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ORIGINAL PAPER

Voltammetric studies of a novel bicopper complex modified glassy carbon electrode for the simultaneous determination of dopamine and ascorbic acid

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Abstract A novel modified glassy carbon electrode (GCE) with a binuclear copper complex was fabricated using a cyclic voltammetric method in phosphate buffer solution. This modified electrode shows very efficient electrocatalytic activity for anodic oxidation of both dopamine (DA) and ascorbic acid (AA) via substantial decrease in anodic overpotentials for both compounds. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) using this modified electrode show two well-resolved anodic waves for the oxidation of DA and AA in mixed solution, which makes it possible for simultaneous determination of both compounds. Linear analytical curves were obtained in the ranges 2.0–120.0 μM and 5.0–160.0 μM for DA and AA concentrations by using DPV methods, respectively. The detection limits were 1.4×10^{-6} M of DA and 2.8×10^{-6} M of AA. This electrode was used for AA and DA determinations in medicine and foodstuff samples with satisfactory results.

Keywords Binuclear copper complex · Modified electrode · Determination · Dopamine · Ascorbic acid

1 Introduction

Dopamine is an important neuron-transmitter compound widely distributed in the brain for message transfer in the mammalian central nervous system. Low levels of DA are related to neurological disorders, such as schizophrenia, Parkinson's disease and to HIV infection [1, 2]. Therefore, determination of DA has attracted much attention from researchers. Up to now, the ability to detect DA with high selectivity and sensitivity is still a major target of electroanalytical research. The major problem encountered in detecting DA is interference from ascorbic acid (AA), a vital vitamin in the human diet, which largely coexists with DA in brain issue and has an overlapping oxidation potential on solid electrodes. Consequently, it is very difficult to determine DA directly. In order to solve this problem, chemically modified electrodes are widely used to eliminate the interference of AA with the determination of DA [3–7]. Many mixed valent compounds such as oxids, complexes, or alloys of copper [8–10], cobalt [11–13] and ruthenium [14–16] have shown electrocatalytic properties. Transition metal complexes as catalysts have received much attention because of their almost exclusive biological function in living systems. However, the literature for analytical applications by copper complex modified electrodes is still limited by the instability caused by the changed structure during the redox process [12, 17–19]. Our group has synthesized a novel five-coordinated binuclear copper (II) complex ($[L_2Cu_2biPy](ClO_4)_4$), which was self-assembled with dipyridine, Cu^{2+} center and tripodal polyamine, 2-[bis(2-aminoethyl)amino]ethanol (L). The ligand L with appropriate flexibility provided enough variability to adapt the different configuration requirement of Cu^{2+}/Cu^{+} in the redox process. The present study focuses on the fabrication of this newly synthesized dicopper complex on a glassy carbon electrode to develop a novel sensor for selective and sensitive detection of DA and AA

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simultaneously. The ability to determine DA and AA in a mixture is of significance in biological and chemical research.

2 Experimental

2.1 Apparatus

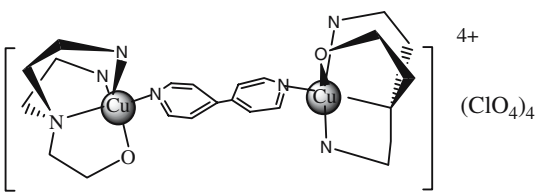
All electrochemical experiments including cyclic voltammetry and differential pulse voltammetry were carried out with a CHI 660A electrochemistry workstation (CHI, USA) connected to a Pentium 200 MHz PC. A conventional three-electrode electrochemical system was used for all electrochemical experiments, which consisted of a working electrode, a platinum wire auxiliary electrode and a saturated calomel reference electrode (SCE). A glassy carbon disk electrode of formal surface area 0.071 cm^2 was used as the working electrode. All potentials reported are versus SCE.

2.2 Chemicals and solutions

The ligand dipyridine was obtained from Aldrich. Dicopper complex was prepared according to the literature [20]. Its structure is shown in Scheme 1. Dopamine hydrochloride was obtained from Sigma (USA). Ascorbic acid was obtained from Chemical Reagent Company of Shanghai (China). All other chemicals were of analytical grade. DA and AA were prepared in water. Phosphate-buffer solutions (PBS) of different pH were prepared by mixing four stock solutions of 0.2 M H_3PO_4, KH_2PO_4, K_2HPO_4 and K_3PO_4. All aqueous solutions were prepared in double distilled, deionized water. High purity nitrogen was used for deaeration.

2.3 Preparation of binuclear copper complex modified GCE

The bare GCE was polished successively with 1.0, 0.3 and 0.05 μm aluminum oxide powder on chamois leather. Then it was rinsed with doubly distilled water and sonicated in ethanol and doubly distilled water for 5 min. After cleaning, the electrode was firstly electrochemically pretreated in 0.1 M H_2SO_4 by scanning the electrode repeatedly between –1.0 V and 1.8 V at 100 mV s^{-1} for 15 min. Then the electrode was treated in PBS (pH 5.0) containing 0.1 mM $[L_2Cu_2biPy]$ $(ClO_4)_4$ complex by scanning between –0.5 and 0.6 V at a rate of 100 mV s^{-1} for 7 cycles. After the modification, the electrode was successively rinsed with ethanol and distilled water and sonicated for 15 min in water to remove any physically adsorbed materials. The binuclear copper complex modified GCE was stored at 4 °C in a refrigerator after use. This electrode was denoted as CuLB/GCE.



Scheme 1 Structure of $[L_2Cu_2biPy]$ $(ClO_4)_4$ complex

3 Results and discussion

3.1 Electrochemical modification of binuclear copper complex on GCE

The modification was conducted under cyclic voltammetric conditions, as shown in Fig. 1A. It can be seen from the figure that the dicopper complex exhibited two anodic and cathodic peaks corresponding with two redox systems of the metal center [21, 22]. The growth of the modified film was observed by monitoring the increase in the charge attributed to the two-redox couples. The CV almost reached a steady state after 7 cycles. The two-redox peak remained constant after the modified electrode was subjected to sonication in PBS for 15 min, showing that the dicopper complex residues have immobilized on the GCE surface (Fig. 1B).

3.2 Electrocatalytic oxidation of single DA and AA

Figure 2 shows the cyclic voltammograms of AA and DA at the surface of unmodified (curve b) GCE and CuLB modified GCE (curve a) in pH 6.9 PBS. As can be seen in Fig. 2A, the direct oxidation of AA at the surface of the unmodified electrode shows a relatively broad and irreversible anodic wave with a peak potential around 0.62 V (dashed line). On the other hand, at the surface of modified electrode the oxidation peak shifted to 0.25 V with well-defined peak shape and a considerable enhancement in the peak current. The 370 mV negative shift and enhanced current of the anodic peak indicates that the CuLB modified GCE has a strong catalytic effect on the AA oxidation. The anodic peak current was proportional to the square root of scan rate in the range 40–200 mV s^{-1}

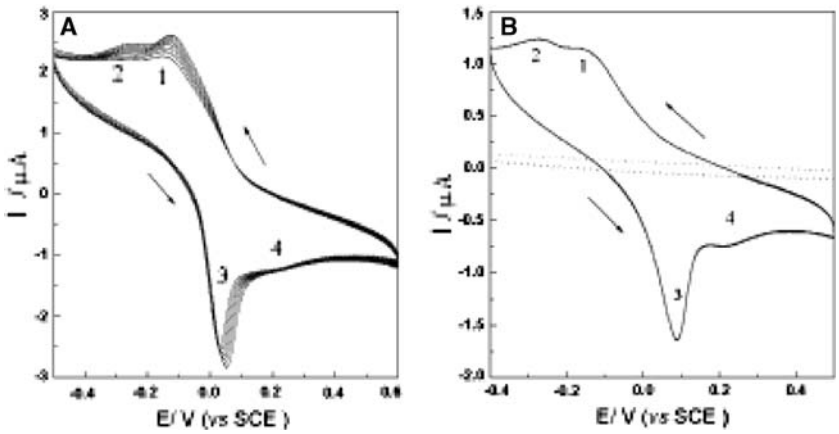


Fig. 1 (**A**) The multi-cycle CVs of GCE in PBS (pH 5.0) containing 0.1 mM $[L_2Cu_2biPy](ClO_4)_4$. (**B**) Cyclic voltammogram of CuLB/GCE in PBS solution without dicopper complex. (Dashed curve indicates bare GC electrode) Scan rate 100 mV s^{-1}

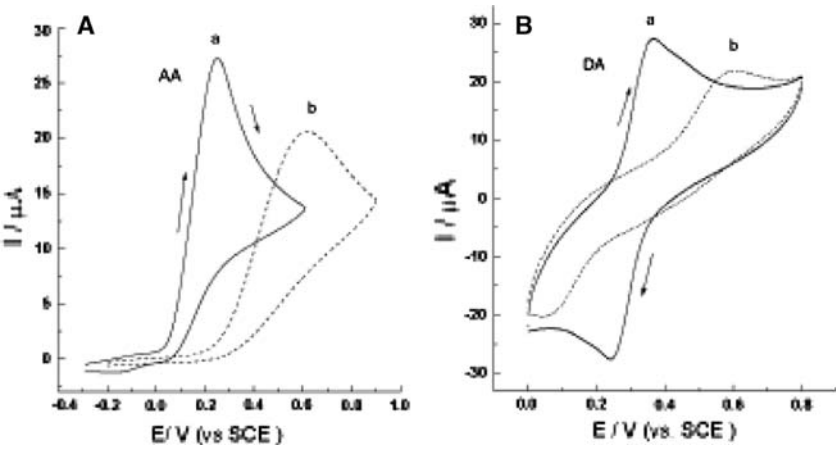


Fig. 2 CVs of 0.05 mM AA and 0.08 mM DA at CuLB/GCE (a) and bare GCE (b) in 0.2 M PBS (pH = 6.9). Scan rate: 100 mV s^{-1}

($i_{pa}(\mu A) = 1.419 + 2.613v^{1/2}$(mV s^{-1}), r = 0.9995), showing diffusion-controlled kinetics [23].

As can be seen in Fig. 2B, the cyclic voltammogram of dopamine in pH 6.9 PBS at the surface of a bare GCE showed quasi-reversible behavior and a much smaller CV peak response with a ΔEp of 0.56V. But at the surface of CuLB/GCE, the peak current increased greatly and the anodic peak potential shifted negatively. The voltammograms show a pair of reversible redox peaks with a ΔEp of only 0.13 V, indicating the increased reaction reversibility at CuLB/GCE. Also, the anodic peak current was proportional to the square root of scan rate in the range 5–200 mV s^{-1} ($i_{pa}(\mu A) = 0.849 + 0.724v^{1/2}$(mV s^{-1}), r = 0.9989), showing diffusion-controlled kinetics. In the above two experiments we found that the current responses due to AA and DA electrooxidation had an approximately 5% decrease after six scan, then the current responses reached to steady state. The second cycles of the CVs are presented in Figs. 2 and 3.

3.3 Electrocatalytic oxidation of DA and AA

DA and AA coexist in the extra cellular fluid of the central nervous system and serum. Since they have similar oxidation potential at most solid electrodes, separate determination of these species is a major problem due to their overlapped signals. In order to establish a sensitive and selective method for the quantification of DA and AA, the CuLB/GCE was studied. Figure 3 shows the CV and DPV response of DA and AA in mixture solution at CuLB/GCE in comparison with that at a bare GCE. Figure 3 (A) shows that the AA and DA responses were resolved into two well separated CV peaks at 0.30 V and 0.48 V at CuLB/GCE (curve a), and one broad and overlapped anodic peak at about 0.64 V at the bare GCE (curve b). However, as shown in Fig. 3 B, much better resolved peaks were obtained by the DPV technique, which gave two peaks at 0.24 and 0.45 V with almost flat base-line for the oxidation of AA and DA

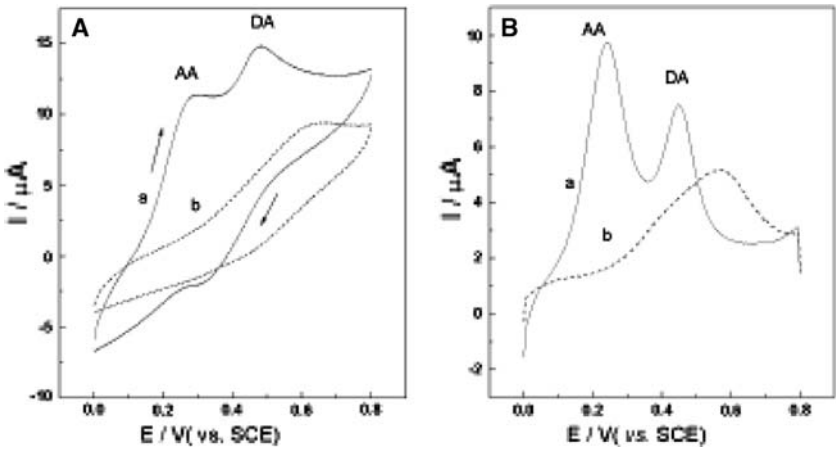


Fig. 3 (**A**) CVs of a mixture of 0.01 mM AA and 0.02 mM DA in PBS (pH 6.9) at CuLB/GCE (a) and a bare GCE (b). Scan rate 100 mV s^{-1}. (**B**) DPV of 0.01 mM AA + 0.02 mM DA at CuLB/GCE (a) and a bare GCE (b) in PBS (pH 6.9). Amplitude, 50 mV; pulse width, 50 ms; pulse period, 200 ms

respectively. The 210 mV peak separation was even larger than the separation of the CV peak, which were large enough to determine AA and DA individually and simultaneously.

3.4 Effect of pH on the oxidation of DA and AA in mixture

Figure 4 shows the pH effect on the DPV peak current in the pH range 3–8. The current maximum appeared at pH 6.9 for both the DA and AA determinations. Further increase of pH caused a slight decrease of the peak currents. Therefore, the optimum solution pH selected was 6.9. In addition, all the anodic peak potentials for the oxidation of AA and DA shifted negatively with increase in pH, demonstrating that protons participated in the electrode reactions.

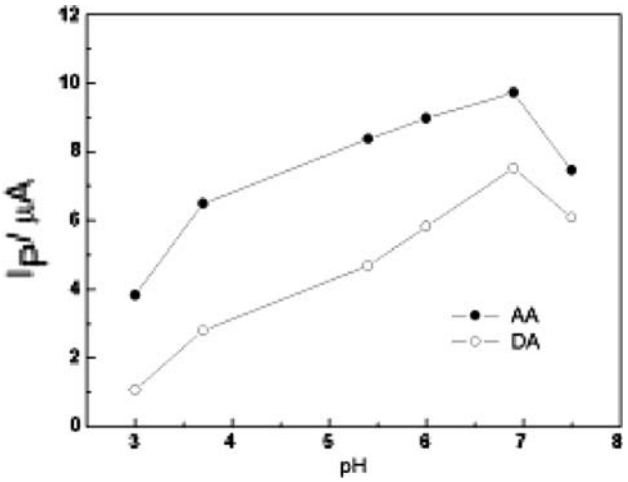


Fig. 4 Effect of pH on the peak current for the oxidation of AA and DA. Concentrations: AA, 0.01 mM, DA, 0.02 mM; scan rate: 100 mV s^{-1}

3.5 Simultaneous determination of DA and AA

Figure 5 A gives the DPV recordings at various AA concentrations with 70 μM DA under the optimized conditions at the CuLB/GCE. From Fig. 5, it can be seen that the peak current of AA increased with increase in AA concentration when the concentration of DA was kept constant. Similarly, as shown in Fig. 6, when the concentration of AA remained constant the

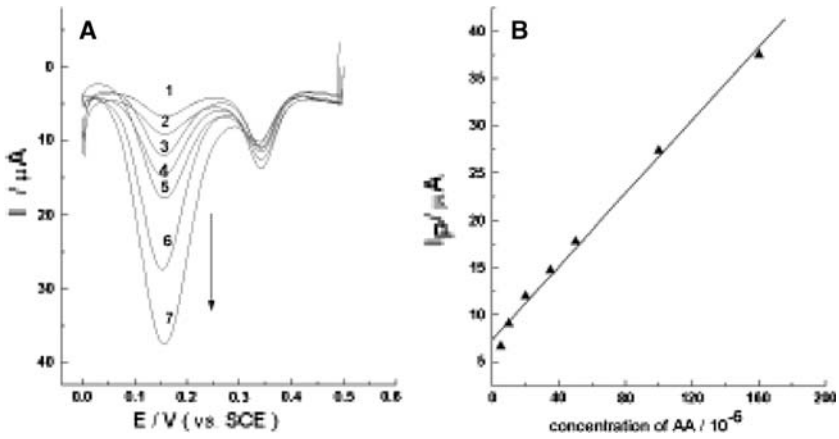


Fig. 5 (**A**) Differential pulse voltammogram of ascorbic acid at various concentrations 1. 5; 2. 10; 3. 20; 4. 35; 5. 50; 6. 100; 7. 160 μM in presence of 70 μM dopamine at the surface of GuLB/GCE. (**B**) Plot of electrocatalytic peak currents (from A) vs. ascorbic acid concentration. Solution conditions: pH 6.9 PBS. Amplitude, 50 mV; pulse width, 50 ms; pulse period, 200 ms

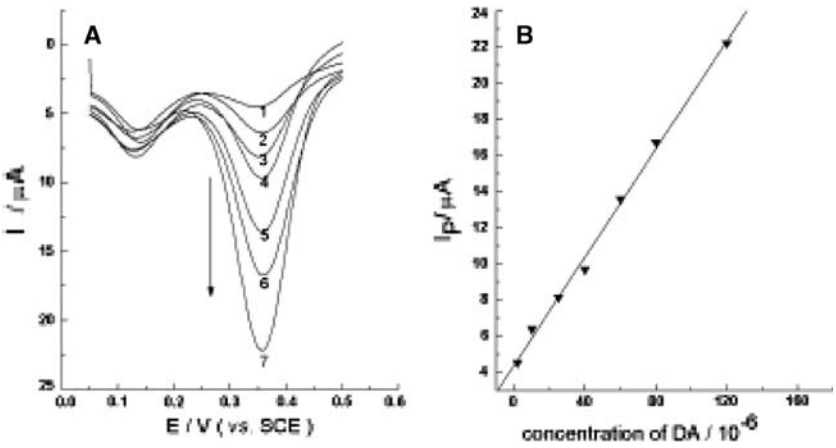


Fig. 6 (**A**) Differential pulse voltammogram of dopamine at various concentrations 1. 2; 2. 10; 3. 25; 4. 40; 5. 60; 6. 80; 7. 120 μM in presence of 20 μM ascorbic acid at the surface of CuLB/GCE. (**B**) Plot of electrocatalytic peak currents (from A) vs. dopamine concentration. Solution conditions: pH 6.9 PBS. Amplitude, 50 mV; pulse width, 50 ms; pulse period, 200 ms

Table 1 Analytical parameters for simultaneous determination of AA and DA

Analyte	Linear range (μM)	Linear regression equation I (μA), C (μM)	Correlation coefficient	Detection limit (μM)
AA	5.0–160.0	i_{Pa} (μA) = 7.40 + 0.19C	0.9964	2.8
DA	2.0–120.0	i_{Pa}(μA) = 4.42 + 0.15C	0.9980	1.4

oxidation peak current of DA was proportional to its concentration.

Under the optimum conditions with the use of the DPV mode the catalytic current peak was linearly related to AA and DA concentrations (Fig. 5B and Fig. 6B). The analytical parameters for the simultaneous determination of AA and DA are listed in Table 1.

From the depicted above, it can be seen that the electrochemical response peaks for AA and DA oxidation at the CuLB/GCE are clearly separated from each other when they co-exist in pH 6.9 PBS. It is therefore possible to determine AA and DA simultaneously in samples at a CuLB/GCE.

Repeatability in the construction of the modified electrode was evaluated by constructing four modified electrodes and determining the sensitivity obtained for each one. The repeatability expressed as relative standard deviation (RSD) was 3.2%. This indicates good repeatability in the modified electrode construction.

3.6 Interferences

The influence of various species on the determination of 10 μM DA and 50 μM AA was investigated. The tolerance limit was determined as the concentration causing ±5% relative errors on the determination. This was 0.03 M Na^+, K^+, Cl^-; 0.01 M Mg^{2+}, Ca^{2+}, SO_4^{2-}; 0.01 M citric acid, lysine; 0.005 M glucose, L-cysteine. Additionally, the CVs for oxidation of 50 μM DA in pH 6.9 PBS solution with a large excess of AA were determined. The voltammetric peak corresponding to the oxidation of DA remained constant until the AA concentration increased to 20 times that of DA. When the same experiment was performed with 50 μM AA, we found that almost 30 times the DA concentration has no effect on the voltammetric signal of AA oxidation.

3.7 Stability

The CuLB/GCE was stable and reproducible. However, the electrode had to be well treated to remove adsorption contaminations to maintain reproducibility. It was found that the electrode can be renewed by CV scans in 0.1 M PBS in the potential window 0.0–0.8 V after each experiment. Generally 20 cycles could regenerate clean background CV curves, and the electrode was ready for the next experiment or for storage.

Table 2 Determination of DA in hydrochloride injection solution (n = 5)

Samples	Labeled (mg ml^{-1})	Added (mg ml^{-1})	Found (mg ml^{-1})	RSD (%)	Recovery (%)
1	10	0	9.87	2.4	–
		0.5	10.39	1.9	104
		1.0	10.82	3.0	95
2	10	0	10.10	2.8	–
		0.5	10.61	2.1	102
		1.0	11.06	2.7	96

Table 3 Determination of AA in food samples ($n = 5$)

Sample	Found (mg per 100 g)	Added (mg per 100 g)	Total (mg per 100 g)	RSD (%)	Recovery (%)	Amount in fruits[24] (mg per 100 g)
Orange juice	30.52	10	40.64	1.9	101.2	34
Peach juice	8.94	10	18.72	1.6	97.8	8
Strawberry	59.45	10	69.55	3.4	101	59
Watermelon	5.63	10	15.84	2.1	102.1	7
Tomato	18.68	10	28.98	2.2	103	20

The CuLB/GCE had high storage stability. For storage in 0.1 M PBS (pH 7.0), the current response decreased 3% over the first 2 days, 7% for 5 days and 20% for the following one month.

3.8 Sample analysis

3.8.1 Determination of DA in dopamine hydrochloride injection

The dopamine hydrochloride injection solution (standard concentration of DA 10 mg ml^{-1}, 2 ml per injection) was diluted to 100 ml with water. 20 μL of this diluted solution or an amount of standard DA solution was injected into each of a series of 10 ml volumetric flasks and made up to volume with 0.2 M PBS (pH 6.9). An aliquot of 2.0 ml of this test solution was placed in an electrochemical cell for the determination of DA using the above DPV method. The results are listed in Table 2.

3.8.2 Determination of AA in foodstuffs

Foodstuffs were selected to analyze the contents of AA by the proposed method. In order to fit into the linear range of AA, all the samples used for detection were diluted with PBS (pH 6.9). The results are listed in Table 3. In order to evaluate the validity of the modified electrode for the determination of AA, recovery studies were carried out on samples to which known amounts of AA standards were added. The results obtained by the proposed method agree well with the table of components in fruits.

4 Conclusions

A novel dicopper complex modified glassy carbon electrode was fabricated by electrodeposition. The modified electrode showed good electrocatalytic activity for the oxidation of DA and AA. Moreover, a better separation of oxidation peaks of DA and AA can be achieved, indicating that the CuLB/GCE facilitates the simultaneous determination of DA and AA with good stability, sensitivity and selectivity. The proposed method can be applied to the determination of DA and AA in real samples with satisfactory results.

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