electrochemical grade tetrabutylammonium perchlorate ($TBAClO_4$) was purchased from Fluka. Integrated DNA Technologies (IDT), Inc. synthesized the ODNs including probe $NH_2$-GAT GAG TAT TGA TGC CGA-3′, complementary target 5′-TCG GCA TCA ATA CTC ATC-3′ and noncomplementary 5′-TAT GCT GGT GCG TCG CAC-3′. All aqueous solutions were prepared using Milli-Q water (18.2 MΩ cm).

### 2.2. Synthesis of 4-hydroxyphenyl thiophene-3-carboxylate (HPT)

A reaction mixture of 3-thiophenecarboxylic acid (7.81 mmol), of thionyl (220.50 mmol) and of hydrokinon (10 mmol) was stirred for 24 h under reflux. Then the reaction mixture was cooled and extracted with diethylether. The combined organic layer was washed with water, sodiumbicarbonate and dried with anhydrous magnesium sulfate. Finally, the solvent was removed by rotary evaporation. 4-Hydroxyphenyl thiophene-3-carboxylate solid was obtained in a 43% yield (M.p. 240 °C) $^1$H NMR (400 MHz, $CDCl_3$, $\delta$-ppm): 8.32 (m, α-2H, Th), 7.61 (m, β-2H, Th), 7.48 (m, α-2H, Th), 6.67 (m, 2H, aromatic), 6.46 (m, 2H, aromatic), 3.1 (s, 1H, hydroxyl); IR $V_{max}^{KBr}$ cm$^{-1}$: 3207 (aromatic C–H), 3188, 3170, 3030, 2956, 2922, 2850, 1880, 1517, 1452, 1361, 1246, 1195, 1095, 833 (β-H), 758 (α-H).

### 2.3. Electropolymerization of HPT, immobilization ODN and detection of hybridization

The electropolymerization was carried out by cyclic voltammetry from 0 to 1.7 V at a scan rate of 50 mV/s using a Gamry 300 Model potentiostat. PHPT was performed onto glassy carbon electrode in acetonitrile solution containing 5 mM monomer and 5 mM $TBAClO_4$ using a three-electrode cell comprising Ag/AgCl reference electrode and using Pt counter electrode. Prior to electropolymerization, glassy carbon electrode was polished with 0.5 μM alumina slurry and then washed with acetone, ethanol, and Milli-Q water.

For the FTIR and experiments, the polymer was electrochemically deposited on an indium tin oxide (ITO)-coated glass electrode. FTIR spectra were recorded on a Perkin Elmer, Spectrum BX II spectrophotometer. The surface topology of the electrodes was characterized using an AFM in contact mode under a constant force (NanoSurf), the roughness was obtained from the 9.8 μm × 9.8 μm scan.

The PHPT/glassy carbon electrode was upturned and 10 μL of ODN-probe (1.35 nmol) was pipetted over the electrode surface and kept at room temperature for 1 h. The resulting ODN probed PHPT/glassy carbon bioelectrode was thoroughly washed with a phosphate buffer solution (PBS) of 7.4 to rinse off any loosely bound ODN-probe from the electrode. The hybridization reaction was carried out by pipetting 10 μL of target ODN (1.49 nmol) in PBS solution (pH 7.4). After hybridization, the electrode was washed with PBS to remove any nonhybridized ODNs. The similar procedure was adopted for both a different molar concentration of target ODN and the mismatched target ODN.

## 3. Results and discussion

The HPT can be electropolymerized in the electrolyte acetonitrile/$TBAClO_4$ to the corresponding PHPT. A typical polymerization voltammogram is presented in Fig. 1 and an anodic oxidation wave is obtained at 1.2 V vs. Ag/AgCl.

This cycle shows the "nucleation loop" where the scan is reversed on the rising part of the peak, which is typical for a voltammogram of monomers yielding conducting polymer [18]. The color of the polymer changed from brown to dark green-red with increasing film thickness. Unlike alkyl substituted polythiophene [19], the PHPT was not soluble in common organic solvents such as acetonitrile and chloroform.

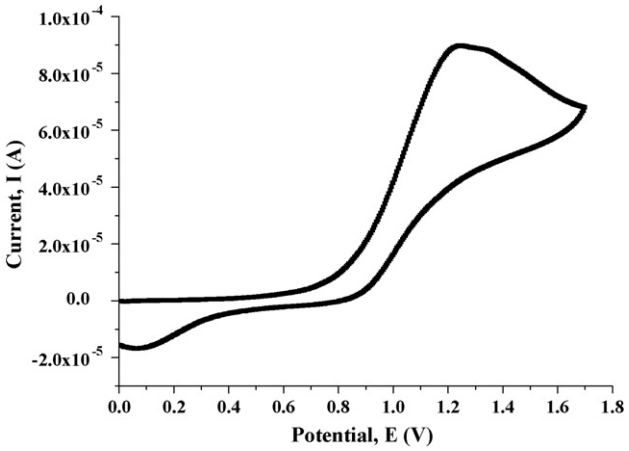


**Fig. 1.** Electropolymerization cyclic voltammogram of HPT on a glassy carbon electrode at 50 mV/s in 5 mM acetonitrile/$TBAClO_4$.

After repeated scans, the coated electrode was removed from the monomer solution and rinsed with acetonitrile to remove the monomer and oligomeric species. Then, electrode was placed in monomer-free electrolytic solution containing 5 mM $TBAClO_4$ into acetonitrile. The polymer film exhibited a high electroactivity in acetonitrile/$TBAClO_4$ media with an almost reversible oxidation wave at 1.2 V (vs. Ag/AgCl Fig. 2a) and a linear dependence of peak current of CV with scan rate. The anodic (Iac) peak current responses

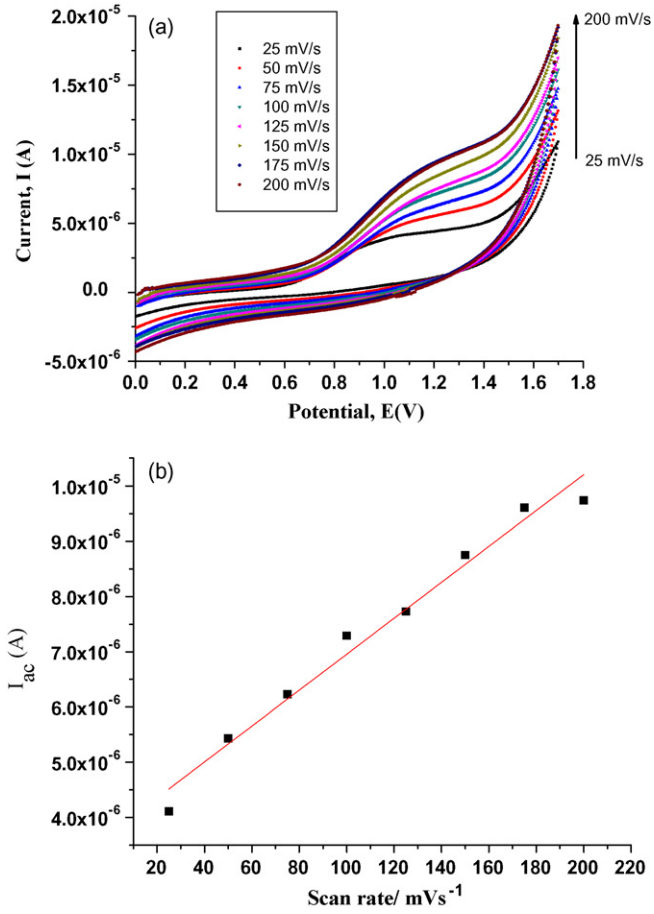


**Fig. 2.** (a) Cyclic voltammogram of PHPT film in acetonitrile/$TBAClO_4$ with different scan rates between 25 and 200 mV/s and (b) relationship of anodic current peaks as a function of scan rate for PHPT film in acetonitrile/$TBAClO_4$.

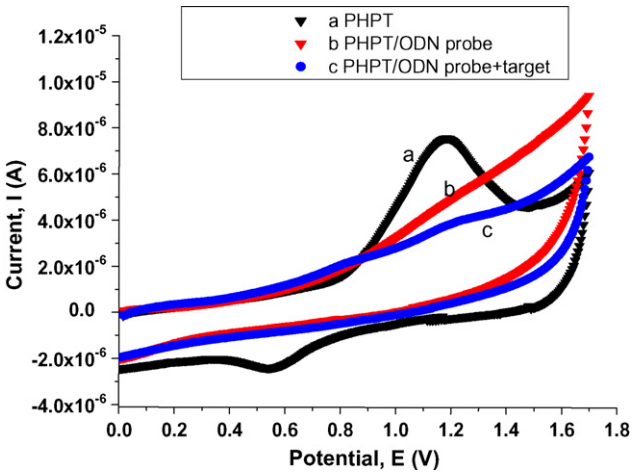


**Fig. 3.** Cyclic voltammograms of the (a) PHPT, (b) PHPT/ODN and (c) ODN hybridized PHPT/ODN electrodes in acetonitrile/$TBAClO_4$ (scan rate: 50 mV/s).

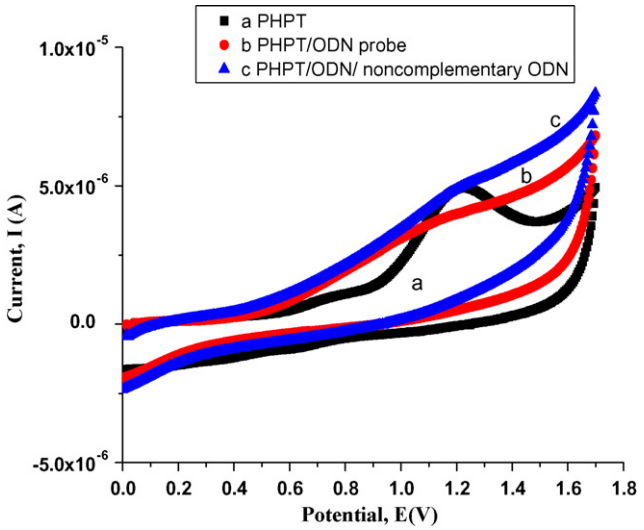


**Fig. 5.** Cyclic voltammograms of the (a) PHPT, (b) PHPT/ODN-probe and (c) after immobilization of noncomplementary ODN onto PHPT/ODN electrodes in acetonitrile/$TBAClO_4$ (scan rate: 50 mV/s).

were recorded and it was found that anodic current responses increase linearly as the scan rate increases (Fig. 2b). It was clear that the redox process was non-diffusional and the electroactive polymer was well adhered to the working electrode surface [20].

Fig. 3 shows the electrochemical characteristics of bare, ODN-probe immobilized, and ODN target hybridized PHPT/glassy carbon electrodes. The PHPT/glassy carbon electrodes illustrated admirable electrochemical performance. It was observed that the intensities of the oxidation current of ODN-probe and ODN target immobilized electrodes decreased, which could be attributed to prompt redox behavior of probe ODN [21]. The probe ODN and target ODN with hybridization could form a potential barrier of the rotation of polymer backbone and bulky conformational modifications made along the conjugated polymer backbone. The decrease in the peak current measured with the ODN hybridization PHPT/glassy carbon electrode may be related to the electrostatic repulsion between the polyanionic hybridized ODN on the cationic PHPT electrode and the anionic redox couple ions [22,23]. This can be explained by the formation of ODN duplexes that further distort the electronic properties of the polymer backbone and/or a additional increase in the amount of electrostatic changes and thus the repulsion of the oppositely charged redox probe (Fig. 4). Additionally, the voltammogram of PHPT in the presence of target ODN indicated a shift in the peak position.

The selectivity of the biosensor was investigated by the hybridization of the probe ODN immobilized PHPT/glassy carbon bioelectrode with noncomplementary ODN sequences. After incubation with noncomplementary ODN sequences, the bioelectrode indicated increasement in the oxidation current at 1.2 V, indicating that the biosensor had good selectivity only for the target ODN (Fig. 5). Also, one can see that the oxidization current of the bioelectrode that was incubated in the solution of noncomplementary ODN does not differ significantly from initially obtained than that of PHPT/glassy carbon electrode.

To investigate sensitivity of the sensor, different amounts of target ODN varying 1.49 and 7.45 nmol were incubated with electrochemical sensors. Fig. 6 exhibits cyclic voltammograms of PHPT/ODN bioelectrode with increasing target ODN concentration and the variation of oxidation peak current as a function of the amount of target ODN physisorbed onto PHPT film using cyclic voltammetry technique. It was found that the peak oxidation current observed at about 1.2 V decreased with an increasing concentration of the target ODN. This result indicates that binding

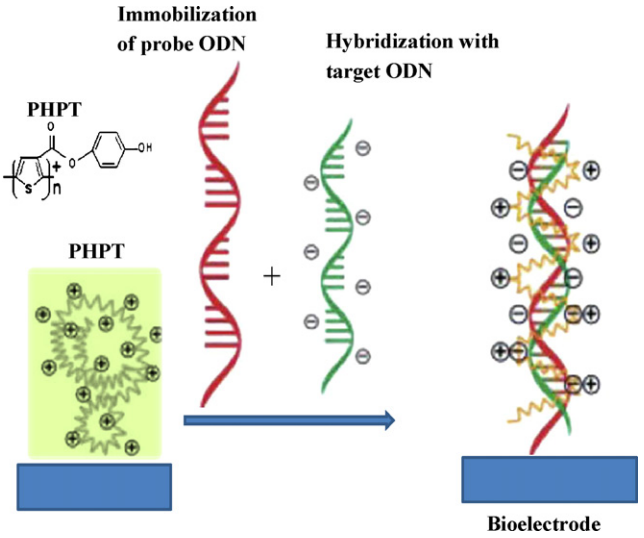


**Fig. 4.** Schematic presentation of the fabrication of PHPT/ODN bioelectrode.

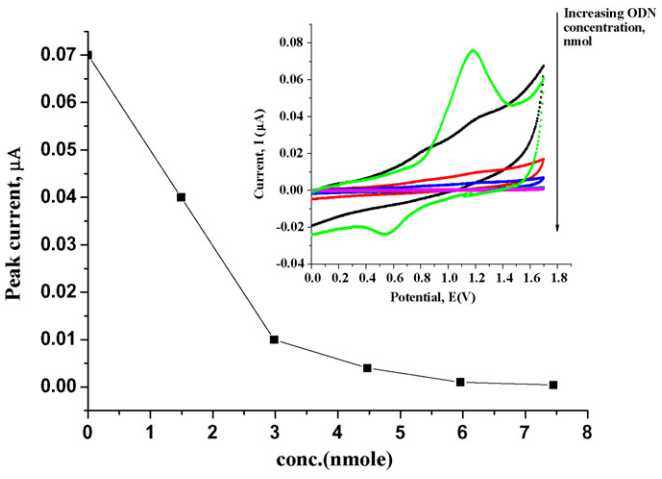


**Fig. 6.** Variation of electrode current at constant potential $E = 1.2$ V, as a function of concentration of target ODN (inset: cyclic voltammograms of PHPT/ODN bioelectrode with increasing target ODN concentration, mmol).

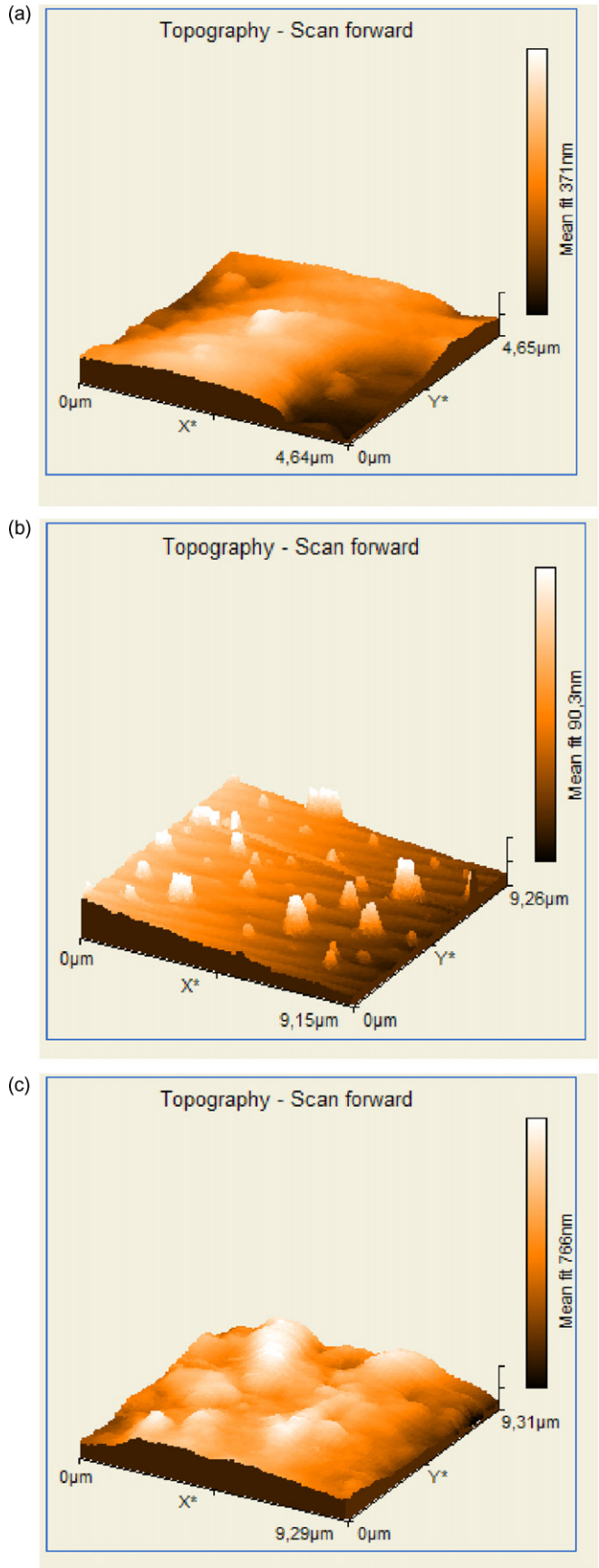


**Fig. 7.** AFM micrographs of the (a) PHPT, (b) PHPT/ODN-probe and (c) ODN hybridized PHPT/ODN electrodes onto ITO.

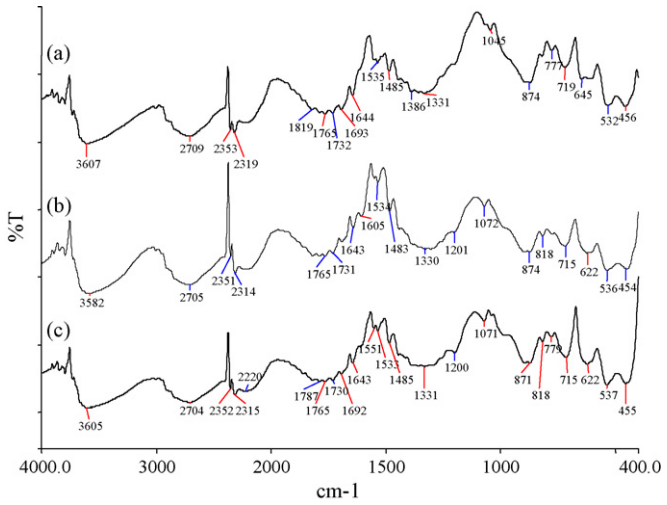


**Fig. 8.** FTIR spectra of the (a) PHPT (b) PHPT/ODN-probe and (c) ODN hybridized PHPT/ODN electrodes onto ITO.

of probe ODN with the target ODN at the probe PHPT/ODN electrode surface had occurred. From Fig. 6, the sensitivity of electrochemical sensor was evaluated 0.02 μA/nmol from the slope at the origin of this curve. The detection limit for the system is 1.49 nmol of ODN in solution. Compared to the detection limit of 10 nmol by Park et al. [24], 1 and 0.5 nmol by Peng et al. [25], 1 nmol by Cha et al. [20] and 2 nmol by Garnier et al. [26], the value of 1.49 nmol by ours appears promising for application.

Fig. 7a–c shows the AFM pictures obtained for bare PHPT/ITO electrode, after immobilization of probe ODN in the case of PHPT/ITO and after the hybridization of the target ODN in the case of probe ODN/PHPT/ITO electrodes, respectively. It may be noted that the roughness in PHPT/ITO surface is due to the immobilization of ODN-probe. The network structure of bioelectrode seen in Fig. 7c arises due to the hybridization of the target ODN to electrode resulting in the rougher topography of the polymeric surface [27].

Fig. 8a–c shows FTIR spectra of PHPT, ODN/PHPT and the ODN hybridized PHPT/ODN films coated onto ITO electrodes. In the spectrum of pure PHPT (Fig. 8a), the bands between 1500 and 1300 $cm^{-1}$ originated from the stretching modes of C=C and C–C in the thiophene ring [28]. The bands at 1066 and 1045 $cm^{-1}$ are assigned to stretching of the C–O–C bonds of PHPT. Vibrations from the C–S bond in the thiophene ring of PHPT are shown at 893 and 871 $cm^{-1}$ [29,30]. The peaks at 1692, 1644 and 1534 $cm^{-1}$ are assigned to the stretching and bending modes of benzene ring in the structure of PHPT. A strong absorption peak at 1732 $cm^{-1}$ is characteristic of the stretching vibration of the carbonyl group of PHPT. FTIR spectrum of the ODN/PHPT and the ODN hybridized PHPT/ODN (Fig. 8b and c) showed additional peaks at 1602, 1200, 1072 and 982 $cm^{-1}$ due to the attachment of ODN-probe and ODN target with PHPT polymer [31].

## 4. Conclusions

In this work, novel functionalized thiophene monomer was synthesized and characterized by FTIR, CV and AFM measurements. It was shown that ODN can be physisorbed onto electrochemically deposited PHPT film onto glassy carbon and ITO electrodes. The PHPT/ODN bioelectrode was employed for the hybridization of the target ODN, with detection limit of 1.49 nmol and showed good sensitivity and selectivity. The sensitivity of the biosensor was 0.0059 μA/nmol. The PHPT bioelectrode is advantageous of low price, ease of preparation without using any grafting agent, good

sensitivity and selectivity. Our further improvement in sensitivity and selectivity will be to prepare nanocomposites of PHPT.

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