Journal of Electroanalytical Chemistry 709 (2013) 1–9

Contents lists available at ScienceDirect

Journal of Electroanalytical Chemistry

journal homepage: www.elsevier.com/locate/jelechem

Electrochemical behavior of sophoridine at a new amperometric sensor based on L-Theanine modified electrode and its sensitive determination

Yinfeng Li [a,b], Lina Zou [a], Ge Song [a], Kunjing Li [a], Baoxian Ye [a,*]

[a] College of Chemistry and Molecular Engineering, Zhengzhou University, Zhengzhou 450001, PR China
[b] School of Chemical and Material Engineering, Henan University of Urban Construction, Pingdingshan 467036, PR China

ARTICLE INFO

Article history:
Received 1 July 2013
Received in revised form 27 September 2013
Accepted 30 September 2013
Available online 10 October 2013

Keywords:
Sophoridine
L-theanine
Electro-polymerization
Amperometry

ABSTRACT

The electro-polymerization of L-Theanine was investigated for the first time on glassy carbon electrode and the polymeric conditions and mechanism were discussed in detail. Hereafter, a simple and sensitive electrochemical sensor based on the poly(L-Theanine) film was established for sophoridine sensing. The electrode process of sophoridine was then studied by cyclic voltammetry and its determination was achieved by amperometry. The results revealed that the poly(L-Theanine) membrane effectively decreased the oxidation potential of sophoridine and greatly increased its response current. And this sensor, fabricated easily and simply as well as very easy surface update, showed good response for sophoridine with a wide linear range from 1.0×10^{-6} to 1.4×10^{-4} mol L^{-1} and a low detection limit of 5×10^{-7} mol L^{-1}. The application of proposed method in analysis of real sample was also evaluated with good performance. This work promoted the potential applications of amino acid materials in electrochemical sensors.

1. Introduction

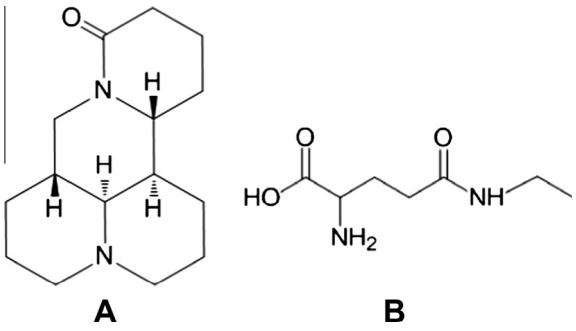
Sophoridine, white or light yellow crystalline alkaloid monomer with molecular formula of $C_{15}H_{24}N_2O$, is one of the quinolizidine alkaloids, which is extracted from seeds of the traditional medicine herb *Sophora alopecuroides* L. [1,2]. Its molecular structure is shown in Scheme 1(a). Sophoridine is used in treating cancer for decades with low drug side-effects [3]. In particular, it exhibited the potential therapeutic efficacy on malignant tumors in the digestive tract such as colorectal carcinoma, gastric cancer, and esophageal cancer [2,4,5]. Furthermore, it has been reported that sophoridine has antiviral effects [6], anti-arrhythmic effects [7] and protective effects on acute myocardial infarction [8]. Recently, Liu has reported the neuroprotective effect of early and short-time applying sophoridine in pMCAO rat brain [9]. Accordingly, sensitive analytical method for sophoridine is necessary and some determination techniques have been reported, such as high performance liquid chromatography (HPLC) [10], GC–MS [11], nonaqueous capillary electrophoresis (CE) [12] and electrogenerated chemiluminescence (ECL) [13]. As far as we know, there is few report about the determination of sophoridine by electrochemical techniques because of its poor electroactivity on conventional electrodes. However, electrochemical method is simple, sensitive, rapid, inexpensive and conducive identifying the redox mechanism of sophoridine, providing important information about its pharmacological actions. Therefore, it is valuable to develop an electroanalytical method for sophoridine.

To date, conducting polymer modified electrodes have received great attention due to their excellent characteristics, including high stability and selectivity, good reproducibility and conductivity, more active sites and good homogeneity. Plenty of conducting polymers have been employed in the fields of ion recognition [14], electron transfer [15] and electroanalysis [16,17]. Among them, there are quite a few reports about amino acid as material to build sensing interface for analytical application [18–20], which reveal that amino acids are promising material for electrochemical sensor. It is in this context, L-Theanine leaps into our field of vision.

L-Theanine ($C_7H_{14}N_2O_3$, Scheme 1(b)), 2-amino-4-(ethylcarbamoyl) butyric acid, a water-soluble amino acid structurally related to glutamic acid, was almost solely found in tea plants, accounting for 1–2% in the dry tea leaves [21,22]. Current researches about L-Theanine mainly focus on its physiological and pharmacological activities, such as neuroprotection in transient ischemic neuronal death [23], relaxing effects under resting conditions [24], hypolipidemic functions [25], regulation of systemic blood pressure [26], suppression of the stimulatory action of caffeine [27], modulation of neurotransmitter [28], and protection against hepatic toxicity [29]. For our knowledge, there have no reports about L-Theanine applied to electrode modified material so far. Hence, developing a kind of L-Theanine modified electrodes has great potential.

* Corresponding author. Tel.: +86 0371 67781757; fax: +86 0371 67763654.
E-mail address: yebx@zzu.edu.cn (B. Ye).

1572-6657/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jelechem.2013.09.030



Scheme 1. Chemical structure of sophoridine (a) and L-Theanine (b).

In this work, L-Theanine was electropolymerized on glassy carbon electrode (GCE) surface to form poly(L-Theanine) modified GCE, marked as poly(L-Theanine)/GCE. The electro-polymerization parameters were discussed in detail. Afterwards, a simple and sensitive electrochemical sensor based on the poly(L-Theanine) film was established for sophoridine sensing. Using this sensor, the electrochemical behavior of sophoridine were investigated systematically and dynamic parameters of electrode process were determined using various electrochemical techniques. It was found that the poly(L-Theanine) film exhibited improving sensitivity for sophoridine detection. A simple, sensitive and stable electroanalytical method of sophoridine was proposed with wide linear range and low detect limit.

2. Experimental

2.1. Apparatus and reagents

Electrochemical measurements were performed on a RST5000 electrochemical workstation (Zhengzhou Shiruisi Instrument Co., Ltd., Zhengzhou, China) with conventional three-electrode cell. A bare GCE (3 mm diameter) or modified GCE was used as the working electrode. An Ag/AgCl and a platinum (Pt) wire were used as reference and counter electrodes, respectively. All the potentials in this paper refer to Ag/AgCl. Electrochemical experiments were performed in 10 mL supporting electrolyte at room temperature.

L-Theanine was purchased from Aladdin (http://www.aladdin-e.com/). Sophoridine was purchased from National institutes for food and drug control (Beijing, China) and used as received. Standard stock solution of sophoridine (1×10^{-2} mol L^{-1}) was prepared with double distilled water and kept under 4 °C. It was diluted to necessary concentration before use. 0.2 mol L^{-1} Phosphate buffer solutions (PBS) were prepared by mixing the stock solutions of 0.2 mol L^{-1} NaH_2PO_4 and Na_2HPO_4. A pH 6.4 aqueous L-Theanine solution was used for electro-polymerization. All the other chemicals were of analytical reagent grade and used as received. Double distilled water was used for all preparations. Human blood samples were obtained from healthy volunteers and treated prior to usage.

2.2. Fabrication of the poly(L-Theanine)/GCE

Prior to modification, the GCE was polished to a mirror finish using finer emery-paper and 0.5 μm alumina slurry respectively. After rinsing thoroughly with water, the GCE was washed ultrasonically in absolute alcohol and double-distilled water again. Then L-Theanine (2.0×10^{-3} mol L^{-1} in PBS, pH 6.4) was electrodeposited on the cleaned GCE surface by cyclic scanning between −1.5 and 2.5 V with 100 mV s^{-1} for five cycles. This was the optimal polymeric condition for fabricating the poly(L-Theatine)/GCE from test. Prior to use, the poly(L-Theatine)/GCE was pretreated in a 0.2 mol L^{-1} PBS by cyclic scans between potentials of 0.4 and 1.0 V (20 cycles).

The electroactive area of poly(L-Theatine)/GCE was calculated using $K_3Fe(CN)_6$ as electrochemical probe by cyclic voltammetry and compared with that of bare GCE. For a reversible process, the following Randles–Sevcik formula was used: $i_{pa} = 2.69 \times 10^5 n^{3/2} A D_0^{1/2} C_0 v^{1/2}$, where i_{pa} refers to the anodic peak current; n is the electron transfer number; A is the surface area of the electrode; D_0 is the diffusion coefficient; C_0 is the concentration of $K_3Fe(CN)_6$ and v is the scan rate. For a 1.0×10^{-3} mol L^{-1} $K_3Fe(CN)_6$ solution containing 0.1 mol L^{-1} KCl electrolyte, $n = 1$ and $D_0 = 7.6 \times 10^{-6}$ - cm^2 s^{-1} [30]. Hence, the electroactive area (A) could be obtained from the slope of voltammetric peak current (i_{pa}) vs. the square root of scan rate ($v^{1/2}$) plot. Based on this theory, the electroactive surface areas of the poly(L-Theanine)/GCE and bare GCE were calculated to be 0.066 cm^2 and 0.056 cm^2, respectively. The effective area of poly(L-Theanine)/GCE was larger than that of bare GCE.

2.3. Analytical measurements

All electrochemical measurements were carried out in PBS buffer solution (pH 7.4) at room temperature unless otherwise specified. After each determination, the poly(L-Theanine)/GCE undergoes stir in PBS (pH 7.4) containing analyte for 30 s to give a regenerated electrode surface. For establishing the analytical method, the amperometry (standard solution titration) was employed and the current–time curve was recorded at potential of 0.85 V within 3000 s under continuous stirring. The serum sample was centrifuged and then after filtering, diluted with PBS (pH 7.4) without any further treatment. The diluted serum sample was spiked with different amounts of sophoridine.

3. Results and discussion

3.1. Electro-polymerization of L-Theanine on GCE

3.1.1. Effects of electro-polymerization potential window

For a polymer film modified electrode, its electrochemical response to the analyte was greatly affected by its polymeric conditions: polymeric potential window, pH of polymeric solution and thickness of polymer film. For current research, the polymeric conditions of poly(L-Theanine) were investigated in detail. The evaluation criteria was its electrochemical response for sophoridine (5.0×10^{-5} mol L^{-1} in 0.2 mol L^{-1} PBS, pH 7.4) by cyclic voltammetry.

At first, the electropolymerization of L-Theanine on GCE surface was performed by repetitive cyclic voltammetry under potential window between −1.5 V and +2.5 V. As shown in Fig. 1A, three relatively weak anodic peaks (marked P1, P2 and P3) at +0.2 V, +1.5 V and +2.15 V and a large cathodic peak (P4) at −0.65 V were observed at the first cycle. From the second cycle on, the P3 disappeared and the P1, P2 and P4 increased concurrently in subsequent cycles, suggesting that the amount of electroactive polymer increased on GCE surface. At the same time, it was also observed that the film growth was faster in the initial six cycles than that in subsequent cycles. From the tenth cycle on, the film grew more slow. After electropolymerization of the L-Theanine, a uniform and greenish blue film of poly(L-Theanine) could be seen by bare eyes on the GCE surface. Furthermore, Fig. 1B–F showed the electro-polymerization voltammograms obtained under different potential window. Here, they were used to demonstrate the importance of potential during the L-Theanine electropolymerization. When the potential window was set between −0.6 V and +2.5 V,