HIGHLY SENSITIVE BIOSENSOR BASED ON ORGANIC ELECTROCHEMICAL TRANSISTORS

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Highly Sensitive Biosensor Based on Organic Electrochemical Transistors

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Certificate of Originality

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MAK CHUN HIN

Abstract

Different biological and chemical sensing methods based on solution gated field-effect transistor have been investigated in recent decades due to their many advantages, including miniaturization without the loss of signal to noise ratio, signal amplification on applied gate voltage and operation in solution environment. However, conventional inorganic field-effect transistors (FET) are usually not bio-compatible. Furthermore, the high temperature processing in device fabrication and the rigid architecture of the devices make it difficult to integrate the devices with biological systems and hinder the applications in biological or chemical sensing. Recently, organic electrochemical transistors (OECTs), a special kind of organic thin film transistor (OTFTs), can operate in various solution or electrolytes at low working voltages with stable performance. The devices can be prepared by solution process such as spin-coating on various substrates at low temperatures. Therefore, OECTs have become promising transducers/sensors for biological and chemical sensing. In this thesis, OECTs with poly(3,4-ethylenedioxythiophene)–poly(styrene sulfonic acid) (PEDOT:PSS) channels have been introduced to detect three analytes, including epinephrine, cholesterol and Adenosine triphosphate (ATP), based on different mechanisms and showed high performance in comparison with conventional electrochemical approaches.

Epinephrine, as an important neurotransmitter, was successfully detected by OECTs with platinum (Pt) gate electrodes. Epinephrine molecules can undergo direct

electro-oxidation on the Pt gate electrode and lead to obvious changes of the channel current of the OECTs. The device performance was optimized by modifying Nafion and carbon-based nanomaterials (graphene, graphene oxide and single-wall carbon nanotubes) on the gate electrodes. It was found that Nafion and single–walled carbon nanotube co-modified gate electrodes can lead to the lowest detection limit of 0.1nM for epinephrine, which covers the normal range in human body (~0.2nM). Compared to the previously reported sensors based on ion-sensitive field effect transistor (ISFET) and cotton-based OECTs, the detection limit of our OECTs is much lower than the reported results due to the different sensing principle and device architecture.

A high cholesterol level in human blood plasma will lead to a high risk in having heart disease and high blood pressure. Therefore, highly sensitive cholesterol biosensors based on different methods have been extensively investigated. In this thesis, OECT-based cholesterol sensors were realized by functionalizing Pt gate electrodes with cholesterol oxidase (ChOx), nanomaterials and the biocompatible polymer Nafion. The sensing mechanism of the cholesterol sensor is as follows. Hydrogen peroxide (H_2O_2) molecules are produced by the reaction of cholesterol molecules catalyzed by ChOx near the functionalized Pt gate electrode and undergo electro-oxidation on the gate surface, which can influence the gate surface potential and thus the effective gate voltage applied on the device. Since the amount of H_2O_2 produced is directly proportion to the concentration of cholesterol, the indirect cholesterol sensing based on the detection of H_2O_2 is thus realized. The cholesterol

sensor can show a detection limit down to 10nM, depending on the surface modification of gate electrodes.

Besides the above sensing principles based on the direct electro-oxidation of biomolecules or indirect sensing by producing H_2O_2 of decomposed biomolecules, OECT can be operated by testing reduced signal. In ATP sensing, dual enzyme (glucose oxidase (GOx) and hexokinase (HEX)) co-modified gate electrodes were employed. GOx can decompose glucose and produce H_2O_2 while HEX can combine ATP and glucose to generate electrochemically inactive molecules and reduce the glucose concentration and thus the concentration of H_2O_2 that can be detected by the device. So the concentration of ATP can be decided according to the decrease of the signal generated by H_2O_2 at a controlled condition. The ATP sensor can show a detection limit down to 10μM, depending on the surface modification of the gate electrodes.

In summary, OECTs are promising transducers for biological and chemical sensing because of high sensitivities and convenient fabrication process. The devices can be prepared on flexible substrate by solution process at low temperature with low cost, making it possible to integrate the devices with various systems, such as wearable electronics and healthcare products. Furthermore, the device performance should be optimized by improving the stability and uniformity of the devices before being considered for commercial applications, which will be the future work of this field.

List of Publications

1. **Chun Hin Mak**, Caizhi Liao, Ying Fu, Meng Zhang, Chun Yin Tang, Yuen Hong Tsang, Helen L. W. Chan and Feng Yan, "Highly-sensitive epinephrine sensors based on organic electrochemical transistors with carbon nanomaterials modified gate electrodes." *Journal of Materials Chemistry C*, 2015

2. Meng Zhang, Caizhi Liao, **Chun Hin Mak**, Peng You, Chee Leung Mak, Feng Yan, "Highly sensitive glucose sensors based on enzyme-modified whole-graphene solution-gated transistors." Scientific reports, 5, p. 8311, 2015

3. Caizhi Liao, **Chun Hin Mak**, Meng Zhang, Helen L. W. Chan, Feng Yan, "Flexible Organic Electrochemical Transistors for Highly Selective Enzyme Biosensors and Used for Saliva Testing." Advanced Materials, 27, p. 676–681, 2015

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Chapter 1 Introduction

1.1 Background

Due to low temperature process, easy to modify the electrical, optical and mechanical properties, easy deposition and low-cost[1], organic electronics have been intensively studied for different applications such as organic light emitted diode (OLED), Organic solar cells (OSCs)[2] and organic thin film transistors (OTFTs)[3], [4]. Organic field effect transistors (OFETs) and organic electrochemical transistor (OECTs) were the main types of OTFTs. In an OECT, an electrolyte analogously replaces the insulator in an OFET.[3] The device can detect the analyte concentration in the electrolyte with high sensitivity based on various sensing principles. Meanwhile, the aqueous environment allows low working voltage of the device because of the high gate capacitance that is mainly the double-layer capacitance at the electrolyte/semiconductor interface. Therefore, OECT is an ideal platform for biological sensing.

The first OECT was a transistor with polypyrole as organic channel reported in 1984.[5] Then different channel materials such as polyaniline and PEDOT:PSS were applied[3, 6] and showed stable performance in chemical and biological sensing (e.g. glucose or pH).[3] Various tactics of sensing different biomolecules, DNA or cells were proposed for different purposes. In addition, device performance is greatly influenced by

fabrication conditions and surface modification, which should be optimized for practical applications.

1.2 Working mechanism of PEDOT:PSS -based OECTs

The working mechanism of PEDOT:PSS -based OECT was investigated by Bernards et al. [7, 8] The channel current of the OECT is changed by the ion doping and de-doping process and shows on and off states at different gate voltages. As shown in Fig. 1.1, when the PEDOT:PSS OECT was immersed in electrolyte with ion, its hole density will be decreased by injected positive ions into PEDOT:PSS if a positive gate voltage is applied. This process is named as de-doping process and the electrochemical reaction to describe the process is shown below[9]:

$$n(PEDOT^+ : PSS^-) + M^{n+} + ne^- \leftrightarrow nPEDOT^0 + M^{n+} : nPSS^- \ ,$$

where M^{n+} is a cation from electrolyte, n the number of charge of the cation, and e^- is the electron from the source electrode. Hence, when the ion moves into PEDOT:PSS layer the PEDOT will change from its oxidation state to neutral state and vice versa.

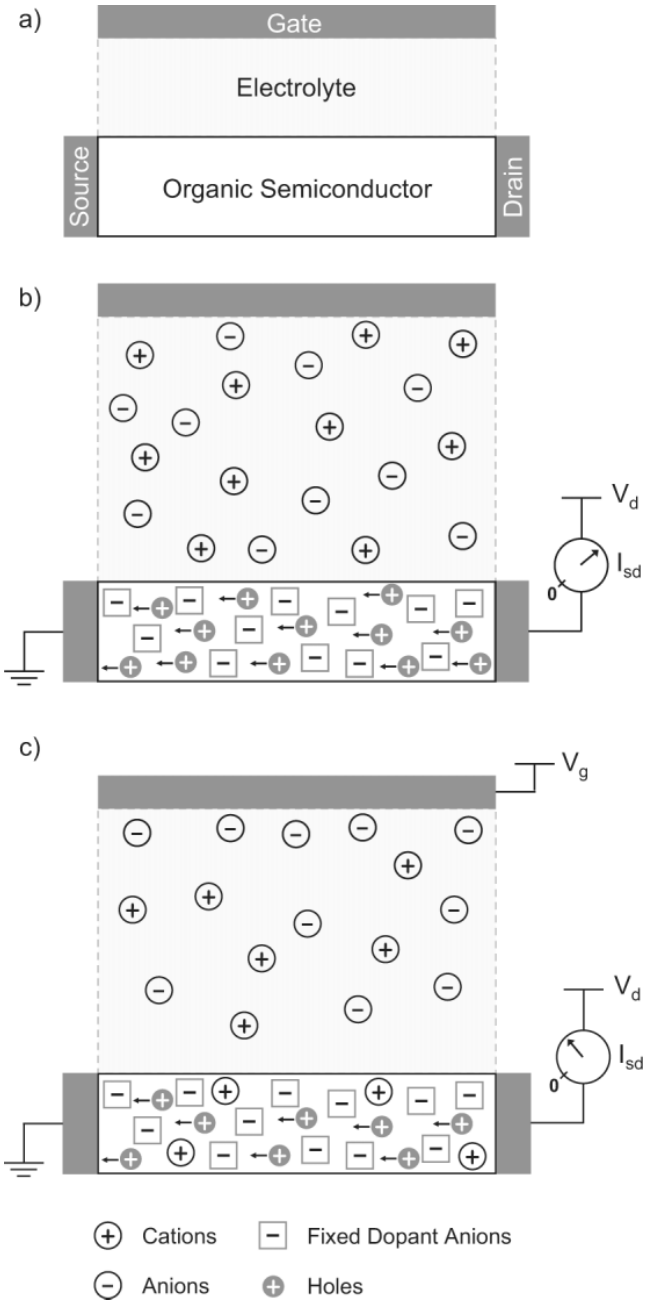


Fig 1.1 The behavior of PEDOT:PSS OECT. a) OECT architecture. b) OECT with no gate voltage applied. The organic semiconductor PEDOT:PSS have no doping and current is high. c) The organic semiconductor PEDOT:PSS have doping and current is low in OECT when gate voltage (Vg) applied. [7]

The above mechanism can be viewed as one ionic circuit combined with one electronic circuit. The ionic circuit was formed by the gate electrode and drain-source electrode by considering the channel is an electrode. The electronic circuit was formed between the drain and source electrodes with the organic semiconductor channel.

The channel current is given by these equations [3]:

$$I_{DS} = \frac{q\mu p_0 tW}{LV_p}(V_p - V_G^{eff} + \frac{V_{DS}}{2})V_{DS}, \quad (\text{when } |V_{DS}| << |V_p - V_G^{eff}|)$$
$$V_p = qp_0 t / c_i, \qquad (1.1)$$
$$V_G^{eff} = V_G + V_{offset},$$

where q is the electronic charge, μ the hole mobility, and p_o is the initial hole density in the organic semiconductor when gate voltage is zero. W and L are the channel width and length, respectively. t is the thickness of the active layer. C_i is the effective gate capacitance of the transistor, V_p the pinch-off voltage, V_g^{eff} the effective gate voltage, and V_{offset} is the offset voltage related to the potential change at the two interfaces: gate/electrolyte and electrolyte/channel.

According to the equation 1.1, the channel current (I_{DS}) changes with the effective gate voltage (V_G^{eff}) changes. When the drain source voltage (V_{DS}) unchanges, then I_{DS} is a bijective function of V_G^{eff}, therefore we can found out the change in effective gate voltage by comparing the I_{DS} in current-time measurement and the transfer characteristic curve to obtain the corresponding effective gate voltage value after adding the analyte.

1.3 Research objective

Previously there were many different modification techniques on the organic semiconductor channels of OECTs to sense various biomolecules. Those tactics were similar to that of an ordinary FET-based sensor. However, recent studies indicated that gate modifications with electrochemically active materials could also give good or even better sensing performance in some biological detections. Hence, we try to develop several types of biosensors based on OECTs by specific gate modifications on the devices, which can lead to electro-oxidation of target analytes on the gate electrodes and thus high channel current responses.

In this thesis, PEDOT:PSS -based OECTs have been prepared and employed for various biosensors. The first sensor is to detect the epinephrine concentration based on the direct-oxidation of epinephrine on gate electrode. The second one is the cholesterol sensors with the enzyme cholesterol oxidase (ChOx) modified on the gate electrodes of OECTs and perform indirect sensing of H_2O_2 generated by the analyte reaction. The third sensor is for the detection of adenosine triphosphate (ATP) with dual enzyme hexokinase (HEX) and glucose oxidase (GOx) co-modified on gate electrode to perform indirect sensing by reducing the signal molecules H_2O_2.

Chapter 2 Review of Organic Electrochemical Transistor Sensors

In this Chapter, the sensors based on organic electrochemical transistors (OECTs) will be reviewed. Until now, various biological and chemical sensors based on OECTs were successfully realized by different research groups. The representative devices include pH/Ion, bacteria, glucose, neurotransmitters, DNA, cells, and others sensors. Usually the OECT-based sensors gave low detection limits and high sensitivity for the target analytes. As the thesis is mainly focusing on biological and chemical sensing of OECTs, other types of sensors such as gas sensors will not be included in this Chapter.

2.1 OECT based biological/chemical sensors

2.1.1 pH and ion sensors

In 1985, a polyaniline based OECT was reported by Wrighton et al.[10], which could be used for sensing redox reagents, such as $Ru(NH3)_6^{3+/2+}$ and $Fe(CN)_6^{3-/4-}$ and the detection range of pH from 1 to 6 in the solution. Later they made the OECT with poly(3-methylthiophene) channel, the device showed stable performance in aqueous

electrolyte over the range of pH from 1 to 9.[11] The device was successfully used to detect the chemical oxidant of $IrCl_6^{2-}$ at 10^{-15} mol. Moreover, the same group used a device based on platinized poly(3-methylthiophene) to detect H_2 and O_2 in aqueous electrolytes and could detect the pH value in electrolyte from 0 to 12 under aerobic conditions.[12] Then the OECT-based pH sensors were further optimized for broader pH ranges with higher sensitivities by several techniques. Another pH sensor based on polypyrrole was demonstrated by Nishizawa et al., which showed the pH sensing range from 3 – 11.[13]

Dabke et al. demonstrated an ion sensor based on polyaniline OECT to detect metal ions.[14] The channel was doped with 18-crown-6 ethers and the OECT could detect K^+ ion with the detection limit of about 100 nM. On the other hand, by the fact that polyaniline could act as an effective catalyst to oxidize SO_2, Gaponik et al. used an OECT based on polyaniline to detect the dissolved SO_2 since the oxidization of SO_2 can change the conductivity of the channel.[15] Saxena et al. reported a Cu^{2+}-selective sensor based on polycarbazole-based OECT with the detection limit of 2.5 μM.[16] The sensing response was due to the conductivity change of polycarbazole by the conformational change in the polymer phase in the presence of Cu^{2+} ions diffused from the electrolyte and no ion selective layer was employed.

Berggren et al. demonstrated ion-selective OECTs by using PEDOT:PSS as the active layers. [17]They coated a Ca^{2+} selective polymeric membrane layer on top of PEDOT:PSS to detect Ca^{2+} down to 10^{-4} M. Similar ion selective sensors such as K^+,

Ca^{2+}, and Ag^{+} with similar concept were developed by Mousavi et al.[18] The detection limits for K+, Ca^{2+} and Ag^{+} were 10^{-4} M, 10^{-4} M, and 10^{-5} M, respectively. Moreover, they found that PEDOT:PSS was sensitive and selective to Ag^{+} in the solution and similar response was found when the sensor was without an Ag^{+} selective membrane. Besides, Bernards et al. reported a PEDOT:PSS-based OECT integrated with a bilayer lipid membrane (BLM) to distinguish monovalent and divalent cations in the solution and used this concept to develop the channel selectivity to monovalent cations.[19]

Yan et al. has investigated the ion-sensitive properties of OECTs[9] based on PEDOT:PSS using three kinds of gate electrodes, Ag/AgCl, Pt, and Au. As shown in Fig. 2.1, the transfer characteristic curves could shift to lower gate voltage when they increased the cation concentration such as H^{+}, K^{+}, Na^{+}, Ca^{2+}, and Al^{3+} separately in the electrolyte, also the curves could be shifted horizontally to merge a universal curve. Furthermore, the gate electrode was found to be an important role in the ion-sensitive properties of the OECTs. Nernstian relationships between gate voltage shift and ion concentration were obtained when the devices using Ag/AgCl electrodes as the gate electrode. However, due to the devices having electrical double layer at the gate/electrolyte interface when a Pt or Au gate electrode was applied, higher ion sensitivities in Pt or Au electrode compared to Ag/AgCl electrode were shown. The devices could detect metal ions down to 1μM. This work provided a clear explanation of the device physics and the electrochemical mechanisms of OECTs.

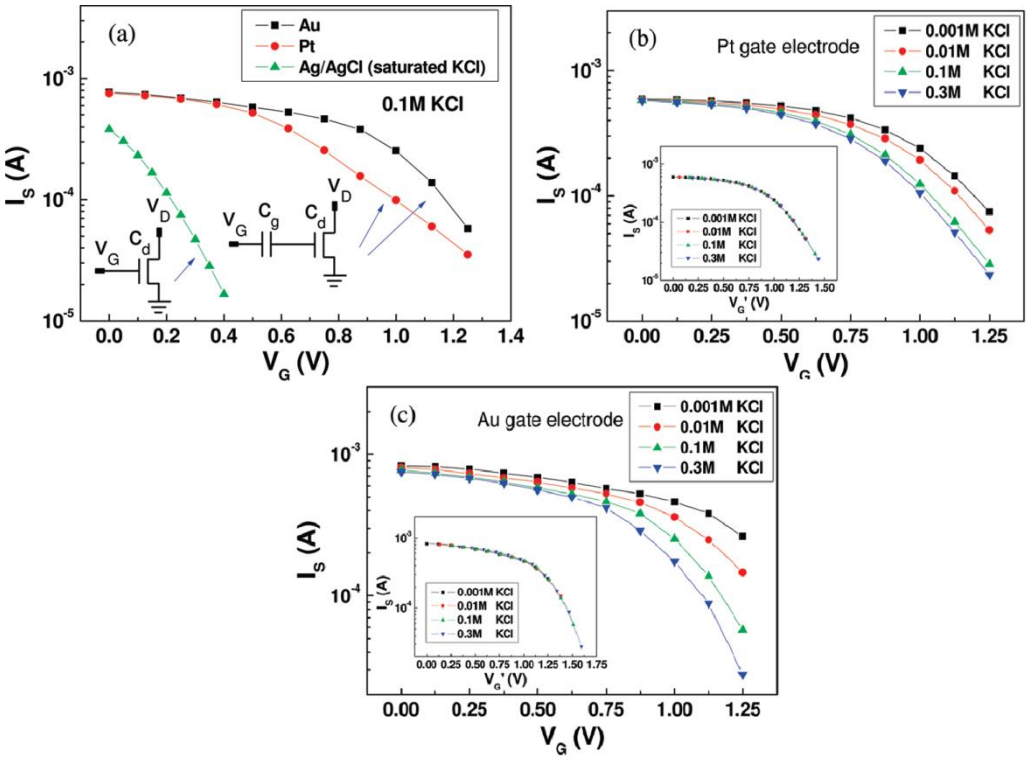


Fig 2.1 (a) Transfer characteristics of the PEDOT:PSS OECT measured in 0.1M KCl solutions with Au, Pt, Ag/AgCl gate electrodes. (b,c) Transfer characteristics of OECTs in different KCl concentrations with Pt and Au gate electrodes, respectively. Insets show transfer characteristics of the OECTs merge a universal curve for different KCl concentration.[9]

2.1.2 Antibody-antigen sensors

Kanungo et al. demonstrated a reagentless OECT immunosensor [20] with PEDOT channel using the binding pair of antibody-antigen because they have high affinity to combine each other. They immobilized the goat anti-rabbit IgG with various concentrations on the channel to detect the antigen rabbit IgG concentrations in PBS

solution. Two antibody immobilization approaches, the physical adsorption and polymerization with PEDOT, were employed and they found that polymerization with PEDOT method was the better one. As shown in Fig 2.2, the sensors detected the antigen down to 0.1ng/mL. Reversely, they immobilized the antigen on PEDOT to detect antibody in the solution. The sensing mechanism of the above device was attributed to conformational change of PEDOT channel by the formation of antibody-antigen pair on PEDOT.

Kim et al. demonstrated a highly sensitive PEDOT:PSS OECT immunosensor [21] for the detection of prostate specific antigen (PSA). The PSA monoclonal antibody (PSA mAb) was immobilized on the channel surface and the OECT could detect prostate specific antigen/α1-antichymotrypsin (PSA–ACT) complex down to 100 pg/mL. The negative surface charge of PSA-ACT complex induced doping effect in PEDOT:PSS, which is the key sensing mechanism in this work. Besides, the detection limit was further improved to 1 pg/mL by using gold nanoparticles (AuNPs) conjugated with PSA polyclonal antibody(PSA pAb). More binding of AuNPs- PSA pAb with PSA–ACT complex were made and this led to more negative charges on the surface and so the sensitivity of the device increased.

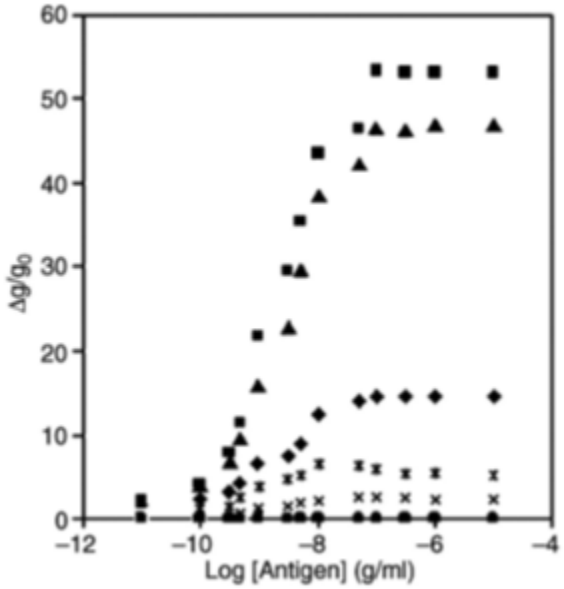


Fig 2.2 PEDOT:PSS OECT antigen sensing.. [20]

2.1.3 DNA sensors

A label-free DNA sensor based on OECT was reported by Krishnamoorthy et al.. The device was using PEDOT channel.[22] Single-strand probe DNA was immobilized on the active layer during the polymerization of PEDOT. The device could detect the complementary target DNA in PBS solution down to 80 ng/mL. The sensing mechanism is based on the conformational change of PEDOT chains due to the hybridization of probe DNA and complementary DNA.

Then Lin et al. demonstrated a label-free PEDOT:PSS OECT DNA sensor [23] which was integrated in a microfluidic system on flexible polyethylene terephthalate (PET) substrates. Fig 2.3 shows the performance of the device did not have obvious change when the device was bent. They immobilized single-strand DNA probes on the Au gate electrode. The device was able to detect complementary DNA targets down to 1 nM. By using pulse-enhanced hybridization process of DNA, the

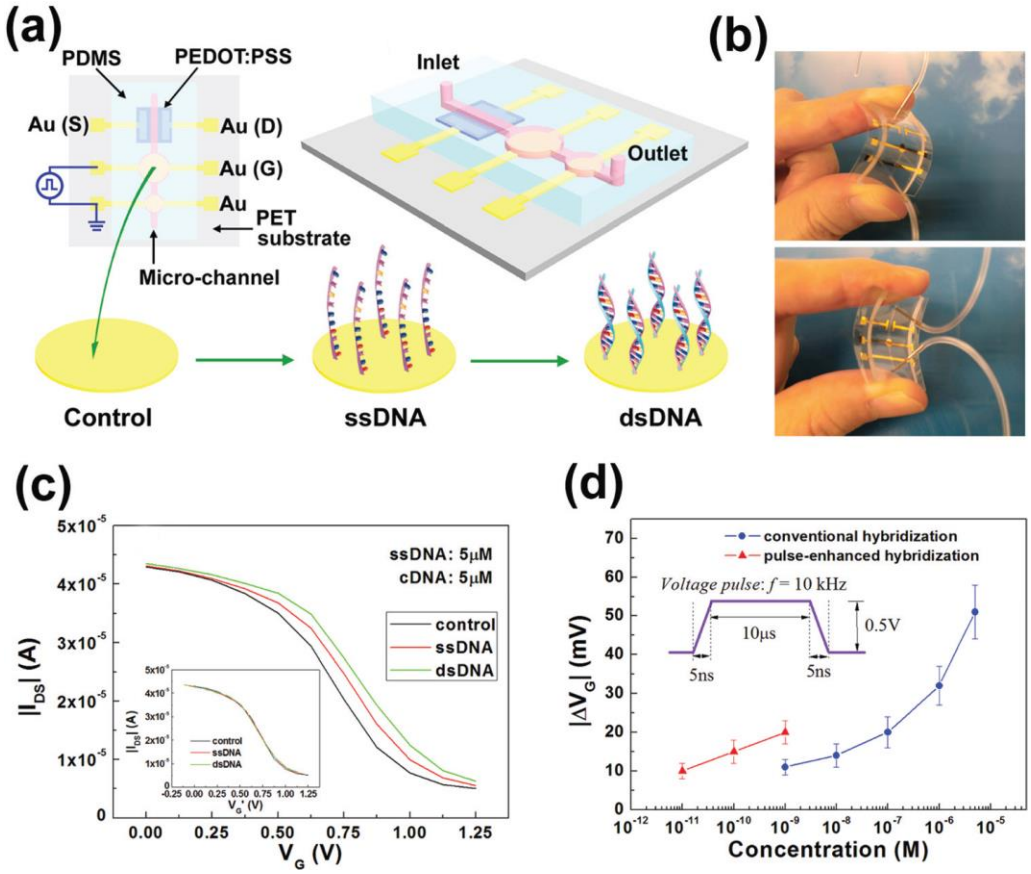


Fig 2.3 a) Schematic diagram of an OECT DNA sensor integrated in a flexible microfluidic system. b) Photographs of a bent device. c) Transfer characteristics of an OECT measured at immobilization state of DNA. d) Voltage shift of different target DNA in PBS solution.[23]

detection limit of OECT DNA sensor was further decreased to 10 pM. The sensing mechanism was attributed to the decrease of the work function of the Au gate electrode caused by the surface dipole formed by the intrinsic negative charge of DNA molecules.

2.1.4 Cell-based sensors

Lin et al. established the first OECT cell-based biosensor by using PEDOT:PSS.[24] The OECT showed excellent biocompatibility and was stable in culture medium. Cancers cells and fibroblasts were cultivated on the surface of PEDOT:PSS layers in the OECTs and the devices were characterized in culture medium. Fig 2.4 shows the behaviors of the OECT was characterized before and after the detachment of cancer cells from the PEDOT:PSS layer by trypsin treatment and the transfer characteristic of the device was found to shift to lower gate voltages after the detachment. When the devices were integrated with fibroblasts, similar result was obtained. These results confirmed that the electrostatic interaction between the cells and PEDOT:PSS layer could affect the transfer characteristic curve in the OECTs.

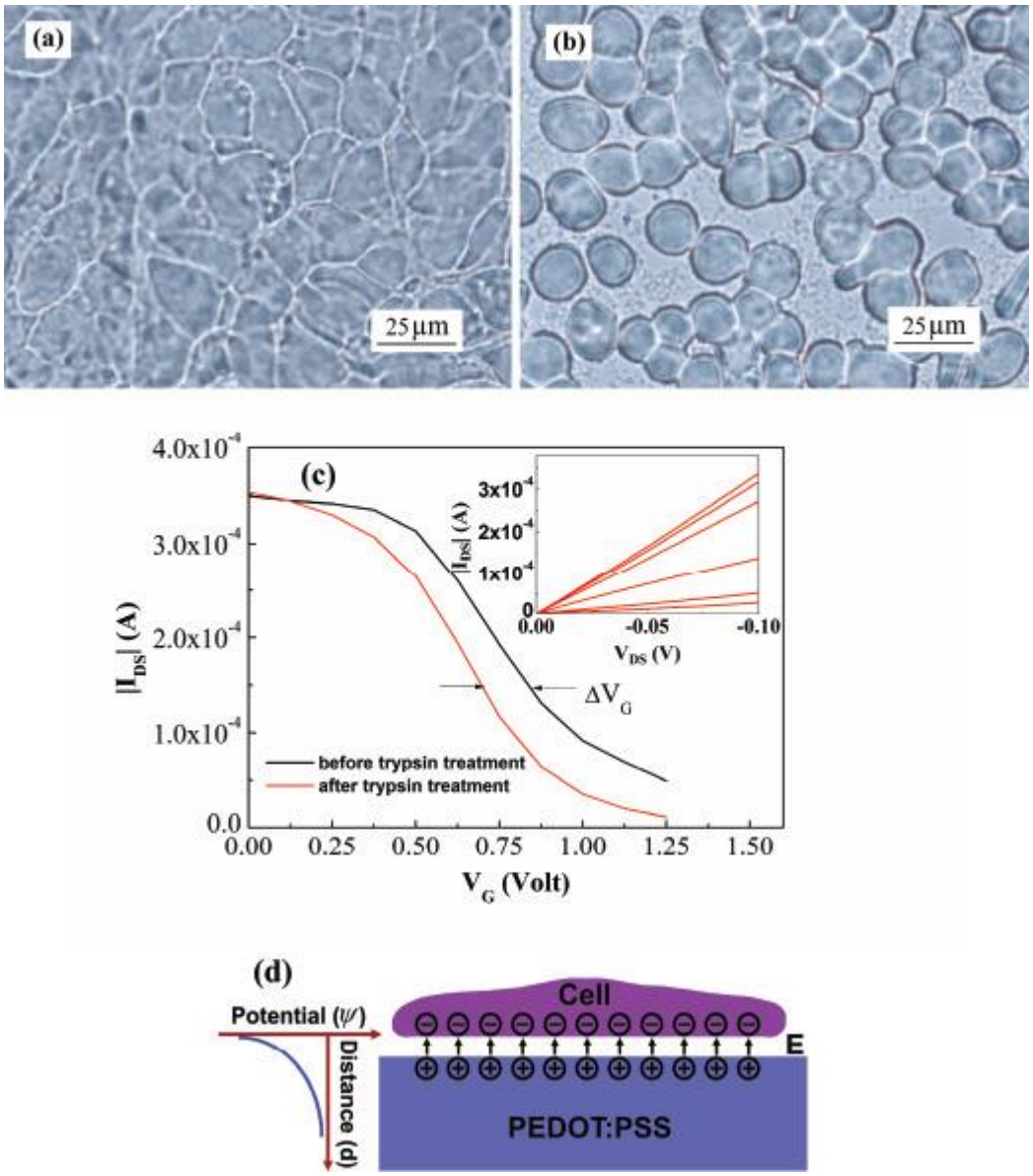


Fig 2.4 a,b) Optical images of cancer cells cultured on PEDOT:PSS films before and after detachment. c) Transfer characteristics of an OECT with cancer cells before and after trypsin solution treatment. Inset: Output characteristics of the OECT. d) Schematic diagram of the electrostatic interaction between an attached cell and PEDOT:PSS film.[24]

2.1.5 Neurotransmitter sensors

Neurotransmitters are the chemical compounds which transmit an impulse from a nerve cell to a target cell such as nerve cell, muscle cell or gland cell.[25] Different neurotransmitter has different psychological effect and too much or too few of them may cause some diseases. Hence several groups have focused on using OECTs to detect different neurotransmitter by various tactics, which will be introduced in this section.

2.1.5.1 Dopamine sensors

In 2011, Tang et al. published an article about the dopamine sensor based on PEDOT:PSS channel OECTs with the functionalized gate electrodes.[26] The dopamine molecules underwent electro-oxidation on the gate electrode and increased the effective gate voltage. As the first time of detecting dopamine based on OECTs, they used Au, Pt, graphite electrode, multi-walled carbon nano-tube to modifiy graphite electrode and multi-walled carbon nano-tubes to modifiy Pt electrode, and in the systematical work they found that the clean Pt electrode gave the highest current response among all the modification under the same condition. In Fig 2.5, this work showed that, in the electro-oxidation-based detection scheme, the electro-catalytic activity of the gate electrode as well as the conventional parameter of FET such as width to length ratio (W/L) and channel to gate area ratio (A_{ch} / A_g) all play important roles on optimizing the device performance. Therefore, the gate electrode applied in such electrochemical sensing should be designed very carefully. In 2014, Liao et al. applied the same sensing

concept and used a bio-compatible polymer Nafion to attract dopamine to the gate electrode by electrostatic interaction in the aqueous environment and repulse main interferences including uric acid and ascorbic acid molecules to realize a selective OECT-based dopamine sensor [27]. In this work, graphene based nano-materials were used to improve the performance including detection limit and slope in effective gate voltage against dopamine concentration.

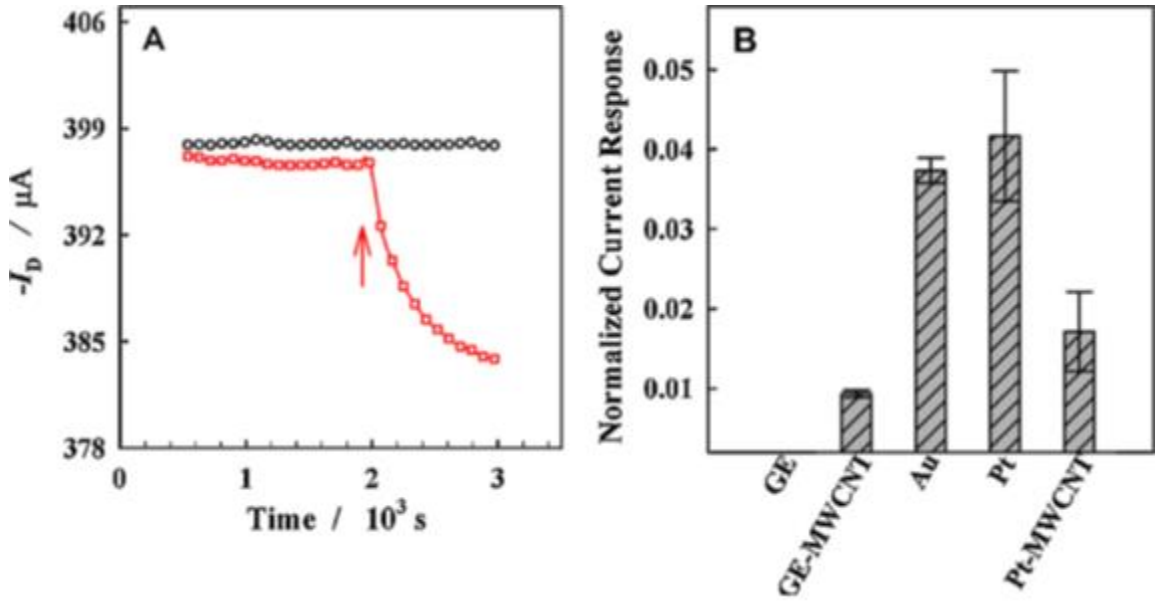


Fig 2.5 (A) ID vs. time curve before and after an addition of 50 nM dopamine in PBS solution. (B) Normalized current responses (NCR) of the OECT with different gate electrodes of 50 nM dopamine. [26]

2.1.5.2 Glutamate and acetylcholine sensor

The glutamate and acetylcholine OECT sensors were demonstrated by Berggren et al. in 2014.[28] In their work, the enzymes, choline oxidase and L-glutamate oxidase, were employed to detect the acetylcholine and glutamate respectively. The Pt-NPs was dissolved in DMSO and mixed with the PEDOT:PSS to enhance the conductivity and employed as both gate electrode and active layer. The enzymes were immobilized on the gate electrode by a crosslinking technology.

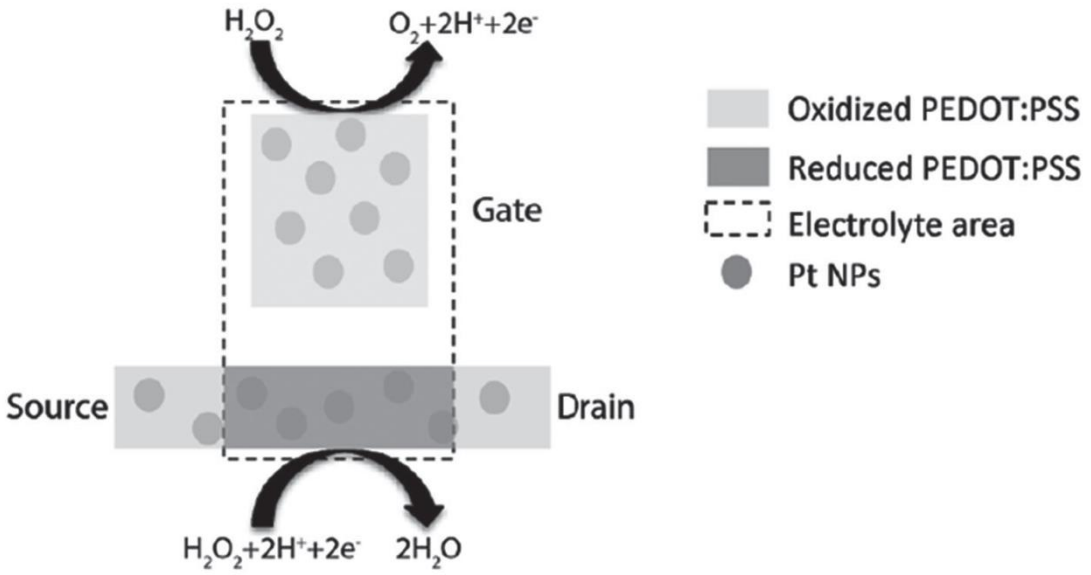


Fig 2.6 Schematic representation of the glutamate and acetylcholine with PEDOT:PSS/Pt NPs OECT sensor.[28]

The enzymes could lead to the reaction of the aforementioned neurotransmitters on the gate electrodes and produce H_2O_2, as shown in Fig. 2.6. Then H_2O_2 underwent

electro-oxidation on the gate electrode and electro-reduction on the channel and acted as signal molecule for the detection of both neurotransmitters. The two types of biosensors showed the detection limits of 5 μM to glutamate and acetylcholine, respectively and the chemical reactions were shown in Fig. 2.7.

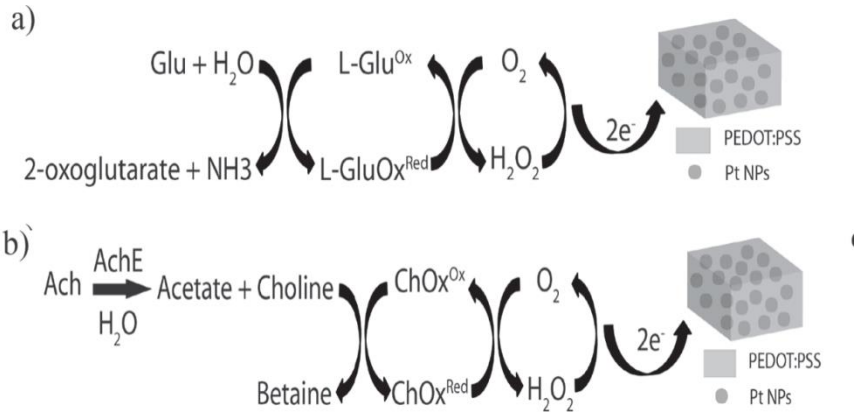


Fig 2.7 Enzymatic chemical reaction of a) glutamate and b) acetylcholine on the PEDOT:PSS electrode [28]

2.1.5.3 Epinephrine sensor

Even though it has no systematic studies the epinephrine sensing, Copped`e et al. fabricated PEDOT:PSS -based OECTs on cotton fibers for stress monitoring.[29] The architecture of the device is shown in Fig. 2.8. They claimed that the device could detect the epinephrine concentration in the human sweat and showed that the detection limit of the epinephrine sensing was 1μM with a Pt gate electrode. The device's selectivity was tested by applying two electrodes, the Pt and Ag gate electrodes. However, the performance of the devices cannot meet the requirements in practical applications since

the epinephrine concentration in human plasma was very low (~0.2 nM). The high detection limit may be due to the poor stability of their devices, as evidenced by the big error bars of the current responses of the devices. Also the selectivity based on changing Ag gate electrode and Pt gate electrode was not convincing as Pt was shown as catalyst in electro-oxidation for many bioanalytes before.

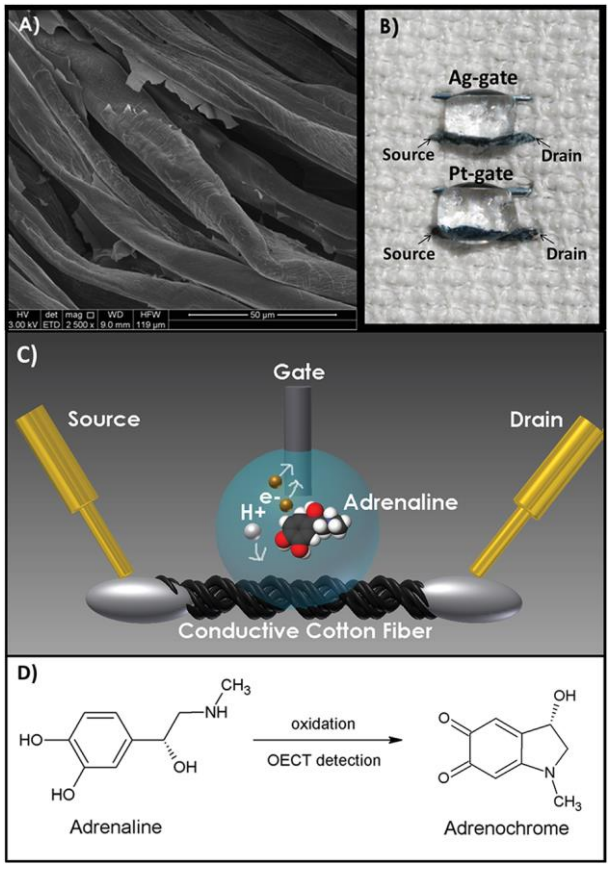


Fig. 2.8 (a) Field emission SEM image of the PEDOT:PSS cotton wire. (b) the cotton-OECT with Ag and Pt electrode. (c) Concept diagram of the cotton-OECT device with a Pt gate and an epinephrine (adrenaline) molecule in its sensing process. (d) Epinephrine (adrenaline) oxidation reaction [29]

2.1.6 Glucose sensors

Glucose sensing has very important applications in the diagnosis of diabetes. Therefore, one of the popular research and application of OECTs was the highly sensitive glucose sensor. Many groups tried to use different active layers or different methods to sense glucose as introduces in this section.

Contractor et al. demonstrated the first polyaniline OECT glucose sensor in 1992.[30] The sensing mechanism was based on the modulation of pH in the electrolyte by adding glucose, which can change the conductivity of polyaniline. Then Malliaras et al. used PEDOT:PSS as the active layer to detect glucose with glucose oxidase in 2006.[31] The devices showed detection limit in micro-molar region. They confirmed that the glucose sensing was based on the oxidation of H_2O_2 generated by the added glucose in the solution. In 2007, the same group reported a detailed work on the enzymatic sensing based on PEDOT:PSS OECTs for detecting glucose.[8] As shown in Fig. 2.9, Nernst equation was used to explain the concept of effective gate voltage, potential distribution along the gate to channel, and offset voltage when the oxidized species (e.g. H_2O_2) was added into the electrolyte. Then they verified that the sensing mechanism was attributed to the Faradaic current generated on the gate electrode that can change the voltage

distribution between the gate, electrolyte and channel. As a result, the transfer curves at different glucose concentrations can be shifted to merge into a universal curve.

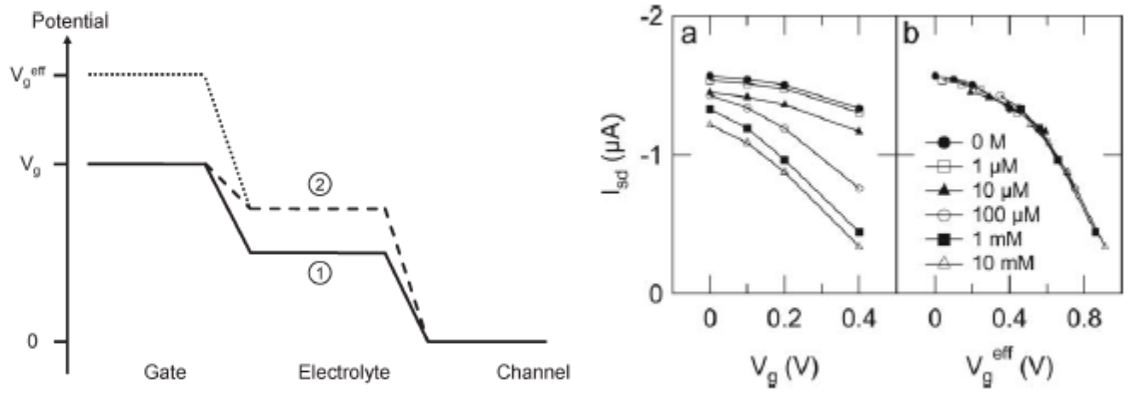


Fig. 2.9 (Left) Potential distribution across the gate to channel when the PEDOT:PSS OECT positive gate bias. Solid line: base solution. Dash line: In the presence of glucose. Dot line: The gate potential which give the same potential in electrolyte in dash line (Right) The shift of the transfer curves in different concentration and merge an universal curve.[8]

In 2011, Tang et al. reported glucose sensors with high sensitivity based on OECTs.[32] They used chitosan (CHIT) to immobilize the enzyme glucose oxidase on the gate electrode to sense the glucose concentration. The nano-materials, MWCNT and Pt-NPs, were integrated to enhance the sensitivities of the devices. The best gate modification showed 5nM to glucose while the Pt electrode with CHIT and GOx showed only 10 μM. The result indicated that nano-materials could dramatically enhance the device sensitivity presumably due to the large surface to volume ratio and the unique electro-catalytic properties of the nano-materials. Two years later, they integrated more graphene based nano-materials to detect glucose with GOx.[33] CHIT and Nafion with different

concentrations were used to immobilize the enzyme GOx and nano-materials on the gate electrodes. Similar performance was resulted and the best device gave the detection limit of 10nM to glucose with good selectivity. The major improvement on the selectivity was mainly attributed to the negatively charged Nafion and chitosan on the gate electrodes.

2.1.7 Other biomolecules sensing

Uric acid (UA) and cholesterol enzymatic sensors were then demonstrated on flexible substrates by Liao et al.[34] The UA sensor was also successfully used in the detection of UA levels in saliva. Similar to the previously report OECT-based sensors, the bending test did not affect the performance after the device was bent for 1000 times. The novelty of this work is to use bilayer films with immobilized enzyme on the gate electrode and it is shown in Fig. 2.10. The bilayer was composed of a negatively charged Nafion with graphene followed by a positively charged layer polyaniline (PANI). The bilayer could repulse both positive (such as Dopamine) and negative (such as UA, AA) charged molecules and only neutral charged H_2O_2 could go through the bilayer. Finally, different kind of enzymes (such as uric acid oxidase, cholesterol oxidase, glucose oxidase) were immobilized on the bilayer with GO as GO showed good ability on immobilizing enzymes. H_2O_2 will be generated once the enzymes were in contact with their corresponding bioanalytes in the electrolyte. Therefore, highly selectivity enzymatic biosensors were obtained by modifying specific enzymes on the gate.

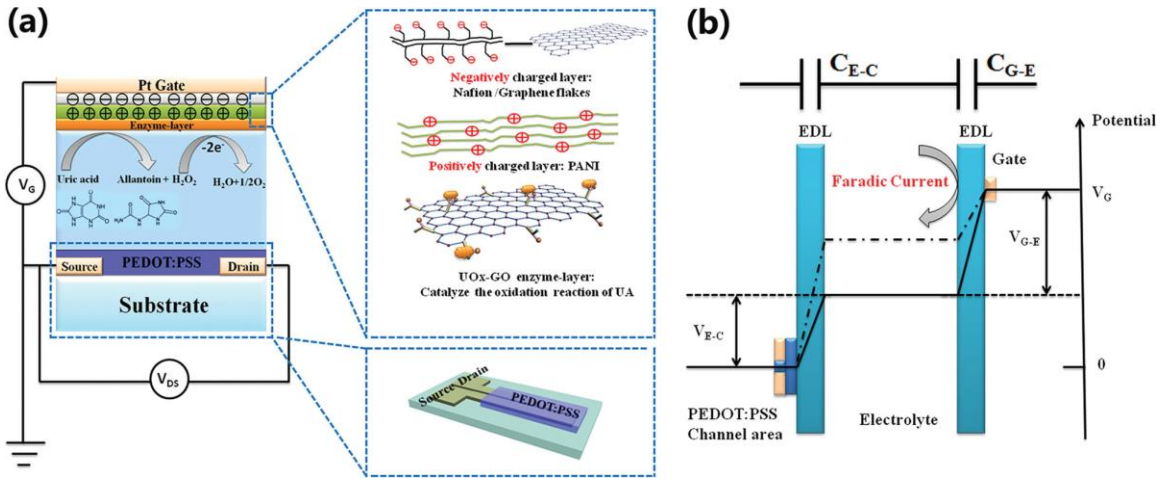


Fig 2.10 a) Schematic diagram of an OECT with a UOx-GO/PANI/Nafi on-graphene/Pt gate. b) Potential drops between the gate and channel of the OECT before (solid line) and after (dash line) the addition of UA in the PBS.

2.2 Summary

In summary, different architecture's OECTs were widely demonstrated for the applications in chemical and biological sensing. Different organic materials such as polypyrrole, polyaniline and PEDOT:PSS were successfully used for different bio-sensing applications. The ion, pH, DNA, antibody-antigen, neurotransmitters, glucose and uric acid were detected by PEDOT:PSS based OECTs. Many groups have developed various approaches to realize high performance biosensors based on channel modification, gate modification and pH adjustment. More importantly, some of the aforementioned OECTs have demonstrated good selectivity, which is important for the practical applications of the OECT-based sensors.

Chapter 3 Epinephrine sensing of Organic Electrochemical Transistors

In this Chapter, we studied the epinephrine sensing of OECTs (PEDOT:PSS channels) with different surface modifications on Pt gates. OECTs based on PEDOT:PSS prepared on cotton fibers have been used for sensing epinephrine due to the electro-oxidation of epinephrine on Pt gates and gave a detection limit of ~1μM.[29] To enhance the performance of epinephrine sensors, systematic studies on the OECT-based epinephrine sensors have been carried out. The OECTs were modified with different nano-materials together with Nafion on Pt gates and have shown much lower detection limit and selectivity. This investigation pave a way for realizing high-performance and low-cost epinephrine sensors suitable for practical applications.

3.1 Introduction

Epinephrine (EPI) is a neurotransmitter and hormone, and its metabolism can lead to glycogenolysis in liver and skeletal muscle, mobilization of free fatty acid, plasma lactate and rate of heart contraction increase.[35,36] Due to these physiological effects, epinephrine is banned in world class games.[37,38] However these effects have medical applications for patient such as cardiac arrest in emergency. Therefore, disposal, highly sensitive and selective epinephrine sensors would find important medical applications.

Different approaches such as liquid chromatography (HPLC),[39,40] polarography[41] and electrochemical methods[42-46] have been developed to detect epinephrine successfully. Compared with these methods, electrochemical sensors are low cost, convenient, portable and feasible for miniaturization. For example, Tsai et al. reported epinephrine electrochemical sensors with a detection limit of 50μM by using Pd-Au nano-particles modified glassy carbon electrodes.[47] Valentini et al. used single-wall carbon nanotubes -modified glassy carbon and stainless steel microelectrodes in cyclic voltammetry (CV) measurements and gave a detection limit of 2μM.[48] Furthermore, OECTs prepared on cotton fibers reported by Coppede et al. were used to detect epinephrine in NaCl solution and showed obvious response when the concentration is above 1μM.[29] However, the epinephrine concentration in human plasma normally is very low (~0.2nM),[49] so the sensitivity of electrochemical methods have to be significantly improved for practical applications.

OECTs have been used in many applications since it was introduced from 1984.[3] Its electrolyte environment and low working voltage made it very useful for biological and chemical sensing. So OECTs have already performed ions, bacteria, glucose, neurotransmitters, DNA, cells and so on (see Chapter 2). It normally showed low detection limit due to the amplification function of the transistors. Therefore highly sensitive epinephrine sensors could be achieved by OECTs. OECTs with PEDOT:PSS as the active layers were prepared by solution process. To enhance their sensitivity,

nanomaterial and Nafion mixtures were modified on the gate electrodes of the OECTs and pronounced effects were obtained. Different potentially low-cost carbon-based nanomaterials, including graphene flakes (Gr), graphene oxide (GO) and single walled carbon nanotubes (SWNTs), were used in the gate modification. The device with Nafion and SWNTs modified on the gate electrode shows a detection limit down to 0.1nM, which is sensitive enough for practical uses. Considering that OECTs can be easily prepared with solution process, the OECT-based epinephrine sensors are promising for disposal clinical applications or medical safety check.

3.2 Fabrication, preparation and measurement of OECT epinephrine sensors

3.2.1 Fabrication of OECTs

1cm × 1cm glass slides were cut from the microslides and washed by acetone, IPA, ethanol, and DI water thoroughly. Then they were stacked on a metal shadow mask firmly by taped and Ti/Pt electrodes (~100nm) were sputtered on them. Then O_2 plasma treatment was done and PEDOT:PSS (Clevios PH500) was coated on the substrates by a spincoater. The PEDOT:PSS which was not on the channel area would be removed by cotton swab with ethanol. The substrates were then annealed in a nitrogen-filled glove box at 185^{o}C for one hour. Silicone seal was painted on the parts of the electrodes

without PEDOT:PSS to avoid short circuit in electrolyte. After the seal was dried, the OECTs is ready for the next steps.

3.2.2 Preparation of OECTs epinephrine sensor

For the gate modification, Nafion(5%) and nanomaterials (graphene, SWNT, GO) were mixed with a volume ratio of 1:1 and drop-coated on the 3mm × 3mm clean Pt gates. Then the electrodes were dried in a 4^{o}C refrigerator for 4 hours. To obtain epinephrine solution, epinephrine hydrochloride powder was dissolved in 1x PBS to obtain 10mM solution and diluted to different concentrations required in experiments.

3.2.3 Measurement of OECTs

All measurements were measured by two labview controlled Keithley 2400 sourcemeters with a USB to transfer and monitor the experimental data. The channel and gate electrodes were rinsed with PBS solution to remove the undesired residue on the channel and gate and immerse in the 10ml PBS solution which was stirred in a beaker. The transfer characteristic curve (I_{DS} vs V_G) was first measured with V_{DS}=0.1V and V_G varied from 0V to 1.2V. Then the channel current (I_{DS}) was measured as a function of time (t) with fixed voltages V_{DS} = 0.1V and V_G = 0.6V. V_{DS} is designed to have relatively high current value but still much smaller than the V_G = 0.6. V_G is designed to have enough

oxidation voltage in OECT and much higher than the V_{DS} but not enough to electro-oxidize the electrolyte. Different volumes of 10mM epinephrine solution were added in the beaker to modulate the epinephrine concentration in PBS solution from 0.1nM to about 3μM for many steps and the corresponding channel current changes were recorded simultaneously.

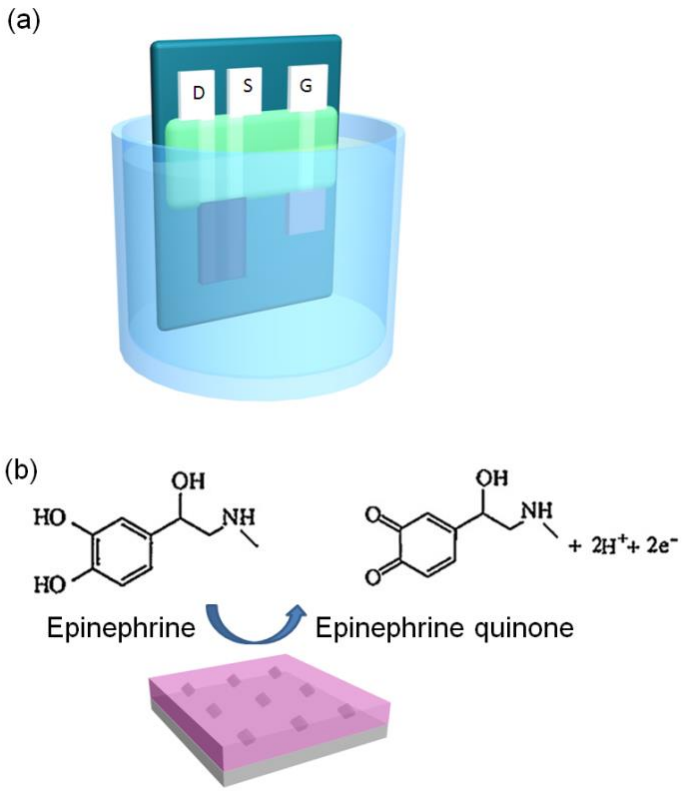


Fig. 3.1 (a) The schematic diagram of an OECT-based epinephrine sensor with a Nafion and nanomaterial-modified gate electrode. (b) The oxidation of epinephrine at the gate electrode modified by Nafion and carbon-based nanomaterials.

3.3 Working principle of OECT-based epinephrine sensors

As discussed before, the conductance of the PEDOT:PSS channel of an OECT can be modulated by the gate voltage due to the electrochemical doping by cations from the electrolyte.[9]The channel current I_{DS} of the device at different source-drain voltage V_{DS} and gate voltage V_G is given by the following equation:[3][8] [see also eq. 1.1]

$$I_{DS} = \frac{q\mu p_0 tW}{LV_p}(V_p - V_G^{eff} + \frac{V_{DS}}{2})V_{DS}, \quad (\text{when } |V_{DS}| << |V_p - V_G^{eff}|)$$
$$V_p = qp_0 t / c_i, \qquad (3.1) =$$
$$V_G^{eff} = V_G + V_{offset},$$

(1.1)

where q and μ are electronic charge and hole mobility, respectively; p_0 is the initial hole density in the channel; V_p and $V_G{}^{eff}$ are the pinch-off voltage and the effective gate voltage on the transistor, respectively; V_{offset} is an offset voltage at gate/electrolyte interfaces; t is the thickness of the active layer; W and L are the channel width and length of the OECT, respectively; c_i is the effective gate capacitance.

The working mechanism of the OECT-based epinephrine sensors is shown in Fig. 3.1. The direct electro-oxidation of epinephrine released two electrons per epinephrine

molecule and produce Faradaic current between the gate electrode and the electrolyte interface. The direct electro-oxidation of epinephrine in PBS is given by the formula:[50][51]

$$epinephrine \rightarrow epinephrine\ quinone + 2H^+ + 2e^- \qquad \textbf{(3.2)}$$

The Faradaic current produced by the direct electro-oxidation of epinephrine at the gate electrode could change the potential difference between the electrolyte and the gate electrode and hence change the effective gate voltage $V_G{}^{eff}$ of the transistor given by: [8][9]

$$V_G^{eff} = V_G + \alpha \log[EPI] + constant \quad ,$$

$$\alpha = 2.3(1+\gamma)kT/2e \quad , \qquad (3.3)$$

where γ is the ratio between the electrical double layer capacitances of the electrolyte/channel interface (C_C) and the electrolyte/gate interface (C_G), $\gamma = C_C/C_G$; k is Boltzmann constant; T is room temperature and [EPI] is the concentration of epinephrine. Therefore, the increase of the epinephrine concentration in the beaker will increase the effective gate voltage of the OECTs and thus decrease the channel current. In other words,

the transfer curve of the OECT will shift to lower gate voltage after the addition of epinephrine in the solution, which will be discussed in section 3.4.

3.4 Performance and discussion on OECTs epinephrine sensors

The low cost, highly sensitive epinephrine sensors with low detection limit have been realized using OECT by optimizing the performance with Nafion and nano-material modified gate electrode. The best device modification gave the detection limit of 0.1 nM and covered the normal concentration level in human plasma and medical safety check.

3.4.1 Device performance with clean Pt gate

The clean Pt gate electrode without modification was employed to observe the OECT's output characteristic and transfer curve. Fig. 3.2(a) showed an obvious voltage shift was observed by comparing the transfer curves in 1x PBS solution and 1x PBS solution with 10μM epinephrine. It was because the epinephrine underwent electro-oxidation at gate voltage and the effective gate voltage increased. Therefore for the transistor needed smaller gate voltage to obtain the same current and then the curve shifted to lower the gate voltage.

Fig. 3.2(b) shows the output curve and the PEDOT:PSS based OECT is a p-type transistor in PBS solution as it has decreasing current with increasing gate voltage (V_G) under the same drain current (V_{DS}).

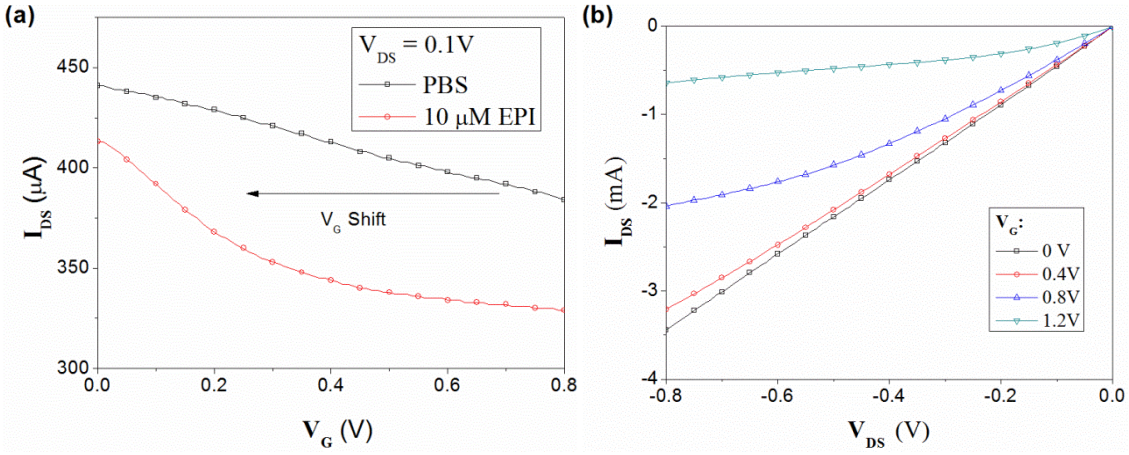


Fig 3.2. (a) Output curves (I_{DS} - V_{DS} at different V_G) of the OECT characterized in PBS solution. (b) Transfer curves (I_{DS} - V_G) of an OECT with a Pt gate electrode characterized in PBS solution before and after the addition of epinephrine with the concentration of 10μM.

3.4.1.1 Epinephrine sensing with clean Pt gate

After the transfer curve is obtained, the channel current (I_{DS}) was measured as a function of time (t) by increasing the EPI concentration in the beaker. The EPI molecules undergo electro-oxidation at Pt gate electrode surface and produce Faradaic current.

According to the Nernst equation there are a potential difference change between electrolyte and electrode when electro-oxidation is happened.[8] Therefore the

electrolyte's potential is increased and more cation in the PBS solution de-doped the channel PEDOT:PSS to decrease the hole concentration and hence decrease the charge carriers' concentration and current.

The device showed the channel current noise level of ~0.05μA, which is much lower than the channel current (>100μA) of the device. As shown in the inset of Fig. 3.3(a), the detection limit (signal/noise ratio > 3) of the device to epinephrine is ~30nM and the slope α for the effective gate voltage change ΔV_G^{eff} versus epinephrine concentration in logarithmic axis (log[EPI]) is 210mV/decade. The channel current change is due to the change in the effective gate voltage V_G^{eff} of the OECT given by Equations (3.1). The effective gate voltages corresponding to different epinephrine concentrations can be decided according to the transfer curve (I_{DS} vs. V_G) of the OECT shown in the inset of Fig. 3.3 (b). By subtracting the corresponding gate voltage before adding epinephrine, we can calculate the changes of the effective gate voltage (ΔV_G^{eff}). Therefore, the response of the device to epinephrine can be presented as ΔV_G^{eff} versus [EPI].

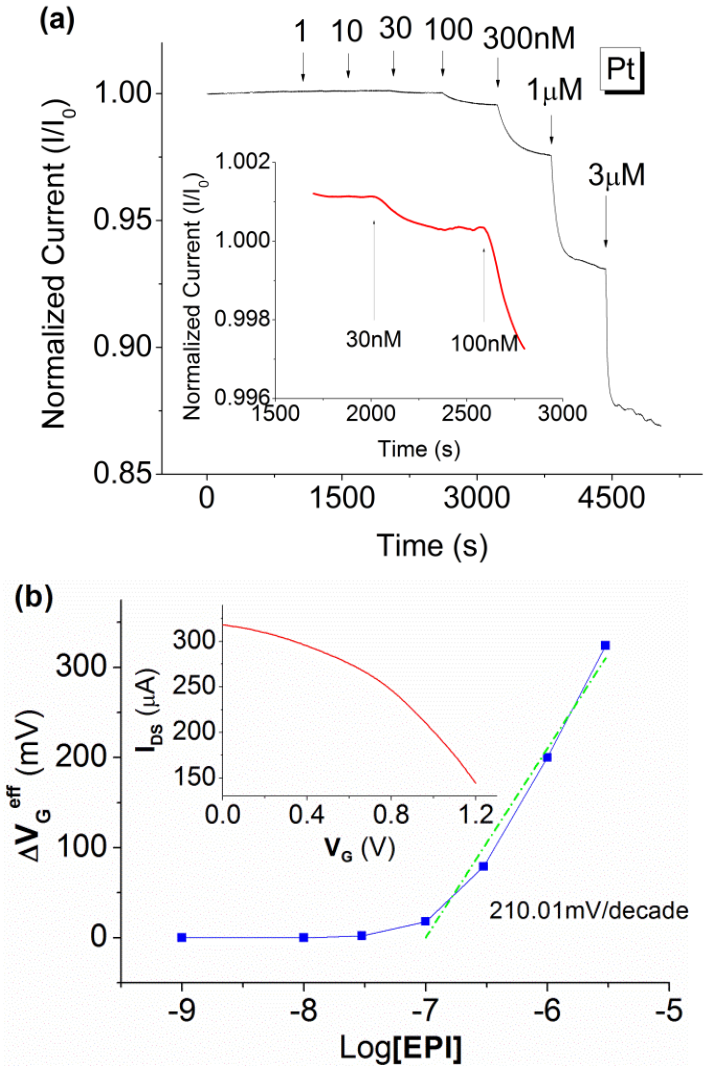


Fig 3.3 (a) The normalized current response of an OECT with a Pt gate to the increasing epinephrine concentration in PBS solution measured at V_{DS}=0.1V and V_G=0.6V. I_0=291.67μA. Inset: the enlarged current response at the detection limit of the device (30nM). (b) The offset voltage change as a function of the logarithmic value of epinephrine concentration ([EPI]). Inset: Transfer curve (I_{DS} versus V_G) of the OECT measured in PBS solution with V_{DS}=0.1V.

3.4.2 Nafion/nano-materials mixture modification and electrocatalytic effect

Nafion, an acidic polymer with the stable Teflon backbone and the acidic sulfonic groups,[52]was employed as a matrix to modify the Pt gate electrodes together with the carbon based nano-materials SWNT, GO, graphene (Gr) separately to enhance the sensitivity[46,48] of epinephrine sensing due to its biocompatible and porous structure. Moreover the carbon based nano-materials had large surface to volume ratio, conductive and relatively cheap, commercial available and have already shown their superior function in other electrochemical sensing method. As shown in Fig.3.1 (b), due to Nafion is negatively charged and epinephrine is positively charged (pKa=8.02) [50] in neutral PBS buffer solution, it is believed that epinephrine would be attracted to the Pt gate when porous Nafion film is on the top of the Pt gate electrode. That would increase the sensitivity and enhance the detection limit. Hence, the surface modification on the gate electrodes is important to the performance of the devices, which has been optimized in this experiment. The homogeneous composite droplets were uniformly, fully coated on the Pt gate surface by drop coating the mixture on the gate surface. The following modification was tested for the epinephrine sensing: (a) Nafion film (average thickness: 1.2 μm), (b) Nafion film (2.3 μm), (c) Nafion+SWNT film (2.1 μm), (d) Nafion+Gr film (2.0 μm) and (e) Nafion+GO films (2.2 μm). By the AFM the surface roughness values (r.m.s.) of all films are ~3.8 nm, as shown in Fig 3.4.

The electrodes were used to observe the electrocatalytic effect by running the cyclic voltammetry (CV). CHI660B electrochemical workstation (CH Instruments, Inc) and a standard three-electrode electrolytic cell were employed for the CV measurements of Pt electrodes which were with/without Nafion-nanomaterials modified (area size: 3mm × 3mm) in an epinephrine (1mM) PBS solution. The voltage scan rate is 50mV/s relative to a standard Ag/AgCl reference electrode and Au counter electrode (size: 3mm × 3mm). As shown in Fig 3.5, the Nafion/nanomaterial modified electrodes and clean Pt electrode had the oxidation peaks at ~ 0.6 V and ~ 0.3 V respectively. The increased oxidation peak voltages after the modification of Nafion can be attributed to the Nafion film that limits the diffusion rate of epinephrine. Besides, the modification of Nafion on the Pt electrode can obviously increase the peak value of the redox current due to the fact that the negatively charged Nafion can attract positively charged epinephrine molecules in the PBS solution to increase the concentration of epinephrine near the electrode.

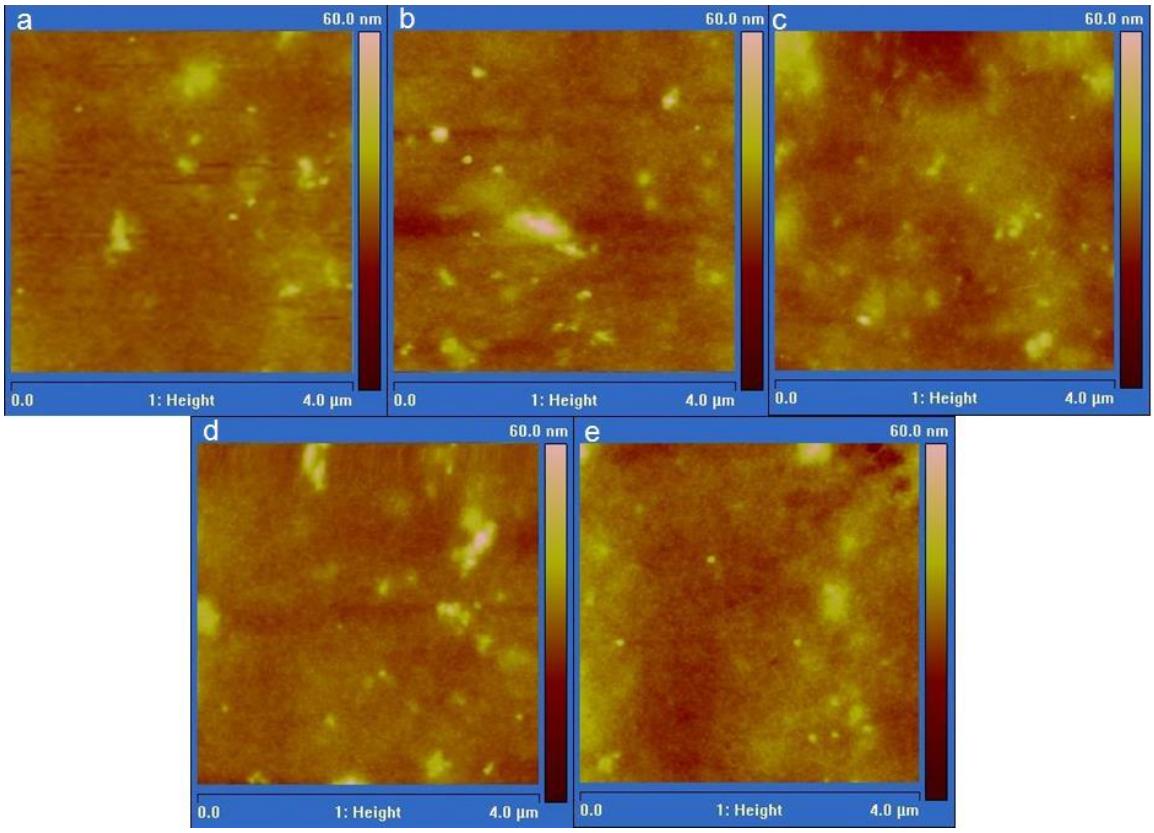


Fig 3.4 AFM images of Pt gate electrodes modified with different films, including (a) 1.2µm thick Nafion; (b) 2.3µm thick Nafion; (c) Nafion+SWNT composite; (d) Nafion+Gr composite; (e) Nafion+GO composite

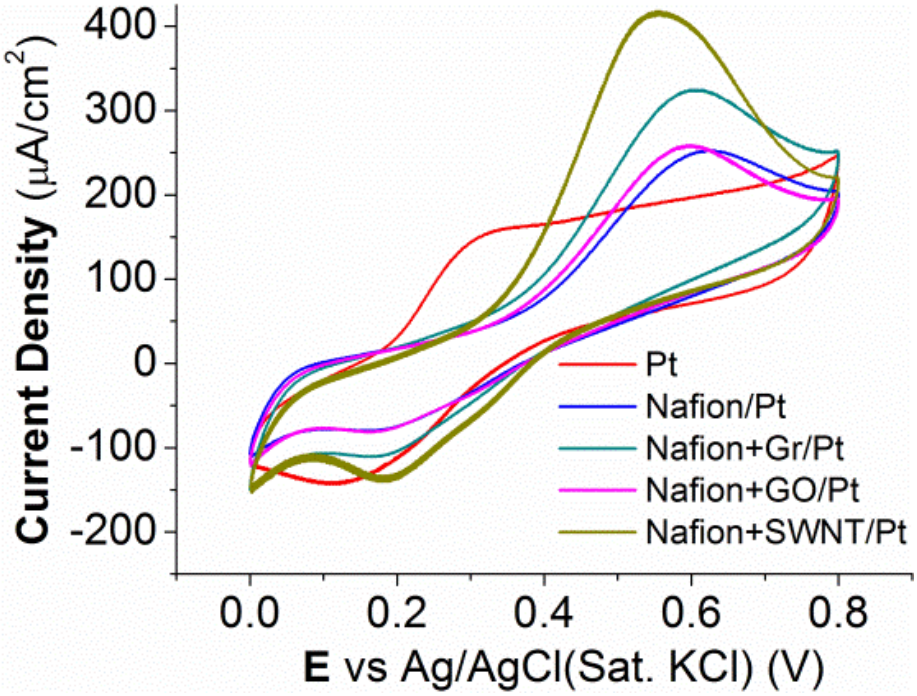
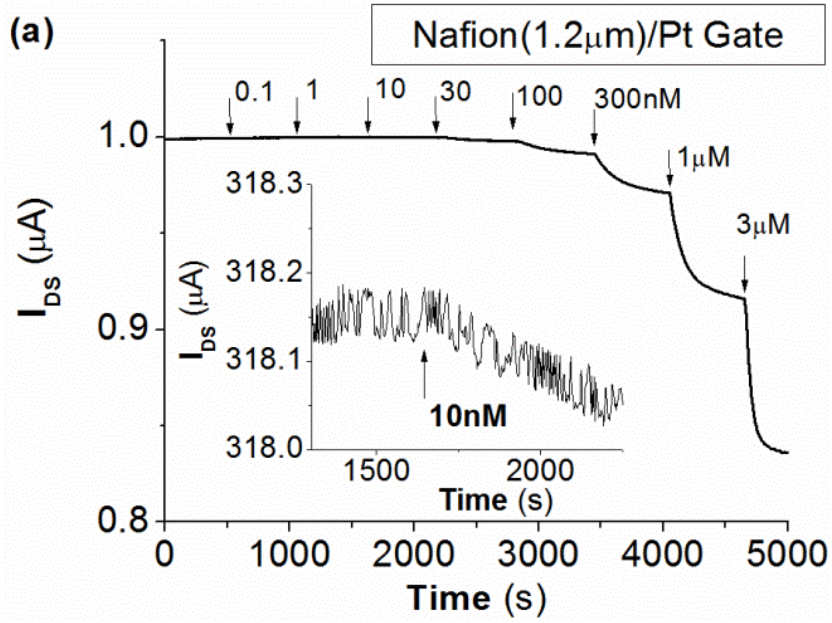


Fig 3.5 Cyclic voltammograms of Pt electrodes modified with different films measured in 1mM epinephrine PBS solution. Voltage scan rate: 50mV/s.

3.4.2.1 Epinephrine sensing with Nafion modified Pt gate

To optimize the Nafion's thickness for epinephrine sensing, Pt gate electrodes with Nafion (1.2 μm) and Nafion (2.3 μm) modification were used for epinephrine sensing. With the same characterization method, the detection limits were found to be 10nM for both and the slope α for the effective gate voltage change ΔV_G^{eff} versus epinephrine concentration in logarithmic axis (log[EPI]) are 136mV/decade and 194mV/decade respectively, as shown in Fig. 3.6. The enhancement on detection limit compare to clean

Pt gate electrode could be attributed to the increased local concentration of epinephrine near the gate attracted by Nafion. The negatively charged Nafion attract the positively charge epinephrine so that the epinephrine diffused across the porous Nafion and contact the Pt surface to undergo electro-oxidation. It was also shown that the slope α was higher for the device with a thicker Nafion film. The higher slope meant higher current response in Faradaic region therefore in the mixture with nano-material, Nafion (~2.3 μm) was chosen to mix the nano-material.



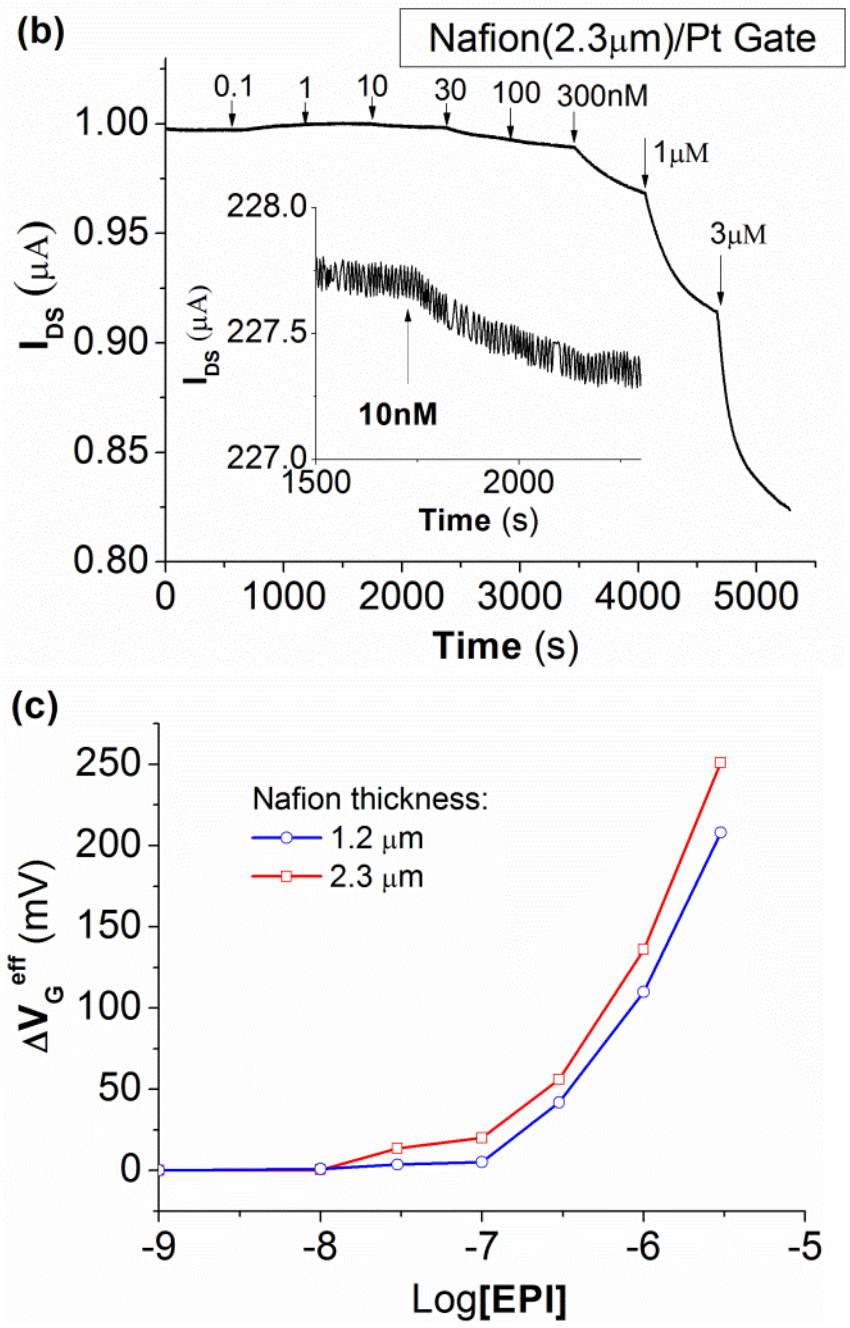


Fig 3.6 (a) The normalized current responses of OECTs with (a) 1.2μm thick (I_0=317.79μA) (b) 2.3 μm thick Nafion films modified on Pt gates to the increasing epinephrine concentration in PBS solution (I_0=227.20μA). V_{DS}=0.1V, V_G=0.6V,. Insets: The enlarged current responses at the detection limits (10nM). (c) The effective gate voltage change (ΔV_G^{eff}) of the two OECTs as a function of the logarithmic value of epinephrine concentration (Log[EPI]).

3.4.2.2 Epinephrine sensing with Nafion/nano-materials modified Pt gate

After optimizing the Nafion quantity for epinephrine sensing, the Nafion was mixed with the carbon based nano-material SWNT, graphene and GO and drop-coated on the gate surface. The Pt gate electrode with different Nafion/nano-materials modification were used to perform the epinephrine sensing. The experimental conditions were the same as the previous experiment.

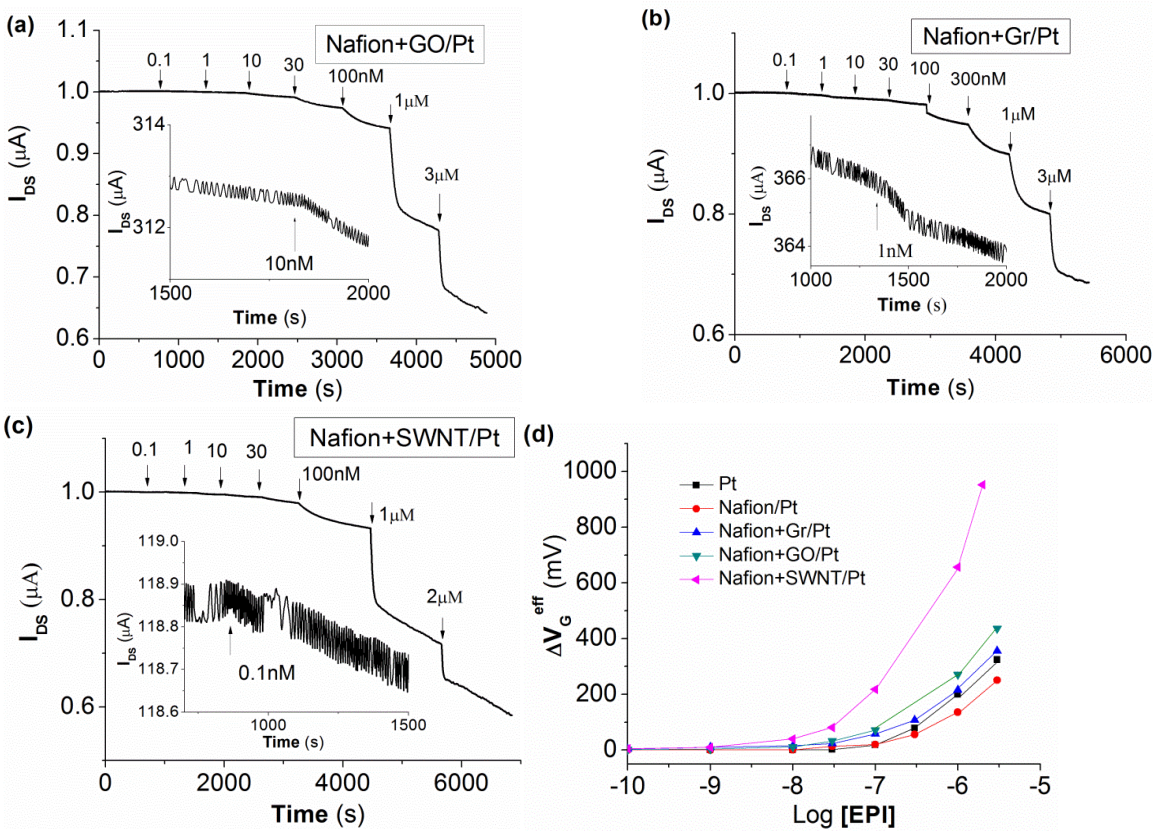


Fig 3.7 The normalized current responses of OECTs with (a) Nafion+GO (I_0=313.13μA); (b) Nafion+Gr (I_0=367.53μA) and (c) Nafion+SWNT films modified on Pt gates to the increasing epinephrine concentration in PBS solution (I_0=119.02μA). V_{DS}=0.1V, V_G=0.6V. Insets: the enlarged current responses at the detection limits ((a) 10nM, (b) 1nM; (c) 0.1nM). (d) The effective gate voltage change (ΔV_G^{eff}) of the OECTs with different gate electrodes as a function of the logarithmic value of epinephrine concentration (Log[EPI]).

Since the OECTs co-modified with Nafion and different nanomaterials (SWNT, Gr and GO) gate electrodes were characterized at the same conditions. Fig. 3.7(a) shows the normalized channel current responses of the OECT with Nafion+GO/Pt gate to epinephrine addition in PBS solution. The detection limit is about 10nM, which is similar to that of the device with Nafion/Pt gate. Therefore, the modification of GO on the Pt gate has little effect on the OECT-based epinephrine biosensors. Fig. 3.7b shows the normalized channel current response of the OECT with Nafion+Gr modified on the Pt gate. The device exhibits an obvious channel current response down to the concentration of 1nM. This enhancement could be due to the enhanced electrocatalytic activity of the gate electrode to epinephrine. Fig 3.7(c) shows the normalized channel current response of the device modified with Nafion+SWNTs on the Pt gate to additions of epinephrine. The detection limit of the device is as low as 0.1nM, which is much lower than those of the aforementioned devices. This result is consistent with the CV measurements of the gate electrodes in epinephrine PBS solution. So the lowest detection limit of the device can be attributed to the highest electrocatalytic activity of the Nafion+SWNT modified Pt gate.

The selectivity of the device is an important issue in practical use as it has been reported that OECTs can be used as many types of electrochemical biosensors. Therefore in this work the major interferences including ascorbic acid (AA) and uric acid (UA) were tested by the device with the Nafion+SWNT/Pt modified gate electrode. We found that the device showed obvious responses to AA and UA at the minimum concentrations of 0.1μM and 1μM respectively, as shown in Fig. 3.8.

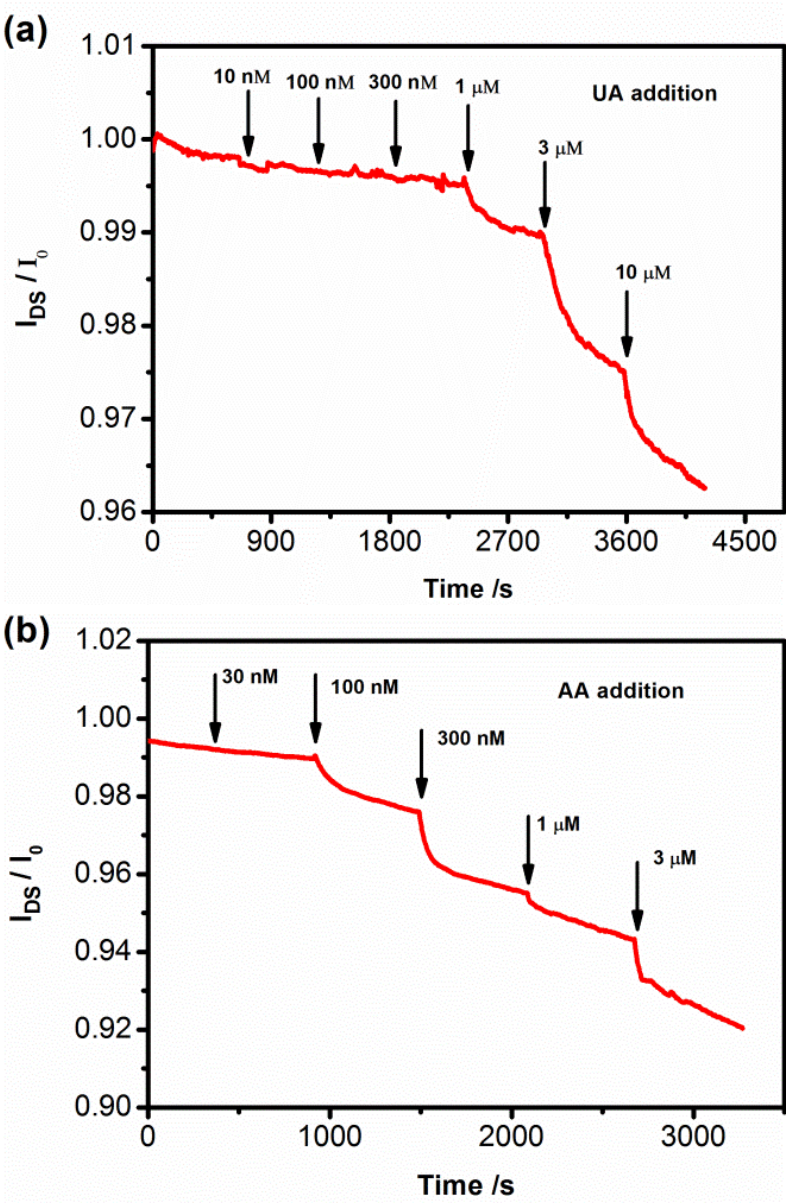


Fig 3.8 Normalized current responses of the OECT with a Nafion/SWCN modified Pt gate to additions of (a) uric acid (UA) and (b) ascorbic acid (AA) measured at V_{DS}=0.1V and V_G=0.6V

Table 3.1 The detection limit and the slope of effective gate voltage per decade of the epinephrine concentration (α) of OECT-based epinephrine biosensors.

Gate of OECT	Detection limit (nM)	α (mV/decade)
Pt	30	210
1.2μm-thick Nafion/Pt	10	136
2.3μm-thick Nafion/Pt	10	194
Nafion+GO/Pt	10	239
Nafion+Gr/Pt	1	248
Nafion+SWNTs/Pt	0.1	533

Table 3.2 Comparison of previously reported epinephrine biosensors by different electrochemical methods

Method	Modification	Detection limit(nM)	Reference
Square wave voltammogram	pyrolytic graphite electrode	170	42
ISFET	Au nanoparticles immobilized on the electrolyte/Al_2O_3 sensing interface	1000	43
CV	White rot fungi cells immobilized on Pt electrode	1040	44
Differential pulse voltammetry	Over-oxidized polypyrrole/multi-walled carbon nanotube composite on glassy carbon electrode	40	45
Amperometric detection	Multiwall carbon nanotubes on basal plane pyrolytic graphite electrode	20	46
Cotton-fiber OECT	Pt gate with no surface modification	~1000	29
OECT	Nafion and different carbon based nanomaterial immobilized on the Pt gate surface.	0.1-30	This Work

3.4.3 Comparison of other transistor based epinephrine biosensors

Table 3.2 shows the results of different electrochemical method, the best device in this work gave the detection limit of 0.1nM while the Cotton-fiber OECT and the ISFET both gave 1μM.[29][43] It should be noted that all the sensors, including our sensors, were transistor based sensors. This gave a chance to compare the inorganic thin film transistor and organic thin film transistor, and both organic transistor with different architecture. Compared with the epinephrine sensors based on conventional ion-sensitive field effect transistors (ISFETs), the improved performance can be attributed to the following two factors. One is the low operation voltage (less than 1 V) of the OECT and the other is the sensing mechanism of the OECT based on the detection of the electrochemical reaction of epinephrine on the gate. For a Si-based ISFET, the operational voltage is around 10V that is one order of magnitude higher than that of the OECT. Therefore the OECT-based sensor is much more sensitive to potential change in the interface than typical ISFET-based transistors. Also for the electrochemical reaction of epinephrine on the gate, the sensitivity of the device can be enhanced by improving the electrocatalytic activity of the gate electrode.

Compared the performance of cotton-fiber OECT and OECTs in our work, the different performance might be due to the poor stability of their devices, as evidenced by the big error bars of the current responses of the devices. Also the cotton may be too thick compare to the usual channel thickness, so that it might not be a thin film transistor even it showed the transistor behavior.

3.5 Summary

In summary, OECTs with Pt gate electrodes modified with biocompatible polymer (Nafion) and carbon-based nano-materials (SWNT, graphene flakes or GO) show high sensitivity and low detection limit to epinephrine. Nafion can enhance the sensitivity of the device by attracting epinephrine molecules to the gate electrode due to their opposite charge. Compare to the Nafion/Pt gate electrode, SWNT and graphene flakes modified on the gate electrode can further improve the sensitivity and decrease the detection limit of the device due to the enhanced electrocatalytic activity of the gate electrode. The device modified with Nafion and SWNTs shows the lowest detection limit of 0.1nM, which is sensitive enough for practical applications. Considering that the OECT-based epinephrine sensors can be prepared by low-cost and convenient solution process, this type of devices are promising transducers for disposable applications in the future.

Chapter 4 Cholesterol Sensing of Organic Electrochemical Transistors

In this chapter, cholesterol sensors based on OECTs were introduced and systematically studied. The device sensitivity was improved when the enzyme cholesterol oxidase and graphene/graphene oxide were immobilized on the gate electrode together with biocompatible polymer Nafion. The optimized devices gave the linear region cover the concentration levels of cholesterol much lower than that in human plasma. Therefore, highly sensitive cholesterol sensors were fabricated by functionalizing the gate electrodes of OECT by enzymes and carbon based nano-materials. In addition, because the devices are fabrication easy, relatively small size and low-cost, they are suitable for one-use disposable bio-sensing applications. We also studied the indirect cholesterol sensing of OECTs by using the enzyme cholesterol oxidase (ChOx). Nafion was employed as the matrix for immobilizing the enzyme ChOx and nano-material to enhance the device sensitivity. ChOx could decompose cholesterol and produce hydrogen peroxide (H_2O_2), which can be easily detected by OECTs.

4.1 Introduction

Cholesterol is a biomolecule frequently found in the food. Normally the cholesterol level in human plasma is 1.3 - 2.6mg/ml (3.36mM to 6.72mM).[53] High cholesterol level in human blood plasma will lead to a high risk in having heart disease and high blood

pressure.[54] So the accurate and rapid detection of cholesterol level is important to human health and food industries. The research on highly sensitive cholesterol biosensors based on different methods have been carried out for many years and found some practical applications with satisfactory performance.[55]

Many different cholesterol sensors based on electrochemical methods, such as amperometric, potentiometric and conductometric cholesterol detections,[55]were demonstrated by the aid of the enzyme ChOx, which can enhance its selectivity and decrease the operational voltage. The signals in the electrochemical detections were mainly produced by electro-oxidation of H_2O_2 generated by cholesterol, similar to the glucose sensors based on OECTs [32][33] and solution-gated graphene transistors reported before.[56] It has been recognized that the integration of enzyme with nano-materials such as graphene flakes and graphene oxide and Nafion/Chitosan biocompatible polymers can dramatically improve the sensitivities and detection limit of such enzyme biosensors. Therefore, similar result would be expected in the enzymatic cholesterol sensing based on OECTs.

In this Chapter, OECT-based cholesterol sensors with enzyme-nanomaterial co-modified gate electrodes were prepared and characterized. Nafion, graphene/graphene oxide and the cholesterol oxidase (ChOx) were mixed and deposited on the gate electrodes to improve the sensitivity and decrease the detection limit of the devices. The best device with Nafion, GO and ChOx modified on the Pt gate electrode showed a detection limit of 10nM and linear response from 1μM. So the devices are sensitive enough to detect the

cholesterol level of human plasma since the cholesterol level is 1.3 - 2.6mg/ml (3.36mM to 6.72mM) in human plasma normally. We also noticed that the performance of the devices are better than that of the cholesterol biosensors reported before.[57][58][59][60]

4.2 Fabrication, preparation and measurement of OECT cholesterol sensors

The preparation and measurements were similar to the sections 3.2.1 and 3.2.3, therefore in this section only the modification procedure of the gate electrodes is introduced.

4.2.1 Preparation of OECTs cholesterol sensor

For the gate modification, Nafion(5%), ChOx (stock solution 3.9 mg /mL) in 0.1x PBS solution and nanomaterials (graphene, GO) were mixed with the volume ratio of 2:1:1 to obtain mixture solutions. The mixtures were drop-coated on 3mm × 3mm clean Pt gates and then the electrodes were dried in 4^{o}C refrigerator for 4 hours.

Cholesterol solution was prepared by dissolving cholesterol powder into the 1x PBS solution with 1% v/v Triton X-100 to obtain 1mM cholesterol solution and then diluted with 1x PBS to obtain the other concentration solutions.

4.3 Working principle of OECT-based cholesterol sensors

The conductance of the PEDOT:PSS channel of an OECT can be changed by the gate voltage due to the electrochemical doping by cations from the electrolyte. So the channel current is sensitive to the offset voltage at the gate surface of the OECT. In sensing applications, the reaction of an analyte can lead to the change of offset voltage on the gate surface and thus a channel current response, such as the epinephrine sensor introduced in Chapter 3. Therefore, the cholesterol indirect sensing was based on the oxidation of H_2O_2 generated by cholesterol on the gate electrode.

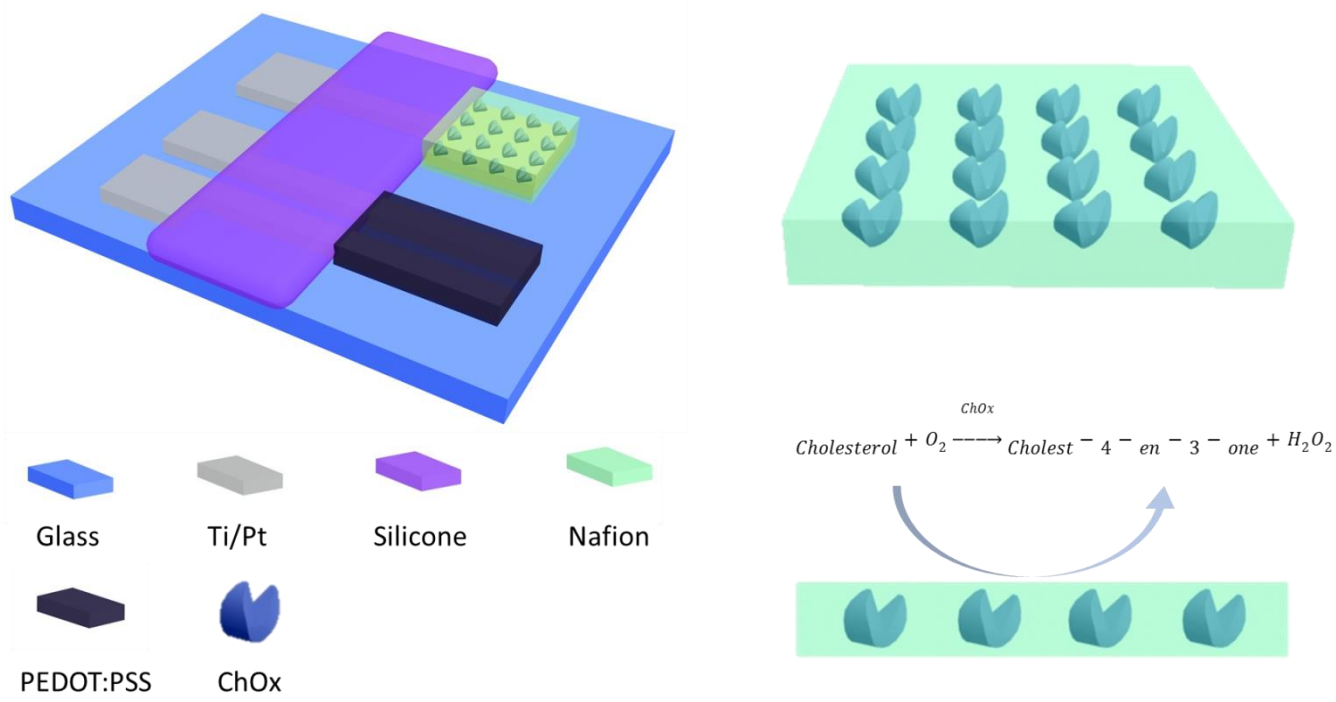


Fig 4.1 The device structure of OECT cholesterol sensor and the reaction cycle

The working mechanism of the OECT-based cholesterol sensors is shown in Fig. 4.1. Nafion was used to immobilize the enzyme ChOx and nano-material on the gate electrode. ChOx can decompose the cholesterol into Cholest-4-en-3-one and H_2O_2 and H_2O_2 undergoes direct electro-oxidation to release two electrons per H_2O_2 molecule and produces Faradaic current between the gate electrode and the electrolyte interface. The whole biochemical reactions of cholesterol sensing in PBS is given by the formulas: [55][57][59][60]

$$Cholesterol + O_2 \xrightarrow{ChOx} Cholest - 4 - en - 3 - one + H_2O_2 \qquad (4.1\ a)$$

$$H_2O_2 \xrightarrow{electro-oxidation} 2H^+ + O_2 + 2e^- \qquad (4.1\ b)$$

The Faradaic current produced by the direct electro-oxidation of H_2O_2 at the gate electrode could change the potential difference between the electrolyte and the gate electrode and hence change the effective gate voltage $V_G{}^{eff}$ of the OECT given by:

$$V_G^{eff} = V_G + \alpha \log[H_2O_2] + constant \quad ,$$

$$where\ \alpha = 2.3(1+\gamma)kT/2e \quad , \qquad (4.2)$$

where γ is the ratio between the electrical double layer capacitances of the electrolyte/channel interface (C_C) and the electrolyte/gate interface (C_G), $\gamma=C_C/C_G$; k is Boltzmann constant; T is room temperature and [H_2O_2] is the concentration of hydrogen peroxide.

However, from equation 4.1a, the H_2O_2 concentration in the beaker should be directly related to the cholesterol concentration added in the beaker. Therefore, the increase of cholesterol concentration would increase the effective gate voltage of the OECT given by:

$$V_G^{eff} = \alpha_{chol} \log[chol] + constant \quad . \quad (4.3)$$

In other words, the transfer curve of the OECT will shift to lower the gate voltage after the addition of cholesterol in the solution.

4.4 Performance and discussion on OECT cholesterol sensors

The Low cost, highly sensitive cholesterol sensors with low detection limit have been realized by using OECTs with Nafion, ChOx and graphene/graphene oxide -modified gate electrodes. The optimum device modification led to the detection limit of 10nM and covered the normal concentration level in human plasma. Therefore, it is sensitive enough for medical or food checking applications.

4.4.1 Nafion with enzyme matrix modification on Pt gate

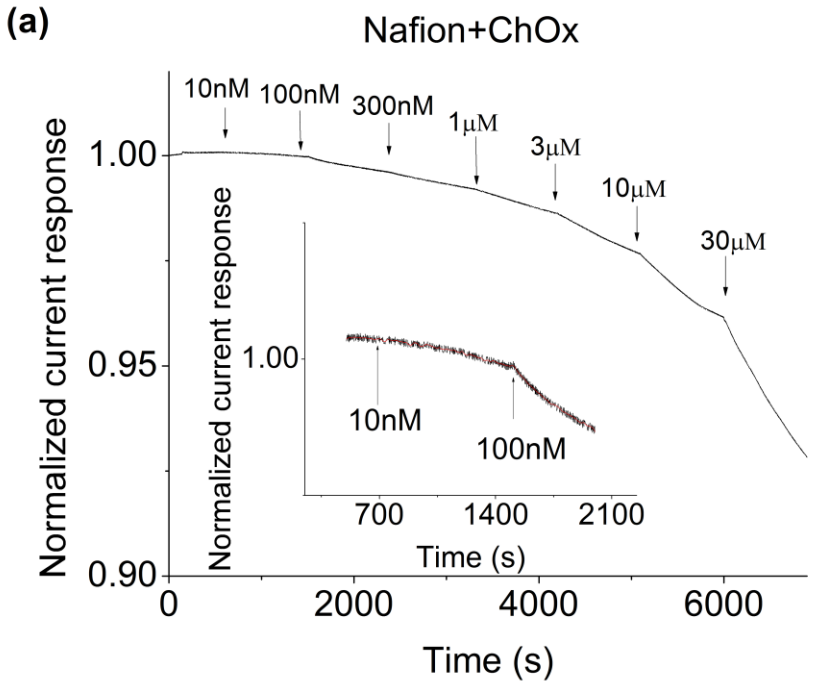
Nafion, as introduced in the Chapter 3, was a porous polymer with stable Teflon backbone and acidic sulfonic groups.[52] It was employed as an enzyme matrix for immobilizing the enzyme ChOx on the gate electrodes of OECTs, being similar to other transistor-based enzyme biosensors reported before.[32][33][56] The Nafion of about 2μm thickness was modified on the Pt electrode, which is thick enough to effectively immobilize the enzyme on the gate electrode. In the sensing process, H_2O_2 molecules were produced by ChOx and underwent electro-oxidation immediately on the gate while the negatively charged Nafion would not affect the sensing performance.

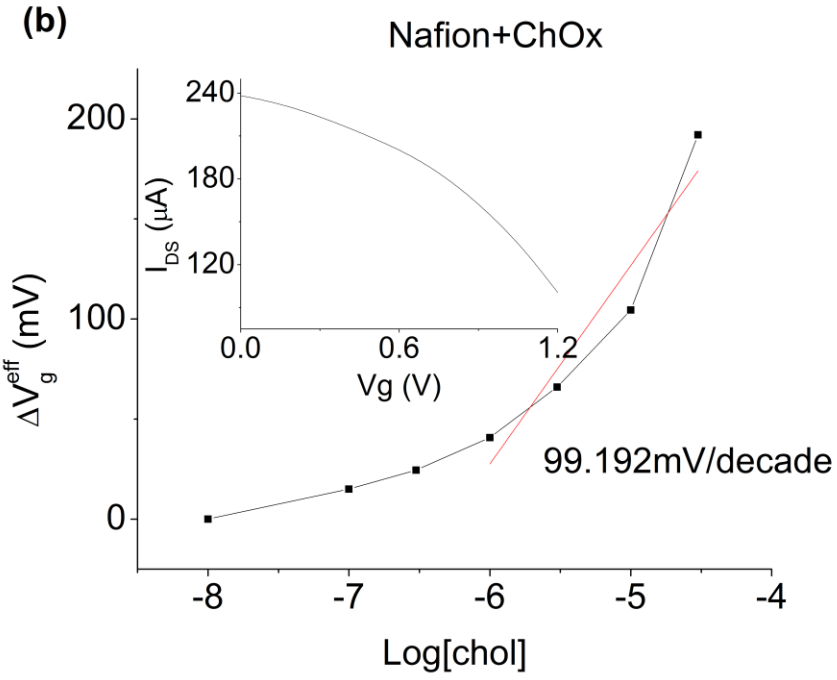
4.4.2 Device performance with Nafion mixture modified Pt gate

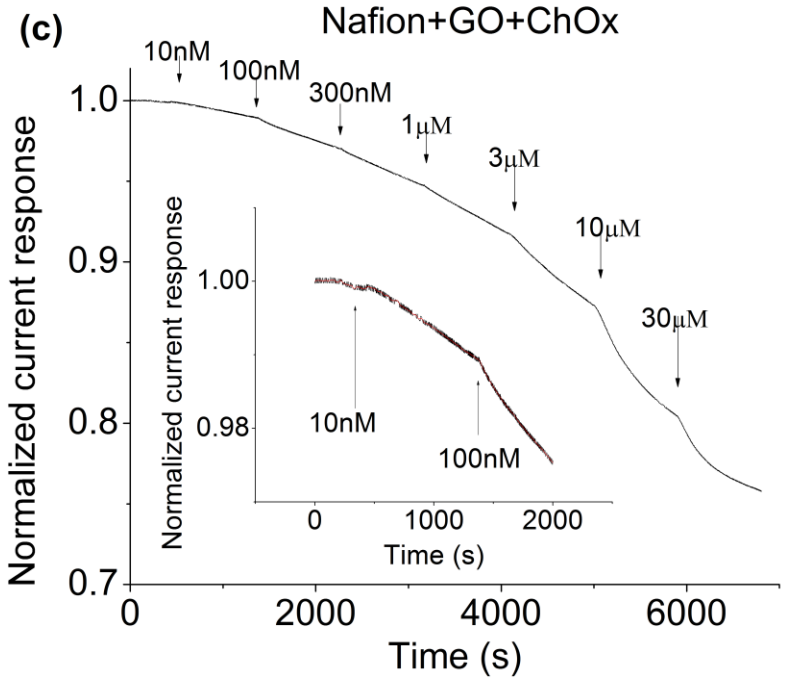
The Nafion and ChOx with/without nano-materials (graphene or graphene oxide) modified Pt gate electrode were employed to detect the cholesterol concentrations in PBS solutions. After the transfer curve was obtained, the channel current (I_{DS}) was measured as a function of time (t) by increasing the cholesterol concentration in the beaker. Channel current responses to additions of cholesterol in the solution were obtained immediately.

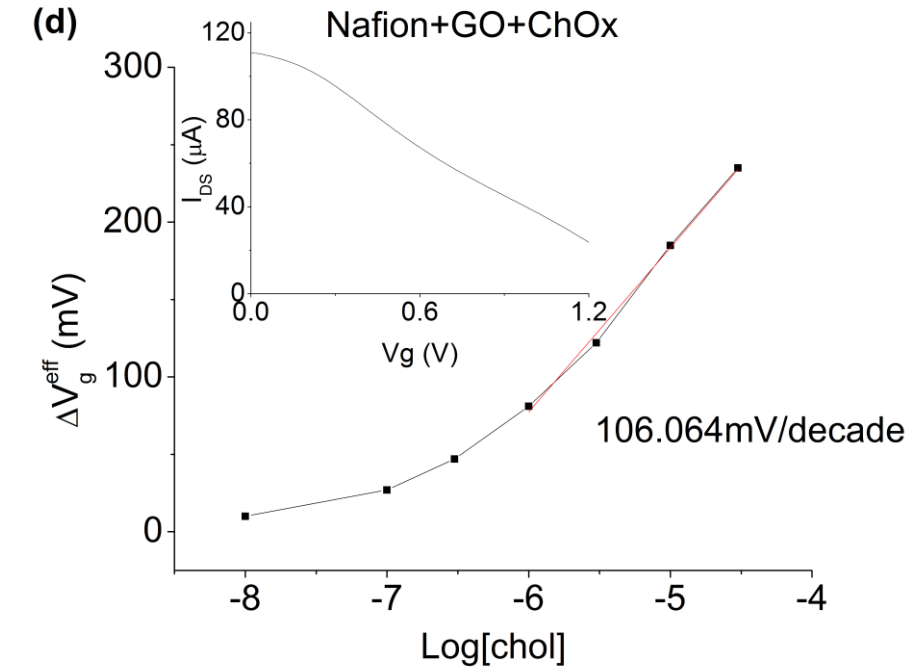
Fig. 4.2 shows ΔV_G^{eff} against the cholesterol concentration for different surface modifications. The device with Nafion-GO/ChOx/Pt gate has the gate voltage change of

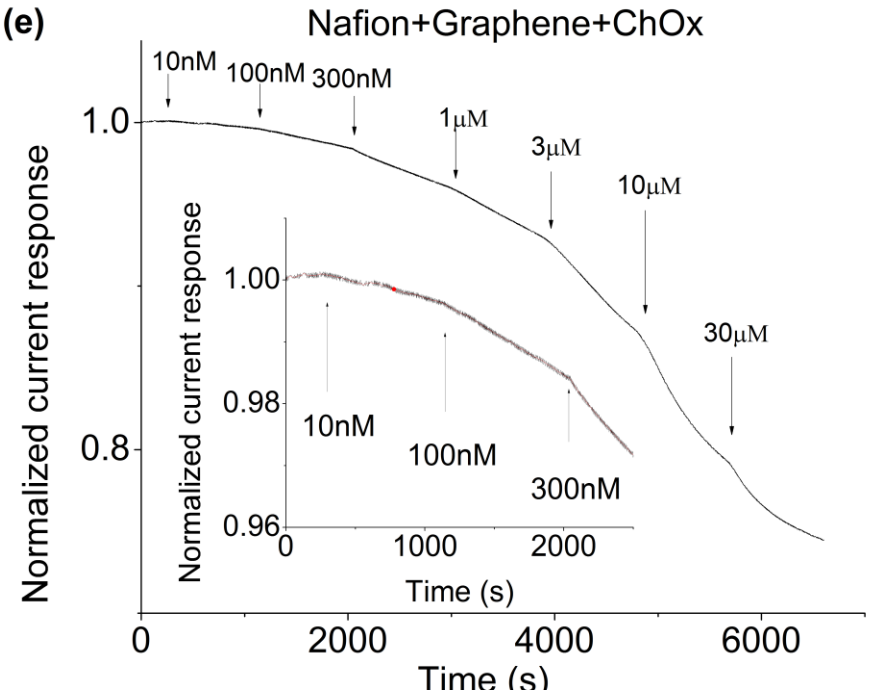
about 106.064mV for increasing one decade cholesterol concentration. The one with Nafion-graphene/ChOx/Pt gate shows the slope of 96.028mV/decade and another one with Nafion/ChOx/Pt gate shows the response for 99.192mV/decade. The results suggest that the method for immobilizing the enzyme ChOx on the gate was successful and nano-materials can enhance the sensitivity of the OECTs.




(b)
Nafion+ChOx
200
100
0
ΔV_g^{eff} (mV)
240
180
120
I_{DS} (μA)
0.0
0.6
1.2
Vg (V)
99.192mV/decade
-8
-7
-6
-5
-4
Log[chol]


(c)
Nafion+GO+ChOx
10nM
100nM
300nM
1μM
3μM
10μM
30μM
1.0
0.9
0.8
0.7
Normalized current response
1.00
0.98
10nM
100nM
0
1000
2000
Time (s)
0
2000
4000
6000
Time (s)


(d)
Nafion+GO+ChOx
300
200
100
0
ΔV_g^{eff} (mV)
120
80
40
0
I_{DS} (μA)
0.0
0.6
1.2
Vg (V)
106.064mV/decade
-8
-7
-6
-5
-4
Log[chol]


(e)
Nafion+Graphene+ChOx
10nM
100nM
300nM
1μM
3μM
10μM
30μM
1.0
0.8
Normalized current response
1.00
0.98
0.96
10nM
100nM
300nM
0
1000
2000
Time (s)
0
2000
4000
6000
Time (s)

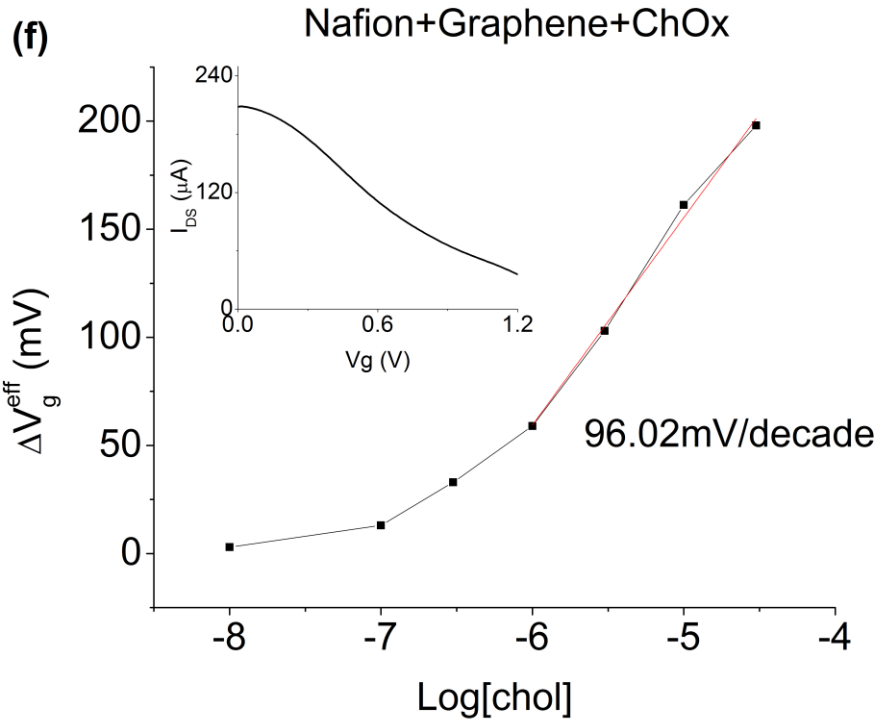


Fig 4.2 (a)(c)(e) The normalized current response of the OECTs with (a) Nafion /ChOx, (c) Nafion/GO/ChOx, (e) Nafion/Graphene/ChOx modified Pt gate to the increasing cholesterol concentration in PBS solution measured at V_{DS}=0.1V and V_G=0.6V. Inset: the enlarged current response at the detection limit of the device of (a) 100nM, (c) 10nM, (e) 10nM.

(b)(d)(f) The offset voltage change as a function of the logarithmic value of cholesterol concentration with (b) Nafion /ChOx, (d) Nafion/GO/ChOx, (f) Nafion/Graphene/ChOx modified Pt gate. Inset: Transfer curve (I_{DS} versus V_G) of the OECT measured in PBS solution with V_{DS}=0.1V

Table 4.1 Detection limit and the change of effective gate voltage (α) of the OECT-based sensors to cholesterol solution

Modification on Pt Gate electrodes	**Cholesterol**	
	Detection limit	α(mV/decade)
Nafion+GO+ChOx	10	106.064
Nafion+Gr+ChOx	10	96.02
Nafion+ChOx	100	99.192

Table 4.1 indicates that the carbon based nano-materials could decrease the detection limit and increase the slope of ΔV_G^{eff} versus cholesterol concentration in logarithmic axis (log[chol]). As the linear response of the cholesterol sensor ranged from 1μM to 40μM, it is sensitive enough for detecting the cholesterol levels in blood plasma.

In addition, the detection limits of the OECT cholesterol sensors were much lower than those of the other electrochemical approaches. The devices could have broader applications rather than medical application, such as cholesterol-free checking for food, snack, milk and other beverages.

In practical applications, OECTs' responses were also dependent on the ion concentration of the working electrolyte,[9] so it is necessary to dilute the isolated cholesterol of plasma or food in buffer solution in the detection. For example, the typical cholesterol level in human plasma is between 3.36mM and 6.72mM, so isolated cholesterol from the blood

plasma is needed to be diluted to the concentration of about 1% by PBS for characterization and it would give the concentration between 33.6μM and 67.2μM, which can be easily detected by the OECT-based sensors.

It has been reported that graphene oxide (GO) could retain proteins' biological activity when they were mixed, indicating promising applications of graphene oxide in OECT biosensors.[61] More importantly, plentiful reactive sites are presented on GO sheets, making it an excellent material for immobilizing enzyme. This may be the reasons that Nafion-GO/ChOx/Pt gate electrode gives the best performance in cholesterol sensing. This result also indicates that not only the electrochemical activity but also enzyme immobilization ability of the nano-materials play important roles on the device performance.

4.4.3 Comparison of other cholesterol biosensors

Compared to the previous cholesterol sensors,[57][58][59]-60] especially the transistor-based sensors, the OECT-based cholesterol sensors showed much lower detection limits down to 10 nM. For the carbon nanotube field effect transistors (CNTFETs) and enzyme field effect transistors (ENFETs) with graphene channel mentioned above, even the similar enzyme modifications on the devices were performed, the detection limits of the two devices were 500 and 1400 μM, respectively, which are nearly ten thousand times

higher than that of our OECT sensors. Table 4.2 list some cholesterol sensors' detection limits compare with our group's OECTs.

Table 4.2 Comparison of different electrochemical based cholesterol biosensors

Method/Device	Modification	Detection limit(μM)	Reference
Chronoamperometry	Silver nanoparticles modified glassy carbon electrode with ChOx	25.8	[57]
Molecularly imprinted polymer capacitive (MIPC) sensor	Electropolymerization of 2-mercaptobenzimidazole on a gold electrode in the presence of cholesterol	0.42	[58]
CNTFET	ChOx immobilized on the PANI/ZnO membrane by physical adsorption technique	500	[59]
ENFET	ChOx has been immobilized on K/PPy/CNT membrane via physical adsorption technique	1400	[60]
OECT	ChOx immobilized on Pt gate surface by mixing ChOx with Nafion and different carbon based nano-material	0.01	This Work

There are several main reasons for the large difference in the three types of devices. The first one is that the enzyme immobilizations in their works were on the channel rather than on the gate electrode. Because the electro-oxidation of H_2O_2 is mainly on the gate electrode, if the H_2O_2 production area is on the channel like their work, H_2O_2 needs to

diffuse to the gate electrode through the electrolyte and the efficiency is lower than that produced on the gate electrode directly.

The second reason is the different gate electrodes employed in the devices. The Pt gate electrode has shown better electro-catalytic effect than Ag/AgCl on electro-oxidation of H_2O_2.

The third reason is the detection method. In their work, they claimed that one sensing mechanisms was due to "protons affect the potential of the gate interface and consequently affect the potential difference between the gate and the source and modulate the channel current"[60]for the releasing of protons H^+ by electro-oxidation of H_2O_2, and the other mechanism was due to "the interfacial potential (ΔV_{in}) developed at the interface between electrolyte solution and oxide layer of the FET"[59] by a Nernst-like equation. So the sensing mechanism is completely different from that of the OECT-based sensors. On the other hand, the working voltage of an OECT is much lower than that of the field effect transistors, making the OECT-based sensors more sensitive to potential changes induced by the analytes.

4.5 Summary

In summary, OECTs with Pt gate electrodes modified with biocompatible polymer (Nafion), ChOx and carbon-based nano-materials (graphene flakes or GO) show high sensitivity and low detection limit to cholesterol. Nafion was successfully used to immobilize the enzyme ChOx and nano-materials (graphene and GO) on the gate to

enhance the device performance. Compared to the Nafion/ChOx/Pt and Nafion/Gr/Pt gate electrodes, Nafion/GO/Pt gate electrodes can further improve the response of voltage shift, which is presumably due to GO that can immobilize enzyme ChOx more effectively for the existence of plentiful reactive sites. The devices have shown much better performance than other transistor-based cholesterol sensors. The optimized device modified with Nafion and GO shows the lowest detection limit of 10 nM, which was sensitive enough for both food safety and clinical applications.

Chapter 5 Adenosine Triphosphate (ATP) sensing of Organic Electrochemical Transistors

In this Chapter, we studied the indirect ATP sensing of OECTs by the aid of the two enzymes: glucose oxidase (GOx) and Hexokinase (HEX). GOx decomposes glucose and produced signal molecule hydrogen peroxide (H_2O_2) for sensing. On the other hand, HEX can combine ATP and glucose to form non-signal molecules adenosine diphosphate (ADP) and D-glucose 6-phosphate and reduce the signal molecules H_2O_2. Nafion was used as the enzyme immobilization matrix in the dual enzyme OECTs and excellent performance has been obtained.

5.1 Introduction

Adenosine triphosphate (ATP) is a big molecule composed of a base adensine, a ribose sugar and three phosphate groups. When ATP undergoes hydrolysis, it will break and give adenosine diphosphate (ADP), HPO_4^{2-} and energy (31kJ/mol).[62] Therefore ATP is a "high-energy" compound and can release energy to cells for any purpose. As one kind of neurotransmitter,[63] previous literature indicated that a human's consumption of ATP in weight is in the order of kilogram per day.[64] In food factory, ATP concentration can be used for monitoring microbiological processes and as an indicator in food

safety.[65] Moreover, the normal ATP concentrations in cells were in milli-molar range,[66] therefore a fast, accurate ATP sensing may be useful in both research and food safety. Here, OECTs are applied to test ATP for the first time and show some potential applications in the future.

5.2 Fabrication, preparation and measurement of OECT ATP sensors

The preparation was similar to sections 3.2.1. Therefore, in this section only the gate modifications and measurements are introduced.

5.2.1 Preparation of OECT ATP sensors

For the gate modification, the Nafion(5%), HEX (stock solution 0.5kU/mL) and GOx (stock solution 0.5kU/mL) in 0.1X PBS were mixed with the volume ratio 2:1:1 to have different mixtures. 5 µL or 10 µL mixtures were drop-coated on the 3mm × 3mm clean Pt gates and then dried at 35ºC for 10-15 mins.

ATP solution was prepared by dissolving the adenosine 5’ -triphosphate (ATP) disodium salt hydrate powder into the de-ionized water solution to obtain 10mM ATP solution and then diluted with de-ionized water to obtain the other solutions with different concentration.

1M glucose solution was prepared by dissolving the glucose powder into PBS solution and heated at 90ºC overnight to speed up the transformation of α - glucose to β - glucose due to the enzyme GOx can only decompose β - glucose.

In this experiment 0.1x PBS solution with 5mM $MgCl_2$ was employed as the base solution because Mg^{2+} was needed to participate in the enzymatic function of HEX.[67]

5.2.2 Measurement of OECT ATP sensors

All measurements were performed by two labview controlled Keithley 2400 sourcemeters with a USB to transfer and monitor the data. The channel and gate electrodes were rinsed with PBS solution to remove the undesired residue on the channel and gate and immerse in 10ml 0.1x PBS solution with 5mM $MgCl_2$ stirred in a beaker. The transfer characteristic curve (I_{DS} vs V_G) with V_{DS}=0.1V and V_G varied from 0V to 1.2V was measured. Then the channel current (I_{ds}) was measured as a function of time (t) with fixed voltages V_{DS} = 0.1V and V_G = 0.6V. 10μM glucose solution was added to the beaker to increase the glucose concentration in the beaker and wait for the stable current. After that the ATP solutions was added in the beaker with increasing concentration to obtain the current responses.

5.3 Working principle of OECT-based ATP sensors

The channel current I_{DS} of the device at different source-drain voltage V_{DS} and gate voltage V_G is given by the following equation: (see also eq(1.1) and (3.1))

$$I_{DS} = \frac{q\mu p_0 tW}{LV_p}(V_p - V_G^{eff} + \frac{V_{DS}}{2})V_{DS}, \quad (\text{when } |V_{DS}| << |V_p - V_G^{eff}|)$$

$$V_p = qp_0 t / c_i, \qquad (5.1)\ [=$$

$$V_G^{eff} = V_G + V_{offset},$$

(1.1)]

where q and μ are electronic charge and hole mobility, respectively; p_0 is the initial hole density in the channel; V_p and $V_G{}^{eff}$ are the pinch-off voltage and the effective gate voltage on the transistor, respectively; V_{offset} an offset voltage at gate/electrolyte interfaces; t the thickness of the active layer; W and L are the channel width and length of the OECT, respectively; and c_i is the effective gate capacitance.

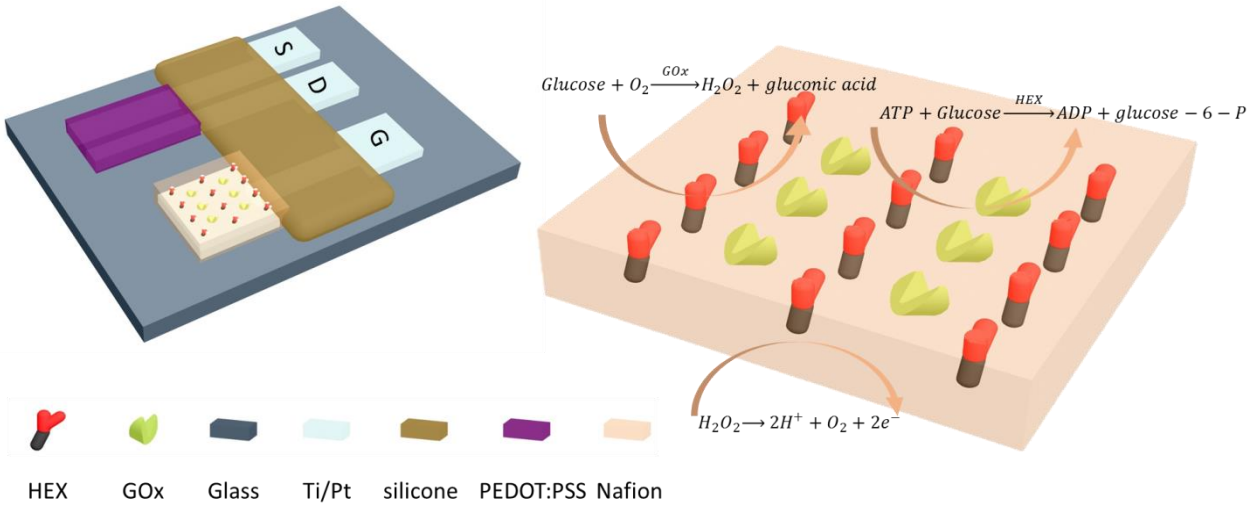


Fig 5.1 The ATP sensor and the gate modification

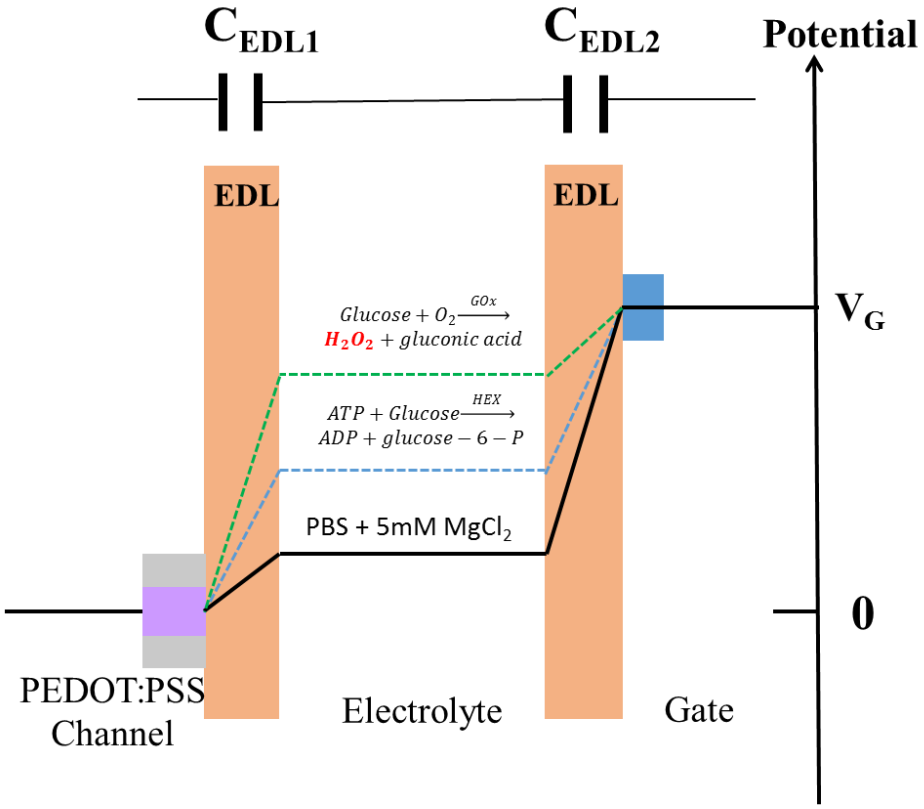


Fig 5.2 Potential distribution of the OECT for ATP sensing.

The working mechanism and potential distribution of the OECT-based ATP sensors is shown in Fig. 5.1 and Fig. 5.2. The enzyme GOx and HEX are immobilized on the gate electrode. GOx can decompose glucose into gluconic acid and H_2O_2 by equation 5.2 (a) and H_2O_2 undergoes direct electro-oxidation to release two electrons per H_2O_2 molecule by equation 5.2 (b) and produces Faradaic current between the gate electrode and the electrolyte interface. After the ATP is added into the beaker, glucose and ATP would have chemical reaction and give out ADP and glucose – 6 – p by equation 5.2 (c).

Because of the concentration of glucose was reduced by the increased ATP concentration, H_2O_2 concentration is reduced and hence the channel current is modulated. The whole biochemical reactions of ATP sensing in PBS solution is given by the formulas: [67]

$$Glucose + O_2 \xrightarrow{GOx} H_2O_2 + gluconic\ acid$$

5.2(a)

$$H_2O_2 \xrightarrow{electro-oxidation} 2H^+ + O_2 + 2e^-$$

5.2(b)

$$ATP + Glucose \xrightarrow{HEX} ADP + glucose - 6 - P$$

5.2(c)

The Faradaic current produced by the direct electro-oxidation of H_2O_2 at the gate electrode could change potential difference between the electrolyte and the gate electrode and thus change the effective gate voltage $V_G{}^{eff}$ of the transistor given by:

$$V_G^{eff} = V_G + \alpha \log[H_2O_2] + constant \quad ,$$

$$\alpha = 2.3(1+\gamma)kT/2e \quad ,$$

(5.3)

where γ is the ratio between the electrical double layer capacitances of the electrolyte/channel interface (C_C) and the electrolyte/gate interface (C_G), $\gamma=C_C/C_G$; k the Boltzmann constant; T the room temperature and [H_2O_2] is the concentration of hydrogen peroxide.

Similar to the cholesterol sensing shown in chapter 4, from equation 5.2(a), the H_2O_2 concentration in the beaker should be proportional to the glucose concentration in the beaker. So the effective gate voltage is given by: [32][33]

$$V_{G,glu}^{eff} = \alpha_{glu} \log[glu] + A$$

(5.4)

On the other hand, the addition of ATP can reduce the glucose level by the enzyme HEX and ATP, as shown in equation 5.2(c). Hence, ATP will decrease the effective gate voltage with a value by reducing the glucose concentration in beaker and given by:

$$V_{G,ATP}^{eff} = \alpha_{ATP} \log[ATP] + B$$

(5.5)

where A, B are constants.

Therefore, the total effective gate voltage is given by:

$$V_g^{eff} = V_{G,glu}^{eff} - V_{G,ATP}^{eff} \quad .$$

(5.6)

As the initial glucose concentration is unchanged and only ATP concentration is increased, we can regard $V_{G,glu}^{eff}$ as a constant and after the ATP addition, the effective gate voltage is given by:

$$\Delta V_g^{eff} = (V_{G,glu}^{eff} - V_{G,ATP}^{eff})_{new} - (V_{G,glu}^{eff} - V_{G,ATP}^{eff})_{initial} = (V_{G,ATP}^{eff})_{initial} - (V_{G,ATP}^{eff})_{new} \quad .$$

(5.7)

Here "initial" is used to imply the state that the beaker contains glucose and give stable current in OECT without ATP and "new" implies the state that the beaker contains glucose and give stable current in OECT after the a quantity of ATP is added.

Because initially there was no ATP in the beaker therefore $(V_{G,ATP}^{eff})_{initial}$ = 0 and

$$\Delta V_g^{eff} = -\Delta(V_{G,ATP}^{eff}) = -\alpha_{ATP} \log[ATP]$$

The obtained equation indicates the relationship of effective gate voltage change after ATP is added into the beaker. Therefore, the increase of ATP concentration in the beaker will decrease the effective gate voltage of the OECTs and thus increase the channel current.

$$|\Delta V_g^{eff}| = |\alpha_{ATP} \log[ATP]| \quad (5.5)$$

5.4 Results and discussion

The OECTs were modified with different amount of Nafion + GOx + HEX mixtures (5 and 10µL). After the OECT was stabilized, 10µL 1M glucose solution was added into the PBS solution to have 1mM glucose concentration in the 10mL beaker. Fig. 5.3 shows the 5µL Nafion + GOx + HEX gate modification for ATP sensing. Fig. 5.3 (a) shows the device current response when glucose was added. The response of the device to glucose shows that the room temperature immobilization method for GOx was good enough for glucose sensing. Then ATP was added in the beaker for several steps with increasing concentrations after the change of current was stabilized at each step. It is notable that the response to ATP was nearly stable after ~ 2 minutes. The device shows a detection limit of 10 µM, which can cover the normal ATP range in cells. The linear slope = 93.531 mV/decade is obtained in the plot of ΔV_G^{eff} against log[ATP].

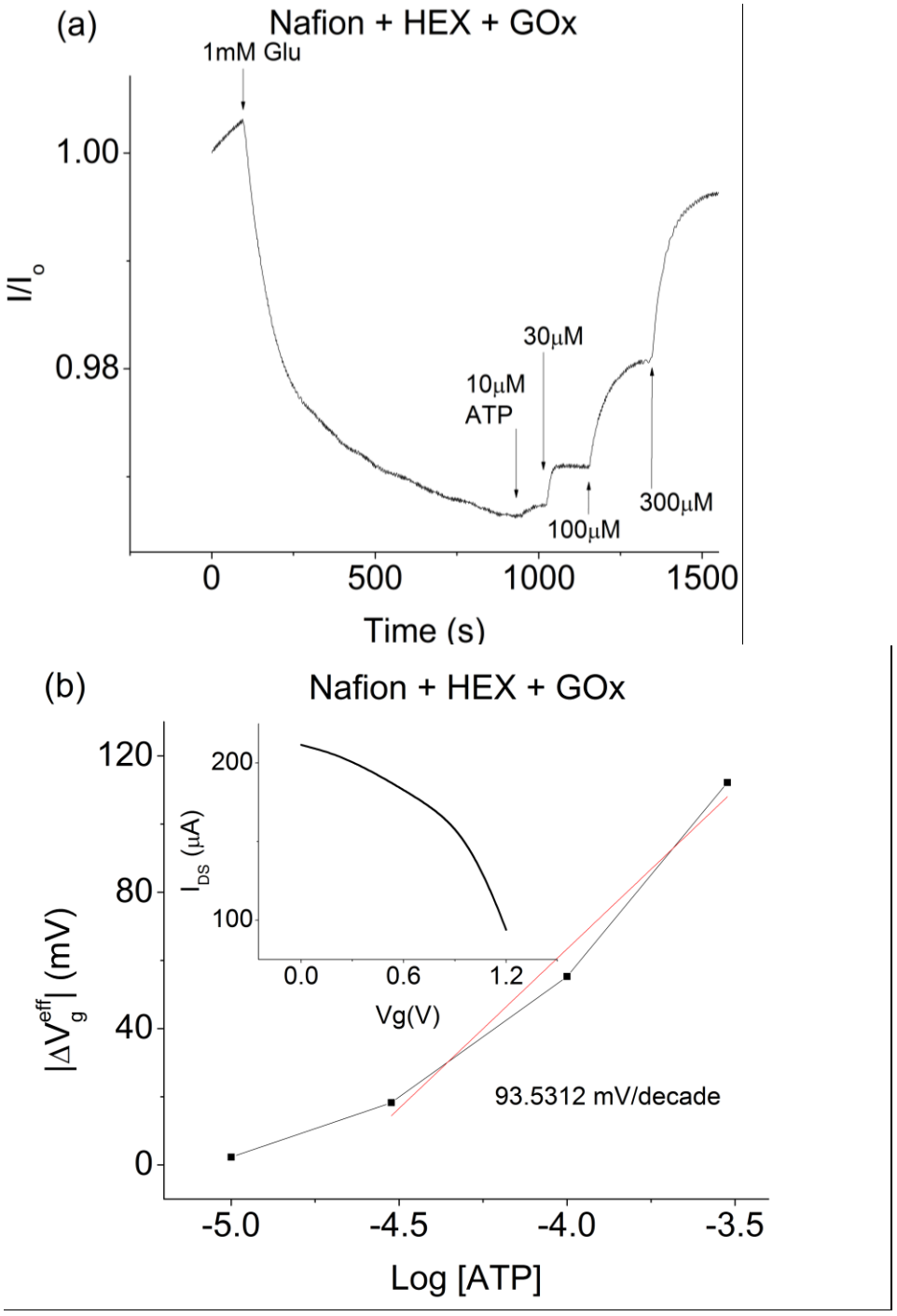
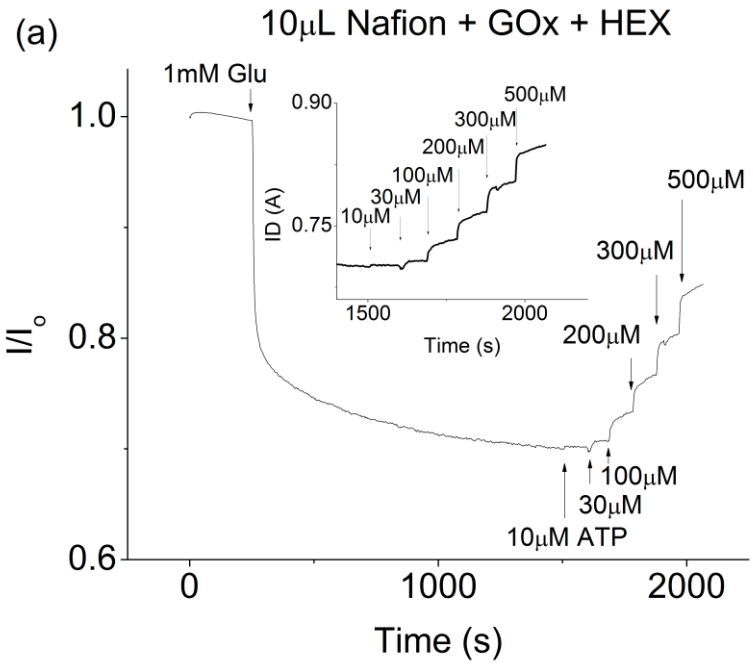


Fig. 5.3 (a) The normalized current response of an OECT with 5μL Nafion/HEX/GOx/Pt gate to the increasing ATP concentration in PBS solution measured at V_{DS}=0.1V and V_G=0.6V. (b) The offset voltage change as a function of the logarithmic value of ATP concentration ([ATP])

Similarly, the modification on Pt gate electrode with 10 μL mixture gives the same detection limit (10 μM) and enhanced current response to ATP, as shown in Fig 5.4. The result indicates that the quantities of two enzymes GOx and HEX can influence the current response and the slope (176.35mV/decade) for the effective gate voltage change ΔV_G^{eff} against log[ATP] although the detection limit has not been improved.



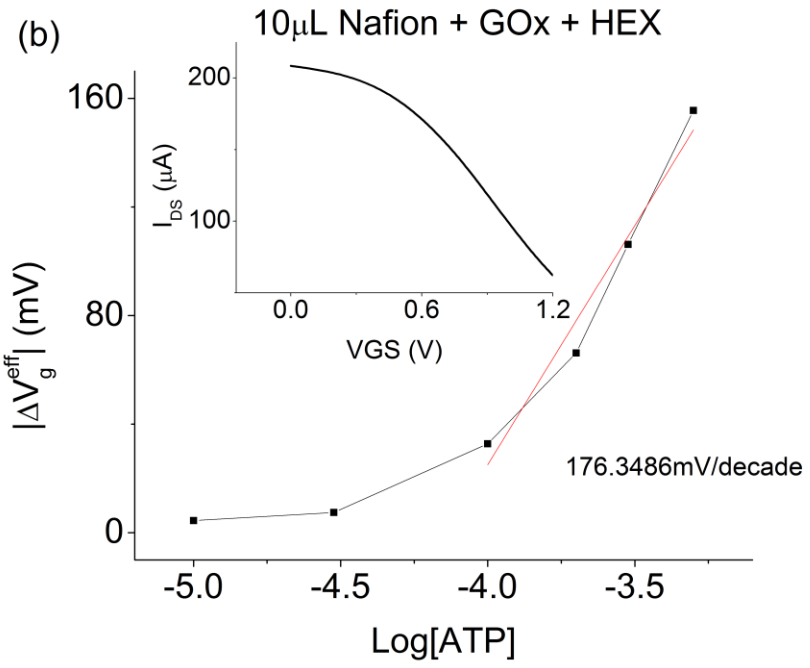


Fig. 5.4 (a) The normalized current response of an OECT with 10μL Nafion/HEX/GOx/Pt gate to the increasing ATP concentration in PBS solution measured at V_{DS}=0.1V and V_G=0.6V. (b) The offset voltage change as a function of the logarithmic value of ATP concentration ([ATP])

These results indicate that ATP sensing can be realized by OECTs with dual enzyme immobilization. Besides, we notice some interesting issues as follows:

(1) The stabilized current in OECT enzymatic ATP sensing was due to the signal molecules (H_2O_2) production equal to the signal molecules decomposition rather than the bioanalytes (glucose) being fully converted to signal molecules. Otherwise the ATP could not affect the result if there was no glucose in the beaker. If all glucose fully converted to H_2O_2 to have stable current, the added ATP cannot control the H_2O_2 concentration anymore.

(2) The ATP sensing time was much faster than the glucose in both devices, indicating that the reaction of ATP is much faster than glucose.

(3) The dual enzymes immobilization at room temperature was successfully realized by simply placing the gate electrode on a 35^{o}C hot plate. Both enzymes GOx and HEX can work properly and give the performance comparable to previous OECT-based enzymatic sensors. Room temperature processing made the sensors to be more easily fabricated.

5.5 Future work of ATP sensors

Even though ATP was successfully detected by OECTs with dual enzymes immobilization on gate electrode, there are still work on optimizing the OECT ATP sensors. To have a very high selectivity to ATP, the disturbance signal from other biomolecules should be avoided. By considering that the main sensing molecule was H_2O_2, a mechanical H_2O_2 filter would be effective in improving the selectivity. Prussian Blue (PB) and its analogs shows high selectivity on H_2O_2 sensing [68][69][70], which can be easily deposited on electrodes by electrodeposition. High crystallinity of Prussian Blue can allow H_2O_2 to penetrate its film and prevent other relatively large biomolecules such as ascorbic acid (AA), uric acid (UA), dopamine and epinephrine to penetrate.

Finally, some nano-materials such as GO[61] showed enzyme immobilization abilities and might improve the sensitivity in ATP sensing when they are employed in the devices.

5.6 Summary

In summary, the OECT ATP sensing based on reduced signal of H_2O_2 was successfully realized. Biocompatible polymer Nafion was successfully used to immobilize the enzymes HEX and GOx on the devices at room temperature within 15 minutes and showed good stability. OECTs with Pt gate electrodes modified with Nafion, GOx and HEX showed high sensitivity and the detection limit of 10 μM to ATP. Future plan of ATP sensor includes the optimization of both sensitivity and selectivity of the devices.

Chapter 6 Conclusion and Future Work

6.1 Conclusion

To conclude, OECTs with Pt gate electrodes modified with biocompatible polymer (Nafion) and carbon-based nano-materials (SWNT, graphene flakes or GO) show high sensitivity to epinephrine. Nafion can enhance the sensitivity of the device by attracting epinephrine molecules to the gate electrode. SWNTs and graphene flakes modified on the gate electrode can further improve the sensitivity and decrease the detection limit of the device due to the enhanced electrocatalytic activity of the gate electrode. The device modified with Nafion and SWNTs shows the lowest detection limit of 0.1nM, which is sensitive enough for practical applications. Considering that the OECT-based epinephrine sensors can be prepared by low-cost and convenient solution process, this type of devices are promising transducers for disposable applications in the future.

Furthermore, the enzymatic sensing of cholesterol was successfully realized by immobilizing the enzyme ChOx, Nafion as well as the conductive nano-materials graphene flakes or graphene oxide (GO) on the gate electrode. ChOx can decompose cholesterol and produce H_2O_2 as the signal molecules. The nano-materials can enhance the current response but not the detection limit. The detection limits of the sensors were ~10nM, which can cover the normal range in human plasma and are suitable for food

safety test. The gate modified with GO showed the best performance on cholesterol sensing probably due to the better enzyme immobilization by GO.

Finally, the enzymatic sensing of ATP was realized by decreasing the signal generated by glucose. Unlike the ordinary enzymatic sensing such as glucose sensing or cholesterol sensing that the sensing mechanisms were based on increasing effective gate voltage by increasing the concentration of mentioned bioanalytes, ATP's sensing mechanism was based on decreasing the effective gate voltage by increasing ATP concentration because ATP can decrease the concentration of glucose and thus the concentration of the signal molecule H_2O_2. The device can be prepared at room temperature, making it suitable for low-cost manufacturing. The devices show the detection limit of 10μM, which can cover the normal ATP concentrations in cells and other systems.

6.2 Future Outlook

The OECTs sensors in this thesis were operated mainly based on the oxidation of biomolecules to achieve sensing. Even though the detection limit was usually very low and the sensitivity was extremely high, it is still not ready to commercialize OECTs as a product for biosensing due to some reasons, such as the poor uniformity and stability of the OECTs.

Recently, 2D-materials such as graphene,[61] MoS_2,[71] WS_2 [72] were used as FET-based sensors and could detect many biomolecules successfully. Graphene was not

only used as biosensors in FET platform, but also showed itself as a good candidate in solution gate transistors. In addition, the 2D materials can be easily used to crosslink the biomolecules such as DNA, RNA, antibody without the complicated surface treatment used on metal electrodes and are thus suitable to immobilize the aforementioned bio-molecules in sensing applications. Therefore, more 2-D materials can be employed in the o OECT-based biosensors to further improve the device performance.

Moreover, other organic semiconductors other than PEDOT:PSS can be used in OECTs and better performance is expected if the materials can show high carrier mobility and better stability in aqueous solutions.

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