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Directly Immobilized DNA Sensor for Label-free Detection of Herpes Virus

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Abstract-**This paper reports the direct immobilization of deoxyribonucleic acid (DNA) sequences of Herpes simplex virus (5'–AT CAC CGA CCC GGA GAG GGA C–3') on the surface of DNA sensor by using the cyclic voltammetric method with the presence of pyrrole. The potential was scanned from -0.7 volt to + 0.6 volt, the scanning rate was at 100 mV/s....This kind of DNA sensor was developed to detect Herpes virus DNA in samples. The FTIR was applied to verify specific binding of DNA sequence and conducting polymer, the morphology of conducting polymer doped with DNA strands was investigated by using a field emission scanning electron microscope (FE-SEM). The results showed that output signal given by co-immobilized DNA/PPy membrane sensor was better than that given by APTS immobilized membrane sensors. The sensor can detect as low as 2 nM of DNA target in real samples.**

I. INTRODUCTION

The detection of specific DNA/RNA sequences is of great importance in numerous applications of modern life science, including identification of medical research and clinical diagnosis [1], [2], controlling the food quality [3], [4], environmental analysis [5], [6]. Many methods have been used for this purpose such as polymerase chain reaction (PCR) [7]-[9], quartz crystal micro-balance (QCM) [10], [11], fluorescence [12], surface plasmon resonance [13], microfluidic system [14], cell culture and real-time PCR, etc... These methods are precise, and allow a wide, dynamic range of detection. However, they are complex, costly and time consuming. In addition, it is impossible to carry the on-site/in-field tests. Thus, development of a cheap, reliable device allowing rapid detection is always the challenge for scientists and engineers. In this context, DNA sensor based on electrochemical detection is one of the feasible and promising tools.

We reported, in this paper, the direct co-immobilization of DNA sequence of Herpes simplex virus and polypyrrole onto the surface of a sensor by cyclic voltammetry to determine the herpes DNA target sequence in the sample. The herpes simplex virus (HSV) is an enveloped double-stranded DNA virus. There are two distinct forms of HSV, serotype 1 and serotype 2 (HSV-1 and HSV-2). HSV-2 is the most common cause of genital herpes, whereas HSV-1 is the most common cause of facial herpes or cold scores. HSV-1 is transmitted through contact with oral secretions. Diseases caused by Herpes virus are commonly found in patients in Vietnam.

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II. EXPREMENTENT

A. Chemical reagents

DNA probe, with a specific sequence to HSV-1 of 5'–AT CAC CGA CCC GGA GAG GGA C–3' and complementary DNA target sequence for HSV-1 of 3'-TA GTG GCT GGG CCT CTC CCT G-5' was supplied by Invitrogen Life Technologies Company through National Institute of Hygiene and Epidemiology of Vietnam. Pyrrole was purchased from Merck. Other chemicals are of analytical grade.

B. Sensor fabrication

The DNA sensor based on microelectrode with various configurations was designed and fabricated at clean room (ITIMS). The sensor consists of pairs of microelectrodes on the surface of silicon substrate, one of which acts as working sensor and the other as a reference electrode. The dimension of the inter-electrodes was 20 µm x 20 µm. The detailed fabrication process was discussed in [15].

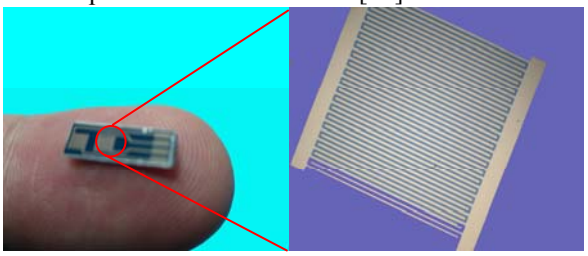


Fig.1. 20 µm x 20 µm microelectrode sensor was fabricated at ITIMS.

C. Cyclic Volttametry electropolymezation

Electro-polymerization was carried out by using IM6EX (Germany) impedance analyzer at room temperature in which the microsensor acted as working electrode while auxiliary electrode was a platinum wire. Reference electrode is Ag/AgCl in saturated KCl.

The sensor was first surface cleaned by KCr_2O_7 in H_2SO_4 98% followed by cyclic voltammograms (swept potential

from -1.5V to +2.1V, scan rate: 25mV/s) in 0.5M H_2SO_4 to activate the surface of the sensors. Finally, the potential was swept on the working electrode from -0.7 volt to 0.6 volt versus standard counter electrode (SCE). The scanning rate was 100mV/s

D. Measurement

Differential measurements were realized to determine the changes in conductance of DNA membrane. AC reference signal (10 KHz, 100mV sine wave), generated by the generator of Lock-in Amplifier SR830, was applied on two identical micro-electrodes of DNA sensor. The output signal was acquired by measuring the voltage drop on two 1 KΩ resistances by the A and B channels of the Lock-in Amplifier and processed by a PC through RS 232 interface. All measurements were performed at room temperature. In this experiment, five DNA sensors were used to test the hybridization of DNA sequences.

III. Results and Discussion

A. The polymeration of Ppy/DNA

Normally, pyrrole is polymerized with the presence of an anionic dopant which contributes to film conductivity. Variety of anions can be used as dopant for polypyrrole (Ppy) polymerization such as Cl^-, NO_3^-. In this work ClO_4^- and DNA sequence were used.

According to Wang et al [16], DNA can be considered as sole counter anion in the electropolymerization process at the working electrode. This allowed maximum possible incorporation of DNA in the conductive polymer throughout the film thickness and full contribution of oligonucleotides charged phosphates to the polymer conductivity.

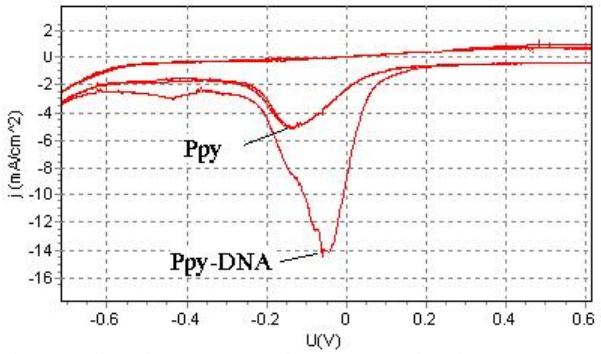


Fig.2. Cyclic voltammograms of 0.5mM Ppy doped 0.05 µM DNA probe sequence in $LiClO_4$ solution. Swept potential from -0.7 V to 0.6V, scanning rate is at 100mV/s.

The cyclic voltammograms of synthesized Ppy and Ppy/DNA film is shown in figure 2 where the oxidation of pyrrole monomer leads to the formation of radical cation, subsequent oxidation of the dimer and coupling will result in the formation of an insoluble polymer, positively charged on the surface. This electrochemical procedure allowed the formation of a copolymer which is a mixture of polypyrrole and an oligonucleotides that shows an increasing current along with conducting film growth which corresponds to incorporation of oligo into the Ppy film. The film was rinsed and used for detection of DNA hybridization.

B. FTIR spectrum of DNA/ Ppy

In this work, the FTIR spectroscopy was used to verify the existence of polypyrrole and DNA sequence on the microelectrode surface after the polymerization process. The infrared spectrum of the DNA/Ppy complexes and pure Ppy were performed on Niconet 6700 FT-IR machine with the effective range from 400 cm^{-1} to 4000 cm^{-1} at room temperature. As shown in figure 3, the absorption band at 1889 cm^{-1}–1629 cm^{-1} vibration plane implied G-C and A-T base pairs while the backbone phosphate group at 1095 cm^{-1} was perturbed upon Ppy interaction [17], [18].

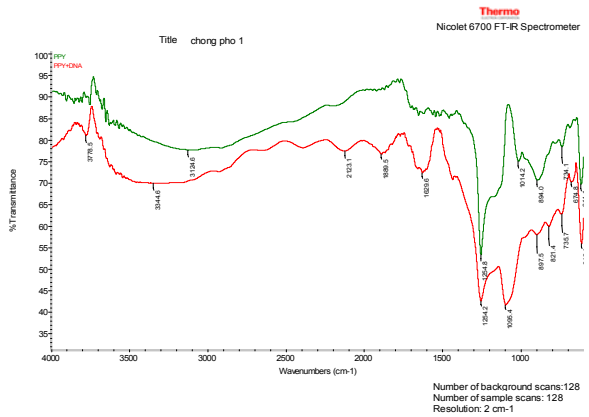
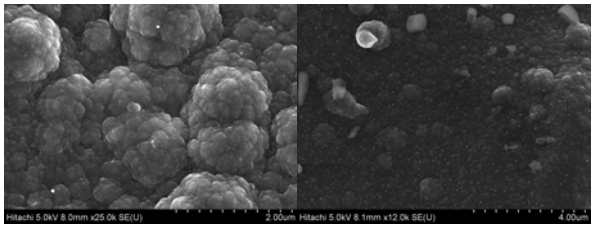


Fig. 3. FTIR spectra of Ppy/ DNA and Ppy (Upper curve: Ppy/Dopant, lower curve: Ppy/Dopant/ DNA).

The absorption band at 1254 cm^{-1} was assigned to the biopolaronic species formed in the over oxidation process of Ppy [19]. The C-H and N-H bonds were also observed at 735 cm^{-1} (for DNA/Ppy film); 734 cm^{-1} for Ppy film; 894 cm^{-1} for DNA/Ppy and 897 cm^{-1} for Ppy membrane, respectively. These results show very good agreement with earlier reported work [20].

C. Morphology of conducting polymer film

The morphology of sensor surface coated with Ppy film was studied by FE-SEM. Figure 4 indicated micrographs of polypyrrole doped with LiClO4 (4a) and with both 0.1 M $LiClO_4$ and 0.05 µM DNA sequence (4b) membrane given by direct electropolymerization method.



(a) (b)

Fig.4. The FE-SEM of Ppy and Ppy – DNA coated onto microelectrode surface. a) Ppy doped $LiCLO_4$, b) Ppy doped $LiCLO_4$ and DNA

In figure 4(a), the pure PPy doped with $LiClO_4$ was cauli-flower structure matching other works [21]. This structure is related to the dopant intercalation in the polymeric chain. As in figure 4(b) the DNA strands was observed as white dots in host polymer membrane. Good distribution of DNA in PPy membrane makes it advantage for hybridization process of the probe in target solution.

D. The hybridization of DNA sensor

As above-mentioned, the probe-attached sensor is commonly soaked into solution containing target DNA. A DNA helix sequence is formed on the surface of the sensor when target/immobilized DNA matching occurred.

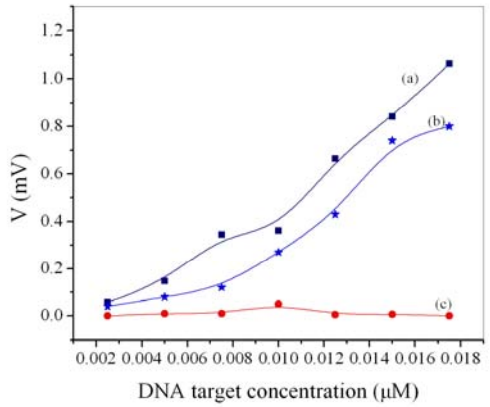


Fig.5. DNA sequence hybridization curve. Room temperature, 0.05 μM DNA probe: (a) DNA/Ppy, (b) DNA/APTS, (c) Non-

Such hybridization is detected by changes in the conductance of the conductive membrane on the surface of sensors leading to the change in output signal of the system. In figure 5, the hybridization illustrated by linear curve that described the relation between the target DNA concentration and output signal of the DNA sensor. It can be seen that, for both APTS and Ppy/DNA attachment method, the sensor can detect as low as 2 nM of target DNA. However, the intensity of the output signal found to be better when direct immobilization was used than that given by APTS. This is explained by the contribution of Ppy and dopant which improve the conductivity of the membrane namely enhancing the electric charge transfer within the film.

IV. CONCLUSION

This paper described the direct immobilization of DNA strand on the surface of sensor by electrochemical method. The DNA sensor was used to determine the Herpes simplex virus DNA (5'–AT CAC CGA CCC GGA GAG GGA C–3') in the sample. The results showed that, DNA/Ppy was strongly bonded to the surface of the sensor. The DNA sensor can detect as small as 2 nM of herpes virus concentration at room temperature and the intensity of the output signal is better than by using APTS attachment method. At the current time, the DNA sensor can be reusable twice heating up the cell to T_m then cooling down quickly. Sub-micron configuration of sensors will be carried out for better sensitivity. In addition, various numbers of mismatch, longer DNA sequence and different samples will be investigated for better selectivity. The authors, in collaboration with the National Institute for Hygiene and Epidemiology of Vietnam, are trying the first analysis to detect influenza virus type A from chicken which is considered as a serious problem in Viet Nam.

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