

Glassy carbon electrode modified with poly (dibromofluorescein) for the selective determination of dopamine and uric acid in the presence of ascorbic acid

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Abstract We are presenting an electrochemical sensor for the simultaneous determination of dopamine (DA) and uric acid (UA) in the presence of even high concentrations of ascorbic acid (AA). It based on a glassy carbon electrode modified with an electroactive film of polymerized dibromofluorescein. The electrochemical behaviors of DA and UA were studied by cyclic voltammetry using the modified electrode. It exhibits excellent electrocatalytic activity towards the oxidation of the two analytes. Most notably, the oxidation potentials differ by 180 and 200 mV between AA-DA and DA-UA, respectively. Thus, excellent selectivity towards the oxidation of DA and UA in the presence of even high concentrations of AA is accomplished. Under the optimum conditions, the anodic peak currents are linearly related to the concentrations of DA and UA in the range from 0.2 to 200 $\mu\text{mol L}^{-1}$ and from 1.0 to 250 $\mu\text{mol L}^{-1}$, respectively. The detection limits for DA and UA are 0.03 $\mu\text{mol L}^{-1}$ and 0.2 $\mu\text{mol L}^{-1}$, respectively (at an S/N of 3). The method has good selectivity and sensitivity and was successfully applied to the simultaneous determination of DA and UA in spiked human serum.

Keywords Dopamine · Uric acid · Dibromofluorescein · Chemically modified electrode · Simultaneous determination

Introduction

Dopamine (DA) is a well-known catecholamine neurotransmitter in the mammalian central nervous system. It plays an important role in the regulation of physiological processes in living systems. Abnormal DA transmission is associated with neurological disorders such as schizophrenia, Parkinson's disease, and Huntington's disease. As a cholinergic drug, DA is widely applied to the treatment of circulatory collapse syndrome caused by myocardial infarction, trauma, renal failure, cardiac surgery, or congestive cardiac failure [1, 2]. Uric acid (UA) is the terminative oxidation product of purine degradation metabolism in human beings. Its concentration level in body fluids such as human serum and urine is marker of many clinical conditions, including hyperuricemia, gout, and the Lesch-Nyan disease [3, 4]. Since DA and UA usually coexist in real biological samples, the development of a selective and sensitive method for their simultaneous determination is highly desirable for analytical and diagnostic applications. The fact that DA and UA are easily oxidized makes their detection feasible by electrochemical methods. However, their simultaneous electrochemical determination remains a challenge because of the coexistence of relatively high levels of ascorbic acid (AA) in body fluids. This compound is oxidized at a potential close to that of DA at bare electrodes, resulting in overlapped voltammetric responses which severely hinder the accurate determination of DA [5]. Moreover, DA and UA at

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bare electrodes yield poor electrochemical responses which interference to each other for the determination of any one of the analytes [6]. Therefore, improvement of selectivity and sensitivity of the working electrode towards DA and UA has been a long-standing issue in electroanalysis. In recent years, chemically modified electrodes (CMEs) have become important electrochemical detection methods for the determination of biologically important compounds because of its good sensitivity, selectivity and stability [7–9]. There have been many reports on the application of CMEs for the selective determination of DA and UA in the presence of high concentration of AA [10–23]. Among them, polymer modified electrodes prepared by electropolymerization method have received extensive interest because chemically stable homogeneous film with controlled thickness can be prepared by this method. It has been demonstrated that electropolymerized films showed excellent selectivity and sensitivity towards DA and UA. For example, poly (evans blue) [18], poly (eriochrome black T) [19], poly (vinyl alcohol) [20], poly (oracet blue) [21], poly(methylene blue) [22], poly (L-arginine) [23] modified electrodes have been used for the determination of DA and UA in the presence of main interferent AA. In this paper, we prepared a new poly (dibromofluorescein) modified electrode to catalyze the oxidation of DA and UA, which provided a simple and sensitive voltammetric method for determining the concentrations of the two compounds simultaneously.

In recent years, organic dyes have been used for fabricating conducting polymer modified electrode. Dibromofluorescein (DBF) is an organic dye of the fluorescein family, which has electrochemical activity. Its electropolymerization at the electrode surface and its function as an electrocatalyst have never been reported in the literature. In this work, we reported for the first time on a conducting polymer film of DBF to modify glassy carbon electrode (GCE) and described the electrochemical behaviors of the novel poly (DBF) modified glassy carbon electrode. Because the poly (DBF) modified electrode possessed the larger real surface area, redox active centre, π - π conjugated bond and better conductivity, the electrochemical reversibility of the oxidation of DA and UA may be greatly improved in the presence of the poly (DBF) film by accelerating the rate of electron transfer, which indicated that the poly (DBF) film had excellent electrocatalytic activity for oxidation reactions of DA and UA at the surface of the modified electrode. Moreover, the modified electrode showed good sensitivity, selectivity and reproducibility for the simultaneous determination of DA and UA in the presence of high concentration of AA. Based on its excellent characteristics compared to other electrochemical sensors reported in terms of high sensitivity, wide linearity and good stability, the poly (DBF) modified electrode was satisfactorily used for the simultaneous determination of DA and UA in human serum by differential pulse voltammetry (DPV).

Experimental

Reagents

DA and UA were purchased from Sigma (USA <http://www.sigmaaldrich.com>). 4', 5'- Dibromofluorescein and AA were obtained from Sinopharm Chemical Reagent Co. Ltd. (China <http://www.reagent.com.cn>). All chemicals were of analytical grade and were used without further purification. The 0.067 mol L⁻¹ phosphate buffer solutions with various pH values were prepared by mixing the stock solutions of 0.067 mol L⁻¹ KH₂PO₄ and Na₂HPO₄. The working solutions of DA and UA were prepared just prior to use. All solutions were prepared with double distilled water.

Apparatus

Electrochemical measurements were performed with a LK2005 Microcomputer-based electrochemical system (LANLIKE, Tianjin, China) (<http://www.lanlike.com>). A conventional three-electrode cell was used, including a saturated calomel electrode (SCE) as reference electrode, a platinum sheet electrode as the counter electrode and a bare or modified glassy carbon disk electrode (GCE) with a diameter of 3 mm used as working electrode. All pH measurements were made with a PHS-3 C digital pH meter (Shanghai Lei Ci Device Works, Shanghai, China) (<http://www.lei-ci.com/>) with a combined glass electrode. A KQ-250B ultrasonic washer (Kunshan Ultrasonic Instrument Works, Kunshan, China) (<http://www.ks-csyq.com/>) was used to wash the electrode.

Preparation of the poly (DBF) modified electrode

Cyclic voltammetry (CV) was used to form polymerization film. Prior to its modification, the bare GCE was polished with 0.05 μ m α - alumina powder and rinsed with 1:1 HNO₃ solution, ethanol, and doubly distilled water for 10 min successively. Then the poly (DBF) modified electrode was prepared by electropolymerization. The polymeric film was deposited by cyclic sweeping from -1.2 to 1.5 V at 100 mV s⁻¹ for 20 cycles in pH 7.0 phosphate buffer solution containing 1.0 $\times 10^{-3}$ mol L⁻¹ DBF. After polymerization, the modified electrode was washed with doubly distilled water, and then air-dried.

Experimental methods

Cyclic voltammetric and differential pulse voltammetric measurements were carried out in phosphate buffer solution. The cyclic voltammograms were recorded by cycling the potential between -0.2 and +0.6 V at a scan rate of 100 mVs⁻¹. The differential pulse voltammetric measurements

were performed by applying a sweep potential from -0.2 to +0.6 V at pulse amplitude of 50 mV and pulse width of 0.1 s. All experiments were carried out at room temperature. The poly (DBF) modified electrode could be used repeatedly after rinsed with doubly distilled water and blotted with filter paper.

Sample preparation

Blood samples were collected from healthy volunteers at the Hospital of Shandong Normal University. A 1.0 mL of fresh blood sample was obtained and centrifuged at 3,000 rpm for 20 min to remove all precipitating materials. The spiked serum samples were prepared as follows: 0.5 mL of the separated serum was transferred to a color comparison tube. After spiked with different quantities of DA and AA, the mixture solution obtained was diluted to 10 mL with pH 5.6 phosphate buffer. Then the synthesized serum samples were placed in the electrochemical cell to detect DA and UA simultaneously in the presence of AA by the proposed DPV method.

Results and discussion

Preparation and characterization of electropolymerized DBF film at the GCE surface

Cyclic voltammetry was used to form the electropolymerization film. Compared with other supporting electrolyte such as acetate buffer solution and borax buffer solution used in electrodeposition process, the obtained polymeric film was observed to be more complete, uniform and compact, which showed better electrocatalytic activity to the oxidation of DA and UA when pH 7.0 phosphate buffer solution was used as supporting electrolyte during polymer formation. Thus it was chosen as supporting electrolyte for electropolymerization in this work. The potential scan range was the most important factor in preparing poly (DBF) film. If the positive potential value for polymerization was below 1.0 V or if the negative one was above -1.0 V, no polymer reaction occurred. Therefore, the potential scan window from -1.2 to 1.5 V was selected as the electropolymerization potential scan range in this paper.

The consecutive cyclic voltammograms of 1.0×10^{-3} mol L⁻¹ DBF in pH 7.0 phosphate buffer solution at bare glassy carbon electrode were shown in Fig. 1. In the first cycle, two anodic peaks were observed at +0.536 V (peak A) and +0.762 V (peak B), which might correspond to the oxidation of DBF monomer. A cathodic peak also appeared at about -0.846 V (peak C) in the first cycle. In the subsequent cycles the larger peaks were observed upon continuous scanning, which was reflecting the continuous growth

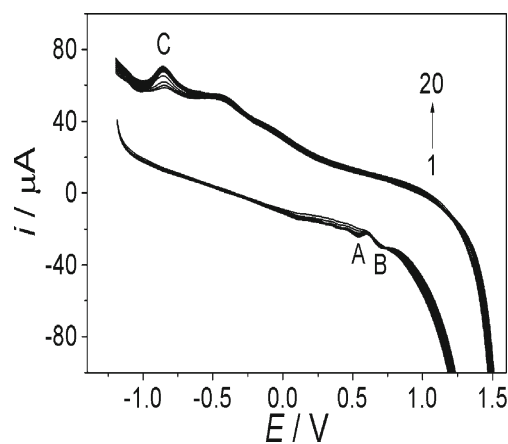


Fig. 1 Cyclic voltammograms of DBF in electropolymerization process from 1 to 20 cycles. DBF: 1.0×10^{-3} mol L⁻¹; Supporting electrolyte: pH 7.0 phosphate buffer solution; Scan rate: 100 mV s⁻¹

of the film. It could be observed that the film growth was faster for the first five cycles than for the other cycles. From the eighteenth cycle on, the film hardly grew, which showed that polymerization had reached saturation. So the total number of the electropolymerizing scans was selected as 20 for forming stable polymer film. After modification, a uniform adherent blue-violet polymer was formed on the GCE surface, which demonstrated that DBF was deposited on the surface of GCE by electropolymerization.

The electrochemical behavior of the poly (DBF) modified electrode was studied in phosphate buffer solution by CV. Figure 2 showed the cyclic voltammograms of the modified electrode recorded in pH 7.0 phosphate buffer solution at different scan rates. In the potential range from -0.3 to 0.3 V, a well-defined pair of stable redox peaks located at ca. 0.0 V (peak A and peak B) were obtained in each cycle. Both the anodic peak current (peak A) and

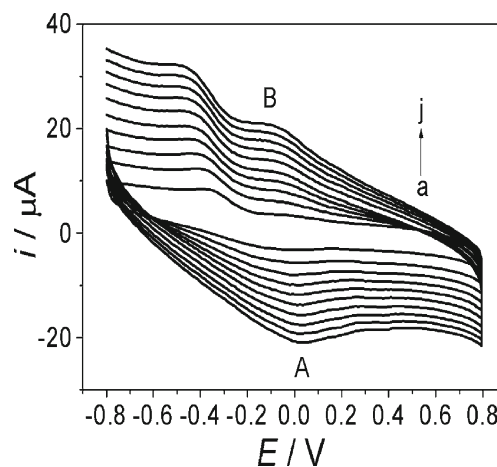


Fig. 2 Cyclic voltammograms of poly (DBF) modified glassy carbon electrode in pH 7.0 phosphate buffer solution at different scan rates. (a) 20, (b) 40, (c) 60, (d) 80, (e) 100, (f) 120, (g) 140, (h) 160, (i) 180, (j) 200 mV s⁻¹

cathodic peak current (peak B) increased linearly with the increase of scan rate (ν) over the range of 20–200 mV s^{-1} , indicating a surface-controlled electrode processes. Moreover, the ratio of anodic peak current to cathodic peak current ($i_{\text{pa}}/i_{\text{pc}}$) was almost equal to unity. With increasing scan rate, the separation of peak potentials ($\Delta E_{\text{p}}=E_{\text{pa}}-E_{\text{pc}}$) would not be changed. The above results suggest that the electrode reaction is reversible [24]. ΔE_{p} ($= 56.3 \text{ mV}$) is close to $2.3RT/nF$ (or $59/n \text{ mV}$ at $25 \text{ }^{\circ}\text{C}$), which was in accordance with a Nernst reversible behavior. So the number of transferred electrons in the electrode reaction was identified as one ($n \approx 1.05$). The surface coverage of poly (DBF) modified electrode was made by adopting the method used by Sharp et al. [25]. According to this method, the peak current is related to the surface concentration of electroactive species, by the following equation:

$$i_{\text{p}} = \frac{n^2 F^2 A \Gamma \nu}{4RT}$$

where n represents the number of electrons involved in reaction ($n=1$), A is the surface geometrical area of electrode (0.0706 cm^2), Γ represents the surface coverage concentration (mol cm^{-2}) and other symbols have their usual meaning. The anodic peak current (i_{pa}) was linearly dependent on the scan rate (ν) over the range of 20–200 mV s^{-1} , with the regression equation $i_{\text{pa}} (\mu\text{A}) = 0.0363 + 0.04368\nu$ (mV s^{-1}) (correlation coefficient, $r=0.9981$). From the slope of anodic peak current versus scan rate, the surface concentration of poly (DBF) was calculated as $7.23 \times 10^{-8} \text{ mol cm}^{-2}$. The result in Fig. 2 proved that DBF had been deposited on the surface of GCE by electropolymerization.

The effect of pH on the electrochemical behavior of the poly (DBF) modified electrode was studied in phosphate buffer solution over the pH range from 4.5 to 7.2. The results showed that both E_{pa} and E_{pc} shifted towards the negative potentials direction with increasing pH, which indicated that protons have taken part in the electrode processes. The anodic peak potentials exhibited a linear dependence on pH values with a slope of 59.8 mV pH^{-1} , which suggested that the ratio of the participated protons to the transferred electrons through the poly (DBF) film is 1:1. Thus, the possible reaction mechanism of poly (DBF) film on GCE can be expressed as follows: In the potential range from -0.3 to 0.3 V , the redox reaction of electrodeposited DBF on GCE involved the oxidation of phenolic hydroxyl group in electrodeposited DBF and back reduction of produced benzoquinone.

Ferricyanide/ferrocyanide redox couple was used as diffusion controlled redox probe to characterize the conductive and the electron-transfer features of the modified electrode. Compared with the unmodified GCE, the peak currents of $\text{Fe}(\text{CN})_6^{3-/4-}$ increased and ΔE_{p} decreased at the modified electrode. It is assumed that these electrochemical properties were related to the improved electrical efficiency of poly

(DBF) film, the increase of geometric area of the electrode and the high electrocatalytic activity of the modified electrode. The prepared poly (DBF) film is multilayer conductive polymer film with redox active centre, and the phenolic hydroxyl functional group as redox active centre in poly (DBF) film had good redox activity.

The electrochemical behaviors of DA and UA at poly (DBF) modified electrode

Figure 3a was the CVs of DA at the bare GCE (curve a) and the modified electrode (curve b) in pH 5.6 phosphate buffer solution. At bare GCE, DA exhibited poor current responds and irreversible electrochemical behavior with 60 mV of ΔE_{p} , the difference between the anodic potential (E_{pa}) and the cathodic peak potential (E_{pc}). However, a well-defined redox wave of DA was observed at the modified electrode with 30 mV of ΔE_{p} . The oxidation peak potential shifted negatively to 0.240 V , and the reduction peak potential shifted positively to 0.210 V . Furthermore, the peak currents increased significantly. These results indicated that poly (DBF) film could accelerate the rate of electron transfer of DA and have good electrocatalytic activity for redox reaction of DA. Figure 3b illustrated the CVs of $1.0 \times 10^{-4} \text{ mol L}^{-1}$

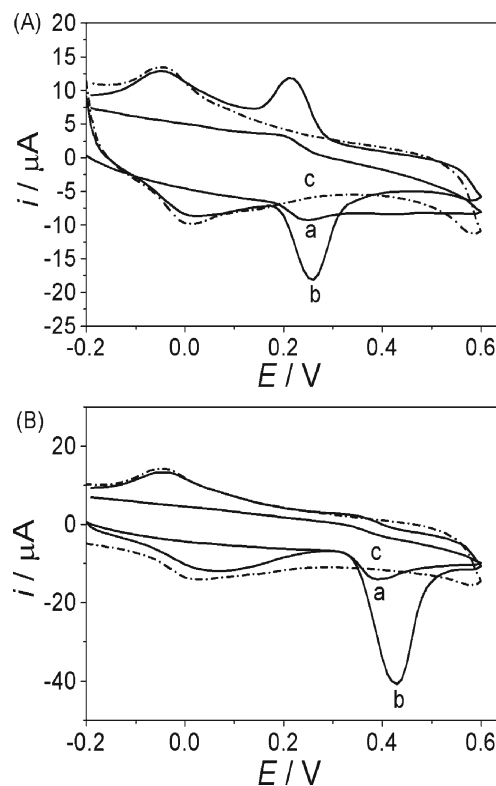


Fig. 3 Cyclic voltammograms of DA (A) and UA (B) at different electrodes in pH5.6 phosphate buffer solution. DA: $1.0 \times 10^{-5} \text{ mol L}^{-1}$, UA: $1.0 \times 10^{-4} \text{ mol L}^{-1}$, Scan rate: 100 mV s^{-1} , (a) bare glassy carbon electrode; (b) poly (DBF) modified electrode; (c) CV of poly (DBF) modified electrode in the absence of DA and UA

¹ UA at the bare electrode (curve a) and modified electrode (curve b). As can be seen, a small and broad oxidation peak was observed at the bare electrode indicating a slow electron transfer kinetic. By comparison, a well-defined oxidation peak response at 0.430 V was observed at the modified electrode. Moreover, the oxidation peak current was about six times higher than that at the bare electrode. The increase in the peak intensity of UA might be linked to the increase of the geometric area of the modified electrode.

The above results demonstrated that the poly (DBF) modified electrode exhibited excellent electrocatalytic activity towards the oxidation of DA and UA. The postulated electrocatalysis mechanism was discussed as follows: The poly (DBF) film is stable redox-active electrodeposited film. As charge mediator, the redox active centre in poly (DBF) film can catalyze the oxidation of DA and UA by accelerating the rate of electron transfer. Moreover, the poly (DBF) film possessed the larger real surface area, π - π conjugated bond, a great deal of active sites and better conductivity, which led to the dissimilar conjugation effect of the two analytes with the electrode interface. Therefore, the electrochemical reversibility of the oxidation of DA and UA may be greatly improved in the presence of the poly (DBF) film by accelerating the rate of electron transfer.

Effect of scan rate on the oxidation of DA and UA at the modified electrode

For the two compounds, the oxidation peak current linearly increased with the square root of the scan rate, suggesting that the system presents features corresponding to diffusion-controlled process for the oxidation of DA and UA [26]. The linear regression equation relating i_{pa} with the square root of scan rate over the range of 20–200 mV s^{-1} , was found to be: $i_{pa} (\mu\text{A}) = -5.265 + 1.857 \nu^{1/2} (\text{mV s}^{-1})$ ($r = 0.9994$) for DA and $i_{pa} (\mu\text{A}) = -8.563 + 2.732 \nu^{1/2} (\text{mV s}^{-1})$ ($r = 0.9989$) for UA, respectively. In addition, the oxidation and reduction peak potentials of DA shifted with the increase of scan rate, which suggested that the electrode reaction of DA is quasi-reversible [24]. The oxidation peak potential (E_{pa}) shifted to more positive potentials with increasing scan rate for UA. The analysis of these data showed that the plot of E_{pa} vs. the logarithm of scan rate presented a linear relation, indicating that the oxidation of UA on the modified electrode surface is irreversible [24].

Effect of pH on the oxidation of DA and UA at poly (DBF) modified electrode

The dependence of the anodic peak potential (E_{pa}) and anodic peak current (i_{pa}) for DA ($2.0 \times 10^{-5} \text{ mol L}^{-1}$) and UA ($1.0 \times 10^{-4} \text{ mol L}^{-1}$) on pH of buffer solution was studied by CV. The oxidation peak potentials (E_{pa}) for DA and

UA showed a same trend and shift almost linearly towards negative potentials when pH was increased in the range of 4.5–7.4, indicating that protons are directly involved in the rate determination step of the oxidation reaction of the two species. The equation relating E_{pa} with pH was found to be: $E_{pa} (\text{V}) = 0.6061 - 0.0608 \text{ pH}$ ($r = 0.9991$) for DA, and $E_{pa} (\text{V}) = 0.7819 - 0.0612 \text{ pH}$ ($r = 0.9985$) for UA, respectively. The slopes of 60.8 mV pH^{-1} for DA and 61.2 mV pH^{-1} for UA are close to the theoretical value of 59 mV pH^{-1} , suggesting that the electron-transfer step is preceded by a protonation with an equal number of protons and electrons involved in their oxidation.

The effect of pH on oxidation peak current for DA and UA was shown that the anodic peak current of DA increased with increasing the pH up to 5.6, then the peak current decreased. However, the oxidation peak current of UA decreased with increasing of pH in a range of 4.5–7.4. The maximum peak current value can be observed at pH 5.6 for DA and pH 4.5 for UA, respectively.

Simultaneous Determination of DA and UA in the presence of high concentration of AA

According to the above experimental results, the modified electrode showed good electrocatalytic activity for oxidation reaction of DA and UA in wide pH range. The maximum peak current value can be observed at pH 5.6 for DA and pH 4.5 for UA, respectively. Because pH 5.6 is closer than pH 4.5 to the physiological pH value and the oxidation of the two compounds had high electrochemical response at this pH, it was selected as the optimum pH for the simultaneous determination of DA and UA in this study.

It is well known that AA and UA were present along with DA in physiological fluids and the concentrations of them were much higher than that of DA. Moreover, the oxidation potential of both AA and UA was nearly same as that of DA, resulting in overlapped voltammetric responses at almost all conventional bare electrodes. Figure 4 showed the cyclic voltammetric response of DA ($1.0 \times 10^{-5} \text{ mol L}^{-1}$) in the presence of UA ($1.0 \times 10^{-4} \text{ mol L}^{-1}$) and AA ($1.0 \times 10^{-3} \text{ mol L}^{-1}$) in phosphate buffer solution of pH 5.6 at both bare GCE and poly(DBF) modified GCE. The voltammogram obtained for mixture of sample at bare GCE (Fig. 4a) was broad, less sensible and overlapped anodic peak, and the peak potentials for AA, DA and UA were totally indistinguishable. Therefore, it is impossible to separate DA, AA or UA from each other using the voltammetric peaks by the unmodified GCE. In great contrast, when the poly (DBF) modified electrode was used, the mixture displayed three well-defined and sensitive oxidation peaks (Fig. 4b). The peaks located at 0.90, 0.270 and 0.470 V, corresponding to the oxidation of AA, DA and UA, respectively. The differences of the oxidation peak potentials for AA–DA and DA–UA were 180 mV and 200 mV,

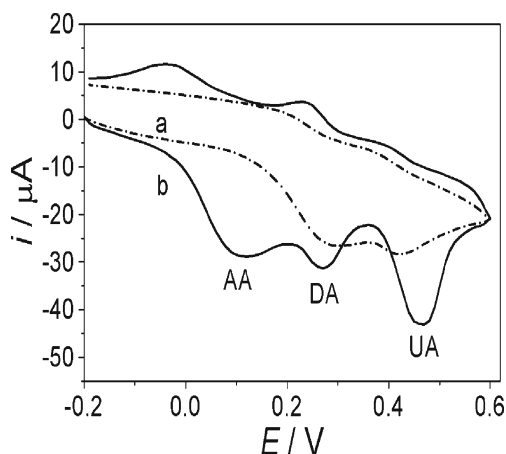


Fig. 4 Cyclic voltammograms of the mixture containing DA, UA and AA at the bare glassy carbon electrode (a) and the poly (DBF) modified electrode (b) in pH 5.6 phosphate buffer solution. DA: 1.0×10^{-5} mol L⁻¹, UA: 1.0×10^{-4} mol L⁻¹, AA: 1.0×10^{-3} mol L⁻¹; Scan rate: 100 mV s⁻¹

respectively, which were enough large separations to allow the simultaneous determination of DA and UA in the presence of AA. The possible reason was that AA (pKa=4.10), UA (pKa=5.7) and DA (pKa=8.87) existed as anionic form, protonated (neutral) form and cationic form at pH 5.6, respectively [16]. Hence, the electrostatic interaction between DA cations and the high electron density of carboxylic group in poly (DBF) film would lead to the increase in concentration of DA around the surface of the modified electrode and accelerate the rate of electron transfer of DA. Protonated form of UA may form hydrogen bonds with OH groups of poly (DBF) film. However, anionic form of the AA was repelled from the negatively charged surface of poly (DBF) film. So, the different interaction between the three compounds and poly (DBF) film would lead to effective separation of AA, DA and UA.

Since differential pulse voltammetry (DPV) has higher sensitivity and better resolution than cyclic voltammetry, DPV was used for simultaneous determination of DA and UA. The DPV parameters were selected as pulse amplitude of 50 mV and pulse width of 100 ms for quantitative determination of the two species in order to obtain maximum peak current. The simultaneous determination of DA and UA in the presence of AA was carried out at poly (DBF) modified GCE when concentration of one species changed, whereas the others remained constant. The results in Fig. 5 showed that the oxidation peak current of DA increased linearly with a correlation coefficient of 0.9991 while increasing DA concentrations in solutions containing a fixed concentration of UA and AA. In addition, no obvious changes in the peak currents and potentials of both UA and AA could be found while varying the concentration of DA. Similarly, in Fig. 6, it can be seen that the oxidation peak current of UA was proportional to its concentration while keeping the concentrations of the other two compounds constant. No interference can be

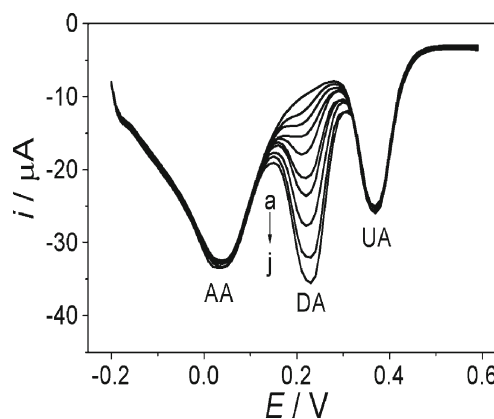


Fig. 5 Differential pulse voltammograms for DA with different concentrations in the present of 200.0 μmol L⁻¹ UA and 800.0 μmol L⁻¹ AA at the modified electrode. DA concentrations: (a) 0, (b) 2.0, (c) 4.0, (d) 10.0, (e) 20.0, (f) 30.0, (g) 40.0, (h) 60.0, (i) 80.0, (j) 100 μmol L⁻¹; Pulse amplitude: 50 mV; Pulse width: 100 ms; pH=5.6 phosphate buffer solution

observed for the determination of UA by the coexisting other two species. All the above results identified that the oxidation processes of DA, AA and UA at poly (DBF) modified electrode are independent from each other, so it is possible to simultaneously determine DA and UA in the presence of AA using the modified electrode in real samples without any interference with each other. Under the optimum conditions, the anodic peak currents are linearly related to the concentrations of DA and UA in the range from 0.2 to 200 μmol L⁻¹ and from 1.0 to 250 μmol L⁻¹, respectively. The detection limits for DA and UA are 0.03 μmol L⁻¹ and 0.2 μmol L⁻¹, respectively (at an S/N of 3).

To test the repeatability of the modified electrode, 5.0×10^{-5} mol L⁻¹ of the two species were measured for six times. The relative standard deviations (RSD) of the peak currents

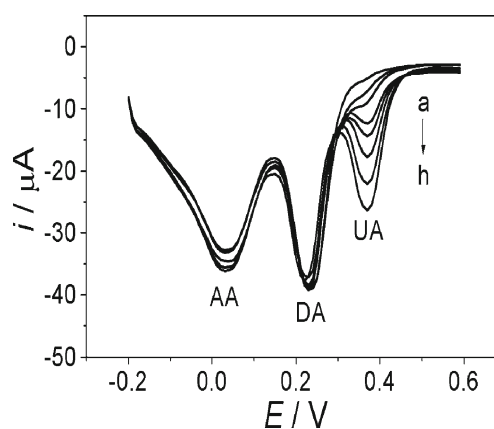


Fig. 6 Differential pulse voltammograms for UA with different concentrations in the present of 50.0 μmol L⁻¹ DA and 800.0 μmol L⁻¹ AA at the modified electrode. UA concentrations: (a) 0, (b) 10.0, (c) 20.0, (d) 40.0, (e) 60.0, (f) 100.0, (g) 180.0, (h) 250.0 μmol L⁻¹; Pulse amplitude: 50 mV; Pulse width: 100 ms; pH=5.6 phosphate buffer solution

Table 1 Comparison of the proposed method with other electrochemical methods for the simultaneous determination of DA and UA

Electrode	Linear range	Detection limit	Ref.
LaFeO ₃ nanoparticles	DA: $1.5 \times 10^{-7} \sim 8.0 \times 10^{-4}$ UA: $1.0 \times 10^{-4} \sim 6.0 \times 10^{-4}$	DA: 3.8×10^{-8}	[13]
Zinc oxide/redox mediator composite film	DA: $1.0 \times 10^{-6} \sim 2.0 \times 10^{-5}$ UA: $2.5 \times 10^{-6} \sim 3.0 \times 10^{-5}$	DA: 1.1×10^{-7} UA: 1.4×10^{-6}	[14]
2-(3,4-Dihydroxyphenyl) -1,3-dithialone self-assembled monolayer film	DA: $2.0 \times 10^{-6} \sim 2.5 \times 10^{-4}$ UA: $1.4 \times 10^{-5} \sim 6.0 \times 10^{-4}$	DA: 7.2×10^{-8} UA: 1.0×10^{-5}	[15]
Palladium nanoparticle -loaded carbon nanofibers	DA: $6.0 \times 10^{-6} \sim 9.6 \times 10^{-4}$ UA: $5.0 \times 10^{-5} \sim 8.0 \times 10^{-4}$	DA: 7.0×10^{-7} UA: 4.5×10^{-6}	[16]
Functionalized-graphene	DA: $5.0 \times 10^{-7} \sim 5.0 \times 10^{-5}$ UA: $1.7 \times 10^{-6} \sim 9.0 \times 10^{-5}$	DA: 2.5×10^{-7} UA: 4.5×10^{-7}	[17]
Poly (evans blue)	DA: $1.0 \times 10^{-6} \sim 1.0 \times 10^{-5}$ UA: $3.0 \times 10^{-5} \sim 1.1 \times 10^{-4}$	DA: 2.5×10^{-7} UA: 2.0×10^{-6}	[18]
Poly(eriochrome black T)	DA: $1.0 \times 10^{-7} \sim 2.0 \times 10^{-4}$ UA: $1.0 \times 10^{-5} \sim 1.3 \times 10^{-4}$	DA: 2.0×10^{-8} UA: 1.0×10^{-6}	[19]
Poly (vinyl alcohol)	DA: $2.0 \times 10^{-6} \sim 7.0 \times 10^{-5}$ UA: $2.0 \times 10^{-6} \sim 5.0 \times 10^{-5}$	DA: 1.4×10^{-6} UA: 6.0×10^{-7}	[20]
Poly (dibromofluorescein)	DA: $2.0 \times 10^{-7} \sim 2.0 \times 10^{-4}$ UA: $1.0 \times 10^{-6} \sim 2.5 \times 10^{-4}$	DA: 3.0×10^{-8} UA: 2.0×10^{-7}	This work

for DA and UA were 1.8 % and 2.5 %, respectively. The same solutions were also determined with six electrodes made independently, and the interelectrode RSD for peak currents DA and UA were 2.4 % and 3.2 %, respectively.

The above results demonstrated that the proposed method had high sensitivity, wide linear range and good repeatability. The comparison of this method with other electrochemical methods for the simultaneous determination of DA and UA was listed in Table 1.

Interferences study

The influence of various foreign species on the determination of 2.0×10^{-5} mol L⁻¹ DA and UA was investigated. The tolerance limit was taken as the maximum concentration of the foreign substances that caused an approximately ± 5 % relative error in the determination. The tolerated ratio of the foreign substances was 500 for NaCl and KCl; 200 for glucose; 100 for cystine, citric acid; 50 for acetaminophen. Due to the excellent property of poly (DBF) modified GCE to substantially separate the peak potentials of AA, DA and UA, the interference from AA could easily be neglected. The high concentration of AA (2.0×10^{-3} mol L⁻¹, 100 times relative

to DA) did not result in any noticeable interference for the detection of DA and UA. Because the interference from AA for determining DA and UA was effectively eliminated, the proposed method can be used for selective detection of DA and UA in the presence of high concentration of AA.

Stability of the modified electrode

The modified electrode exhibited high storage stability when it was kept in dry state at room temperature. The relative lifetime of the modified electrode was evaluated by periodically measuring the response sensitivity of DA (2.0×10^{-5} mol L⁻¹) by differential pulse voltammetry. If the electrode lost 10 % of the initial signals, they were considered disabled. The results showed that the modified electrode can remained more than 95 % of the initial signal after 4 weeks storage, which indicated that the modified electrode was not deteriorated even for 1 month.

Analytical applications

The practical analytical utility of the modified electrode was illustrated by simultaneous determination of DA and UA in

Table 2 Simultaneous determination of DA and UA in human serum samples ($n=6$)

Sample	Original (μmolL^{-1}) UA	Added (μmolL^{-1})			Found (μmolL^{-1})		Recovery (%)	
		DA	UA	AA	DA	UA	DA	UA
1	9.26	4.0	8.0	400	3.93	17.52	98.2	101.5
2	13.10	8.0	10.0	800	7.81	22.93	97.6	99.3
3	20.85	10.0	20.0	1000	10.07	41.66	100.7	102.0

spiked human serum. The synthesized samples contained diluted 20-fold human serum, spiked interferent (high concentration of AA) and spiked various amounts of DA standard solution. The standard addition technique was used to detect DA and UA simultaneously in the presence of high concentration of AA by the proposed DPV method. The obtained results were summarized in Table 2. It can be seen that all spike recoveries were accurate and precise, which indicated the good applicability of the poly (DBF) modified electrode to simultaneous determination of DA and UA in the real biological samples.

Conclusions

In this paper, the novel poly (DBF) modified electrode had been prepared by simple and fast electropolymerization method, and used as electrochemical sensor for the simultaneous determination of DA and UA by DPV. The modified electrode had good stability, sensitivity and selectivity. The interference of AA was eliminated effectively. The method was applied to detect DA and UA simultaneously in human serum samples with satisfactory results. Hence, we believe that the poly (DBF) modified electrode can become a useful tool for the assay of DA and UA in biological samples due to its rapid speed, good precision and low cost of analysis.

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