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Electrochemical behavior of a covalently modified glassy carbon electrode with aspartic acid and its use for voltammetric differentiation of dopamine and ascorbic acid

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Abstract Aspartic acid was covalently grafted on to a glassy carbon electrode (GCE) by amine cation radical formation in the electrooxidation of the amino-containing compound. X-ray photoelectron spectroscopic (XPS) measurement and cyclic voltammetric experiments proved the aspartic acid was immobilized as a monolayer on the GCE. Electron transfer to $\text{Fe}(\text{CN})_6^{4-}$ in solution of different pH was studied by cyclic voltammetry. Changes in solution pH resulted in the variation of the charge state of the terminal group; surface p K_a values were estimated on the basis of these results. Because of electrostatic interactions between the negatively charged groups on the electrode surface and dopamine (DA) and ascorbic acid (AA), the modified electrode was used for electrochemical differentiation between DA and AA. The peak current for DA at the modified electrode was greatly enhanced and that for AA was significantly reduced, which enabled determination of DA in the presence of AA. The differential pulse voltammetric (DPV) peak current was linearly dependent on DA concentration over the range 1.8×10^{-6} – 4.6×10^{-4} mol L⁻¹ with slope (nA μmol^{-1} L) and intercept (nA) of 47.6 and 49.2, respectively. The detection limit (3δ) was 1.2×10^{-6} mol L⁻¹. The high selectivity and sensitivity for dopamine was attributed to charge discrimination and analyte accumulation. The modified electrode has been used for determination of DA in samples, in the presence of AA, with satisfactory results.

Keywords Electrocatalytic oxidation · Monolayer · Chemically modified electrode · Aspartic acid · Dopamine

Introduction

Electrochemical techniques have been proved to be advantageous in the determination of DA, especially in brain chemistry. A major problem is, however, the interference of AA, which is also present in biological fluids at very high concentrations (100–500 $\mu\text{mol L}^{-1}$) whereas dopamine (DA) levels are much smaller (< 100 nmol L⁻¹) [1]. It is known that the direct electrooxidation of DA and AA at bare electrodes is irreversible and requires high overpotentials. DA and AA are, moreover, oxidized at very similar potentials at bare electrodes [2, 3] and oxidation often suffers from a pronounced fouling effect, which results in rather poor selectivity and reproducibility. Both sensitivity and selectivity are, therefore, of equal importance in DA determination.

Several strategies aimed at overcoming the problems of AA interference have emerged [4–21]. Among these, a widely used method is to coat the electrode with an anionic film such as Nafion [4–10], which repels the negatively charged AA while attracting the positively charged DA at normal pH values and, therefore, protects the electrode surface from the AA interference. Electrodes coated with Nafion usually suffer from a slow response, however, because of the low diffusion coefficients of analytes in the film, and from memory effects because of the strong binding affinity between cations and Nafion [11, 12]. Another convenient solution is to modify electrodes with negatively charged groups such as poly(ester sulfonic acid) [13, 14], over-oxidized polypyrrole [15, 16], poly(indole-3-acetic acid) [17], polyeugenol [18], and ω -mercapto carboxylic acid [19]. Recently, Downard et al. [20] modified a carbon electrode with *p*-phenylacetate tetrafluoroborate diazonium

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salt and used it successfully for differentiation of DA and AA, and Brajter-Toth's group [21] reported the cyclic voltammetric behavior of DA at a thiotic acid and hexanethiol co-modified gold electrode and indicated that slower kinetics were observed. Very recently, we reported the electrochemical fabrication of covalently modified glassy carbon electrodes with amino acid monolayer films and their application to simultaneous/selective determination of DA and/or AA [22–26].

As a part of our study on the development of new electrochemical sensors for determination of DA and/or AA, this paper describes the preparation of aspartic acid monolayer-modified glassy carbon electrodes by electrochemical oxidation of aspartic acid to its cation radicals to form a chemically stable covalent link between the nitrogen atom of the amine group and the edge plane sites of the electrode surface. Because of its charge exclusion and accumulative properties under suitable pH conditions, the electrode was used to achieve electrochemical differentiation between DA and AA. The amine group of DA is positively charged, whereas the hydroxyl next to the carbonyl group of AA is negatively charged at neutral pH ($pK_b=8.87$ and $pK_a=4.10$, respectively). Consequently, the aspartic acid modified layer, a negatively charged film, repels the negatively charged AA and selective sensing of DA can be achieved. This suggested the potential usefulness of the modified electrodes for selective determination of DA in the presence of AA. Indeed, under optimum conditions, the modified electrode is capable of detecting micromolar concentrations of DA in the presence of millimolar levels of AA.

Experimental

Chemicals and solutions

DL-Aspartic acid was purchased from Sigma (USA). DA hydrochloride was purchased from Fluka. Ascorbic acid and acetonitrile (ACN) were obtained from the Chemical Reagent Company of Shanghai (Shanghai, China). ACN was of analytical quality and dried over 3-Å molecule sieve before use. All other reagents were analytical quality and were used as supplied. A solution of aspartic acid was prepared in absolute ACN containing 0.1 mol L^{-1} NBu_4BF_4 ; DA and AA were prepared in 0.1 mol L^{-1} phosphate buffer solution (PBS, pH 6.0) immediately before use.

Electrochemical measurements

All electrochemical experiments were performed with a CHI 832 electrochemical workstation (USA) in a conventional three-electrode electrochemical cell using glassy carbon ($\phi=3 \text{ mm}$, BAS) as the working electrode, twisted platinum wire as the auxiliary electrode, and a saturated calomel electrode (SCE) as the reference

electrode. The surface area of the GCE was determined by methylene blue adsorption [27, 28]. An average value of 0.092 cm^2 was obtained and indicated that the surface area was approximately 1.3 times more than its geometric area.

XPS measurements were performed on an ESCA-LAB-MK II spectrometer (VG, UK) with a Mo Ka X-ray radiation as the X-ray source for excitation. The data were obtained at room temperature, and typically the operating pressure in the analysis chamber was below 10^{-9} Torr with an analyzer pass energy of 50 eV. The resolution of the spectrometer was 0.2 eV. The elemental nitrogen-to-carbon ratio (N/C) can be used to assess the extent of modifier coverage. Values for N/C were calculated by dividing the total number of counts under the N(1s) band by that under the C(1s) band and multiplying the results by 100 after taking into account the different sensitivity factors [29].

All experiments were performed at room temperature (approx. 21°C). Differential pulse experiments employed a scan rate of 10 mV s^{-1} , a pulse amplitude of 25 mV, a pulse rate of 0.5 s, and a pulse width of 60 ms. In all experiments the solutions and electrodes were kept motionless and solutions were thoroughly deoxygenated by bubbling of high-purity nitrogen. A nitrogen atmosphere was maintained over the solutions.

Preparation of the modified electrode

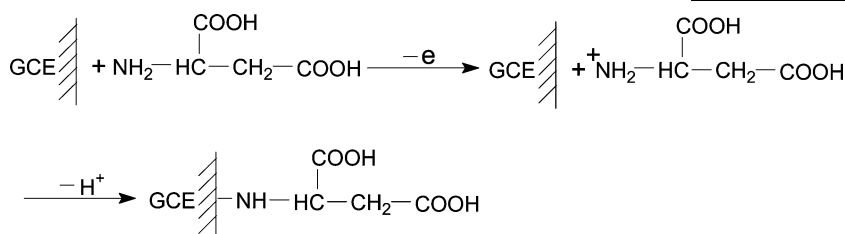
The glassy carbon electrodes (GCE) were prepared by polishing first with fine wet emery paper (grain size 4000) then with 6.0 and $1.0 \mu\text{m}$ alumina slurry on microcloth pads (Buehler, USA), to a mirror-like finish. The GCE were sonicated in water for 15 min after each polishing step. After the initial polishing the GCE were resurfaced using $1.0 \mu\text{m}$ alumina slurry only. All GCE were sonicated for 15 min in water, rinsed with water and ethanol, and dried with a stream of highly purified nitrogen immediately before use. The electrodes were then treated by cyclic scanning in the potential range 0.50–1.70 V at 20 mV s^{-1} for five cycles in $1.0 \times 10^{-3} \text{ mol L}^{-1}$ aspartic acid solution (ACN, 0.1 mol L^{-1} NBu_4BF_4). After rinsing of the electrode with ethanol and water and sonication for 15 min in 0.1 mol L^{-1} PBS (pH 6.0), to remove any physisorbed, unreacted materials from the electrode surface, the aspartic acid-modified electrode (Asp/GCE) was ready for use. The Asp/GCE was stored in 0.1 mol L^{-1} PBS (pH 6.0) at 4°C .

Results and discussion

Electrochemical modification of GCE with aspartic acid

Aspartic acid gives a single broad irreversible oxidation peak at approximately 1.32 V (Fig. 1A) on a GCE in ACN containing 0.1 mol L^{-1} NBu_4BF_4 , no cathodic peak can be observed on the reverse scan when the scan

rate was increased to 2.0 V s^{-1} , indicating that the species obtained after the first electron transfer undergoes a chemical reaction. The one-electron oxidation of the amino group turns into its corresponding cation radical, and these cation radicals can form carbon–nitrogen links at the carbon electrode surface [30–34]. The oxidation peak currents decrease quickly with successive scanning until the fifth cycle. This is attributed to passivation of the GCE, which is related to grafting of the aspartic acid on the surface of GCE [33]. The modification process was almost complete after five cycles. Deinhammar's group [34] reported modification of a GCE with an amine-containing compound and suggested that diffusion rates and steric effects are most significant factors affecting the immobilization of amine-containing compounds at the GCE surface. As a primary amine, the electrochemical behavior of aspartic acid is in agreement with the literature [34]. Accordingly, we propose the EC-modification process illustrated in Scheme 1

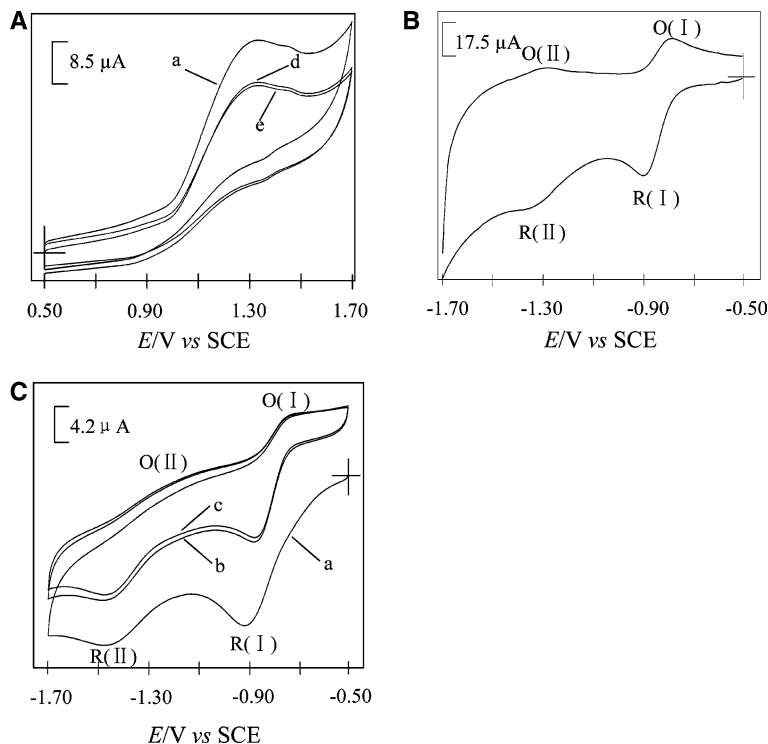


To verify that electrooxidation can immobilize aspartic acid on carbon electrode surface, XPS was used for analysis of the electrode surface by following changes

in the nitrogen content of the GCE surface as a function of anodic voltage limit. In Fig. 2, curve A is the result for a GCE treated before the onset of amine oxidation and curves B–D result from increases in the extent of amine oxidation. For comparison, curve E shows the XPS spectrum in the N(1s) region for a freshly polished but untreated GCE. The XPS for a GCE immersed in solution at open circuit is effectively the same as that of A and E—only trace levels of nitrogen are detected. In contrast, scanning to the upper limit of 0.65 V leads to an increase in nitrogen-containing species at the GCE surface ($\text{N/C}=1.04$). On scanning to more positive voltage limits, the nitrogen content increases further, reaching N/C of 2.10 at 1.00 V and 4.18 at 1.60 V. These data show that oxidation of the amine functionality can immobilize aspartic acid on the GCE surface. On the other hand, the position of the peak maximum at 398.60 eV, which is consistent with the formation of a carbon–nitrogen bond between the amine cation radical

and an aromatic moiety of the GCE surface, also verifies the immobilization of aspartic acid on the electrode surface.

Fig. 1 Cyclic voltammograms obtained from aspartic acid. Solvent: ACN. Electrode: glassy carbon. Supporting electrolyte: $0.1 \text{ mol L}^{-1} \text{ NBu}_4\text{BF}_4$. Scan rate: 20 mV s^{-1} . **A** and **B** GCE in aspartic acid solution ($1.0 \times 10^{-3} \text{ mol L}^{-1}$); **C** Asp/GCE in solution containing only ACN and $0.1 \text{ mol L}^{-1} \text{ NBu}_4\text{BF}_4$. *a*, *b*, *c*, *d*, and *e* are the first, second, third, fourth, and fifth cycles, respectively



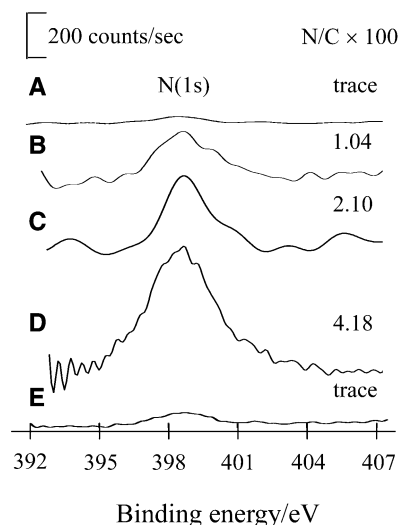


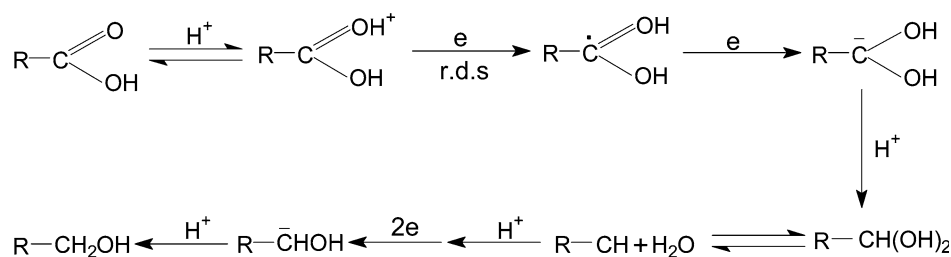
Fig. 2 XPS in the N(1s) region for freshly prepared GCE immersed in aspartic acid solution ($1.0 \times 10^{-3} \text{ mol L}^{-1}$) and held at 0.00 V for 5 min (A), or cycled for five scans at 20 mV s^{-1} between 0.00 and 0.65 V (B), 1.00 V (C), and 1.60 V (D). The spectrum in part (E) was obtained from a freshly polished GCE

Furthermore, testing of the stability of the immobilized aspartic acid layer also supported the formation of a covalent linkage. For example, after the

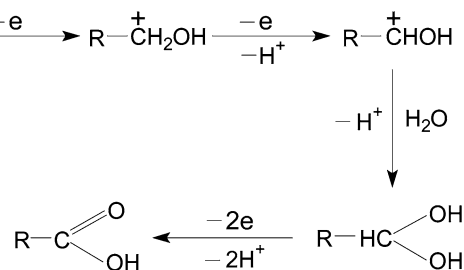
thermally desorbed under high vacuum (10^{-9} Torr) for 40 min at 110°C . After all this treatment the XPS results were not observably different from those in Fig. 2D.

To further investigate the structure of the modification layer, the surface density of amines on the electrode surface was also studied by cyclic voltammetry. Oxidation of the amino group with a carbon electrode should enable the molecules to be bonded to the carbon. When the molecule was bonded it should be possible to observe its reduction. From the area of the reduction peak of the voltammogram one can deduce the number of molecules, which were bonded. The oxidation and reduction voltammograms of aspartic acid at GCE are shown in Figs. 1A and 1B, respectively. It is apparent the electrochemical oxidation of aspartic acid is a mono-electron and irreversible process, whereas the reduction gives two pairs of reversible redox waves (R(I)/O(I) and R(II)/O(II)) with ΔE_p of 0.10 and 0.040 V, respectively. The cathodic wave R(I) at -0.90 V and the anodic wave O(I) at -0.80 V are attributed to reduction and re-oxidation of the carboxyl functionality in the molecules of aspartic acid bonded to the electrode surface [35, 36]. In accordance with the literature [37], we can expect the mechanism of the redox process to be a four-electron process as depicted in Scheme 2.

I. Reduction process



II. Re-oxidation process



GCE were treated as described above they were sonicated for 15 min in a variety of solutions, including water, ethanol, and pH 6.0 PBS, and then

The other pair of redox waves R(II)/O(II), observed at -1.34 and -1.30 V, respectively, correspond to reduction and reoxidation of amine groups in the mol-

ecules of aspartic acid bonded to the electrode surface by a monoelectron process [33]. After binding of aspartic acid to the GCE, the aspartic acid-modified electrode was carefully rinsed in an ultrasonic cleaner and transferred to ACN solution containing 0.1 mol L^{-1} NBu_4BF_4 . The reduction voltammogram of Fig. 1C was then observed. It is apparent that most of the reversibility was lost and that with successive scans the voltammograms were slowly drawn out; increasing the scan rate from 0.02 to 1.0 V s^{-1} slowly reduced the decrease of voltammogram. The loss of reversibility and the disappearance of the voltammogram may be ascribed to protonation of the radical anion by residual water. From the area of the first voltammogram the number of molecules which had undergone monoelectron reduction can be deduced. Knowing the surface area of the electrode a concentration of $1.2 \times 10^{-9} \text{ mol cm}^{-2}$ can be calculated, indicating monolayer surface coverage [27, 38].

Blocking effect of Asp/GCE on the electrochemical behavior of redox probes

$\text{Fe}(\text{CN})_6^{4-}$ and $\text{Ru}(\text{NH}_3)_6^{3+}$, two oppositely charged redox probes, have been used to investigate the effect of

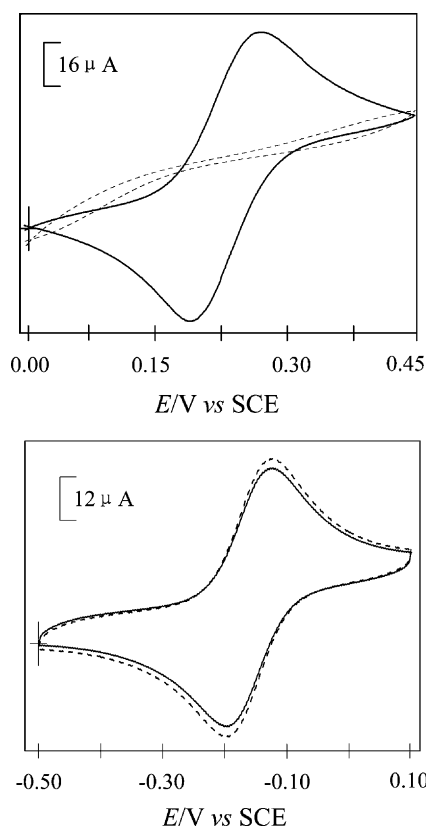


Fig. 3 Cyclic voltammograms obtained from $2.0 \times 10^{-3} \text{ mol L}^{-1}$ $\text{Fe}(\text{CN})_6^{4-}$ (top) and $\text{Ru}(\text{NH}_3)_6^{3+}$ (bottom) in 0.1 mol L^{-1} PBS (pH 7.0) at the GCE (solid line) and the Asp/GCE (dashed line). Scan rate: 50 mV s^{-1}

the aspartic acid film on their electrochemical behavior. Figure 3 shows the cyclic voltammograms obtained from $\text{Fe}(\text{CN})_6^{4-}$ (top) and $\text{Ru}(\text{NH}_3)_6^{3+}$ in neutral aqueous solution at the GCE (solid line) and the Asp/GCE (dashed line). It is apparent that electron transfer of $\text{Fe}(\text{CN})_6^{4-}$ is nearly completely blocked at the Asp/GCE whereas the electrochemical behavior of $\text{Ru}(\text{NH}_3)_6^{3+}$ at the Asp/GCE is almost unchanged compared with that at the GCE, indicating no blocking effects occur at Asp/GCE. The CV results show that the selective response of Asp/GCE to $\text{Ru}(\text{NH}_3)_6^{3+}$ is directly related to both solution pH and probe charge. This can be ascribed to the electrostatic environment at the film–solution surface [21, 39–42]. In neutral solution the surface carboxyl group of aspartic acid is expected to fully dissociate, assuming that its pK_a is near to that of aspartic acid ($pK_a = 2.9$). Thus, on the negatively charged aspartic acid film, $\text{Ru}(\text{NH}_3)_6^{3+}$ should be able to accumulate on the electrode surface, which is consistent with the observation that traces of $\text{Ru}(\text{NH}_3)_6^{3+}$ can be accumulated on the $-\text{COO}^-$ terminated electrode surface [20, 43]. Response of $\text{Fe}(\text{CN})_6^{4-}$ was not observed, however. This indicates that a negative Donnan potential must be established at the film surface as a result of high negative charge density of the $-\text{COO}^-$ group [20, 21, 44, 45]. Electrostatic repulsion thus resists access of $\text{Fe}(\text{CN})_6^{4-}$ to the electrode surface.

To further confirm the electrostatic interaction, $\text{Fe}(\text{CN})_6^{4-}$ was used to investigate the electrochemical properties of the monolayer as a function of solution pH. As shown in Fig. 4, CV reversibility for $\text{Fe}(\text{CN})_6^{4-}$ is better at pH = 1.0–2.5 ($\Delta E_p < 80 \text{ mV}$) and is similar to that at the GCE. Under these conditions the surface carboxyl groups of the aspartic acid are neutral and so the modified film does not prevent $\text{Fe}(\text{CN})_6^{4-}$ from gaining access to the Asp/GCE surface and undergoing electron transfer. With increasing pH the $\text{Fe}(\text{CN})_6^{4-}$ peak current decreases and the peak separation increases markedly, because of increasing dissociation of the carboxyl functionalities and, therefore, the increasing negative charge on the Asp/GCE surface. Because I_p and ΔE_p change with solution pH, they can be used to

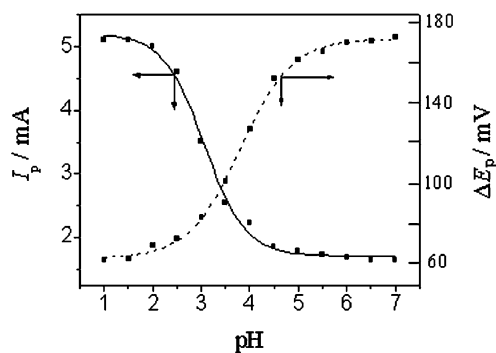


Fig. 4 Plots of I_p (solid line) and ΔE_p (dashed line) as a function of solution pH for cyclic voltammograms of $2 \times 10^{-3} \text{ mol L}^{-1}$ $\text{Fe}(\text{CN})_6^{4-}$ at the Asp/GCE. Scan rate: 50 mV s^{-1}

determine the acid dissociation constant of the $-\text{COOH}^-$ head groups of aspartic acid in the monolayer [38, 45]. From the relationship between I_p or ΔE_p and the corresponding solution pH, the surface pK_a of the aspartic acid film grafted on to the GCE surface can be estimated to be approximately 3.3 and 3.8. These results are higher than that for aspartic acid in solution ($pK_a = 2.9$), which is in agreement with results for alkanethiol SAM bearing dissociable terminal groups—the surface pK_a values of the SAM terminal groups are higher than those of the corresponding molecules in solution [21, 46, 47]. The higher pK_a determined for the carboxyl groups in the monolayer indicates that the surface environment affects the pK_a . As previously proposed, the higher surface pK_a may be because of hydrogen-bonding stabilization of the acid form of the $-\text{COOH}$ groups in the monolayer [48]. Our result is not in line with that of Saby [40], however, who determined the surface pK_a of a 4-carboxyphenyl film on a modified GCE ($pK_a = 2.8$). This pK_a is smaller than the value expected for benzoic acid in solution ($pK_a = 4.2$) [46]. Saby proposed that a specific interfacial effect between the carbon surface and the carboxylate functionality or the phenyl ring of the layer might play an important role.

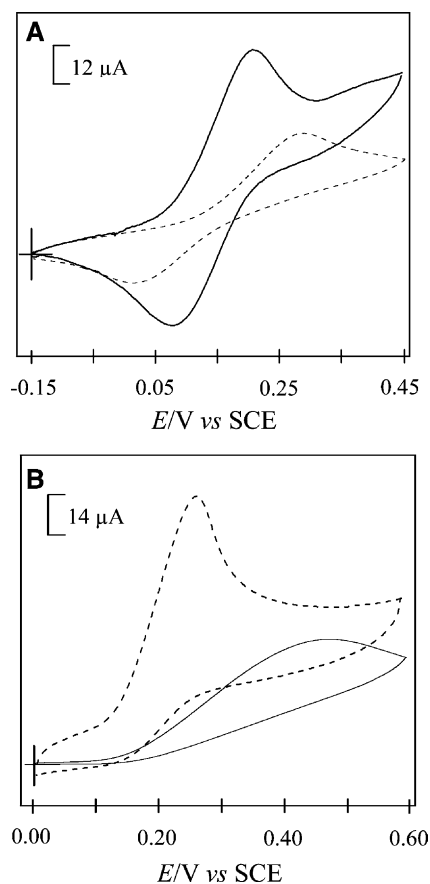


Fig. 5 Cyclic voltammograms obtained from 1.0×10^{-4} mol L^{-1} DA (**A**) and 1.0×10^{-3} mol L^{-1} AA (**B**) in 0.1 mol L^{-1} PBS (pH 6.0) at the GCE (*dashed line*) and the Asp/GCE (*solid line*). Scan rate: 50 mV s^{-1}

Electrochemical responses of DA and AA at Asp/GCE

The cyclic voltammograms of DA and AA at the GCE and Asp/GCE are shown in Figs. 5A and 5B, respectively. As shown in Fig. 5A, a much broader and smaller CV peak response with a ΔE_p of 0.28 V is observed for DA at the GCE (*dashed line*) whereas the Asp/GCE leads to an obvious increase in CV peak current response and more reversible behavior with a ΔE_p of 0.12 V (*solid line*). It is, however, clearly apparent from Fig. 5B that AA gives a much smaller oxidation wave at 0.46 V with an $E_p - E_{p/2}$ of 0.17 V at the Asp/GCE (*solid line*) compared with that ($E_{p,a} = 0.26 \text{ V}$, $E_p - E_{p/2} = 0.058 \text{ V}$) at the GCE (*dashed line*). The oxidation wave of DA is shifted to less positive potential ($E_{p,a} = 0.16 \text{ V}$) and AA is oxidized at more positive potentials at the Asp/GCE, because of a kinetic effect, and thus a substantial increase in the rate of electron transfer from DA is observed. This is attributed to the improved reversibility of the electron-transfer processes. This shift can be attributed to the more favorable interactions between DA and the negatively charged monolayer. At the same time, the rate of electron transfer from AA is reduced as a result of the introduction of the negatively charged monolayer (at pH 6.0, the acid groups on the surface of monolayer are deprotonated and negatively charged, the hydroxyl next to the carbonyl group of AA is also negatively charged ($pK_a = 4.10$), whereas the amino group of DA is positively charged ($pK_b = 8.87$)).

The transport characteristics of DA at the modified electrode were investigated further. The linear scan voltammetric (LSV) current response for DA at the Asp/GCE was found to be linearly proportional to the scan rate in the range from 20 to 280 mV s^{-1} , which is an indication of adsorption behavior. More evidence for the adsorption of DA was demonstrated by the following experiments. When the Asp/GCE was switched to a medium containing only 0.1 mol L^{-1} PBS (pH 6.0) after being used to measure a DA solution, almost the same voltammetric peak signal was observed.

Chronocoulometry was used to determine the concentration of DA adsorbed on the electrode surface. The charge is given by integrated Cottrell equation [49]:

$$Q_{\text{total}} = 2\pi^{-(1/2)} nFA C_0 D_0^{1/2} t^{1/2} + nFA\Gamma_0 + Q_{\text{dl}}$$

where n is the number of electrons, F the Faraday constant, A the electrode area, C_0 the bulk concentration of analyte, D_0 the diffusion coefficient of analyte in solution, t the time, Γ_0 the surface concentration of analyte, and Q_{dl} is the double-layer charge.

The first term of the right-hand side represents the charge of oxidation of the species under diffusion control. The second term is charging of surface-attached species. And the last is the double-layer charge. This equation shows that a plot of Q_{total} against $t^{1/2}$ should be a straight line with an intercept equal to $(nFA\Gamma_0 + Q_{\text{dl}})$. Because Q_{dl} can be easily measured in a separate experiment in the supporting electrolyte alone, the con-

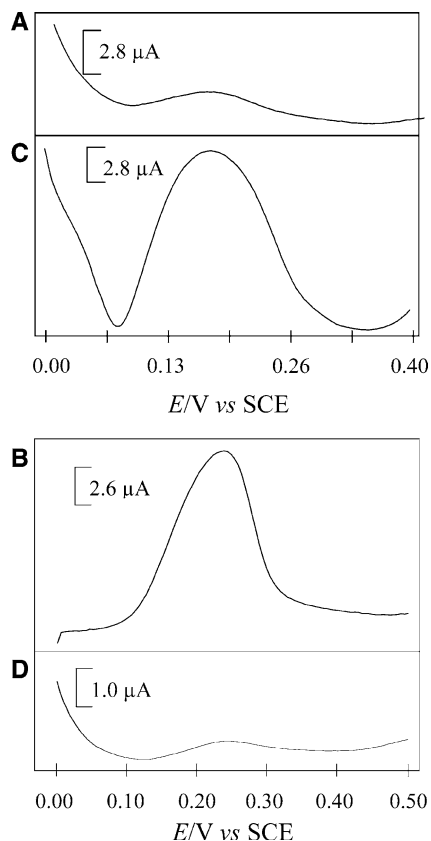


Fig. 6 Differential pulse voltammograms in 0.1 mol L⁻¹ PBS (pH 6.0) obtained for 2.0 × 10⁻⁵ mol L⁻¹ DA (A) and (C) and for 2.0 × 10⁻³ mol L⁻¹ AA (B) and (D) at the GCE (A) and (B) and the Asp/GCE (C) and (D)

tribution of the adsorbed species can be determined and Γ_0 calculated. In the case studied Γ_0 was found to be 1.48 × 10⁻¹⁰ mol cm⁻². Such a value is indicative of monolayer accumulation of DA on/inside the film [18].

Figure 6 shows the differential pulse voltammograms for oxidation of 2.0 × 10⁻⁵ mol L⁻¹ DA and 2.0 × 10⁻³ mol L⁻¹ AA in 0.1 mol L⁻¹ PBS (pH 6.0) at the GCE and the Asp/GCE, respectively. As shown in this figure, the sensitivity to DA increases about ninefold at the Asp/GCE and the response to AA is dramatically suppressed. This property suggests that the Asp/GCE is efficient for voltammetric differentiation between DA and AA.

Effect of pH

The pH range is very critical to the characteristics of DA and AA and also of the modified layer, so the effect of pH was studied in detail. Results show that the peak potential for DA oxidation varies linearly with pH and is shifted to more negative potentials with a slope of -0.057 V per pH unit (not shown). The peak current has very interesting non-linear behavior as a function of pH, as illustrated in Fig. 7A. Initially, the response increases continually as pH increases from 2.0 to 5.0. This is

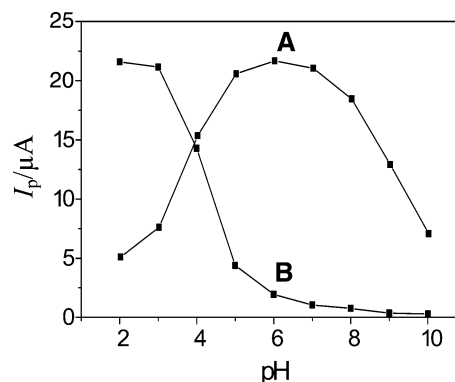


Fig. 7 Dependence on solution pH of the peak current of DA (A) and AA (B) at the Asp/GCE. Conditions as for Fig. 6

probably because of increasing dissociation of surface-attached acid groups and, therefore, a simultaneous increase in electrostatic attraction occurring between DA and the electrode surface. Note that up to pH 8.0 DA occurs in its protonated form and is, therefore, readily preconcentrated on negatively charged coatings. Between pH 5.0 and 7.0, the peak current changes slightly. It then decreases with increasing pH as DA loses protons and becomes a neutral species. A different sensitivity profile is observed for AA, however (Fig. 7B). The peak current is highest in the most acidic solution and then decreases, at first slowly and then dramatically at approximately the pK_a value of AA (4.10). In more basic solutions, AA is an anion and is repelled from the electrode, and the current is approximately one order of magnitude smaller than that at pH < 4.0. Solution of pH 6.0 was therefore selected for determination of DA.

Analytical characterization

Under optimum conditions, using the differential pulse mode, the peak current is linearly related to DA concentration from 1.8 × 10⁻⁶ mol L⁻¹ to 4.6 × 10⁻⁴ mol L⁻¹ in the presence of 1.0 × 10⁻³ mol L⁻¹ AA. For the regression plot of $i_{p,DA}$ against DA concentration the slope is 47.6 nA μmol⁻¹ L, the y-intercept is 49.2 nA, and correlation coefficient (r^2) is 0.997. The detection limit (3δ) for DA is 1.2 × 10⁻⁶ mol L⁻¹.

To characterize the reproducibility of the modified electrode, replicate measurements were performed for a solution of 2.0 × 10⁻⁵ mol L⁻¹ DA in the presence of 1.0 × 10⁻³ mol L⁻¹ AA. A coefficient of variation of 2.6 was obtained for results from 11 successive measurements.

As an example of the analytical performance for the modified electrode, mixtures of DA and AA were analyzed. Dopamine hydrochloride injection (DHI) solution (10 mg DA per milliliter, 2 mL per injection) was diluted to 10 mL with water, 50 μL of the DHI solution and an appropriate amount of AA standard solution (0.025 mol L⁻¹) were added to a series of 10-mL grad-

Table 1 Determination of DA in the presence of AA^a

No.	DA content (10 ⁻⁵ mol L ⁻¹)	AA content (10 ⁻³ mol L ⁻¹)	DA concn found (10 ⁻⁵ mol L ⁻¹)	Relative error (%)
1	5.30	0.25	5.38	1.51
2	5.30	1.25	5.44	2.64
3	5.30	2.50	5.56	4.91
4	5.30	7.50	5.68	7.16
5	5.30	12.5	5.80	9.43

^aNumber of samples assayed, 5

uated flasks and the solutions were diluted to volume with pH 6.0 PBS. These solutions (5.0 mL) were pipetted into the electrochemical cell and the concentrations of DA were determined by the calibration method. The results are shown in Table 1. The good agreement with the standard content is a promising feature for the applicability of the modified electrode for determination of DA in the presence of a high concentration of AA.

Electrode stability and reversibility

Because the electrode-preparation procedure is easy and rapid, it is not very important for the electrode to be stable for a prolonged time. However, we checked its long-term stability by measuring the response from day to day during storage in PBS (pH 6.0) at 4°C. It was found that the current response decreased by approximately 8% of its initial response in 1 day, by 20% in 5 days, and by 25% in 1 month.

For practical work, electrode reversibility is required. It must be possible to use it many times in samples containing different concentrations of analyte. Because of the preconcentration effect, however, the electrode transferred from DA-containing medium to analyte-free medium retained much of its response, which will affect subsequent measurements. It was found, however, that the renewal of the film is easily accomplished by soaking the electrode in PBS (pH 6.0) and cycling its potential over the range 0.0 to 1.6 V (0.1 V s⁻¹). Depending on DA concentration, 30–50 scans are sufficient for efficient renewal. There was only a small difference between the DA peak potentials of new and renewed electrodes (0.008 V shift in the positive direction after 15 renewals).

Conclusions

Glassy carbon electrodes modified with aspartic acid by an electro-oxidation procedure have been characterized by cyclic voltammetry and XPS. Such modified electrodes have very effective exclusion properties for anionic AA with preferential uptake of cationic DA; this has been used for determination of DA in the presence of excess AA.

Importantly, this process simplifies the fabrication of modified electrodes, a process which often involves a

series of pretreatment, activation, and functionalization steps. Also, compared with other modification procedures needed to pretreat carbon electrodes, this process does not damage the surface structure of the carbon electrode and results in a small background current. (Electrochemical or chemical oxidation may damage the carbon surface, which leads to greatly increased background currents [50].)

A practical application of the aspartic acid-modified electrodes was illustrated by determination of the pK_a of the surface carboxyl groups. The method may provide information about the surface structure complementary to that obtained with other techniques, for example use of the electrochemical quartz-crystal microbalance (EQCM) [48].

Finally, the aspartic acid monolayer film with hydrophilic carboxylic acid groups is expected to be useful in sensor design. It is known that the carbon electrode has a wide potential window which is suitable for studying the electrochemical behavior of the redox compounds whose redox reactions occur at negative potential. Taking into account the stability of the monolayer, modified at the surface by the electrooxidation procedure, the Asp/GCE can be used as a suitable charged substrate for fabrication of multilayer films by layer-by-layer assembly based on electrostatic attraction. This is in progress in our laboratory.

With its low cost and easy of preparation, the aspartic acid-modified electrode seems to be of great utility for further sensor development.

References

- O'Neill RD (1994) *Analyst* 119:767
- Wang J, Hutchins LD (1985) *Anal Chim Acta* 167:325
- Deakin MR, Kovach PM, Stutts KJ, Wightman RM (1986) *Anal Chem* 58:474
- Kristensen EW, Kuhr WG, Wightman RM (1987) *Anal Chem* 59:1752
- Feng J, Brazell M, Renner K, Kasser R, Adams RN (1987) *Anal Chem* 59:1863
- Capella P, Ghasemzadeh B, Mitchell K, Adams RN (1996) *Electroanalysis* 2:175
- Gerhardt GA, Oke AF, Nagy G, Moghaddam B, Adams RN (1984) *Brain Res* 290:390
- Zen JM, Chen PJ (1997) *Anal Chem* 69:5087
- Ju H, Ni J, Gong Y, Chen HY, Leech D (1999) *Anal Lett* 32:2951
- Pihel K, Walker QD, Wightman RM (1996) *Anal Chem* 68:2084
- Guadajupe AR, Abruna HD (1985) *Anal Chem* 57:142
- Wang J, Peng T (1986) *Anal Chem* 58:3257
- Lau YY, Chien JB, Wong DKY, Ewing AG (1991) *Electroanalysis* 3:87
- Niwa O, Morita M, Tabei H (1994) *Electroanalysis* 6:237
- Zhang X, Ogorevc B, Tavcar G, Svegl IG (1996) *Analyst* 121:1817
- Hsueh C, Brajter-Toth A (1994) *Anal Chem* 66:2458
- Yu A, Sun D, Chen HY (1997) *Anal Lett* 30:1643
- Ciszewski A, Milczarek G (1999) *Anal Chem* 71:1055
- Malem F, Mandler D (1993) *Anal Chem* 65:37
- Downard AJ, Roddick AD, Bond AM (1995) *Anal Chim Acta* 317:303

21. Cheng Q, Brajter-Toth A (1995) *Anal Chem* 67:2767
22. Lin XQ, Zhang L (2001) *Anal Lett* 34:1585
23. Zhang L, Lin XQ (2001) *Anal Bioanal Chem* 370:956
24. Zhang L, Lin XQ (2001) *Analyst* 126:367
25. Zhang L, Sun Y (2001) *Anal Sci* 17:939
26. Zhang L, Sun Y, Lin XQ (2001) *Analyst* 126:1760
27. Cheng L, Liu J, Dong SJ (2000) *Anal Chim Acta* 417:133
28. Laviron E (1967) *Bull Soc Chim Fr* 3717
29. Scofield JH (1976) *J Electron Spectrosc Related Phenom* 8:129
30. Downard AJ, Mohamed A (1999) *Electroanalysis* 11:418
31. Masui M, Sayno H, Tsuda Y (1968) *J Chem Soc B* 973
32. Barnes KK, Mann CK (1967) *J Org Chem* 32:1474
33. Barbier B, Pinson J, Desarmot G, Sanchez M (1990) *J Electrochem Soc* 137:1757
34. Deinhammer RS, Mankit H, Anderegg JW, Marc P (1994) *Langmuir* 10:1306
35. Luo H, Shi Z, Li N, Gu Z, Zhuang Q (2001) *Anal Chem* 73:915
36. Dai H, Shiu KK (1996) *J Electroanal Chem* 419:7
37. Baizer MM, Lund H (1983) *Organic Electrochemistry*, Marcel Dekker Inc. New York, pp379–507
38. Cheng Q, Brajter-Toth A (1996) *Anal Chem* 68:4180
39. Cheng Q, Brajter-Toth A (1992) *Anal Chem* 64:1998
40. Saby C, Ortiz B, Champagne GY, Belanger D (1997) *Langmuir* 13:6805
41. Molinero V, Calvo EJ (1998) *J Electroanal Chem* 445:17
42. Madoz J, Kuznetsov BA, Medrano FJ, Garcia JL, Fernandez VM (1997) *J Am Chem Soc* 119:1043
43. Downard AJ, Roddick AD (1995) *Electroanalysis* 7:376
44. Redepenning J, Tunison HM, Finklea HO (1993) *Langmuir* 9:1404
45. Liu J, Cheng L, Lin B, Dong SJ (2000) *Langmuir* 16:7471
46. Petrov JG, Mobius D (1996) *Langmuir* 12:3650
47. Doblhofer K, Figura J, Fuhrhop J (1992) *Langmuir* 8:1811
48. Wang J, Frostman LM, Ward MD (1992) *J Phys Chem* 96:5224
49. Kissinger PT, Heineman WR (1996) *Laboratory techniques in electroanalytical chemistry*, Marcel Dekker, New York (Chapter 3)
50. Nagaoka T, Yoshino T (1986) *Anal Chem* 58:1037