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Simultaneous determination of dopamine and serotonin by use of covalent modification of 5-hydroxytryptophan on glassy carbon electrode

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Abstract An electrochemical biosensor was fabricated by covalent modification of 5-hydroxytryptophan (5-HTP) on the surface of glassy carbon electrode (GCE). The electrode, denoted as 5-HTP/GCE, was characterized by X-ray photo-electron spectroscopy, cyclic voltammetry and differential pulse voltammetry. For comparison, tryptophan modified GCE (TRP/GCE) and serotonin modified GCE (5-HT/GCE) were prepared by the same method. It was found that electrocatalytic ability of these electrodes was in the order of 5-HTP/GCE>TRP/GCE>5-HT/GCE for the oxidation of dopamine (DA) and 5-HT. The sensor was effective to simultaneously determine DA and 5-HT in a mixture. It can resolve the overlapping anodic peaks into two well-defined voltammetric peaks at 0.24 and 0.39 V (versus SCE). The linear response is in the range of 5.0×10^{-7}–3.5×10^{-5} mol L^{-1} with a detection limit of 3.1×10^{-7} mol L^{-1} for DA, and in the range of 5.0×10^{-6}–3.5×10^{-5} mol L^{-1} with a detection limit of 1.7×10^{-6} mol L^{-1} for 5-HT ($s/n=3$), respectively.

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Dopamine (DA) is a ubiquitous neurotransmitter in mammalian brain tissues, and it was found that low levels of DA existed in patients with Parkinson's disease [1]. Serotonin (5-HT) is widely distributed in the brain, and plays a crucial role in emotional functions together with other neurotransmitters. There is considerable interest in developing modified electrodes to determine DA and 5-HT. Simultaneous measurement of DA and 5-HT is important, since both of them often occur together in biological systems [2, 3].

Recently, our groups have found that variously bioactive molecules, such as amino acids [4], DNA [5], choline and its derivatives [6–9], can be covalently grafted on the surface of carbon based electrodes. The stratagem of using bioactive species for the surface modification has its advantage for the fabrication of biosensors since the formed interfacial monolayer may serve as a molecular recognition element by its biocompatibility. Investigation along this line is not only for obtaining useful biosensors but also interesting for studies of molecular interactions on solid/liquid interfaces.

Tryptophan (TRP) and its derivatives, such as serotonin (5-HT), 5-hydroxytryptophan (5-HTP) and 5-hydroxyindole-3-acetic acid (5-HIAA) are important compounds related to biological systems [10]. Since this series of molecules all contains an amine group that can be linked on a carbon surface [4], those molecules with similar molecular structure may provide a good example for investigation of molecular interactions at the electrode interface.

In this paper, we have found interesting catalytic activities of the TRP, 5-HT, 5-HTP grafted GCEs for DA and 5-HT determination. This report describes the preparation, characterization and the applications of these modified electrodes. It was found that the 5-HTP modified electrode not only exhibited

strong catalytic ability toward the oxidation of DA and 5-HT, but also resolved their voltammetric responses into two well-defined voltammetric peaks on the interfacially grafted monolayer, which can be used to determine DA and 5-HT simultaneously. The designed sensor was further used to provide little insight into molecular interaction in living systems.

Experimental

Reagents

The 5-hydroxytryptophan (5-HTP), serotonin (5-HT), tryptophan (TRP) and dopamine (DA) were purchased from Sigma (USA, www.sigma-aldrich.com). All other reagents used in this study were all of analytical grade. Solutions of DA and 5-HT were prepared in water, prior to use. The 0.1 mol L^{-1} phosphate buffer solutions of different pH values (PBS) were prepared as the electrolyte solution by mixing four stock solutions of 0.1 mol L^{-1} H$_3$PO$_4$, KH$_2$PO$_4$, K$_2$HPO$_4$ and KOH (Shanghai Chemical Company, Shanghai, China, www.china-reagent.instrument.com.cn). All aqueous solutions were prepared in doubly distilled, deionized water. High purity nitrogen was used for deaeration of the prepared aqueous solutions.

Apparatus

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were obtained at a CHI 832 electrochemical analyser (Chenhua, Shanghai, China). The scan rate dependent CVs were obtained at a CHI 660A electrochemical analyzer of the same company. A conventional three-electrode system was used. Glassy carbon disk electrodes (GCEs, formal disk area of 0.125 cm^2, Chenhua, Shanghai, China) were used as the basal electrodes for fabrication. A saturated calomel electrode (SCE) and a platinum wire electrode were used as the reference electrode and the counter electrode. All potentials reported in this paper are referred to SCE. For accurate potential control in scan rate dependent experiments, ohmic drops in the cell were compensated to about 95%. Experiments were carried out at ambient temperature (25°C). XPS determination was carried out at ESCALAB MK2 spectrometer (VG Corporation, UK) with Mo Kα X-ray source at 50 and 0.05 eV/step.

Preparation of modified electrodes

Prior to modification, the basal GCE was polished repeatedly with 6, 1 and 0.05 μm alumina slurries, followed by successive sonication in ethanol and doubly distilled water for 5 min, and in order to remove any adsorbed substances on the electrode surface. Finally, it was dried

under nitrogen atmosphere ready for use. 5-HTP was electrochemically deposited on the basal GCE by CV from −1.7 and 1.8 V at 20 mV s^{-1} in 0.1 mol L^{-1} PBS (pH 7.0) containing 1.0×10^{-3} mol L^{-1} 5-HTP and 1.0×10^{-2} mol L^{-1} LiClO$_4$ for several cycles. The 5-HTP molecule could be grafted on the carbon surface to from monolayer modified electrode, denoted as 5-HTP/GCE. The prepared electrode was sonicated in water for 5 min to remove any physically adsorbed substances. 5-HT and TRP grafted GCE, was similarly prepared for comparison and denoted as 5-HT/GCE and TRP/GCE, respectively. These electrodes were stored in 0.1 mol L^{-1} PBS (pH 7.0) in a refrigerator at 4°C before use.

Analytical procedure

A 5 mL phosphate buffer (0.1 mol L^{-1} pH 6.0) containing a specific amount of sample solution was transferred to a voltammetric cell, and then purged with purified nitrogen for 10 min to remove oxygen. After accumulation for 30 s, the voltammetric experiments were carried out at room temperature.

After each determination, the modified electrode was subjected to a potential cycling in 0.1 mol L^{-1} pH 7.0 PBS in a potential window of 0–0.8 V for about 25 cycles, which could activate and renew the electrode surface until a clean and stable background CV was obtained.

Results and discussion

Electrochemical grafting of 5-HTP on GCE

5-HTP was grafted on the basal GCE by CV from −1.7 and 1.8 V at 20 mV/s in 0.1 mol L^{-1} PBS (pH 7.0) for several cycles. It is shown that the figure that 5-HTP exhibited an oxidation peak at abut 0.59 V in the anodic sweeping on the first anodic scan (curve 1), and no re-reduction peak appeared in the reverse cathodic sweeping, which suggested a chemically irreversible oxidation process (Fig. 1). The oxidation of the hydroxy group on 5-HTP to the quinonimine should be the cause to this oxidation peak [11]. However, this peak did not appear in the next cycles. Instead, a smaller peak at 0.36 V followed by a broad anodic wave in the potential range 0.8–1.6 V appeared and increased with the increasing of the cycle (curve 2–6), only it can be seen that a small re-reduction wave appeared at about −0.3 V. It is shown that the surface grafting was about saturation from a steady state after sixth scan. Higher electrocatalytic activity and current sensitivity could be provided by the surface coverage of grafting under but close to saturation than at full saturation [12]. Similar phenomena were also observed for the grafting of 5-HT and TRP from our studies, so the optimal cycle number was estimated as 6. The electrochemical behavior of
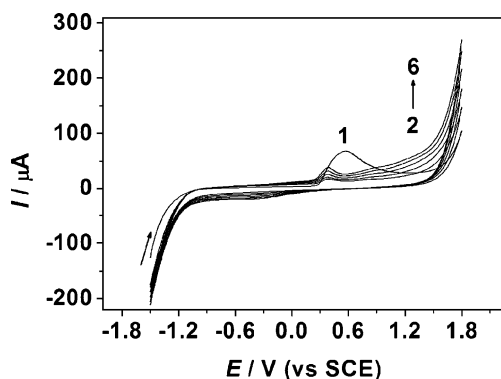
Fig. 1 The multi-cycle CV of GCE in PBS (pH 7.0) containing 50 mmol L^{-1} 5-HTP and 10 mmol L^{-1} LiClO$_4$. Scan rate: 20 mV s^{-1}. 1–6 the first–sixth cycle, respectively

5-HTP is in line with that reported for primary amines [13, 14]. Scheme 1 depicts the reaction mechanism of the surface grafting (see in Supplementary Material).

XPS was used for characterization of the electrode surfaces and shown in Fig. S1 (Supplementary Material). It can be seen that the carbon–nitrogen bond appeared at 399.4 eV, which is agreed with the mechanism of the 5-HTP grafting process. Therefore, the 5-HTP residues have been immobilized successfully on the GCE surface.

Electrocatalytic oxidation of DA and 5-HT

It was found that the 5-HTP/GCE has strong catalytic activity toward the oxidation of DA and 5-HT. Figure 2 a shows the cyclic voltammetric curves (CVs) of 2.0×10^{-5} mol L^{-1} DA in pH 6.0 phosphate buffer solution (PBS) at the 5-HTP/GCE (curve B) and bare GCE (curve A). At a bare GCE, DA shows a broad and small oxidation–reduction peak, which means it cannot be used for any real application for the detection of DA. However, the 5-HTP/GCE gave significantly increased peak currents and a more reversible electron process to DA. Compared with that at a bare GCE, the oxidation peak potential shifts negatively to 0.22 V and the separation between peak potentials (ΔE_p) was 75 mV. The 5-HTP/GCE provided a remarkable enhancement in the DA oxidation response current and a lowering of overpotential, owing to an enhancement of surface accumulation of DA in the 5-HTP grafted layer.

Since both of DA and 5-HT coexist in a biological system, simultaneous determination of DA and 5-HT and elimination interference from each other are very important [7]. The CVs of 2.0×10^{-5} mol L^{-1} 5-HT were also investigated as shown in Fig. 2b. From Fig. 2b, it can be seen that there exists a broad and weak oxidation peak of 5-HT at a potential of about 0.51 V at a bare GCE (curve A). However, the peak current increased about 5-fold and peak potential shifted negatively about 120 mV in comparison with bare GCE (curve B). The CV peak 5-HT oxidation
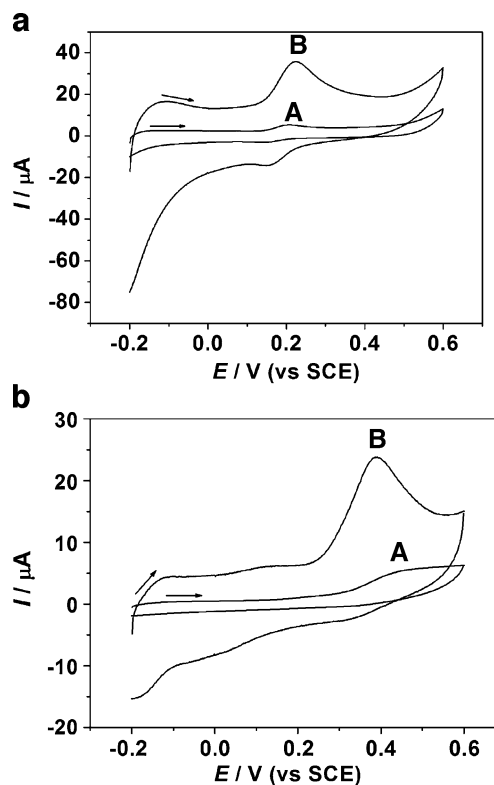

Fig. 2 CVs of 20 μmol L^{-1} DA (**a**) and 20 μmol L^{-1} 5-HT (**b**) at 5-HTP/GCE (*B*) and a bare GCE (*A*) in 0.1 mol L^{-1} PBS (pH 6.0). Scan rate, 50 mV s^{-1}

appeared at 0.39 V, which is 170 mV more negative than the peak potential of DA oxidation. Interestingly, a very small re-reduction peak appeared at about 0.30 V in the reverse scan (curve B) even at a slow scan rate of 50 mV s^{-1}.

The mechanism of the oxidation of DA and 5-HT on the 5-HTP/GCE surface were not clear, we think that, at neutral pH, there exists strong electrostatic interaction between the negative–COO$^-$ groups on the surface of 5-HTP layer and positive–NH$_3^+$ groups in the DA (pK_a 8.9) or 5-HT (pK_a 7.59) molecules, which would accelerate the oxidation process of DA or 5-HT. So it could be found that the overpotential decreased and peak current increased strongly.

Effects of scan rate and solution pH

The influence of scan rate on the oxidation peak current of DA and 5-HT at the 5-HTP/GCE was studied by use of CV mode. As shown in Fig. 3, the peak current of DA varied linearly with the scan rate from 10 to 500 mV/s with a linear regression equation of $I_{pa}=13.56+0.27\ v$ (μA, mV/s, $R^2=0.9980$), indicating an adsorption-controlled process. Meanwhile, the anodic peak current of 5-HT at the 5-HTP/GCE was also proportional to the scan rate over the range of 10–450 mV/s with a linear function of $I_{pa}=7.46+0.14\ v$ (μA, mV/s, $R^2=0.9995$), showing a typical surface adsorption kinetics [15].

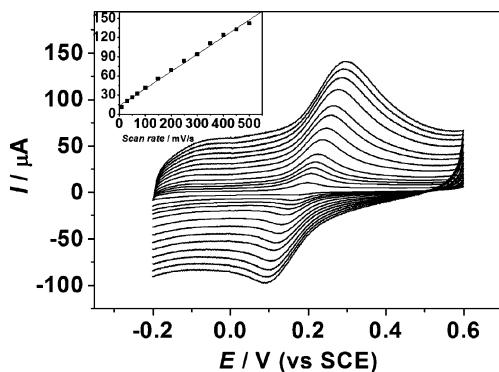**Fig. 3** CVs for the oxidation of 50 µmol L^{-1} DA at 5-HTP/GCE in PBS (pH 6.0). Scan rates: from 10 to 500 mV s^{-1}. *Inset*: plot of anodic peak current vs. scan rate

The effect of pH on the electrode response for the oxidation of DA and 5-HT was also investigated by DPV mode in the pH range 3–9. The peak currents of DA and 5-HT increased with the increase of pH, and the highest oxidation peak currents were observes in pH 6.0 PBS. So the pH 6.0 PBS was chosen as the supporting electrolyte for the experiment. The slope of −55.8 mV/pH for DA and −57.3 mV/pH for 5-HT (close to the anticipated Nernstian theoretical value of 59 mV), which are in agreement with the 2e$^-$/2H$^+$ reaction, respectively [16].

Electrochemical oxidation of DA and 5-HT in a mixture

Figure 4 shows the CV and DPV responses of DA and 5-HT in mixtures at the 5-HTP/GCE in comparison with that at a bare GCE. As shown in Fig. 4a, curve B, the CV of a sample solution containing DA and 5-HT shows a board and overlapped anodic peak at 0.41 V at bare GCE. So it is impossible to deduce any information from the broad and overlapped voltammetric peak. However, the overlapped voltammetric peak is resolved into two well-defined CV peaks (curve A), at about 0.24, 0.39 V and the separation between peak potentials (ΔE_p) was 150 mV.

Furthermore, as shown in Fig. 4b, much better resolved peaks were obtained by DPV technique, the two peaks at 0.19, 0.38 V corresponding to the oxidation of DA and 5-HT. The 190 mV of separation between peak potentials (ΔE_p) was even larger than the separation of CV peak. So the DPV technique benefits to simultaneous determination of DA and 5-HT.

Analytical applications

To learn more about the electrochemical responses under various DA concentrations in the co-existence of 5.0× 10^{-6} mol L^{-1} 5-HT under the optimized conditions by DPV techniques (Fig. 5). It can been seen that a linear response was obtained between the peak current and DA concentration, $I_{pa}=15.88+0.36C$ (µA, µM, $R^2=0.9962$) in
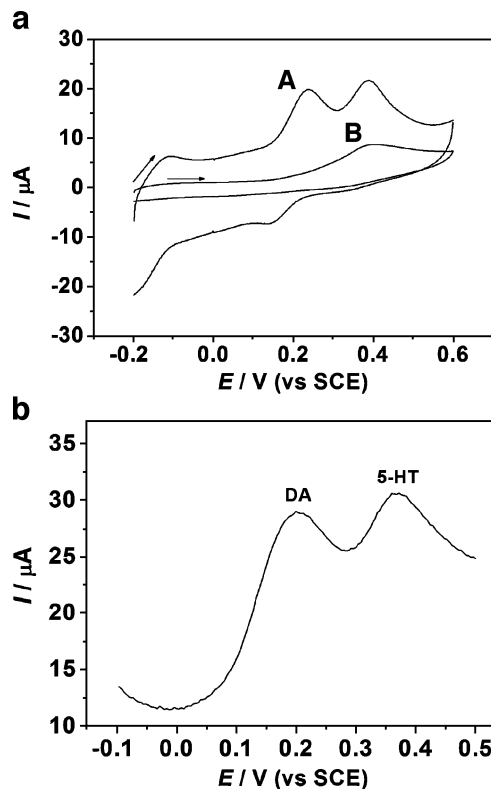

Fig. 4 (**a**) CVs of 10 µmol L^{-1} DA and 10 µmol L^{-1} 5-HT in PBS (pH 6.0) at 5-HTP/GCE (*A*) and a bare GCE (*B*). Scan rate, 50 mV s^{-1}. (**b**) DPVs of the mixture containing 10 µmol L^{-1} DA and 10 µmol L^{-1} 5-HT at 5-HTP/GCE in PBS (pH 6.0). Amplitude, 50 mV; pulse width, 50 ms; pulse period, 200 ms

the range of 5.0×10^{-7}–3.5×10^{-5} mol L^{-1} with a detection limit of 3.1×10^{-7} mol L^{-1} (s/n=3).

Figure 6 showed the DPVs of various concentrations of 5-HT at the 5-HTP/GCE in the presence of 5.0×10^{-6} mol L^{-1} DA. The peak current of 5-HT was linear to the concentration in the range of 5.0×10^{-6}–3.5×10^{-5} mol L^{-1} with a linear
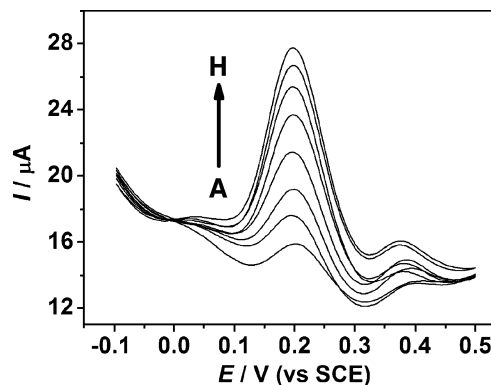

Fig. 5 DPVs of DA at 5-HTP/GCE in the presence of 5 µmol L^{-1} 5-HT in pH 6.0 PBS. DA concentration (from *A* to *H*): 0.5, 5, 10, 15, 20, 25, 30, 35 µmol L^{-1}. Amplitude, 50 mV; pulse width, 50 ms; pulse period, 200 ms
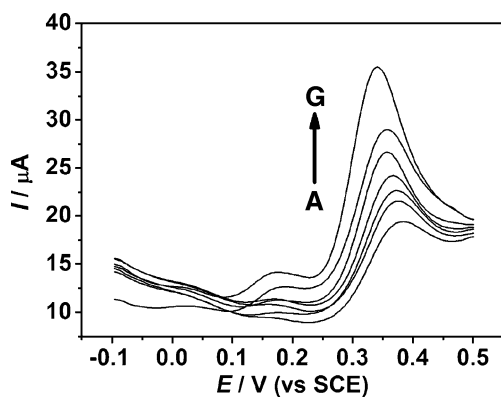
Fig. 6 DPVs of 5-HT at 5-HTP/GCE in the presence of 5 μmol L^{-1} DA in pH 6.0 PBS. 5-HT concentration (from *A* to *G*): 5, 10, 12, 16, 20, 25, 35 μmol L^{-1}. Amplitude, 50 mV; pulse width, 50 ms; pulse period, 200 ms

Table 2 Comparison of CV behavior at TRP/GCE, 5-HT/GCE and 5-HTP/GCE for DA and 5-HT oxidation[a]

Electrodes	DA		5-HT		ΔE_{pa} (mV)
	E_{pa}(V)	i_{pa}(μA)	E_{pa}(V)	i_{pa}(μA)	
TRP/GCE	0.28	7.8	0.41	8.3	130
5-HT/GCE	0.32	6.6	0.43	7.8	90
5-HTP/GCE	0.24	12.4	0.39	13.7	150

[a] At 50 mV/s in 10 μmol L^{-1} DA+10 μmol L^{-1} 5-HT+0.1 mol L^{-1} PBS (pH 7.0)

function $I_{pa}=16.06+0.54C$ (μA, μM, R^2=0.9983). The detection limit was 1.7×10^{-6} mol L^{-1} (s/n=3).

For practical performance, the 5-HTP/GCE was used for direct analysis of the mixture sample of DA and 5-HT. The dopamine hydrochloride injection solution (standard concentration of DA 10 mg ml^{-1}, 2 mL per injection) was diluted to 100 mL with 0.1 mol L^{-1} PBS (pH 6.0). 10 μL of this diluted solution and 1.0 mL of standard 5-HT solution were injected into each of a series of 10 mL volume flasks and made up to volume with 0.1 mol L^{-1} PBS (pH 6.0). The results are listed in Table 1. As seen in this table, the mixture samples were analyzed with DPV mode, and the results was satisfactory.

Stability

In order to investigate the stability of the 5-HTP/GCE, the modified electrode was stored in PBS (pH 7.0) at 4°C, the current response had 2% decrease during the first 2 days, 6% for 5 days and 21% for the following 1 month. In addition, the electrode had to be well treated to remove adsorption contaminations to maintain the reproducibility. The electrode can be renewed by CV scans in 0.1 mol L^{-1} PBS in the potential window 0.0–0.8 V after each experiment. As a result, after 25 cycles of scanning could

regenerate clean background CV curves, the electrode was ready for the next experiment or for storage.

Comparison of TRP/GCE, 5-HT/GCE and 5-HTP/GCE

The CV behaviors of these prepared electrodes are exhibited in Table 2. It is shown that TRP/GCE and 5-HT/GCE could also catalyze the oxidation of DA and 5-HT with significant reductions of overpotentials. The catalytic ability of these electrodes was in the order of 5-HTP/GCE>TRP/GCE> 5-HT/GCE for DA and 5-HT. The negatively charged hydroxyl group of hydroxybenzene and carboxylic groups on 5-HTP could be the key factor for the strong catalytic ability toward DA and 5-HT, so these groups could attract the positively charged DA and 5-HT. The 5-HTP/GCE exhibits excellent catalytic ability.

Conclusions

This work demonstrated 5-HTP grafted GCE not only exhibited remarkable electrocatalytic activity toward DA and 5-HT oxidation with improving the peak current and lowering their oxidation overpotential, but also resolved the overlapping anodic DA and 5-HT peaks into two well-defined peaks. Thus, it becomes possible to detect DA and 5-HT in a mixture solution simultaneously. These were attributed to the negatively charged hydroxyl group of hydroxybenzene and carboxylic groups on 5-HTP.

Table 1 Determination of DA and 5-HT in injections (n=5)

Sample		Concentration (μmol L^{-1})	Detected (μmol L^{-1})	R.S.D. (%)
1	DA	1.06	1.03	2.1
	5-HT	1.00	1.02	3.4
2	DA	1.06	1.09	2.8
	5-HT	1.00	0.97	1.9
3	DA	1.06	1.05	3.1
	5-HT	1.00	1.04	2.0

Table 3 Peak potential (E_p), linear range (LR) and detection limit (DL) of Asp, ACh and 5-HTP modified GCEs for DPV determination of DA and 5-HT

	DA			5-HT			Ref.
	E_p/V	LR/μM	DL/μM	E_p/V	LR/μM	DL/μM	
Asp/GCE	0.22	1.8–460	1.2	_	_	_	[17]
Ach/GCE	0.31	0.7–5	0.3	0.43	1–30	0.5	[7]
5-HTP/GCE	0.19	0.5–35	0.31	0.37	5–35	1.7	This work

In comparison with the acetylcholine, grafted GCE (ACh/GCE) [7], and the aspartic acid, grafted GCE (Asp/GCE) [17], data are exhibited in Table 3. It can be seen from the table that catalytic activity toward the oxidation reactions of DA is the order of 5-HTP/GCE>Asp/GCE>ACh/GCE, which is characterized by shifts of the peak potentials. From analytical point of view, the Asp/GCE is most suitable for DA detection with the widest linear ranges (LR) (1.8–460 μM). However, the 5-HTP/GCE was more suitable for DA analysis for the higher linear detection limit (0.31 μM) in the low concentration. Meanwhile, in comparison with the ACh/GCE, the 5-HTP/GCE had lower oxidation overpotential for the analysis of 5-HT. Thus, the 5-HTP/GCE can be expected to be preferable for modified of microelectrodes for in pharmaceutical analysis with high stability and selectivity.

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