

A novel imprinted electrochemical sensor for dopamine determination based on electron conductivity enhanced by ferrocenyl chalcone derivative film

Received Oct.8, 2018,
Accepted Nov.24, 2018,

DOI: 10.4208/jams.100818.112418a

<http://www.global-sci.org/jams/>

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Abstract. A novel biochemical sensor based on molecular imprinted technology was built through electropolymerization. One of the keystones of the technology is the discovery and application of new conductive polymer. Herein, a new conductive ferrocenyl chalcone derivative: [1-Oxo-3-(3-thienyl)-2-propen-1-yl] ferrocene (OTPyFc) was synthesized and characterized by nuclear magnetic resonance (NMR) and finally adopted in the technology. The OTPyFc and pyrrole mixture was eletropolymerized in this work and showed satisfying result in improving current peaks. The linear relationship was 1.729×10^{-6} M to 6.916×10^{-4} M. It is believed that the structure and their conductive analogs formed co-polymers would have a promising prospect in future research.

Keywords: OTPyFc; Molecular Imprinted Technology; DPV; Electrochemical Sensor;

1. Introduction

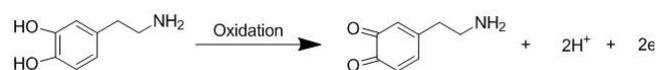
Electropolymerization technique has been used in various electro-conductive monomers such as benzene ring, five-membered heterocycle, alkynes and alkene to fabricate conducting polymers. Among which, polypyrrole (PPy) is one of the most extensively used for it can be easily deposited electrochemically onto a variety of substrate materials forming a stable film and can be used in a neutral pH region[1]. A stable electro-conductive membrane could be ideal candidates for substrate on electrochemical sensor, however, in most instances, the membrane can't show any signals to analysis. Molecularly imprinted polymers (MIPs) technique was brought in to tackle the analysis recognition problem. Generally, MIPs are thin films copolymerized by functional monomers and crosslinked with target molecules. Molecular templates formed via polymerization and elution. Finally, a complementary cave is obtained which shows specific affinity to original molecule. It is reported that the mechanism of MIPs technique is somehow like antigen-antibody or key-lock recognition[2]. Thus, MIPs technique endows the films specific recognition ability and broaden the application of electro-conductive films. It is reported that monomers, such as ethacridine[3], nicotinamide[4], o-aminophenol[5], or monomer mixtures[6] could be applied in DA determination by MIPs technology and showed satisfying results. Nevertheless, Shabi Abbas Zaidi pointed out that MIPs sensors having been typically suffered from low sensitivity for the lacking of conductivity and electrocatalytic activity[7]. An effective way

to deal with this problem is to find some conductive materials to be a mediator. Ferrocenyl chalcone derivative was chosen as the mediator in this work.

2. Experimental

2.1 Reagents and chemicals

3-Thiophenecarboxaldehyde (ThCA>97%) was purchased from Titan (Shanghai, China). Tetra-n-butylammonium hexafluorophosphate (n-Bu₄NPF₆), and Acetylferrocene (AcFc) were provided by Beijing bailingwei Technology Co. (Beijing China). Sodium dihydrogen phosphate (NaH₂PO₄), disodium hydrogen phosphate (Na₂HPO₄) ethanol (EtOH) acetonitrile (MeCN) and sodium hydroxide (NaOH) were bought from Chongqing Chuandong Chemical (Group) Co., Ltd. HNO₃, 98% H₂SO₄, ascorbic acid (AA), urid acid (UA), dopamine (DA), potassium chloride (KCl), and pyrrole (Py) were purchased from Aldrich and all reagents used in experiments were of



Scheme 1 The oxidized process of DA.

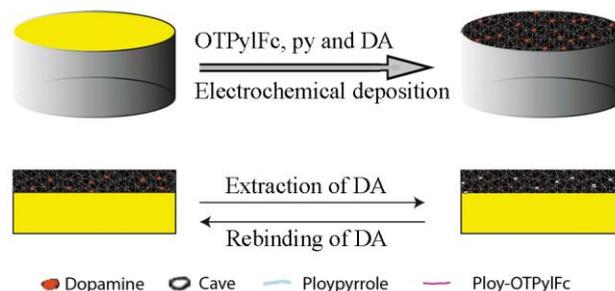


Figure 1. Sketch of organic monomers forming polymers and the process of DA extract and rebind with the polymers.

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analytical grade and without further purification. All aqueous solutions were prepared by ultrapure water.

2.2. Apparatus

The ploy-Ferrocenyl chalcone-pyrrole (OTPyFc-Py) was characterized by a field emission scanning electron microscope (FESEM) using a JSM-7800F operated at 20 KeV. ^1H NMR and ^{13}C NMR spectra (Bruker Avance 600 spectrometer) were used to ensure the structure of [1-Oxo-3-(3-thienyl)-2-propen-1-yl] ferrocene. All electrochemical experiments, cyclic voltammetry and differential pulse voltammetry included, were performed on a LK 2006AZ electrochemical workstation (Tian-jin Lanlike Co., China) with a widely used conventional three electrodes system: golden electrode modified with PFcCP as working electrode, a platinum foil as counter electrode and a saturated calomel electrode (SCE) as reference electrode. All potential values were given below versus the saturated calomel reference electrode. OTPyFc was prepared in microwave reactor (MAS-, sineo microwave technology co. Ltd. Shanghai, China), and monitored by TLC with silica gel coated plates.

2.3 Synthesis of OTPyFc

OTPyFc was synthesized according to Aldol reaction. Briefly, AcFc (456 mg), ThCA (250 mL) and NaOH (160 mg) were

dissolved in 2 mL EtOH by a three-neck round-bottom flask, then stirred vigorously for 5 min. The reaction was carried out in the microwave reactor ($P=200$ W) in 40°C for 5 min. When the reaction was finished, the mixture was extracted in a separatory funnel, then washed with water and saturated NaCl solution for 3 times, respectively. Finally, the washed oil was condensed by rotary evaporator and separated by silica gel column chromatography.

^1H NMR (600 MHz, Chloroform- d): δ (ppm) = 7.79 (d, J = 15.5 Hz, 1H), 7.64 – 7.53 (m, 1H), 7.46 – 7.41 (m, 1H), 7.39 (dd, J = 5.2, 2.8 Hz, 1H), 6.95 (dd, J = 15.5, 1.2 Hz, 1H), 4.90 (d, J = 1.7 Hz, 2H), 4.58 (d, J = 1.7 Hz, 2H), 4.21 (d, J = 1.2 Hz, 5H).

^{13}C NMR (151 MHz, CDCl_3): δ (ppm) = 193.12, 138.35, 134.39, 128.33, 126.89, 125.24, 122.74, 80.62, 72.69, 70.09, 69.69.

2.4 Preparation of OTPyFc-Py modified golden electrode (OTPyFc-Py/GE)

The golden electrode was polished with $0.05\ \mu\text{m}$ alumina slurries on polish cloth, then washed in ultrasonic with ultrapure water, ethanol (1:1/V:V), HNO_3 (1:1/V:V) and ultrapure water, respectively. Finally, reduced in 1 M H_2SO_4 solution for 15 circles by cyclic voltammetry until the peak

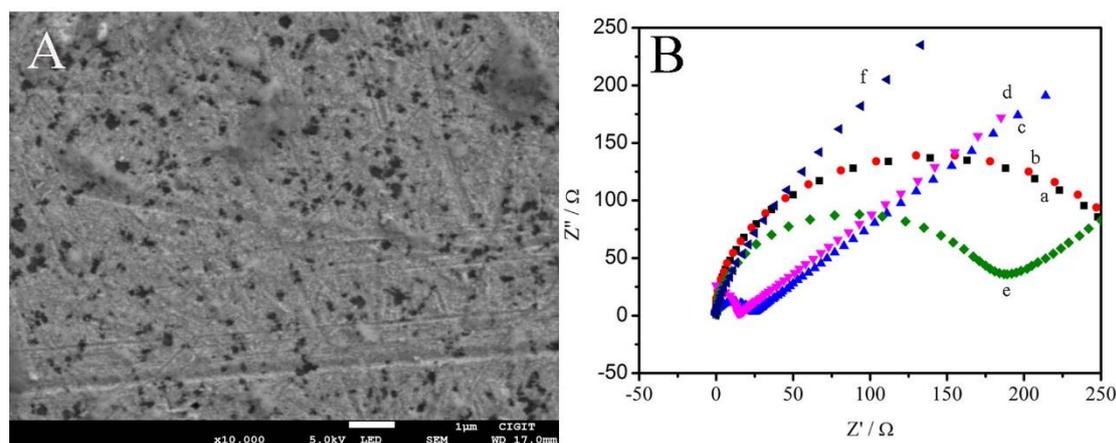


Figure 2. (A) SEM of MIP/GE after washed by acetonitrile and water. (B) EIS recorded in a $0.01\ \text{mol/L}$ $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$ solution: (a) Bare golden electrode; (b) OTPyFc and DA copoly; (c) OTPyFc, pyrrole and DA co-poly; (d) only OTPyFc ploy; (e) pyrrole and DA; (f) OTPyFc, pyrrole and DA co-poly and then washed.

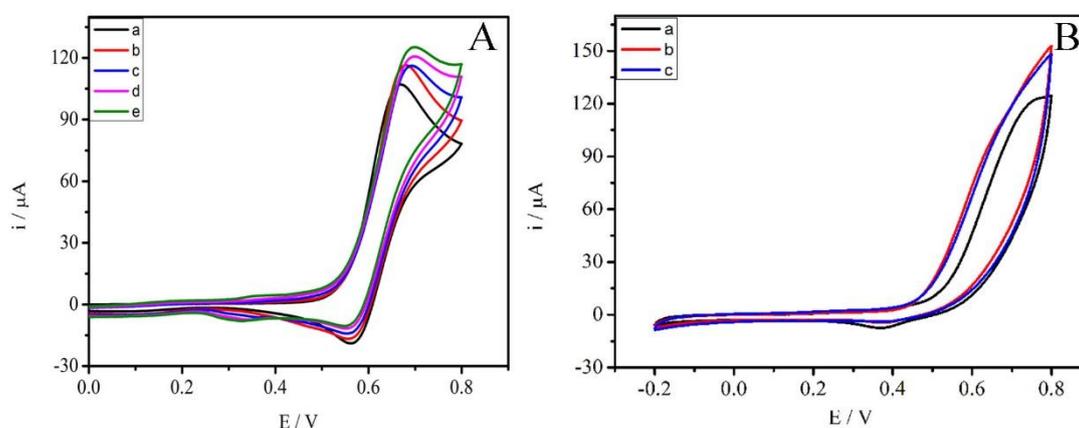


Figure 3. (A) Differential pulse voltammetry for the DA determination. (B) Analytical curve constructed from DPV of (a) 6.916×10^{-4} M; (b) 3.458×10^{-4} M; (c) 6.915×10^{-5} M; (d) 1.383×10^{-5} M; (e) 6.916×10^{-6} M; (f) 3.456×10^{-6} M; (g) 1.729×10^{-6} M.

current have no obvious change. The potential was from -0.2 V to 1.4 V. Pyrrole, n-Bu4NPF6 and OTPyIFc were dissolved in acetonitrile / water solution (VMeCN:Vwater = 9:1) and immersed in ultrasonic to form a homogeneous solution, the concentration was 0.1M, 0.1M and 5mM, respectively. Cyclic voltammetry was employed to ploy the mixture. The electrochemical polymerization was performed from -0.2 V to 0.8 V and the scan rate was 10 mV/s and circled 3 times.

3. Results and discussion

The procedure of preparation of MIPs PPy-OTPyIFc mixed film on golden electrode was vividly depicted in **Figure 1**. Pyrrole can be easily deposited onto working electrode by cyclic voltammetry. But OTPyIFc was not easy to form a stable polymerization in the same condition as pyrrole do. To tackle this problem, we use Mehmet Senel's method[8] to fabricate a copolymer. OTPyIFc can grow up to form ploy-OTPyIFc polymer on the pre-formed PPy film. Furthermore, pyrrole and thiophene derivatives have the possibility in generating copolymers. Therefore, the new film may be composed by PPy, ploy-OTPyIFc and pyrrole-OTPyIFc co-polymer. In the process of polymerization, DA can combine with the film through hydrogen bond and then washed away by hydrogen bond destruction, leaving a cave in the film which could match with DA perfectly. The mechanism of the oxidation of DA(9) in the film is shown in **Scheme 1**. Once the DA was oxidized, the hydrogen bonds of carbonyl group would be destroyed and DA could easily peeled off leaving a molecule hole. And the exfoliation progress can be observed through cyclic voltammetry in 0.1M PBS, the peak current dropped off obviously indicating that the oxidized DA was dissolved into PBS from the electrode.

3.1. SEM of the modified electrodes

As is shown in **Figure 2A**, the morphological structures of PFcCP modified golden electrode (GE) were tested by SEM. The mixture was deposited onto GE by cyclic voltammetry, then a thin conductive film was got. The new film was flat in general, inevitably, there were few big blocks gathered on the thin film during the deposition progress. Also, some defects on

the membrane which might be peeled off by distilled water or acetonitrile in the washing step could be observed in the picture.

3.2 Electrochemical impedance spectroscopy (EIS) of the copolymer

When pyrrole and DA anchored onto the sensor, the Ret value (e) (**Figure 2B**) went down but not evidently indicating PPy facilitates the electron transfer. When OTPyIFc was added, the electron-transfer resistance of (c) and (d) in Figure 2B went down obviously. That means the newly synthesized material is favor of the improvement of sensor conductivity owing to the ferrocene and the conjugation structure. The impedance of (c) is 23 Ω and (d) is 63 Ω , respectively. The phenomenon may be due to pyrrole polymer can help OTPyIFc to adhere on the electrode. When the DA was washed from the organic polymer, the high-frequency area almost disappeared and low-frequency area was prone to be a straight line. The electric resistance is only 0.01 Ω , corresponding to scatter (f). According to Xianwen Kan's theory [1], the MIPs bonding with DA would block the arrival of the $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$ to go through the cavity onto electrode surface. This would explain the sharp fall off of the impedance after the washing process.

3.3 Electrochemical polymerization of pyrrole and OTPyIFc

The electrochemical polymerization of pyrrole and OTPyIFc by cyclic voltammogram was recorded by **Figure 3**. When the solution have OTPyIFc only, the redox peak hardly changed. This indicates the OTPyIFc solution just have redox reaction on the electrode surface instead of deposited onto it. After rinsed by acetonitrile, the electrode surface has no difference with bare one by naked eyes. **Figure 3A** and **Figure 3B** show anodic peak current increase in the polymerization progress. This phenomenon indicates the film is electro-conductive film.

3.4 Determination of LOD of dopamine by DPV.

In order to apply the sensor in practical samples for determination of DA, a calibration curve was got by fitting data points. Those points are oxidation peak current on DA concentration which was pre-dealt by mathematical methods. In detail, take the logarithm of the DA concentration and then multiplied by -1 to be the horizontal ordinate, and oxidation peak current to be vertical ordinate. The linear relationship of

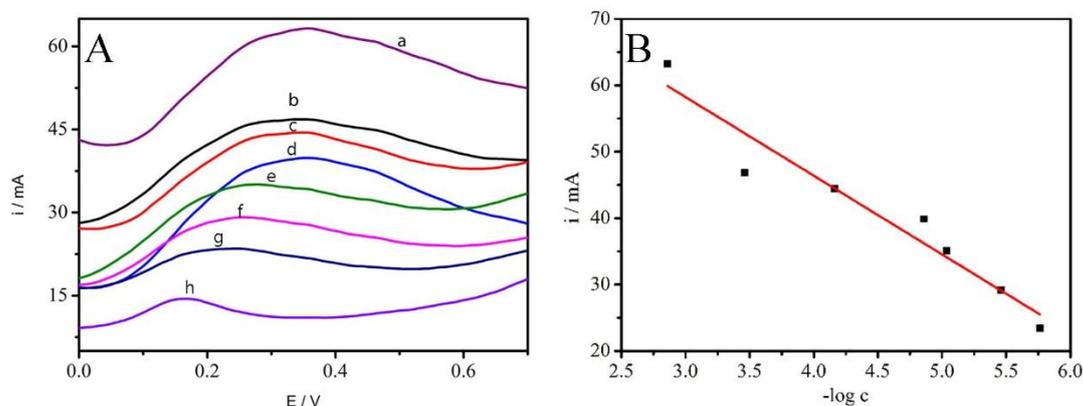


Figure 4. (A) Differential pulse voltammetry for the DA determination. (B) Analytical curve constructed from DPV of (a) 6.916×10^{-4} M; (b) 3.458×10^{-4} M; (c) 6.915×10^{-5} M; (d) 1.383×10^{-5} M; (e) 6.916×10^{-6} M; (f) 3.456×10^{-6} M; (g) 1.729×10^{-6} M

oxidation peak current with DA concentration was from 1.729×10^{-6} M to 6.916×10^{-4} M and the correlation coefficient was -0.95144.

4. Conclusion

In this work, new material OTPyIFc was synthesized and characterized. Owing to its good electro-conductive property, it was added to pyrrole to improve the membrane conductivity and was expected to enhance sensing accuracy. The linear relationship of oxidation peak current with DA concentration was ranging from 1.729×10^{-6} M to 6.916×10^{-4} M. What's more, our work provide a new way in improving MIPs conductivity. The future study can exchange thiophene on OTPyIFc to pyrrole or other conjugate rings. And there would be more choice.

Acknowledgements

This work was supported by the National Natural Science Foundation of China through the Young Program (61503309).

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