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Fabrication of Electrochemical Sensor for Acetaminophen Based on Levodopa Polymer and Multi-walled Carbon Nanotubes Complex

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In this work, we describe the fabrication of electrochemical sensor for acetaminophen (ACOP) based on levodopa (L-Dopa) polymer and multi-walled carbon nanotubes (MWCNTs) complex modified carbon paste electrode (Poly (L-Dopa)/MWCNTs/CPE). L-Dopa Polymer and MWCNTs complex is prepared by in situ electrochemical polymeric deposition and the electrochemical polymeric deposition behavior of L-Dopa on the surface of MWCNTs was investigated by cyclic voltammetry (CV). A novel method for the determination of ACOP is developed based on a well-defined electrochemical response signal of ACOP at Poly (L-Dopa)/MWCNTs/CPE by differential pulse voltammetry (DPV). Under the optimized conditions, the value of the DPV oxidation peak current linearly depends on the concentration of ACOP in the range from 0.06 to 30 μM. The linear equation for ACOP detection is I_{pa} (μA) =0.0718c (μM) +0.0307 (r=0.9990) and the detection limit is 0.02 μM (S/N=3). The recoveries for ACOP are from 98.2% to 99.4% with relative standard deviation (RSD) between 3.9% and 4.6% in diluted serum samples.

Keywords: Acetaminophen; Levodopa; Multi-walled carbon nanotubes; Electrochemical polymeric deposition; Electrochemical sensor.

1. INTRODUCTION

Acetaminophen (N-(4-hydroxyphenyl)acetamide, ACOP) is an effective and widely used drug for the treatment of pain and fever [1]. Its chemical structure is shown in Fig. 1. Unlike non-steroidal

anti-inflammatory drugs (NSAIDs), ACOP is almost unanimously considered to have no anti-inflammatory activity and does not produce gastrointestinal damage or untoward cardio renal effects. [1]. In general, ACOP seems to be safe and appears to have no toxic effects on human's health at normal therapeutic doses. Nevertheless, the overdose of ACOP leads to the accumulation of toxic metabolites that may cause hepatotoxicity and acute liver failure [2-3]. Considering the wide range of therapeutic uses, the development of highly sensitive and selective methods for the determination of ACOP in biological samples is of great significance. [4]

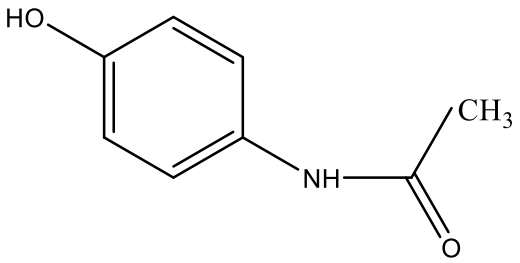


Figure 1. Chemical structure of acetaminophen.

There are various analytical methods such as spectrometry [5-6], chromatography [7-8], thermogravimetric analysis [9] and electrochemistry [10-11], which have been developed for the determination of ACOP. In contrast, the method of electrochemistry is characteristic of high sensitivity and selectivity, simple operations, low costs and fast responses [12].

Carbon nanotubes (CNTs), which contain multi-walled carbon nanotubes (MWCNTs) and single-walled carbon nanotubes (SWCNTs), have been attracting much interest over the years [13-14]. The unique structures and properties, such as good electrical conductivity, high electrocatalytic effect, strong adsorptive ability and low price, make MWCNTs attractive for developing highly sensitive chemical modified electrodes [15-17]. Many studies on fabrication of MWCNTs' modified electrode for the determination of ACOP have been reported in the past years [18-20].

Levodopa ((2S)-2-amino-3-(3, 4-dihydroxyphenyl) propionic acid, L-Dopa) is an amino acid, and it is also the immediate precursor of the neurotransmitter dopamine [21-22]. The chemical structure of L-Dopa is similar to that of dopamine. Furthermore, L-Dopa has a negatively charged carboxyl group and a positively charged amine group, which is a zwitterionic molecule, whereas dopamine has only a positively charged amine group, not a zwitterionic molecule [23]. Although electrochemical polymeric deposition of dopamine and its application for electrochemical detection has witnessed great progress so far [24-27], the researches about electrochemical polymeric deposition of L-Dopa and its application for electrochemical detection are somewhat limited [28]. As is known to all, carbon paste electrode (CPE) is relatively low cost as compared with glassy carbon electrode. However, to the best of our knowledge, Poly (L-Dopa)/MWCNTs complex modified CPE and its application to highly sensitive and selective detection of ACOP have not been reported thus far.

In this regard, we report here the fabrication of electrochemical sensor for ACOP based on levodopa polymer and multi-walled carbon nanotubes complex modified carbon paste electrode (Poly

(L-Dopa)/MWCNTs/CPE). L-Dopa Polymer and MWCNTs complex was prepared by in situ electrochemical polymeric deposition, and the electrochemical polymeric deposition behavior of L-Dopa on the surface of MWCNTs was investigated in detail. After that, the electrochemical sensor was employed in the highly sensitive and selective detection of ACOP by differential pulse voltammetry (DPV). The fabricated electrochemical sensor displayed fast electron transfer and prominent electrocatalytic ability to ACOP with satisfactory results.

2. EXPERMENT

2.1. Chemicals and reagents

All reagents were analytical grade and used as received otherwise specified statement. Acetaminophen (ACOP) and levodopa (L-Dopa) were ordered from Aladdin Reagent (Shanghai, China). Graphite powder and paraffin oil were obtained from Sinopharm Chemical Reagent (Shanghai, China). Dipotassium hydrogen phosphate (K_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4) and potassium nitrate (KNO_3) were provided by Tianjin Damao Chemical Reagent (Tianjin, China). Multi-walled carbon nanotubes (MWCNTs) (>97% purity, ＜2 μm length and 10-20 nm internal diameter) were purchased from Nanotech Port (Shenzhen, China). All required solutions were prepared using ultrapure water (Millipore, USA).

2.2. Instrumentation

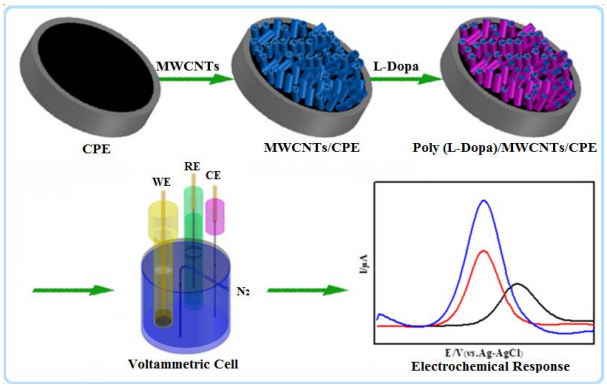
Electrochemical measurements were performed on a CHI830D electrochemical analyzer equipped with data processing software (Shanghai Chenhua Instrument Co., Shanghai, China). Conventional three-electrode system was used for all electrochemical experiments. Bare or modified carbon paste electrode (CPE) (diameter = 3 mm) was used as working electrode (WE), Ag/AgCl (sat. KCl) electrode was used as reference electrode (RE) and platinum wire electrode was used as counter electrode (CE). The pH measurements were performed using a pHS-3C digital pH meter (Shanghai Instrument Electric Science Instrument Limited by Share Ltd, Shanghai, China).

2.3. Fabrication of electrochemical sensor

The procedure for sensor fabrication is depicted systematically in Scheme 1. MWCNTs were pretreated by stirring for 10 h in 3 M HNO_3 at 60℃ to remove the impurities, then filtered and rinsed to neutral pH value with water, followed by drying in a vacuum oven [29-30]. CPE was first prepared by mixing 0.2 g graphite powder and paraffin oil in the ratio 70:30 (w/w) in a mortar to produce a homogeneous paste, and a portion of the resulting paste was inserted into the bottom of a polyethylene tube (inside diameter = 3 mm) [30-31]. After that, CPE was polished carefully on a weighing paper. MWCNTs modified CPE (MWCNTs/CPE) was fabricated according to the literature method [32]. Briefly, the CPE was directly grinded with a suitable amount of pretreated MWCNTs powder, which

was tiled on a weighing paper. A thin MWCNTs layer was formed on the CPE surface by intercalating the MWCNTs into the soft carbon paste with the aid of mechanical force and the action of chemical and physical absorption. Prior to the electrochemical experiment, MWCNTS/CPE was rinsed with water. L-Dopa polymer modified MWCNTs/CPE (Poly (L-Dopa)/MWCNTs/CPE) was obtained by in situ electrochemical polymeric deposition with cyclic voltammetry (CV). MWCNTs/CPE was immerged in a solution containing varied concentration of L-Dopa (0.1 M KNO_3, pH 7.0). Cyclic scans were executed by applying definite consecutive potential cycles at the potential range of -0.4 to +0.8 V (vs. Ag/AgCl), with initial potential of -0.4 V and scan rate of 25 mV s^{-1}[28]. The prepared electrode was rinsed thoroughly with water.

All of the prepared electrodes were stored at 4 ◦C in refrigerator when not in use.



Scheme 1. Schematic illustration of Poly (L-Dopa)/MWCNTs/CPE sensor.

2.4. Electrochemical measurements.

Differential pulse voltammetry (DPV) was used for determination of ACOP, which was performed within a potential window ranging from +0.2 to +0.6 V (vs. Ag/AgCl) with a potential increment of 0.05 V in 0.1 M K_2HPO_4-KH_2PO_4 buffer solution (PBS). Cyclic voltammetry (CV) from +0.1 to +0.8 V (vs. Ag/AgCl), with initial potential of +0.1 V, was carried out in 0.1 M PBS for the electrochemical behavior research of ACOP at Poly (L-Dopa)/MWCNTs/CPE. The test solutions were thoroughly purged with nitrogen before experiments. All electrochemical measurements were performed at room temperature.

3. RESULTS AND DISCUSSION

3.1. Preparation and characterization of Poly (L-Dopa)/MWCNTs/CPE

3.1.1 Electrochemical polymeric deposition of L-Dopa on the surface of MWCNTs/CPE

Poly (L-Dopa) modified MWCNTs/CPE was fabricated by electrochemical polymeric deposition. Carbon paste constituted the basic construction of the electrode, Poly (L-Dopa) and

MWCNTs complex served as electrocatalytic mediators. During the electrochemical polymeric deposition process of L-Dopa, two reversible redox peaks were observed (Fig. 1). Initially, the oxidation peak current decreased gradually with the number of scan cycles increasing, indicating that L-Dopa in the solution (0.1M KNO_3, pH 7.0) was first converted into levodopa quinone under electro-oxidation at about 0.47 V. When the levodopa quinone reaches a certain concentration, polymerization-crosslinking reaction of L-Dopa and levodopa quinone occurred on the electrode surface. Then the oxidation peak potential shifted to positive potential (0.50V), and the oxidation peak current increased gradually. The increase of voltammetry current was consistent with the continuous growth of Poly (L-Dopa) onto the electrode surface. Steady voltammogram was achieved after about 40 scan cycles were performed.

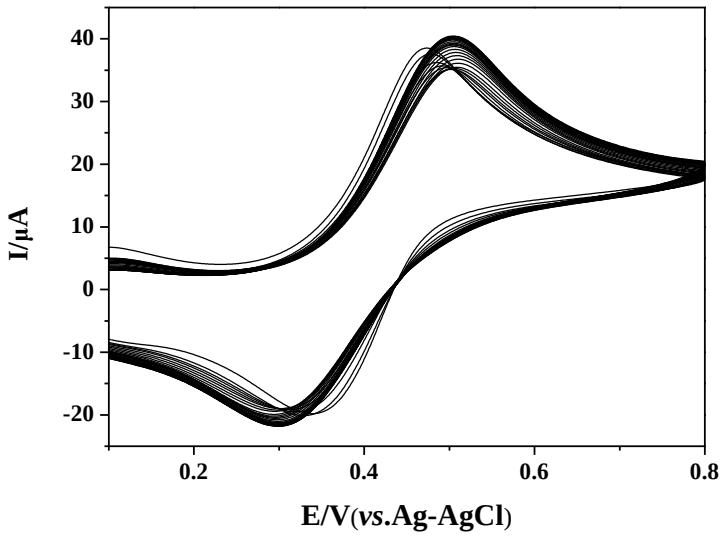


Figure 1. The CV curves of L-Dopa electrochemical polymeric deposition on the surface of MWCNTs/CPE.

3.1.2 Electrochemical characterization of Poly (L-Dopa)/MWCNTs/CPE

DPV of 20 μM ACOP in 0.1 M PBS (pH 6.0) was applied to monitor the electrocatalytic ability of the modified electrode. As displayed in Fig. 2, the unmodified CPE gives an oxidation peak at 0.46 V (curve a). When the CPE was modified with MWCNTs, obvious increase in peak current and a negative peck potential shift can be observed (curve b). As expected, Poly (L-Dopa) modified MWCNTs/CPE (curve c) yielded the highest response for ACOP, and a well-characteristic oxidation peak was observed with the peak potential at 0.40 V. Such a phenomenon can be ascribed to the synergic electrocatalytic effect of Poly (L-Dopa) and MWCNTs [28]. Scheme 2 is the possible electrocatalytic mechanism we inferred for ACOP detection at Poly (L-Dopa)/MWCNTs/CPE according to the literature [33]. Firstly, Poly (L-Dopa) was electro-oxidized to Poly $(L\text{-Dopa})_{OX}$. After that, when ACOP contacted with Poly $(L\text{-Dopa})_{OX}$ on the surface of Poly (L-Dopa) /MWCNTs/CPE, ACOP was oxidized by Poly $(L\text{-Dopa})_{OX}$. At the same time, Poly $(L\text{-Dopa})_{OX}$ was reduced to Poly (L-Dopa). Therefore, Poly (L-Dopa) as an electrocatalytic medium completed the redox cycle.

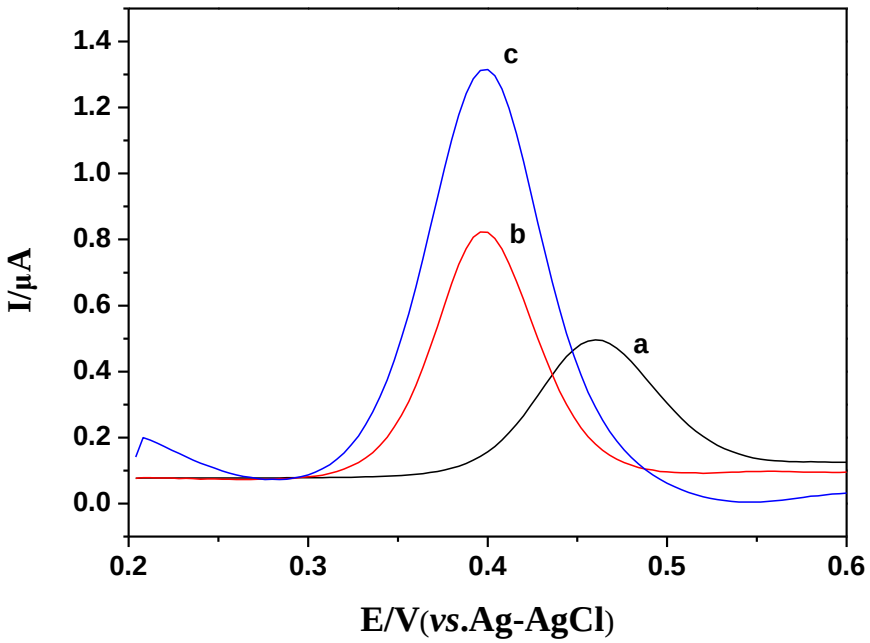
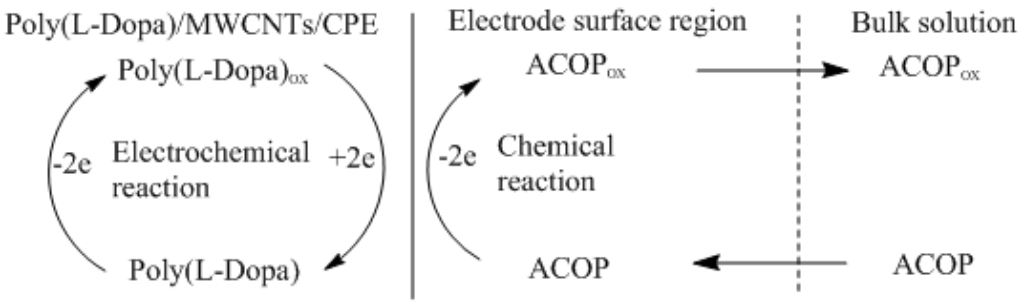


Figure 2. The DPV curves of 20 μM ACOP at CPE (a); MWCNTs/CPE (b) and Poly (L-Dopa)/MWCNTs/CPE(c).



Scheme 2. The electrocatalytic mechanism for detecting ACOP at Poly (L-Dopa)/ MWCNTs/CPE.

3.1.3 The electrochemical behavior of ACOP at Poly (L-Dopa)/MWCNTs/CPE

Cyclic voltammograms of 20 μM ACOP in 0.1 M PBS (pH 6.0) were obtained at different scan rates from 10 to 250 $mV \cdot s^{-1}$ (Fig. 3). As scan rate increased peak potentials shifted to more positive values and peak currents increased, which is a typical characteristic of an irreversible electrochemical reaction [34]. Additionally, the oxidation peak current of ACOP linearly increased with the square root of the scan rate ($\nu^{1/2}$) (*inset* in Fig. 3). The linear regression equation relating Ipa with $\nu^{1/2}$ was found to be: Ipa (μA) $=0.18\nu^{1/2}(mV \cdot s^{-1})-0.1514$ (r=0.9991). This indicates that the electrochemical reaction of ACOP at Poly (L-Dopa)/MWCNTs/CPE is diffusion controlled process [28].

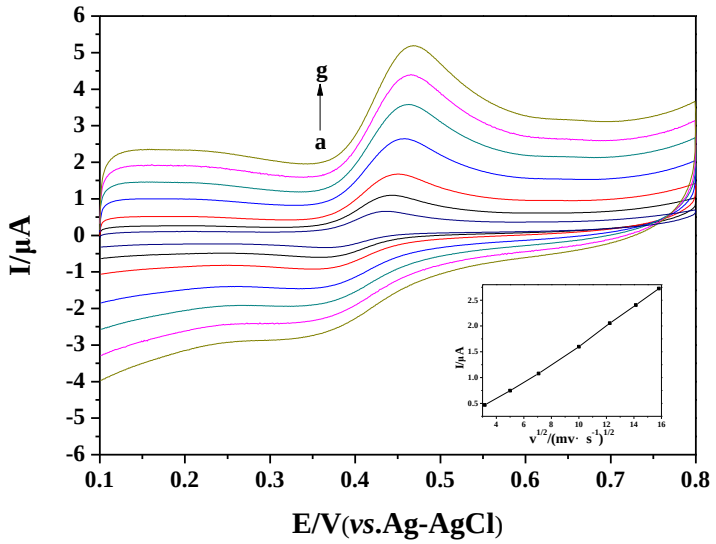


Figure 3. Cyclic voltammograms of 20 μM ACOP at Poly (L-Dopa)/MWCNTs/CPE at different CV scan rates (a→g: 10~250 mV s^{-1}). Buffer solution: 0.1 M PBS (pH 6.0). The inset shows the linear relationship of current and the square root of the scan rate.

3.2. Optimization of experimental conditions

3.2.1 Influence of concentration of L-Dopa in electrochemical polymeric deposition solution

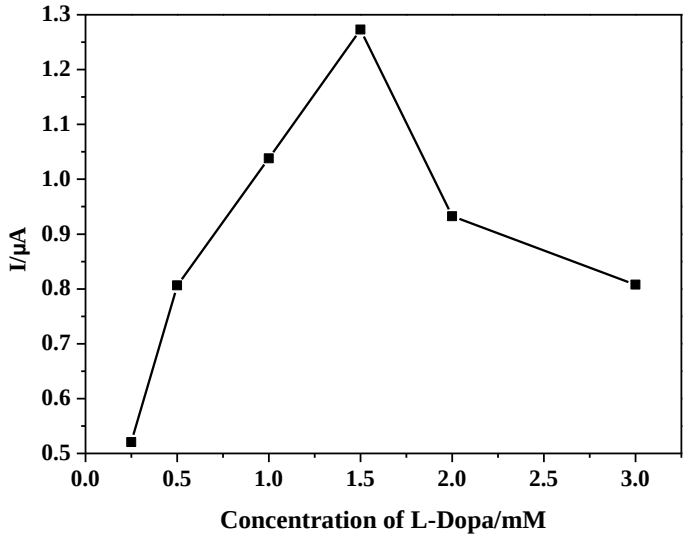


Figure 4. DPV response of 20 μM ACOP at Poly (L-Dopa)/MWCNTs/CPE (0.25~2.5 mM L-Dopa electrochemical polymeric deposition solution; CV scan cycles: 40). Buffer solution: 0.1 M PBS (pH 6.0).

The amount of Poly (L-Dopa) formed on the modified electrode surface is likely to influence the electrocatalytic activity of the modified electrode. To achieve the optimal analytical performance,

the DPV response of ACOP at modified electrodes, which were prepared in different concentration of L-Dopa electrochemical polymeric deposition solution, were investigated firstly. As shown in Fig. 4, DPV response of ACOP at Poly (L-Dopa)/MWCNTs/CPE depended on the concentration of L-Dopa (0.25~2.5 mM). Initially, the increase of L-Dopa concentration brought about the enhancement of DPV response. When L-Dopa concentration was more than 1.5 mM, the DPV response at Poly (L-Dopa)/MWCNTs/CPE was decreased. This is assumably ascribed to the fact that the overmuch Poly (L-Dopa) was deposited on the electrode. Excessive amounts of poly (L- Dopa) are detrimental to the enhancement of electrocatalytic activity, and hinder the electroactive species entering the electron conducting network of MWCNTs [30]. So the optimal concentration of L- Dopa for the electrode preparation is 1.5 mM.

3.2.2 Influence of CV scan cycles of L-Dopa electrodeposition

As same as the influence of L-Dopa concentration in electrochemical polymeric deposition, the CV scan cycles may also have influence on the amount of Poly (L-Dopa) formed on the modified electrode. So, we then examined the DPV response of ACOP at Poly (L-Dopa)/MWCNTs/CPE prepared with different scan cycles of the electrochemical polymeric deposition. Fig. 5 illustrated the variations of DPV response of ACOP with the CV scan cycles (20~60). The maximum DPV response was obtained with the scan cycles of 40. Therefore, the optimum CV scan cycles for Poly (L-Dopa)/MWCNTs/CPE fabrication is 40.

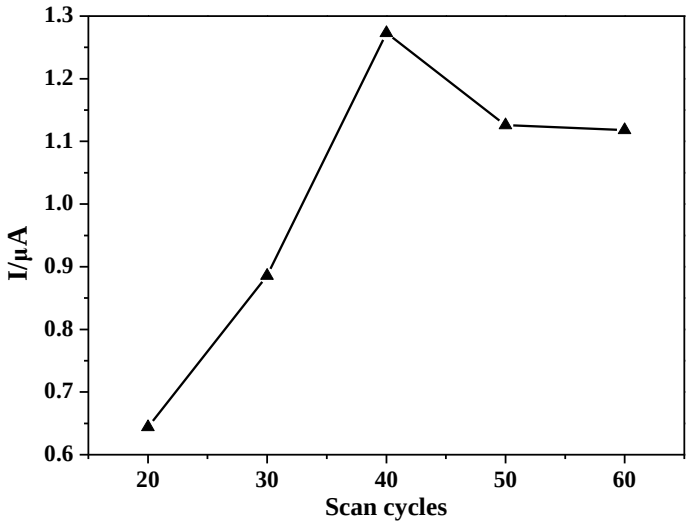


Figure 5. DPV response of 20 μM ACOP at Poly (L-Dopa)/MWCNTs/CPE (1.5 mM L-Dopa electrochemical polymeric deposition solution; CV scan cycles: 20~60). Buffer solution: 0.1 M PBS (pH 6.0).

3.2.3 Influence of supporting electrolyte and pH

Different supporting electrolytes such as 0.1 M Sodium acetate-Acetic acid buffer solution, 0.1 M Sodium citrate-Citric acid buffer solution and 0.1 M PBS were investigated at Poly (L-

Dopa)/MWCNTs/CPE. Well-defined DPV response with stable oxidation peak and high peak current of ACOP was obtained in 0.1 M PBS.

The influence of pH on the determination of ACOP at Poly(L-Dopa)/MWCNTs/CPE by DPV was researched. According to Fig. 6, the oxidation peak currents increased with the pH value increasing from5.0 to 6.0, and then decreased with the pH value increasing from 6.0 to 7.5. These results suggesting that the oxidation reaction of ACOP was kinetically less favorable at higher pH [35]. Hence, 0.1 M PBS (pH 6.0) was chosen as supporting electrolyte in the subsequent experiments.

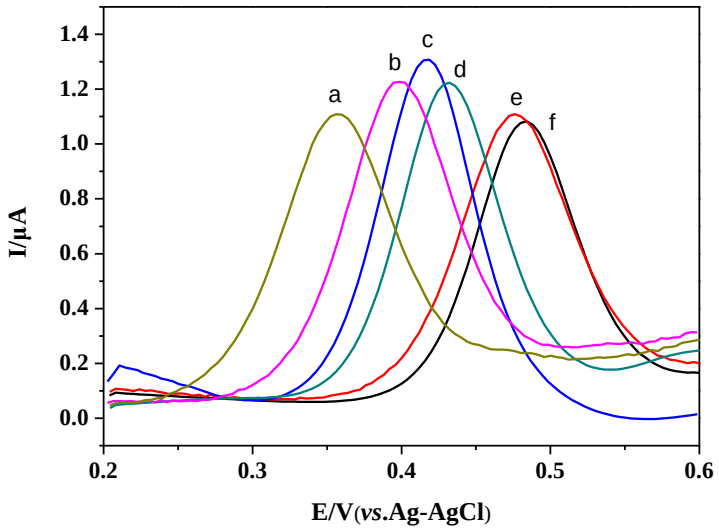


Figure 6. DPV curves of 20 μM ACOP at Poly (L-Dopa)/MWCNTs/CPE in 0.1 M PBS (a→f: pH 5.0~pH 7.5).

3.3. Calibration curve and detection limit

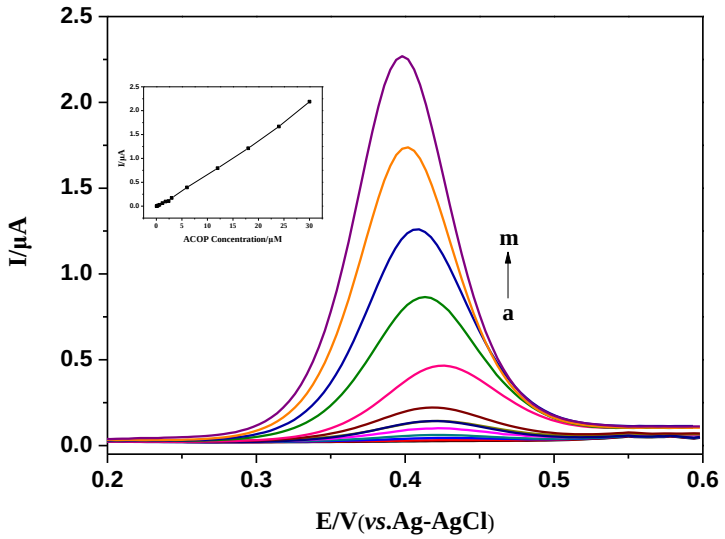


Figure 7. DPV curves of different concentrations of ACOP at Poly (L-Dopa)/MWCNTs/CPE (a→m: 0.06~30 μM).The inset shows the linear relationship of current and the concentration of ACOP.

As shown in Fig.7, the DPV analysis was performed for the different concentrations of ACOP at Poly(L-Dopa)/MWCNTs/CPE in 0.1 M PBS (pH 6.0). The DPV peak current and the concentration of ACOP showed a good linear relationship in the range from 0.06 to 30 μM (*inset* in Fig. 7), the linear regression equation was Ipa (μA) =0.0718c (μM) +0.0307 (r=0.9990). Based on the signal-to-noise of 3(S/N=3), the detection limit for ACOP was obtained as 0.02 μM.

3.4. Comparison between Poly (L-Dopa)/MWCNTs/CPE sensor and other sensors reported for ACOP assay

To better demonstrate the advantage of the proposed sensor for the determination of ACOP, the analytical performances has been compared with other previously reported sensors and listed in Table 1. The Poly (L-Dopa)/MWCNTs/CPE sensor shows superior analytical performance in terms of wide linear dynamic range and low detection limit over other sensors. The results indicate that the proposed Poly (L-Dopa)/MWCNTs/CPE is suitable for the detection of low concentration ACOP.

Table 1. Comparison between the proposed sensor and other sensors reported for ACOP assay.

Electrochemical sensor	Linear range/(μM)	LOD/(μM)	Method	Reference
BiO-SPEs	0.5~97	0.03	DPV	[10]
Nevirapine/CPE	2.0~12	0.77	DPV	[11]
MWCNTs/CTS/GCE	0.1~200	0.024	DPV	[18]
(CMWCNTs-$NHCH_2CH_2NH)_6$/GCE	1.0~200	0.092	DPV	[19]
AuNP/MWCNTs/GCE	0.09~35	0.03	DPV	[20]
Boron-doped Diamond Electrode	2.99~283	0.768	SWV	[34]
Chitosan/CPE	0.8 ~200	0.508	SWV	[36]
IL/ZnO/NPs/CPE	0.1~550	0.07	SWV	[37]
MIP/pABSA/GCE	0.05~100	0.043	DPV	[38]
Ni/CNFs	0.125~12.7	0.05	CV	[39]
GO/XDA/Mn_2O_3/GCE	1.0~1000	0.056	CA	[40]
PEI/fMWCNT/GCE	0.0999~6.95	0.0558	SWV	[41]
Poly(L-Dopa)/MWCNTs/CPE	0.06~30	0.02	DPV	This work

3.5. Stability of the electrochemical sensor

In order to investigate the stability of the fabricated electrochemical sensor, three Poly (L-Dopa)/MWCNTs/CPEs were prepared individually, and the DPV voltammograms of 20 μM ACOP (0.1M PBS, pH 6.0) were recorded for 10 consecutive days. As shown in Fig. 8, although the peak current decreased gradually, it was found to retain 92.6% of its initial peak current response by the end of 10 days. The results indicate a good stability of the sensor.

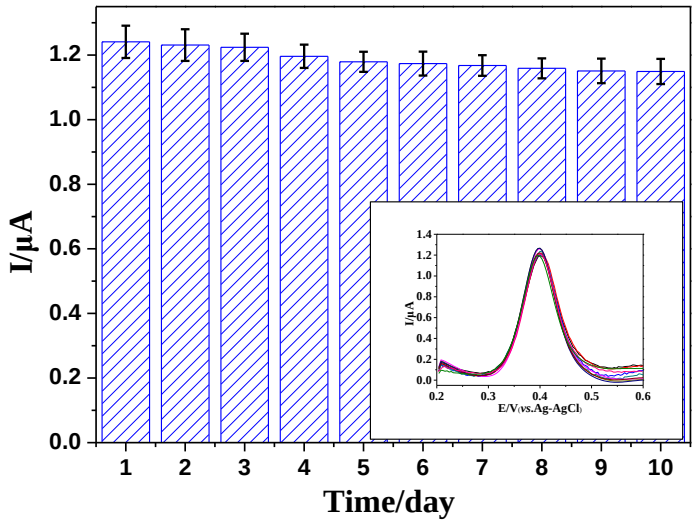


Figure 8. Investigation of the stability of the electrochemical sensor (n=3). The inset shows the DPV curves of one electrode in the three electrodes.

3.6. Analytical applications

The fabricated Poly (L-Dopa)/MWCNTs/CPE was further applied to the determination of ACOP in diluted serum samples. Human blood obtained from healthy volunteers was centrifuged at 5000 rpm for 30 min to separate serum. The serum was diluted 10 times with 0.1 M PBS (pH 6.0) without any pretreatment. The samples were spiked with known amounts of ACOP and measured by DPV as described before. The recoveries for ACOP are from 98.2% to 99.4% with relative standard deviation (RSD) between 3.9% and 4.6% in diluted serum samples (Table 2), which is acceptable for quantitative assays performed in real samples.

Table 2. Analytical results for ACOP in diluted serum samples (n=5).

Method	Added/(μM)	Determined/(μM)	Recovery/%	RSD/%
DPV	4	3.93	98.2	4.3
	8	7.95	99.4	3.9
	12	11.88	99.0	4.6

4. CONCLUSION

In conclusion, the electrochemical sensor based on Poly (L-Dopa)/MWCNTs/CPE has been fabricated by in situ electrochemical polymeric deposition. The proposed electrochemical sensor method for the determination of ACOP showed a good linear relationship and high sensitivity. Furthermore, the test results of ACOP in diluted serum samples have demonstrated the high selectivity

of the proposed electrochemical sensor. Therefore, the developed electrochemical sensor is feasible for the determination of ACOP in real samples.

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