Electrochemical direct immobilization of DNA sequences for label-free herpes virus detection

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Abstract. DNA sequences/bio-macromolecules of herpes virus (5’–AT CAC CGA CCC GGA GAG GGA C–3’) were directly immobilized into polypyrrole matrix by using the cyclic voltammetry method, and grafted onto arrays of interdigitated platinum microelectrodes. The morphology surface of the obtained PPy/DNA of herpes virus composite films was investigated by a FESEM Hitachi-S 4800. Fourier transform infrared spectroscopy (FTIR) was used to characterize the PPy/DNA film and to study the specific interactions that may exist between DNA biomacromolecules and PPy chains. Attempts are made to use these PPy/DNA composite films for label-free herpes virus detection revealed a response time of 60 s in solutions containing as low as 2 nM DNA concentration, and self life of six months when immersed in double distilled water and kept refrigerated.

Keyword: Electrochemically, bio-macromolecules, herpes virus.

1. Introduction
Label-free, electrochemical DNA detection have become an attractive, and a major focus in biotechnology and clinical diagnostics [1-3]. This technique offers high sensitivity, low cost, and fast analysis and can be easily integrated into small devices [4]. Typically, a single-strand DNA is firstly immobilized onto a conducting polymer coated on an electrode, then the microsensor converts the chemical recognition event into a measurable electronic signal. The immobilization of the DNA, normally by means of covalent attachment between the DNA oligos and polymeric membrane, plays a major role in determining the overall performance of an electrochemical DNA sensor [5, 6].

The conducting polymers offers an excellent interface for immobilized DNA probes/microsensor’s surface [7-10] among which, thanks to large surface area and binding ability with DNA, bio-compatible, high hydrophilic, well stable and facile incorporation of many counter ions. Polypyrrole (PPy) is the most commonly used [11]. In addition, PPy has attracted much interest since both its good thermal and mechanical stability and maintains its conductivity for several months or a year [12, 13].

DNA is a large and highly structured biomacromolecule of which single-strand backbone is composed by alternatively a deoxyribose and a phosphate group. The latter confer to DNA its polyanionic character. The rationale hypothesis for the study on interaction of DNA biomacromolecules of herpes virus (HSV-1) and PPy chains is that PPy in their oxidized state...
provides positively charge density for electrostatically interaction with the highly negative charged DNA. In general, the hybridization reaction of a single strand of DNA (target) and an immobilized DNA probe is of extreme importance in the achievement of suitable sensitivity. Shimizu [14] and Livache [15, 16] have proposed an electrochemically directed polymerization which allows preparing PPy/DNA composite films directly or in one-step for immobilization of DNA onto the electrodes. During the preparation of PPy/DNA composite films, deoxyribonucleic acid is well suited to act as a natural polyelectrolyte, meaning as dopant for conducting polymers such as polypyrrole that carry a net positive charge in their oxidized state, as illustrated in figure 1.

Figure 1. Illustration of the covalent interaction of a polypyrrole chain and DNA biomacromolecules of herpes virus.

Cyclic voltammetry (CV), is well known as an excellent analytical technique with CV, one can control the programmable delivery of electrons through the electrode and the potential applied to the electrode to couple the soluble oligomers of pyrrole and the deposited PPy chains during the direct electrosynthesis of PPy/DNA composite films. Also, the DNA probes are reoriented in better order in the field caused by applied potential. It is noted that the DNA uptake is proportional to increasing ionic strength resulting in the observed adsorption of polyelectrolyte onto hydrophobic surfaces [17]. The steady increasing state of potential during anodic potential sweep, usually positive sign, may decrease in the electrostatic repulsion between the DNA probes. Meanwhile cathodic potential sweep, the steady decreasing state of potential appears to make it advantage for the reorientation of the DNA probes. Therefore a more compact structure of the couple of DNA and pyrrole ligomer is obtained, resulting in more favorable adsorption on the adsorbent surface. In this work, the PPy/DNA (herpes virus) films were electrochemically synthesized directly by cyclic voltammetry and grafted onto arrays of interdigitated platinum microelectrodes which are used for the determination of the herpes.

2. Experimental

2.1. Chemicals and reagents
Pyrrole was purchased from Merck. Doubly distilled water in a quartz apparatus was used to prepare aqueous solutions. To remove oxygen from stirred aqueous solutions 0.1 M LiClO₄ (Merck) were bubbled with nitrogen gas for 30 minutes before adding pyrrole monomer (without further purification) and during electrosynthesis of PPy/DNA (herpes virus) composites.

Custom oligonucleotides, specific to herpes virus (5′–AT CAC CGA CCC GGA GAG GGA C–3′), were synthesized by Invitrogen Corp.

2.2. Preparation of PPy/DNA electrodes
0.3 M pyrrole and 0.1 μM single strand DNA of herpes virus was placed in a 5 ml three-electrode cell, at room temperature. The potential applied on the working electrode was relative to an Ag/AgCl (3 M NaCl) reference electrode; and a Pt sheet (1 cm²) serves as auxiliary electrode. The process was controlled by EG&G 362 (Princeton Applied Research) incorporating the Ecuivre-HH5 program. The
microelectrodes were fabricated at International training Institute for Materials Science (ITIMS), Hanoi University of Technology, by using standard microelectronic technology.

Firstly, the microelectrodes were immersed in a mixture of 1 M KCr$_2$O$_7$ and 1 M H$_2$SO$_4$, and washing for several times by double distilled water; followed by cyclic voltammetry in a solution of 0.5 M H$_2$SO$_4$ with potentials ranging of -1.5 and +1.5 V, at a scan rate of 25 mV/s for cleaning and activating the surface. Thereafter the direct immobilization of DNA of herpes virus was done by cyclic voltammetry (potential sweep of 100 mV/s, potential range: -0.3 to +0.9 V).

![Diagram](image)

**Figure 2.** Diagram illustrates measurements of the sensibility of PPy/DNA electrodes. A signal of alternative current that had frequency of 10 KHz and amplitude of 100 mV taken out from generator of the Lock-in Amplifier SR830 was applied on PPy/DNA electrodes (working electrode) and other PPy electrodes (reference electrode).

The morphology of the surface of obtained PPy/DNA films was investigated by a field emission scanning electron microscopy Hitachi-S 4800. The presence of DNA in the PPy matrix was confirmed with FTIR measurements using the Thermo Nicolet 6700. The sensibility of the PPy/DNA electrodes was investigated by a Lock-in Amplifier SR830 (figure 2). A signal of alternative current (10 KHz, 100 mV) was applied on PPy/DNA electrodes serving as working electrodes, another one is the reference electrode. The output signal was acquired by measuring the voltage drop on two 1 KΩ resistances by the channels A and B of the Lock-in Amplifier, which was set on automatically differential measurement mode.

### 3. Results and discussion

A typical voltammogram of a one-step immobilization of DNA of herpes virus in copolymerization with pyrrole was accomplished by repeated potential cycling over the range of -0.3 and 0.9 V. As seen in figure 3, the irreversible oxidation of polymerization of pyrrole in the presence of DNA appears to be more easily, at 0.2 V (figure 3b) than that of polypyrrole, at 0.48 V (figure 3a). It indicates that the existence of covalent/hydrogen bonds between the DNA biomacromolecules and pyrrole monomers makes its advantage for the polymerization process, and plays an important role in decreasing the oxidation potential of pyrrole and expanding the oxidation potential range.

As the results, the oxidation of a conducting polymer increases the density of charge carriers in the polymer film. This oxidation process also creates deep Coulomb traps due to the presence of the dopants. In parallel, the mentioned covalent/hydrogen bonds limit the diffusion of species of soluble oligomers of pyrrole to the electrode surface. Thus, the value of peak current density of the later, 0.08 mA.cm$^{-2}$, is smaller in comparison with the one of the first, 0.1 mA.cm$^{-2}$. To reduce this, a supporting 0.1 M LiClO$_4$ electrolyte was added. In this case an oxidative current density is steadily increasing up to 0.8 mA.cm$^{-2}$, several times higher more than the one of the first two (figure 3c).
Figure 3. Cyclic voltammograms of the electropolymerizations of (a) Pyrrole were conducted in aqueous solutions of 0.3 M pyrrole, at scan rate of 100 mV.s\(^{-1}\); (b) with 0.1 μM single strand DNA biomacromolecules of herpes virus; and (c) with 0.1 μM single strand DNA biomacromolecules of herpes virus and 0.1 M LiClO\(_4\).

Figure 4 shows the scanning electron micrographs of the PPy and PPy/DNA films. In case of PPy film, an irregular and heterogeneous membrane illustrated by numerous cauliflowers like projections and channels between them was observed. Meanwhile, since DNA bio-macromolecules act as template reagents, the PPy/DNA membrane showed an ordered and stacked structure, a continuous film consisting of globular granules that coalesced together into a more smoothing and compact surface covered entirely the surface electrode.

The channels appear to be disappeared, but there are still several aggregates sized in submicron. This can be explained by the linkage of DNA biomacromolecules and soluble species of pyrrole oligomers during copolymerization to form the bi-dimensional growth of the PPy/DNA composite film. In consequences, DNA biomacromolecules may be incorporated into the whole of a PPy matrix for easier hybridization with the DNA target in solutions.

As obtained the PPy/DNA composite films possess the oxidative current density (figure 3c) up to 0.8 mA.cm\(^{-2}\) and the ordered and stacked structure (figure 4, rhs.). It may be considered as a result of an improved interchain hopping of polarons and higher mobility of charge carriers/polarons in the PPy/DNA composite films.

In figure 5, the spectroscopic characteristics for PPy are observed at the vibration bands in a range of 500-1650 cm\(^{-1}\) for both PPy and PPy/DNA samples [18]. The analysis of the FTIR spectra also presents the increase and extension of the absorption band centered at around 3400 cm\(^{-1}\) belonging to asymmetric stretching of NH groups (figure 5, lower) which resulted from the covalent attachment of the DNA biomacromolecules with NH groups of a moiety of PPy matrix (figure 1). Unlike PPy (figure
The spectrum of a PPy/DNA sample showed a continuous increase tail in absorption from 3650 to 4000 cm\(^{-1}\) (figure 5, lower). It is the tail of the \(-1\) eV (\(-8066\) cm\(^{-1}\)) bipolaron absorption band that is the signature for electrical conductivity of conducting polymers [19, 20]. In addition, for the PPy sample, a rippled obtuse peak having featureless shape that was observed in the 1350-1760 cm\(^{-1}\) region, is constructed with several component vibrations owing to the imine/amine species. The bands assigned to \(\nu(C=\text{C})\) stretch are observed in the region of 1400-1620 cm\(^{-1}\). The presence of DNA biomacromolecules of HSV-1 causes the split of spectroscopic characteristics (figure 5, lower). Indeed, the 1350-1760 cm\(^{-1}\) region of PPy/DNA consists of several features sharply, such as the bands in the regions 1450 cm\(^{-1}\) are assigned to the C–N bond stretching. Besides, an intensive band centered at 1629 cm\(^{-1}\) that was constructed with the 1647 cm\(^{-1}\) vibration band due to C=\text{N} stretching and the 1600 cm\(^{-1}\) vibration band due to C=\text{C} bonds associated with C–N stretching and bending vibration [21, 22]. Furthermore, due to the presence of DNA biomacromolecules, the adsorption band expanding from 1800 cm\(^{-1}\) upward that is attributed to free charge carriers [23], is not just split and grouped into smaller vibration bands centered at 1889, 2123, 2397 and 2668 cm\(^{-1}\), but with higher intensity. It reveals that the imine/amine charge carriers and the conjugated structure of PPy chains may be disturbed by the interactions between them and biomacromolecules of HSV-1 and thus limited the extent of charge delocalization along the polymer chains, leading to increase of spectral features.

In the range 1000 to 1300 cm\(^{-1}\), the increase of spectral feature of absorption bands was also observed. These bands present the relationship in population of charge carriers, amine and imine species of PPy. Without DNA strands, a sharp peak at 1254 cm\(^{-1}\) and a shoulder at \(-1200\) cm\(^{-1}\) characterize C–C and C–N stretching vibrations of amine species (aromatic structure), and a peak at 1014 cm\(^{-1}\) is attributed to C–H in plane vibration [24]. Oppositely, as seen in the spectrum of PPy/DNA composite (figure 5, lower), a new sharp peak was observed at 1095 cm\(^{-1}\), corresponding to the in-plane stretching vibration of NH\(^{+}\) of imine species (quinonoid structure) that was performed by protonation of PPy chains [25].
It implies that a part of aromatic structure of PPy chains converts into quinonoid structure, and the formation of quinonoid structure was extended during electropolymerization of pyrrole in the presence of DNA of HSV-1. Furthermore it might be resulting from the Coulomb interaction of $\pi$-conjugated electron in C=C of pyrrole and the lone pairs of electrons in nitrogen atoms with phosphate groups of the DNA biomacromolecules.

![Figure 6](image.png)

**Figure 6.** The change in voltage drop on a resistance connected to a PPy/DNA composite film as a function of DNA target concentration (upper) that is its sensing characteristic in comparing with the constant in the case of PPy film (lower).

The increase of charge carriers in PPy chains and higher mobility of charge carriers/polarons in the PPy/DNA composite films led to the increase in conductivity as seen in their sensing measurements. The voltage drop on a resistance connected to the PPy/DNA film reaches a stable value after 60 seconds and recorded. A quasi linear increase of the voltage drops with DNA target concentration was observed and shown in figure 6. Meanwhile the voltage drop on another film connected to the PPy film appears to be constant. These results are still repeated after the PPy/DNA films immerged in doubly distilled water, and stored in refrigerator for six months.

4. Conclusions
The PPy/DNA composite films having an ordered and stacked structure, as confirmed by FE-SEM studies, were electrosynthesized directly by cyclic voltammetry at 0.2 V onto the surface of the microelectrodes. The analysis of FTIR data mentioned above revealed that DNA bio-macromolecules of herpes virus act as template reagents. The intensive interactions of the DNA bio-macromolecules and PPy chains may disturb imine/amine charge carriers and the conjugated structure of PPy chains and thus limited the extent of charge delocalization along the polymer chains. A part of aromatic structure of PPy chains converts into quinonoid structure and causes an increase of population of charge carriers in PPy chains of the PPy/DNA composite films.

The PPy/DNA based sensors can detect the HSV-1 DNA from 1–18 nM. The response time was 60 seconds. These PPy/DNA sensors can be used for rapid screening in food and medical applications.

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Acknowledgment
This work is financially supported by the Vietnam Ministry of Education and Training (under code B2008-21-09). We are grateful to Vietnam National Center for Hygiene and Epidemiology for HSV-1 sequence samples.