American Journal of Analytical Chemistry, 2011, 2, 289-293
doi:10.4236/ajac.2011.22036 Published Online May 2011 (http://www.SciRP.org/journal/ajac)

Simultaneous Determination of Dopamine and L-Ascorbic Acid by Modified Carbon Paste Electrode with Ni (II) Cyclam Complex

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Received November 8, 2010; *revised January* 10, 2011; *accepted May* 16, 2011

Abstract

The electroanalysis of dopamine (DA) and ascorbic acid (AA) by square wave voltammetry has been performed at a modified carbon paste electrode with macrocyclic ligand 1,4,8,11-tetraazacyclotetradecane (cyclam) and monolayer of Ni (II) cyclam. In pH 7.2 buffer solutions, the electrostatic reaction of AA with di-positive monolayer shifts the oxidation potential to less positive potential, while the electrostatic repulsion of DA with the monolayer shifts the oxidation potential of DA to more positive potential. The separation between the oxidation peaks of AA and DA at the present di-positive monolayer modified electrode (252 mV) was larger than that (187 mV) at the cyclam modified electrode. In addition, the catalytic oxidation of AA by oxidized DA has been advantageously eliminated at the modified carbon paste electrode with cyclam and Ni (II) cyclam complex. Thus, the determination of DA in the presence of an excess of AA is possible with the present modified electrodes.

Keywords: Macrocyclic Compound, Carbon Paste Electrode, Dopamine, Ascorbic Acid

1. Introduction

Dopamine (DA) and ascorbic acid (AA) are compounds of great biomedical and neurochemical interest playing a potential role in human metabolism. DA is one of the most significant catecholamine, functioning as a neurotransmitter in the central nervous system and a medicament to drug addiction and Parkinson's disease [1,2]. It affects brain processes that control movement, emotional response, and ability to experience pleasure and pain. AA is a water-soluble vitamin that is widely required in metabolism. It has been used in the prevention and treatment of common cold, mental illness, cancer and Aids [3]. In mammalian brain DA and AA coexists in the extracellular fluids.

There are various determination methods includes ultraviolet spectroscopy (UV) [4], high performance liquid chromatography (HPLC) [5,6], capillary electrophoresis (CE) [7] and electrochemical approaches [8-10]. Because both DA and AA are oxidisable compounds, their detection can be made by electrochemical methods based on anodic oxidation. When a potential is applied at the electrode, ascorbic acid is also oxidized to dehydroascorbic acid, which undergoes further chemical reaction to form the *gem*-diol (**Figure 1**). As is known the oxidation potential is pH dependent [11] (The first pK_a is at 4.17 and the second is at 11.57).

Dopamine is oxidized to form dopaminequinone with the liberation of two electrons (**Figure 2**). It is generally believed that direct redox reactions of these species at bare electrodes are irreversible and therefore require high overpotentials. Moreover the direct redox reactions of these species at the bare electrodes take place at very similar potentials and often suffer from a pronounced

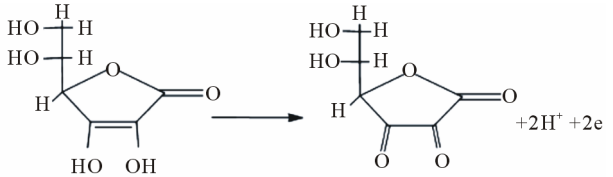

Figure 1. Electrooxidation of ascorbic acid.

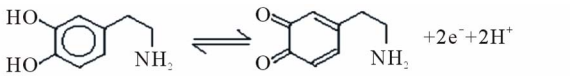

Figure 2. Electrooxidation of dopamine.

fouling effect, which results in rather poor selectively and reproducibility. Thus the simultaneous determination of DA and AA is of critical importance in the field of biochemistry and medical treatment [12].

The use of chemically modified electrodes greatly increases the selectivity and sensitivity toward these analytes. The development of voltammetric sensors for the detection of neurotransmitters in the extracellular fluid of the central nervous system has received much interest in the past few decades. So, many different strategies have been employed for the modification of the electrode surface [13]. A simple method for preparing electrochemical modified electrodes (CME) is based on doping carbon paste with the biocatalyst [14-16]. The most important advantage of the "mixed catalyst-carbon paste electrode" is the substantial reduction of response time owing to the absence of a layer that hinders mass transport. The catalyst is an integral part of the sensing element, and hence the electrode responds rapidly to changes in the level of the substrate.

The goal of this study was to develop a production method for modified carbon paste electrodes with macrocyclic compounds for selective measurement of neurotransmitters in physiological pH (7.2). This modified electrode with Ni (II) 1, 4, 8, 11-tetraazacyclotetradecane has been used for Simultaneous Determination of DA and AA. The electrochemical behavior of a broad family of macrocyclic complexes of nickel has been studied by Busch *et al.* [17].

2. Materials and Methods

2.1. Chemical and Reagents

All chemicals used were of analytical-reagent grade. Double distilled, deionized water (Milli-Q system, Millipore, Japan) was used for preparation of all solutions. 1,4,8,11-tetraazacyclotetradecane (cyclam), dopamine hydrochloride and ascorbic acid were bought from Fluka and used as such. All the voltammetric studies were carried out in phosphate buffer (potassium phosphate were also used as a supporting electrolyte). Buffer solutions were prepared from orthophosphoric acid and its salts in the pH range of 3-9.

2.2. Apparatus

The voltammetric system used for the studies was Autolab PSTAT 10 potentiostat joined to a Metrohm 663 VA. Square wave voltammetry was carried out in a three-electrode cell. Silver/silver chloride (3 mol·dm^{-3} KCl), a platinum wire and a bare or modified electrode were used as reference, counter and working electrodes, respectively. The pH values were measured with a digital pH meter MK VI (systronics).

2.3. Preparation of Bare Carbon Paste Electrode

The bare carbon paste electrode was prepared by hand mixing of graphite powder and silicon oil at a ratio 70:30 (w/w) in an agate mortar until a homogenous paste was obtained. The paste was then tightly packed into a PVC tube (3 mm internal diameter) and the electrical contact was provided by a copper wire connected to the end of tube. The bare carbon paste electrode was polished successively with 0.3 and 0.05 μm Al_2O_3 slurry on emery paper. It was then rinsed with doubly distilled water and sonicated in 1 + 1 HNO_3, acetone and doubly distilled water for 10 min, respectively.

2.4. Preparation 1of Modified Carbon Paste Electrode

The macrocyclic nickel complex (nickel (II) 1,4,8,11-tetraazacyclotetradecane) was synthesized, purified and characterized by elemental analysis and ^{13}C-NMR and IR spectral measurements according to reported procedure [18].

The carbon paste electrodes were prepared as before with 5% of the modifier in graphite-silicon oil matrix and used in conjunction with an Ag/AgCl reference electrode and a platinum counter electrode. The thickness of modified carbon paste was controlled in the range of 4 - 6 mm.

2.5. Procedure

The electrochemical experiments were performed in a 25 cm^3 electrolytic cell with 10 cm^3 solutions. All measurements were conducted at room temperature and under a nitrogen atmosphere. Nitrogen gas was bubbled through the solution for 30 min prior to each electrochemical measurement.

Solutions of various pHs were tested with phosphate buffer. The square-wave voltammograms were recorded for the unmodified electrode and the electrode modified with cyclam (CME-1) in 0.5 M phosphate buffer solution (pH = 7.2) containing DA and AA at different scan rates. Then, another set of experiments was carried out to study the effect of catalysis by the incorporated metal ion in the macrocyclic ring on the electrode modified with nickel (II) macrocyclic complex (CME-2).

3. Results and Discussion

3.1. Electrochemical Behavior of AA and DA on CME-1

The square-wave voltammograms obtained for the oxidation of AA and DA at the CME-1 and the bare electrode are shown in **Figure 3**. At the bare electrode when both AA and DA coexist, only one voltammetric peak is obtained for both analytes. Thus it is impossible to determine the individual concentrations from the broad voltammetric peak. Moreover the catalytic oxidation of AA by the oxidized DA [19] enhances the oxidation peak current of DA at the bare electrode. The precise determination of DA in the presence of AA is not possible because of this catalytic oxidation. As the AA concentration, for example, in the extracellular fluid is very high, this mediated oxidation would affect the accurate determination of DA and this unwanted catalytic oxidation needs to avoid. The present modified electrode clearly separates the merged voltammetric peaks of AA and DA and the mediated oxidation of AA by oxidized DA has been successfully eliminated. At these electrodes the AA oxidation occurs well before the DA oxidation potential and hence the mediated oxidation would not be expected. Since the voltammetric peak of DA is well separated from the AA peak, the determination of DA in the presence of AA is possible with the present modified electrode. The separation between the oxidation peaks of AA and DA at the CME-1 in phosphate buffer solution (pH = 7.2) was 187 mV.

3.2. Electrochemical Behavior of AA and DA on CME-2

Figure 4 shows the oxidation of AA and DA at the bare carbon paste electrode and the modified carbon paste electrode with nickel (II) macrocyclic complex (CME-2). As can be readily seen from **Figure 4** a negative shift in the AA oxidation potential can be the electrostatic interaction of AA with the positively charged monolayer. Since AA is negatively charged in neutral aqueous solution (pH = 7.2), the electrostatic interaction is expected between AA and Ni^{2+} redox centers of the monolayer and it would favor the oxidation of AA.

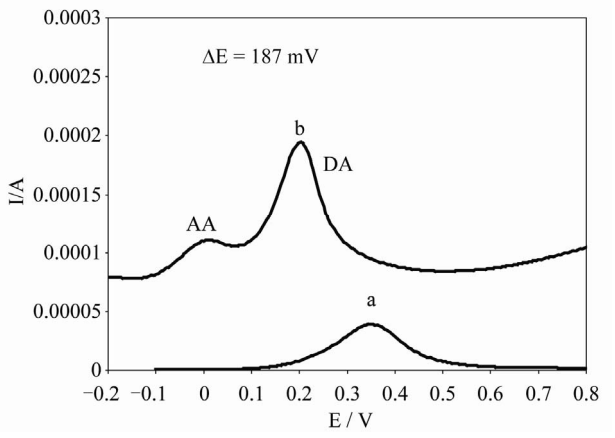

Figure 3. Square-wave voltammograms obtained for the oxidation of DA and AA (2×10^{-5} M) at (a) bare and (b) CME-1 in 0.5 M phosphate buffer (pH = 7.2).

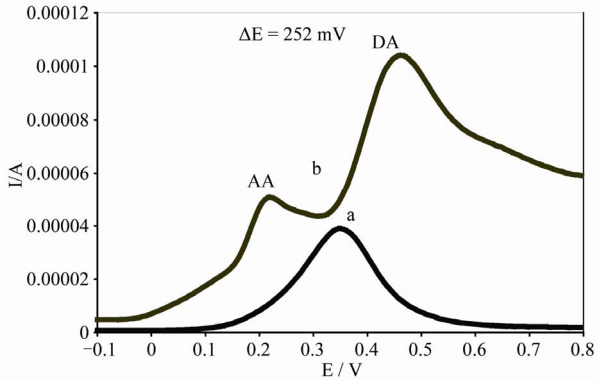

Figure 4. Square-wave voltammograms obtained for the oxidation of DA and AA (2×10^{-5} M) at (a) bare and (b) CME-2 in 0.5 M phosphate buffer (pH = 7.2).

In contrast, DA exists in the cationic form physiological pH (pK_a 8.9). It is repelled by the Ni^{2+} redox centers. Hence, it cannot enter the monolayer to the same extent as AA, and the interference with the determination of DA is diminished. These results indicated that the problem of the overlapped voltammetric responses of DA with AA, due to their coexistence in real biological fluids can be effectively overcome by use of dipositive monolayer of Ni (II) cyclam.

The monolayer modified electrode successfully resolves the merged voltammetric peaks of AA and DA and the peaks are separated enough (252 mV separation) to determine the concentration of each analytic.

3.3. Effect of Film Thickness on the Voltammetric Response

The thickness of complex film directly controls the electrode performance. The optimum film thickness reflects compromise between mechanical stability and residual current.

Nevertheless, the high residual current remains a severe limitation of these modified electrodes. The residual current tends to be high when maximum catalyst is used for modification of carbon paste electrode. The film thickness was varied by preparing the electrodes with different wt% of catalyst. The electrode prepared with 5 wt% macrocyclic complex or ligand, shows the best performance. When the films were too thin, the limited amount of

catalyst loaded apparently affect the sensitivity. Whereas, when the films were too thick, residual current increased remarkably. So, electrodes prepared with the optimum of catalyst (5 wt%), were used in all experiments.

3.4. Optimization of the Solution pH

Figure 5 shows the ΔE_p versus pH plots for CME-2 in the phosphate buffer with various pHs (in range of 3 - 9).

The electrochemical reaction can be induced and monitored by voltammetry to quantify the concentration of ascorbic acid in solution.

The voltammograms obtained with the Ni (II) macrocyclic for solutions containing L-ascorbic acid in strongly acidic media (e.g. pH2) showed that L-ascorbic acid did not couple catalytically with the Ni (II) macrocyclic. Therefore, optimization of the solution pH was necessary in order to obtain a catalytic couple. A variation in the electrolyte pH will result in variations in the formal potential of L-ascorbic acid. Therefore, the thermodynamic driving force for the catalysis will vary with the pH, making the peak currents and the shapes of the voltammograms at different pH values. The anodic peak currents increased with an increase in the pH up to 6.6, and then gradually decreased up to pH 9. So, the most optimized pH for catalytic oxidation of AA is 6.6. However, in this pH, electron transfer kinetic for the oxidation of DA was found to be rather sluggish owing to the electrostatic repulsion between positively charged DA (pK_a 8.9) and Ni (II). So, the electrostatic repulsion of DA and with di-positive monolayer shifts the oxidation potential of DA to more positive potential, while the electrostatic reaction of AA with the monolayer shifts the oxidation potential to less positive potential. In this study, all of the measurements were carried out at the physiological pH (7.2) that it is much near to optimized pH.

3.5. Effect of Scan Rate on Peak Currents of DA and AA

The results show an initial linearity which curves off at higher scan rates. It suggests that the reaction is initially diffusion controlled, but at faster scan rates the electron transfer becomes rate determining. It appears from these data that a scan rate of 100 $mV \cdot s^{-1}$ was used for the purpose of simultaneous determination of DA and AA. In addition, the E_p values are shifted to more positive values.

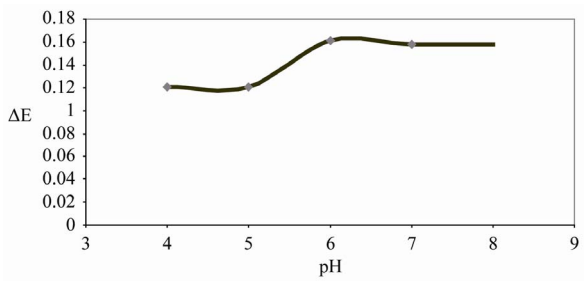

Figure 5. Plot of ΔE_p vs pH for 2×10^{-3} M ascorbic acid and dopamine obtained by CME-2 in the 0.5 M phosphate buffer.

3.6. Calibration Curve and Reproducibility

The calibration plots for the oxidation of AA and DA were linear for a wide range of concentration (1-100 μM for AA and 1.5 - 100 μM for DA at the CME-2).

To characterize the reproducibility of the CME-2, repetitive measurementregeneration cycles were carried out. The results of 15 successive measurements showed a relative standard deviation of 4.1% and 3.8% for 50 μM ascorbic acid and 50 μM dopamine.

4. Conclusions

Redox processes of organic compounds often have slow charge transfer rates, leading to poorly defined voltammetric responses. Modification of the electrode surface by a redox mediator reduces the over-potential for the redox processes. The modified carbon paste electrodes with cyclam and Ni (II) cyclam monolayer have been successfully applied to the determination of ascorbic acid and dopamine. The separation between the oxidation peaks of AA and DA at the CME-2 (252 mV) was larger than that (187 mV) at the CME-1.

The carbon paste approach permits convenient mixing of different ligands and as the ligand is homogeneously mixed in the bulk of the paste, renewal of the surface is done simply by pressing out the paste from syringe, which is easier and faster. Moreover, the electrode so fabricated can be stored for about six months in an air-tight container.

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