



Highly sensitive dopamine biosensors based on organic electrochemical transistors

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ABSTRACT

Organic electrochemical transistors (OECTs) based on poly(3,4-ethylenedioxythiophene):poly(styrene sulfonic acid) (PEDOT:PSS) with different gate electrodes, including graphite, Au and Pt electrode, etc., have been used as dopamine sensor for the first time. The sensitivity of the OECT to dopamine depends on its gate electrode and operation voltage. We find that the device with a Pt gate electrode characterized at the gate voltage of 0.6 V shows the highest sensitivity. The detection limit of the device to dopamine is lower than 5 nM, which is one order of magnitude better than a conventional electrochemical measurement with the same Pt electrode. It is expected that OECT is a good candidate for low cost and highly sensitive biosensor for the detection of dopamine.

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1. Introduction

Dopamine, as one kind of neurotransmitter, has been widely studied since it was discovered in the late 1950s (Wightman et al., 1991a,b). Dopamine plays an important role in the functions of central nervous system, renal, hormonal, and cardiovascular system, etc. (Ikemoto, 2007; Wickens et al., 2007). The imbalance or dysfunction of the dopaminergic neuron process can result in various neurological diseases such as sleeping and eating disorders, Parkinson's disease, addictive behaviors associated with drug abuse, etc. (Tang et al., 2007; Ahlskog, 2007). However, the clinical concentration of dopamine is very low; for example, the dopamine levels in plasma, urine, and single adrenal chromaffin cell are in the range of nM, μ M, and fM, respectively (Hashizume et al., 1995; Peterson et al., 2002; Wightman et al., 1991a,b). Therefore, the development of methods for rapid and sensitive detection of dopamine in biological system is very important for the routine analysis and diagnosis of neurological disorders (Laviolette, 2007). There have been various analytical methods to determine dopamine including high performance liquid chromatography (HPLC) (Chan et al., 2000), mass spectroscopy (Hows et al., 2004), electrochemical detection (Zhang et al., 2000; Zhao et al., 2001), etc. These methods have intrinsic advantages along with some limitations. HPLC and mass spectroscopy allow very low detection limit; however, they are not

suitable for portable and low cost analytical measurement since expensive special instruments are needed. In this regards, electrochemical analytical technique for dopamine determination is an attractive method due to low cost, easy operation, fast response, high sensitivity and feasibility of miniaturization (Zhang et al., 2000; Zhao et al., 2001). Apart from the above methods, field effect transistors (FETs) have been found to be excellent transducers for the detection of dopamine. Recently, the dopamine detection by using ion-sensitive FET devices modified with phenylboronic acid has been reported (Freeman et al., 2007). An ultrasensitive dopamine FET sensor based on polysilicon nanowires has been demonstrated as well (Lin et al., 2008). However, to the best of our knowledge, the utilization of organic thin film transistor (OTFT) for dopamine detection has not been investigated up to date. OTFTs show many advantages over inorganic counterparts, including easy fabrication, low cost, flexibility and excellent biocompatibility, and thus OTFTs are good candidates for disposable biosensors (Sokolov et al., 2009; Yan and Tang, 2010; Lin et al., 2009).

Transistors have shown important applications in label-free and high-sensitivity detection of biomolecules including antigen–antibody (Stern et al., 2007), DNA (Yan et al., 2009), protein (Estrela et al., 2010; Kanungo et al., 2002), enzyme (Yan et al., 2005), cells (Patolsky et al., 2006), etc. Organic electrochemical transistors (OECTs), as one type of OTFT, have attracted much attention in the past decades because the devices can be used in aqueous solutions with very stable performance and low operating voltages (~ 1 V) (White et al., 1984; Bernards and Malliaras, 2007; Berggren and Richter-Dahlfors, 2007; Lin et al., 2010a).

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Therefore, OECTs have broad potential applications in low cost and highly sensitive biosensors. For example, OECTs based on poly(3,4-ethylene dioxithiophene) (PEDOT) has been fabricated and used for DNA sensors (Krishnamoorthy et al., 2004). OECTs have been used as highly sensitive glucose sensors (Zhu et al., 2004; Tang et al., 2011), immunosensor (Kim et al., 2010) and have been integrated in a microfluidic system for multi-analyte biosensing (Yang et al., 2009). More recently, we have systematically investigated the ion-sensitive properties of OECTs based on poly(3,4-ethylenedioxythiophene):poly(styrene sulfonic acid) (PEDOT:PSS) and used the devices in cell-based biosensors (Lin et al., 2010b).

In this paper, we present the determination of dopamine using PEDOT:PSS-based OECTs. Different kinds of gate electrodes, including graphite (GE), Au and Pt electrodes and the GE and Pt electrodes modified with multi-walled carbon nanotubes (MWCNT)–chitosan (CHIT) hybrid, have been used. We find that the device with Pt gate electrode shows the best detection limit (lower than 5 nM), which is one order of magnitude better than a conventional electrochemical detection with the same electrode and also indicates that the proposed OECTs are sensitive enough for potential dopamine detection in real samples such as plasma and urine.

2. Experimental

2.1. Materials and apparatus

MWCNTs aqueous suspension solution was purchased from Shenzhen Nano-Technologies Port Co., Ltd. (China). Dopamine and PEDOT:PSS were purchased from Sigma–Aldrich and stored at 4 °C. Chitosan was purchased from Advanced Technology & Industrial Co., Ltd., Hong Kong and used as received. The dopamine stock solution (10 mM) was prepared with HClO₄ aqueous solution (100 mM) and stored in a refrigerator (4 °C) for future use. All other reagents were of analytical grade or better. De-ionized water and freshly prepared solutions were used throughout. CHIT aqueous solution (5 mg mL⁻¹) was prepared by dissolving 0.5 g CHIT in 100 mL of 50 mM acetic acid solution (pH, 5–6). The solution was sonicated for an hour and then stored in a refrigerator (4 °C). 50 μL of 5 mg mL⁻¹ CHIT acetic acid solution was added into 0.95 mL of 20 mg mL⁻¹ MWCNTs aqueous suspension and then the mixed solution was sonicated for 2 h to prepare MWCNT–CHIT hybrid aqueous suspension. The phosphate buffer saline (PBS, pH 7.2, Invitrogen Inc.) solutions were used as a background electrolyte in all experiments.

Semiconductor parameter analyzer (4156C, Agilent Technologies), CHI660B electrochemical workstation (CH Instruments Inc.) and an electrolytic cell were employed for characterizing the transistors and electrochemical measurements. PEDOT:PSS was used as the active material of OECTs. The GE electrode, MWCNT–CHIT hybrid modified GE electrode, Au electrode, Pt electrode and MWCNT–CHIT hybrid modified Pt electrode served as gate for OECT and working electrodes for electrochemical measurements. A Pt foil served as a counter electrode and an Ag/AgCl (sat. KCl) electrode was used as a reference electrode. All experiments were performed at room temperature.

2.2. Device fabrication

Fig. 1A shows the schematic diagram of the device structure of PEDOT:PSS-based OECT for dopamine sensor. Glass slides were used as substrates for the fabrication of the devices. Au source and drain electrodes were deposited by thermal evaporation through a shadow mask. The width/length (W/L) of the channel is 6 mm/100 μm. Prior to the coating of PEDOT:PSS layer, the glass

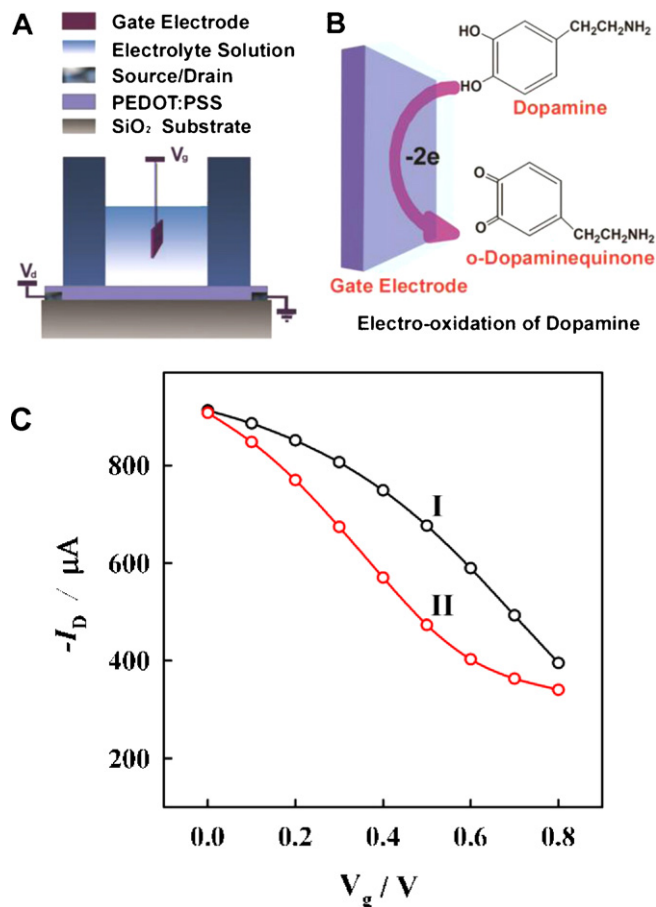


Fig. 1. (A) The schematic diagram of a dopamine sensor based on an organic electrochemical transistor with an active layer of PEDOT:PSS. (B) The electro-oxidation reaction of dopamine at the surface of a gate electrode. (C) Typical I_D vs. V_g curves of an OECT with a Pt gate electrode characterized in blank (line I) and 2 μM dopamine (line II) PBS (pH 7.2) solution. $V_{DS} = -0.1$ V.

slides were treated with oxygen plasma for 60 s to improve the film adhesion. Then PEDOT:PSS solution was spin coated at 3500 rpm onto the patterned substrates and then the samples were annealed at 200 °C for 60 min in a glove box filled with high purity N₂ gas. The thickness of the PEDOT:PSS film was about 80 nm.

For the preparation of MWCNT–CHIT/GE gate electrodes, the graphite (GE) electrode was firstly polished with a grit paper and Al₂O₃ powder, then sonicated in de-ionized water for 5 min and rinsed carefully with ethanol and de-ionized water. Thereafter, 10 μL of MWCNT–CHIT hybrid aqueous suspension was drop-coated on the surface of GE electrode. After the CHIT film was formed, the MWCNT–CHIT/GE electrodes were rinsed thoroughly with de-ionized water for future use. The preparation of MWCNT–CHIT/Pt gate electrodes was similar to that of MWCNT–CHIT/GE as described above.

2.3. Device characterization

Before measurements, all of the as-prepared electrodes were immersed in PBS (pH 7.2) for 15 min to remove the residua. The OECT device was tested at a fixed $V_D = -0.2$ V at different gate voltages (V_g : 0–0.8 V) and PBS (pH 7.2) was used as the electrolyte for all measurements. Additionally, the electrochemical response of Pt electrode to dopamine was investigated by cyclic voltammetry (CV) and amperometric i – t curve. Amperometric measurements were carried out in a stirred PBS (pH 7.2) solution.

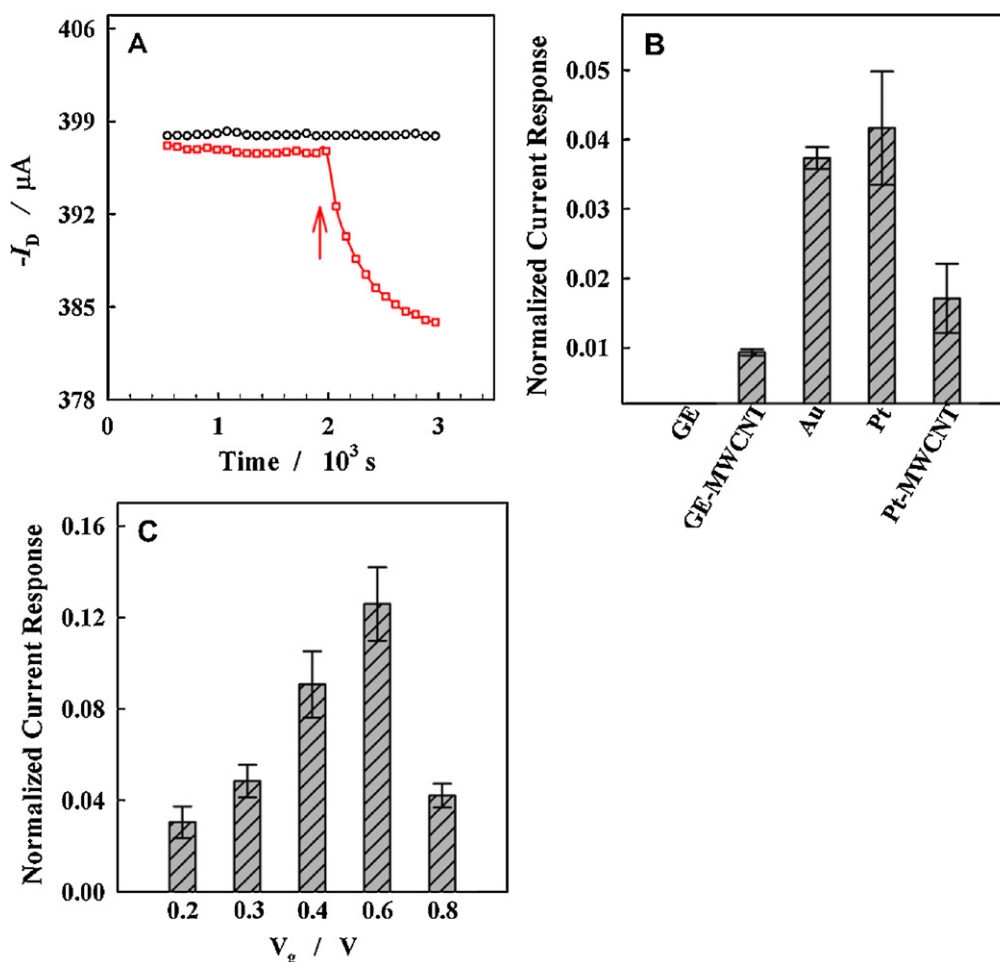


Fig. 2. (A) Bottom (circle) curve is the typical I_D vs. time curve of an OECT with a Pt gate electrode characterized before and after an addition of 50 nM dopamine in PBS (pH 7.2) solution; top (square) curve is I_D of the same device. (B) Normalized current responses (NCR) of the devices with different kinds of gate electrodes to additions of 50 nM dopamine. $V_g = 0.6 V$, $V_{DS} = -0.1 V$. (C) The effect of the applied gate voltage V_g on NCR of the device (Pt gate electrode) to additions of 0.1 μM dopamine. $V_{DS} = -0.1 V$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

3. Results and discussions

Fig. 1A shows the schematic diagram of an OECT based on PEDOT:PSS, which is placed in a container filled with phosphate

buffer saline (PBS, pH 7.2) solution. Fig. 1B shows the working principle of the dopamine sensor based on OECT. The added dopamine is electro-oxidized to *o*-dopaminequinone at the surface of the gate electrode and generates Faradic current, which will decrease the

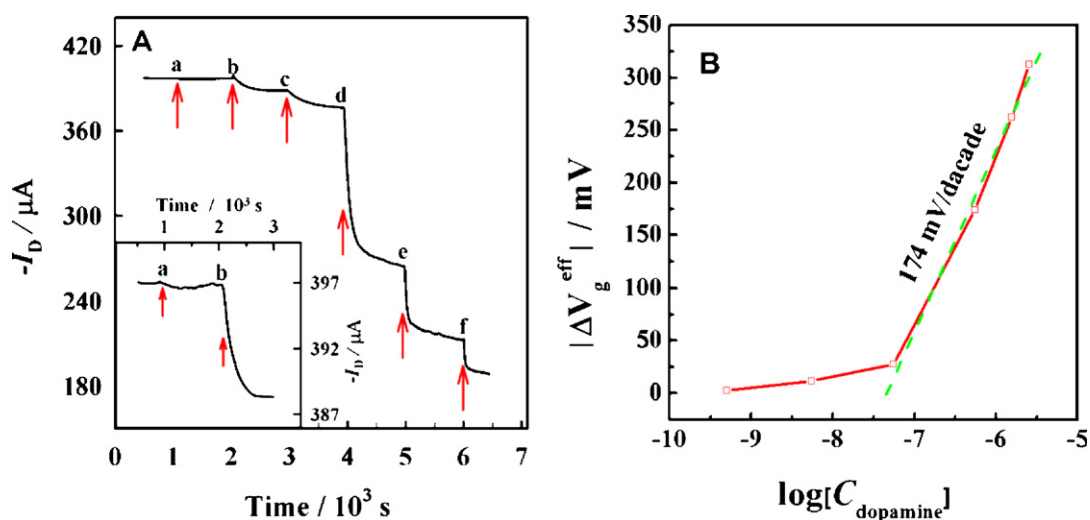


Fig. 3. (A) Typical I_D vs. time curve of an OECT with a Pt gate electrode to additions of dopamine with different concentrations in PBS (pH 7.2) solution. Dopamine additions, a–f: 0.5, 5, 50, 500, 1000, and 1000 nM. $V_g = 0.6 V$, $V_{DS} = -0.1 V$. (B) The change of the effective gate voltage (ΔV_g^{eff}) of the OECT induced by different concentrations of dopamine ($C_{dopamine}$).

potential drop at the electrolyte/gate interface and increase the effective gate voltage (V_g^{eff}) applied on the transistor. Therefore, the sensing mechanism is very similar to the OECT-based H_2O_2 sensors reported before (Bernards et al., 2008).

Fig. 1C shows the transfer curves (I_D vs. V_g) of an OECT with a Pt gate electrode characterized before and after the addition of 2 μM dopamine in PBS solution. The transfer curve shifts to lower gate voltage after the addition, indicating that the effective gate voltage is increased due to the electro-oxidation of dopamine at the Pt gate electrode.

In order to continuously monitor the response of the OECT to additions of dopamine, the real-time I_D response was measured at fixed voltages: $V_g = 0.6\text{ V}$ and $V_{DS} = -0.1\text{ V}$. Fig. 2A shows the typical I_D vs. time curves of the device to the addition of 50 nM dopamine. The device exhibits a good response and the relative change of I_D (ΔI_D) reaches 14.2 μA after long enough time. Compared with the I_D vs. V_g curves in Fig. 1C, the I_D vs. time curve is more intuitive and can demonstrate the real-time response of the sensor to additions of dopamine.

For the determination of dopamine, the common electrodes used in electrochemical detections are carbon or novel metal (Au, Pt) electrodes with or without nano-materials modified on the surfaces (Wightman et al., 1991a,b; Zhang et al., 2000; Zhao et al., 2001). Fig. 2B shows the response of OECTs with different types of gate electrodes to an addition of 50 nM dopamine. The normalized current response (NCR) is used to facilitate comparison among different devices. Normalization is done relative to the zero concentration limit as:

$$\text{NCR} = \left| \frac{I_D^{\text{conc}} - I_D^{\text{conc}=0}}{I_D^{\text{conc}=0}} \right|, \quad (1)$$

where the channel currents (I_D) are considered at zero concentration and at the concentration of interest. Three identical gate electrodes were prepared for each condition. Error bar in Fig. 2B shows the standard deviation of the response of the three identical devices. There is no obvious response at GE gate electrode. The NCR at MWCNT-CHIT/GE gate electrode reaches about 0.01, suggesting that the modification of graphite with MWCNT-CHIT hybrid improves the device response to dopamine. This may be attributed to the good electrocatalytic activity of the MWCNT-CHIT hybrid to electro-oxidation of dopamine (Zhao et al., 2001). On the other hand, the devices with Au and Pt gate electrodes exhibit higher NCR to the addition of 50 nM dopamine than that for MWCNT-CHIT/GE gate electrode, which are 0.037 and 0.042, respectively. However, the modification of Pt gate electrode with MWCNT-CHIT hybrid does not enhance the NCR of the device. The results in Fig. 2B clearly demonstrate that the gate electrode materials have significant influence on the response of the device to dopamine and the Pt gate electrode results in the highest sensitivity of the dopamine sensor. Therefore, pure Pt gate electrode is used in the OECTs in the following experiments unless it is specifically described. The cause of the best effect of the Pt electrode is not fully understood, which may be due to the excellent electrocatalytic activity of the Pt electrode as a dopamine sensor.

Fig. 2C shows the effect of the applied V_g on the NCR of the device to the addition of 0.1 μM dopamine by using a Pt gate electrode. Error bar shows the standard deviation of the NCR of three identical devices. It can be found that the device shows the largest NCR when the applied V_g is 0.6 V and further increase of the gate voltage results in a decrease of the NCR. Therefore, the gate voltage is selected to be 0.6 V in the following experiments.

Fig. 3A shows the real-time I_D curve of a device characterized in PBS solution during the additions of various concentrations of dopamine. We can find that the device exhibits response to the addition of 0.5 nM dopamine, and the relative change of I_D increases

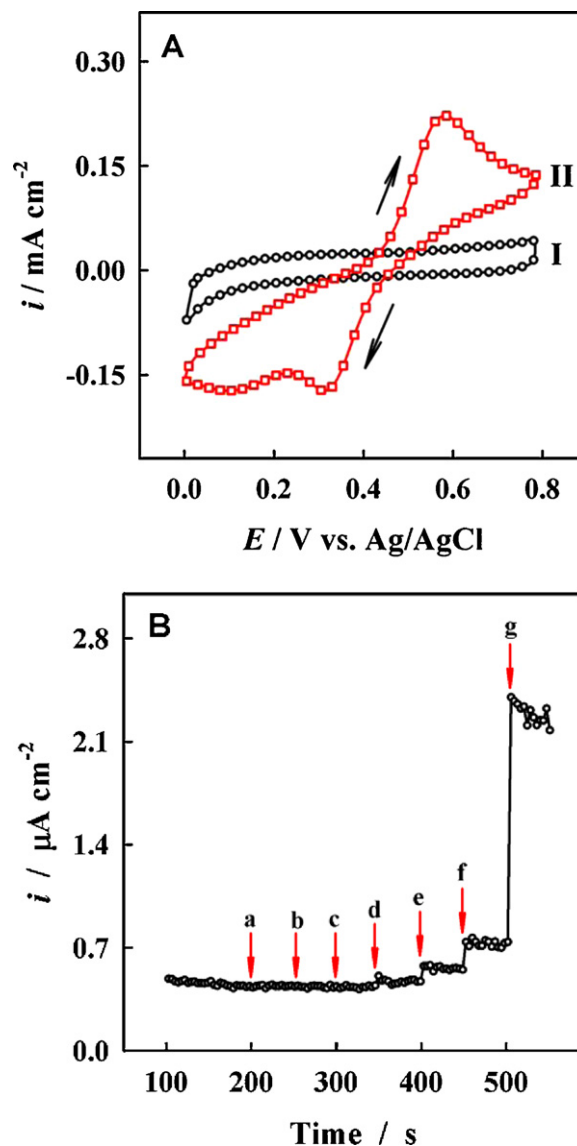


Fig. 4. (A) Cyclic voltammograms (CV) of Pt electrode in blank (line I) and 1 mM dopamine (line II) PBS (pH 7.2) solutions. CV scan rate: 50 mV s^{-1} . (B) Amperometric responses of Pt electrode to the additions of dopamine. Dopamine additions, a–g: 0.25, 0.5, 5, 50, 100, 200 and 500 nM. The applied potential is fixed at 0.6 V vs. Ag/AgCl.

with the increase of dopamine concentration. However, for the detection limit of the device, the corresponding signal to noise ratio should be larger than 3 ($S/N > 3$). Therefore the detection limit of the dopamine sensor is lower than 5 nM.

The change of the channel current can be attributed to the variation of the effective gate voltage (ΔV_g^{eff}) due to the oxidation of dopamine at the gate electrode (Fig. 1B), which results in a horizontal shift of the transfer curve. In order to demonstrate more clearly the sensing mechanism of OECT to additions of dopamine, ΔV_g^{eff} at different concentrations of dopamine (C_{dopamine}) are calculated according to the current change (ΔI_D after 10^3 s) and shown in Fig. 3B. We can find that ΔV_g^{eff} is proportional to $\log[C_{\text{dopamine}}]$ in the range of $5 \times 10^{-8}\text{ M}$ to $3 \times 10^{-6}\text{ M}$, which is very similar to the H_2O_2 sensor reported before by Bernards et al. (2008). Therefore, the effective gate voltage V_g^{eff} can be described as follows:

$$V_g^{\text{eff}} = V_g + 2.30(1 + \gamma) \frac{kT}{ne} \log[C_{\text{dopamine}}] + A, \quad (2)$$

where V_g is the gate voltage; γ the capacitance ratio (defined as $\gamma = C_c/C_g$, where C_c and C_g are the channel and gate capacitance, respectively); k the Boltzmann's constant; T the temperature, e elementary charge; n ($=2$) the number of electrons transferred during the electro-oxidation of dopamine; and A is a constant related to other factors. As shown in Fig. 3B, the slope of the fitting curve in the linear region of ΔV_g^{eff} vs. $\log[C_{\text{dopamine}}]$ is 174 mV/decade and thus $\gamma = 4.8$.

If there is no dopamine in the solution, V_g^{eff} is equal to V_g . After an addition of dopamine, V_g^{eff} is increased and higher than V_g . According to Eq. (2) and the fitting parameters of the linear region in Fig. 3B, the effective gate voltage V_g^{eff} will be equal to V_g when the concentration is lower than the value: $C_{\text{min}} = 10^{-neA/[2.30(1+\gamma)kT]} \approx 5 \times 10^{-8}$ M. However, our experiments show that the additions of dopamine with the concentrations lower than C_{min} can induce pronounced changes of the effective gate voltage, indicating that the oxidation process of dopamine still occurs at such low concentrations. Therefore, Eq. (2) is not applicable when the dopamine concentration is less than 50 nM. In real applications, the sensitivity of the device at low concentration needs to be calibrated.

For comparison, the Pt gate electrode is used to characterize dopamine concentration with the conventional electrochemical method. Fig. 4A shows a pair of redox peak currents observed at about 0.58 and 0.32 V vs. Ag/AgCl. These redox peak currents correspond to the electro-oxidation and electro-reduction of dopamine at the Pt electrode, respectively (Zhang et al., 2000). From the results of amperometric measurements, as shown in Fig. 4B, the Pt electrode has no obvious current response until 50 nM dopamine is added. However, the detection limit of the OECT to dopamine is less than 5 nM by using the same Pt electrode as a gate, indicating that the OECT is much more sensitive for dopamine sensing than the typical electrochemical amperometric method. On the other hand, the detection limit of the OECTs to dopamine is comparable to or better than those reported in other papers, such as ion-sensitive FETs for dopamine sensors (7×10^{-5} M) (Elfstro et al., 2007); electrochemical detection of dopamine by using poly(sulfosalicylic acid) modified glass carbon (GC) electrode (5×10^{-9} M) (Zhao et al., 2001), MWCNTs-Nafion modified GC electrode (1.4×10^{-6} M) (Chen et al., 2009), and hybrid inorganic–organic coating modified Pt electrode (2.5×10^{-5} M) (Lupu et al., 2009), etc. Moreover, it has been reported that the clinical concentrations of dopamine in urine and plasma are in the range of micromolar and nanomole levels (Peterson et al., 2002; Hashizume et al., 1995), respectively. Therefore the OECT-based dopamine sensor is potentially useful for the determination of dopamine in clinical applications. In addition, further experiments are needed to calibrate the sensitivity, stability and selectivity of the devices for real applications.

4. Conclusions

In summary, OECTs with different gate electrodes have been used in dopamine sensing. We find that the sensitivity of the device is related to both the material of its gate electrode and operation voltage. The OECT with a Pt gate electrode shows the lowest detection limit (lower than 5 nM), which is one order of magnitude better than a conventional electrochemical measurement with the same Pt electrode. Therefore, OECT is a promising device that can be used in low cost and highly sensitive biosensors for the detection of dopamine and other biomolecules.

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