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A voltammetric sensor for simultaneous determination of ascorbic acid, noradrenaline, acetaminophen and tryptophan

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ABSTRACT

In this study, poly-Trypan Blue modified glassy carbon electrode was developed for the simultaneous determination of ascorbic acid (AA), noradrenaline (NA), acetaminophen (AC) and tryptophan (Try). Bare glassy carbon electrode (GCE) has limitations for resolving the oxidation currents of these compounds. Poly-Trypan Blue modified electrode not only separates the voltammetric signals of AA, NA, AC and Try with potential differences of 150, 170, and 280 mV between AA–NA, NA-AC, and AC–Try, but it can also enhance the oxidation currents of AA, NA, AC and Try by 11.0, 7.0, 1.4, and 3.8 times, respectively, compared to bare electrode. The diffusion coefficient, D, and the heterogeneous rate constant, k_h, were estimated for the oxidation of noradrenaline at the modified surface. The detection limits of AA, NA, AC and Try were also calculated as 0.10, 0.06, 0.10 and 0.80 μmol L^{-1}, respectively. Finally, the fabricated sensor was satisfactorily used for the simultaneous determination of these molecules in pharmaceutical and human biological samples.

1. Introduction

Noradrenaline (NA) is the most important catecholamine neurotransmitter in the mammalian central nervous system. Many diseases are related to changes in its concentration; thus, the determination of NA in biological fluids helps the diagnosis of some diseases in clinical medicine, evaluation of therapeutic and pharmacodynamic effects of neurological, psychiatric and cardiovascular disorders. To date, several analytical methods such as high performance liquid chromatography [1], gas chromatography [2], chemiluminescence [3] and spectrophotometry [4] have been reported for the determination of NA. The electrochemical determination of NA has been the focus of research because NA is an electroactive compound. However, two problems are involved in electrochemical determination: first, the irreversibility of the electrochemical property of NA results in a large over-potential; and second, the oxidation potential of AA, AC and Try, which largely coexists with NA in biological fluid overlapping with NA oxidation potential. In order to solve these problems, various modified electrodes have been developed [5–11].

Electrochemical methods are among important methods in environmental monitoring, medicine and biotechnology, and industrial process control [12–16]. The chemically modified electrodes are very interesting tools for the analysis of many substances at trace level using sensitive electroanalytical techniques. An important point in chemically modified electrode utilization in speciation work is to choose the most convenient modifier for each analyte because the sensitivity and selectivity of the electroanalytical response depend on the characteristics of the modifier [17–20]. Conductive polymer modified electrodes have been widely used owing to their catalytic ability, good stability and broad potential window [21–26]. These polymers have been used for many applications such as chemical and biological sensors, electronic nanodevices, catalysis and electrocatalysis. Trypan Blue is an acid azo dye commonly used as a stain to distinguish viable from non-viable cells. It contains two amine groups and phenolic O—H, which can form an electropolymerized film on the surface of a glassy carbon electrode. In this study, poly-Trypan modified glassy carbon electrode serves as an excellent electrocatalyst property towards the oxidation of NA, AA, AC and Try. The literature review indicates that no electrochemical method related to chemically modified electrode has been reported for simultaneous determination of these four compounds in biological and pharmaceutical samples. Table 1 shows comparisons of the proposed method and other electrochemical methods reported for the determination of NA, AA, AC and Try.

2. Experimental

2.1. Apparatus and reagents

All electrochemical experiments were performed using a Metrohm instrument, Model 797 VA processor. A conventional three–electrode electrochemical system consists of a working electrode (poly-Trypan Blue modified glassy carbon electrode), a platinum wire counter electrode, and Ag/AgCl (3.0 mol L^{-1} KCl) as a reference electrode. A Corning

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Table 1
Comparison of some characteristics of the different modified electrodes for the determination of AA, NA, AC and Try.

Sensitivity (μA L μmol^{-1})				Limit of detection (μmol L^{-1})				Linear dynamic range (μmol L^{-1})				Interferences	Ref.
AA	NA	AC	Amino acid	AA	NA	AC	Amino acid	AA	NA	AC	Amino acid		
–	0.04	0.03	0.028	–	0.6	–	–	–	1.2–900.0	75.0–600.0	90.0–600	Ascorbic acid	5
–	0.046	0.025	0.017	–	0.21	–	–	–	0.47–5000	15.0–500.0	20.0–900	Not reported	6
–	0.056	0.06	–	–	0.043	0.78	–	–	0.70–100.0	0.9–80.0	–	Not found	7
–	0.322	0.042 0.022	–	–	0.043	–	–	–	0.08–30.0 30.0–700.0	20.0–600.0	–	Ascorbic acid, dopamine, epinephrine	8
–	0.0156	0.0172	–	–	0.09	0.9	–	–	0.7–2000.0	1.0–2500	–	Not reported	9
–	0.067 0.993	0.0095 0.025	–	–	0.14	–	–	–	0.5–65.4 65.4–274.2	12.0–59.1 59.1–261.7	–	Not found	10
–	0.091	0.02 0.035	–	–	8.0	0.9	–	–	15.0–100 15.0–1000.0	220.0–850.0	–	Not reported	11
–	0.05	0.039	–	–	0.05	0.20	–	–	0.08–2000.0	0.9–1900.0	–	Not reported	21
–	–	–	–	0.5	0.1	–	–	1.0–500.0	0.63–62.5	–	–	Cysteine	22
0.026	8.295	–	–	49.8	0.131	–	–	00–1000	0–10.0	–	–	Not reported	24
–	0.093	0.033	0.102	–	0.07	0.1	0.9	–	1.3–230.1	1.9–188.0	3.9–61.8	Epinephrine	26
0.039	0.111	0.280	0.021	0.1	0.06	0.1	0.8	1.0–630.0	0.1–560.0	0.2–530.0	1.0–345.0	Cysteine	This work

pH–meter, Model 140, with a glass electrode (conjugated with an Ag/AgCl reference electrode, Model 6.0232.100) was used to determine the pH values of the solutions.

The Trypan Blue was purchased from Sigma-Aldrich (Dye content, 60%). AA (>99.0%), AC (>99.0%), NA (>98.0%), Try (>98.0%) and other reagents were provided by Merck Company. Stock solutions of AA and AC (0.010 mol L^{-1}) were prepared daily by dissolving a suitable amount of these reagents in water, stock solution of NA and Try (0.010 mol L^{-1}) were prepared daily by dissolving an appropriate amount of NA and Try in a minimum volume of 0.10 mol L^{-1} H_3PO_4 solution, and the resulting solution was diluted with water. Phosphate buffer solutions (PBS) with different pH levels were prepared by mixing 0.10 mol L^{-1} Na_2HPO_4 and 0.10 mol L^{-1} NaH_2PO_4 solutions at different ratios. The solution pH levels were adjusted by adding 1.0 mol L^{-1} H_3PO_4 and/or NaOH solution.

2.2. Preparation of poly-Trypan Blue film modified glassy carbon electrode

Prior to modification, bare GCE was polished using alumina slurries with 0.05 μm. Then, in order to remove any adsorbed substance on the surface of the electrode, it was ultrasonicated in ethanol and doubly distilled water for 5 min. Finally, the electrode was dried under nitrogen flow and was ready to use. The electrode was subsequently placed in a solution containing 0.005 mol L^{-1} Trypan and phosphate buffer pH = 3, and cyclic potential sweep was applied in the potential range of 0 to +1.5 V for 30 cycles at 50.0 mV s^{-1}. The resulting electrode, poly-Trypan/GCE, was activated by several cyclic voltammetry in a potential range between 0 and +0.80 with a scan rate of 100 mV s^{-1} in buffer solution (pH 3.0) until a steady state voltammogram was obtained to increase its reproducibility.

2.3. Preparation of real samples

Six tablets of vitamin C (labeled 500 mg vitamin C per tablet) were completely ground and homogenized, 200 mg of it was accurately weighed and dissolved with ultrasonication in 25 mL of water. Finally, a suitable volume of the resultant solution plus 5 mL of the phosphate buffer solution (pH 3.0) was diluted with water in a 10-mL volume flask, and the resulting solution was used for the analysis of AA.

The noradrenaline bitartrate drug injection solution (specified content of NA is 4.0 mg/4 mL) was analyzed directly after being diluted 100-times with the buffer solution (pH 3.0), and an aliquot of 10 mL of this test solution was placed in the electrochemical cell. The potentials were controlled between 0.0 and +1.0 V at a pulse amplitude of 100 mV, pulse time of 50 ms, and sweep rate of 50 mV s^{-1}. I_{pa} was also measured at the oxidation potential of NA.

Blood samples were put into heparinized tubes and centrifuged at 3000 rpm for 10 min. The plasma was separated and stored in the refrigerator to be analyzed. Plasma samples were deproteinated using acetonitrile according to Aly et al. [27]. 2.0 mL of acetonitrile was added to plasma and it was centrifuged for 10 min at 2000 rpm. The supernatant was transferred to a small conical flask and it was evaporated to dryness under the stream of nitrogen. The dry residue was diluted with phosphate buffer solution of pH 3.0 and it was transferred into the voltammetric cell (20 mL) to be analyzed without any further pretreatment. Standard addition method was used to determine AA, NA, Try and AC in the samples.

Ten tablets of AC (labeled 325 mg acetaminophen per each tablet) were completely ground and homogenized, 60 mg of this powder was accurately weighed and dissolved with ultrasonication in 25 mL of water. Then, 100 μL of the solution plus 5–mL of the buffer (pH 3.0) was diluted with water in a 10–mL volumetric flask, and the resulting solution was used for analysis. Then, the diluted sample solutions were transferred into the electrochemical cell to determine their concentrations by differential pulse voltammetry (DPV).

3. Results and discussion

3.1. Characterization of the poly-Trypan modified GCE

Fig. 1 shows the electrode surface morphology of poly-Trypan film coated GC electrode, characterized by scanning electron microscopy. We can clearly see the existence of electrodeposited polymer on the surface of GCE, which provides an effective surface area for electrocatalytic oxidation of AA, AC, NA, and Try. In addition, energy-dispersive X-ray spectroscopy (EDX) shows the presence of C, N, O and S elements on the prepared electrode, indicating the successful formation of polymer layer on the surface of GCE [28].

Fig. 2A displays 30 continuous CVs of Trypan (0.005 mol L^{-1} in 0.1 mol L^{-1} PBS pH 3.0) polymer formation onto a GCE by scanning potential over the range of 0 to +1.5 V at 50 mV s^{-1}. The CVs showed two anodic peaks appearing at +0.68 V (irreversible oxidation of NH_2 group) and +1.02 V (quasireversible oxidation of phenolic-OH group), which gradually tend to be stable after 30 scans. After the continuous potential scanning, the currents of these peaks related to the oxidation and reduction of the monomer decreased, indicating the deposition and growth of an electroactive layer on the electrode surface to form a conducting film of poly-Trypan at GCE (The mechanism of electropolymerization of Trypan Blue at GC electrode is given in the