Electrochemical Investigation of Tryptophan at a Poly(*p*-aminobenzene sulfonic acid) Film Modified Glassy Carbon Electrode

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A glassy carbon electrode (GCE) modified with poly(*p*-aminobenzene sulfonic acid) [Poly(*p*-ABSA)] film is fabricated by voltammetric technique in phosphate buffer solution (pH 8.0) containing 5.0×10^{-3} mol L^{-1} *p*-ABSA. Electrochemical behaviors of tryptophan at the Poly(*p*-ABSA) film electrode are investigated with voltammetry. The results indicate that the electrochemical response of tryptophan is improved significantly in the presence of poly(*p*-ABSA) film. Compared with the bare glassy carbon electrode, the Poly(*p*-ABSA) film electrode remarkably enhances the irreversible oxidation peak current of tryptophan. Some parameters such as voltammetric sweeping segments for the electrochemical polymerization, pH, accumulation potential and accumulation time are optimized. Under the optimal conditions, the oxidation peak current is proportional to tryptophan concentration in the range of 1.0×10^{-7} to 1.0×10^{-6} mol L^{-1}, and 2.0×10^{-6} to 1.0×10^{-5} mol L^{-1} with a detection limit of 7.0×10^{-8} mol L^{-1}. The proposed procedure is successfully applied to the determination of tryptophan in a commercial amino acid oral solution.

Key Words : Tryptophan, *p*-Aminobenzene sulfonic acid, Electropolymerization, Voltammetry

Introduction

Tryptophan (Trp) is an amino acid essential to humans. It is a vital constituent of proteins and indispensable in human nutrition for establishing and maintaining a positive nitrogen balance. It is sometimes added to dietary, food products, pharmaceutical formulas due to the scarcely presence in vegetables. This compound also is a precursor for serotonin, melatonin, and niacin. It has been implicated as a possible cause of schizophrenia in people who cannot metabolize it properly. When improperly metabolized, it creates a waste product in the brain that is toxic, causing hallucinations and delusions.[1] Therefore, many analytical methods have been established for its determination with a variety of sample matrices.

Spectrophotometry[2-7] is mainly technique for the determination of Trp. Most of the spectrophotometric methods involve laborious and slow procedures with the modification of tryptophan by numerous reagents. Trp has also been analyzed by HPLC with a pre-column derivatization,[8] post-column derivatization,[9] or without derivatization by using its absorbance at ultraviolet region,[10] fluorescence[11] or electrochemical properties.[12] Although sensitive and selective, it is often complex, tedious and time-consuming. Therefore, a simple, sensitive and less expensive procedure for Trp analysis is of great interest. The methods based on electroanalysis might be the most notable and have been widely reported. The mercury electrodes[13] and carbon paste electrodes[14] have mainly been employed in these researches. More recently, carbon paste electrodes with electrochemical pretreatment[15] or special chemical modification[16,17] have been developed in electroanalysis of amino acids with good performance.

It is well known that tryptophan always coexists with tyrosine (Tyr) in humans and herbivores bodies. In the electrochemical investigation, the oxidation potential for the oxidation of Tyr is too near to that of the oxidation of Trp. Therefore, the interference of tyrosine usually can not be eliminated when the electrochemical determination of tryptophan.[18,19]

In this paper, a simple, sensitive and selective method was developed for the determination of Trp in presence of Tyr based on a Poly(*p*-aminobenzene sulfonic acid) film modified glassy carbon electrode. The film electrode exhibits good stability and high sensitivity for the determination of Trp. The successful application of the film electrode in the determination of Trp in a commercial amino acid oral solution without any pretreated steps demonstrate that it is a promise device in practical area.

Experimental

Apparatus and chemicals. Electrochemical measurements were carried out on CHI 660A electrochemical workstation (CH Instruments, Chenhua Corp., Shanghai, China). A conventional three-electrode system was employed with a bare GCE or poly(*p*-ABSA) film modified GCE (3.0 mm in diameter) as the working electrode, a saturated calomel electrode (SCE) as the reference electrode, and a platinum electrode as the counter electrode. All the potentials given in this paper were referred to the SCE. The pH value of phosphate buffer solutions (PBS) was adjusted by pHS-2 pH meter (Leici instrument factory, Shanghai, China). A magnetic stirrer was used for the convective transport when necessary. *p*-ABSA was obtained from Shanghai Chemical Industry Factory (Shanghai, China). All amino acid were purchased from Shanghai Bio Life Science &

Technology Co, Ltd (Shanghai, China). All chemicals were of analytical reagent grade and were used without further purification. Phosphate buffer solutions (PBS) were prepared from KH_2PO_4 and Na_2HPO_4, and adjust the pH with KOH and H_3PO_4. Stock solution of tryptophan (1.0×10^{-3} mol L^{-1}) was prepared by dissolving tryptophan in PBS (pH 3.0). Solutions with concentration below 1.0×10^{-3} mol L^{-1} were freshly prepared before use. All aqueous solutions were prepared in deionized water.

Preparation of poly(p-ABSA) film modified electrode. A glassy carbon electrode was polished to a mirror finished with polish paper and 0.3-0.05 μm alumina slurry, and cleaned thoroughly in an ultrasonic cleaner with 1:1 nitric acid solution, alcohol, and redistilled water sequentially. *p-ABSA* was electrochemically polymerized onto the glassy carbon electrode by voltammetric scanning at 100 mV s^{-1}. The monomer concentration of *p-ABSA* in solution was 5.0×10^{-3} mol L^{-1} and the supporting electrolyte consisted of 1/15 mol L^{-1} $Na_2HPO_4 \pm KH_2PO_4$ (pH 8.0) and 0.1 mol L^{-1} KCl. The polymerization process was carried out under the potential controlled between −1.50 and 2.00 V for 24-segment scanning.

Analytical procedure. Electrochemical experiments were carried out in a conventional electrochemical cell containing 10 mL 1/15 mol L^{-1} phosphate buffer solution and a certain concentration of Trp. After accumulation for 60 s under −0.10 V, linear sweep voltammograms were recorded in the potential range from 0.40 V to 1.00 V at a scan rate of 100 mV s^{-1}.

Results and Discussion

Electropolymerization of *p*-ABSA at the GCE surface. A poly(*p*-ABSA) film modified electrode was fabricated by electropolymerization of *p*-ABSA on a glassy carbon electrode. The experiment was performed in a phosphate buffer solution (pH 8.0) containing 5.0×10^{-3} mol L^{-1} *p*-ABSA and 0.1 mol L^{-1} KCl with cyclic voltammetric sweeps in the potential range from −1.50 V to 2.00 V. As shown in Figure 1, an anodic peak 1 at 0.90 V and a cathodic peak 2 at −0.20 V were observed when in the first scan. From the second scan, an anodic peak 3 appeared with potential at +0.10 V. The oxidation and reduction peak current increase with the segment of voltammetric scan increasing, indicating that an electroconductive polymer film has been formed on the electrode surface. The electrochemical behavior of *p*-ABSA at GCE was similar to the references reported.[20,21] After 24 segments, the electropolymerization procedure was terminated and the polymer film electrode was washed with redistilled water to remove the physically adsorbed material. Then, the film electrode was transferred to an electrochemical cell containing phosphate buffer, and cyclic voltammetric sweeps were carried out to obtain electrochemical steady-state. After dried in air, a chromaticity poly(*p*-ABSA) film could be seen at the electrode surface obviously. Thickness of the poly(*p*-ABSA) film could be adjusted by the segments of voltammetric scan.

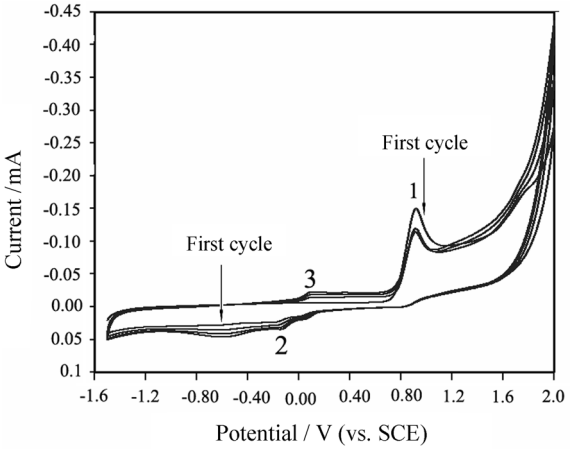

Figure 1. Cyclic voltammograms for the electropolymerization of *p*-ABSA film on a glassy carbon electrode in a phosphate buffer solution (pH 8.0) containing 0.1 mol L^{-1} KCl. *p*-ABSA concentration: 5×10^{-3} mol L^{-1}. Scan rate: 100 mV s^{-1}.

Voltammetric behavior of Trp at the poly(*p*-ABSA) film electrode. Cyclic voltammograms of 2.0×10^{-6} mol L^{-1} Trp at the poly(*p*-ABSA) film modified GCE (a) and a bare GCE (b) in pH 3.0 phosphate buffer were shown in Figure 2. At the bare GCE, Trp exhibited a poor electrochemical response, but at the poly(*p*-ABSA) film modified electrode, the peak current was increased greatly. The oxidation peak current of Trp (peak a) at the poly(*p*-ABSA) film electrode was 17 times of that at the bare GCE. It's well known that there is high electron density of sulfonic group in the chemical structure of *p*-ABSA, so that the surface of the film modified electrode is in negatively charge. In pH 3.0, $-NH_2$ in Trp can be protonated with H^+ and a form of $-NH^{3+}$ is obtained to interact with the fabricated film electrode through electrostatic interaction. Therefore, Trp can be accumulated to the surface of the poly(*p*-ABSA) film electrode effectively, and the sensitivity could be enhanced significantly.

Influence of potential on the electropolymerization of poly(*p*-ABSA) film. Potential is the most important factor

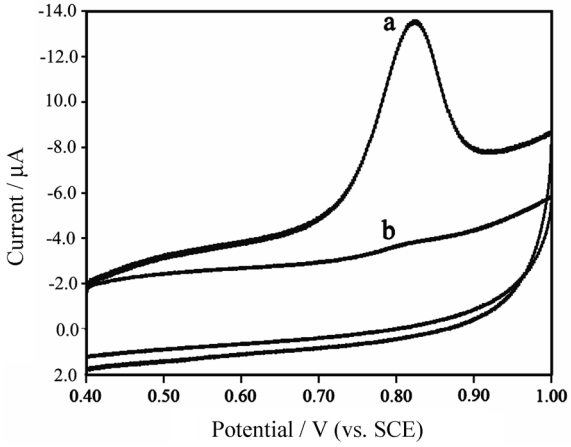

Figure 2. Cyclic voltammograms of 2.0×10^{-6} mol L^{-1} Trp at the poly(*p*-ABSA) film modified GCE (a) and a bare GCE (b) in a phosphate buffer (pH = 3.0).

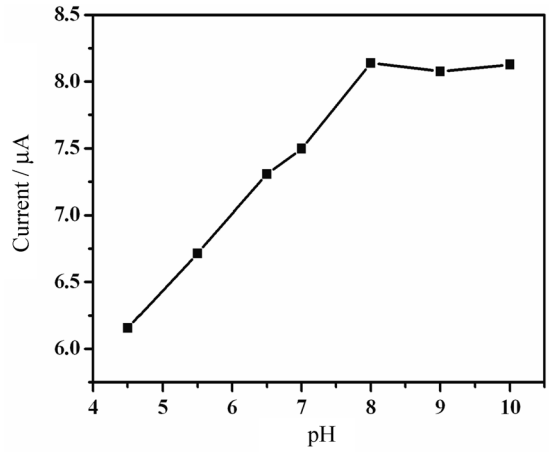

Figure 3. Voltammetric response of 2.0×10^{-6} mol L^{-1} Trp at the poly(*p*-ABSA) film electrode obtained at different pH value. Accumulation potential: −0.1 V. Accumulation time: 60 s. Scan rate: 100 mV s^{-1}.

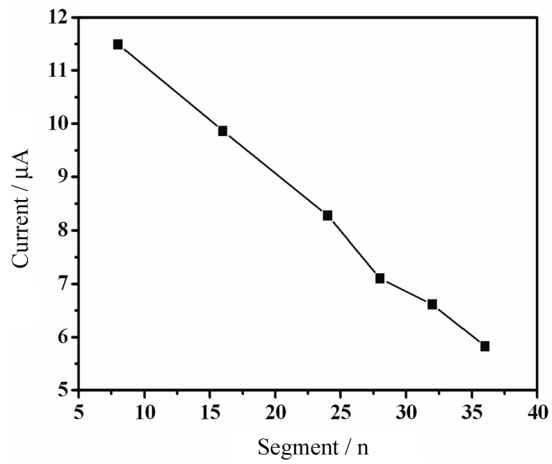

Figure 4. Influence of the segments of the cyclic voltammetric sweeps for the electropolymerization of *p*-ABSA on the oxidation peak current of 2.0×10^{-6} mol L^{-1} Trp.

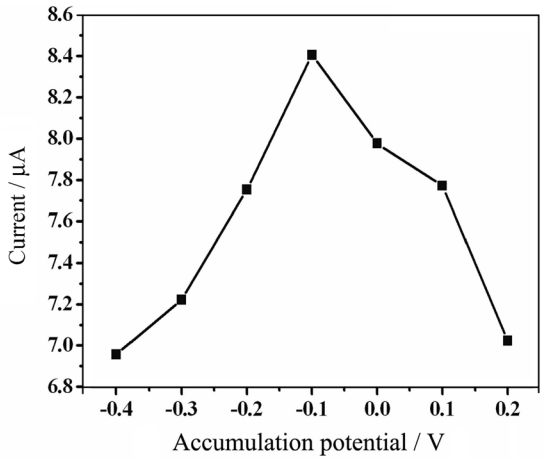

Figure 5. Effect of accumulation potential on the oxidation peak current of 2.0×10^{-6} mol L^{-1} Trp at the poly(*p*-ABSA) film electrode.

when cyclic voltammetry was used to form poly(*p*-ABSA) film through electropolymerization. It was found that the positive potential value for electropolymerization was lower than 1.50 V or the negative one was higher than 0.80 V, there was no polymer film can be observed. When the positive potential value reached 1.80 V, the electropolymerization was occurred. However, the electrode will be destroyed when the positive potential value was higher than 2.00 V. Therefore, the potential range from −1.50 V to 2.00 V was employed for the electropolymerization of *p*-ABSA in this paper.

Voltammetric response of Trp at the Poly(*p*-ABSA) film electrode obtained at different pH Value. Effect of *p*-ABSA solution pH on the current response of Trp is shown in Figure 3. It was found that the oxidation peak current of Trp was increased gradually with pH increase. It main due to the amount of sulfonic group on electrode surface is increased with pH increase, consequently more Trp cations were attracted to the film electrode. When pH ≥ 8.0 the oxidation peak current varied slightly. Thus, pH 8.0 was selected for construction of poly(*p*-ABSA) film electrode in the subsequent analytical experiments.

Effect of poly(*p*-ABSA) film thickness on the electrochemical response of Trp. Poly(*p*-ABSA) film thickness which was controlled by the cyclic voltammetric sweep segments was also an important parameter which we should investigate carefully. Effect of the film thickness on the oxidation peak current of 2.0×10^{-6} mol L^{-1} Trp was investigated. As shown in Figure 4, the peak current was decreased with the film thickness increasing due to the electron transfer rate of Trp on the poly(*p*-ABSA) film electrode was retarded. Nevertheless, the repeatability and stability for the film modified electrode were poor when the voltammetric sweeping segment was less than 24. Taking the sensitivity and stability into account, we selected the film thickness obtained with 24-segment sweeping as the optimal condition in the further experiments.

Influence of accumulation potential and time on the current response of Trp. Influence of accumulation potential (E_{acc}) on the oxidation peak current of Trp was investigated at different potential range from −0.40 V to 0.20 V. As shown in Figure 5, it can be seen that the peak current of Trp increases rapidly in the accumulation potential range from 0.20 V to −0.10 V, then it decreases greatly when the accumulation potential is lower than −0.1 V. Thus, an accumulation potential, −0.10 V, was employed in the further investigation.

Effect of accumulation time (t_{acc}) range from 0 to 150 s on the peak current following accumulation at −0.10 V is demonstrated in Figure 6. The peak current is found to increase with the accumulation time increasing, the oxidation peak current of Trp change slightly when t_{acc} is longer than 60 s. Therefore, an accumulation time of 60 s is found reasonable for the present analytical study.

Influence of pH value. Influence of solution pH on the oxidation of Trp at the Poly(*p*-ABSA) film electrode in the presence of 2.0×10^{-6} mol L^{-1} Trp was investigated with linear sweep voltammometry in the pH range from 2.5 to 7.0. As depicted in Figure 7, the oxidation peak current of

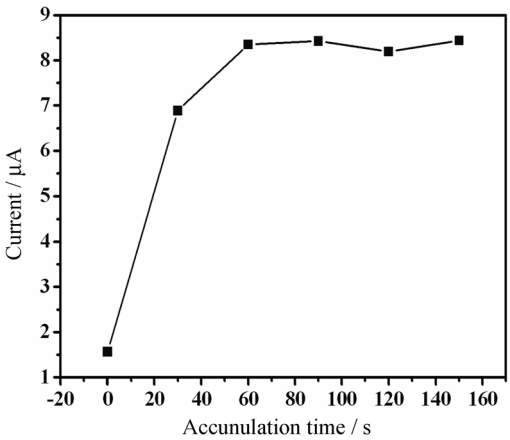

Figure 6. Effect of accumulation time on the oxidation peak current of 2.0×10^{-6} mol L^{-1} Trp at the poly(*p*-ABSA) film electrode.

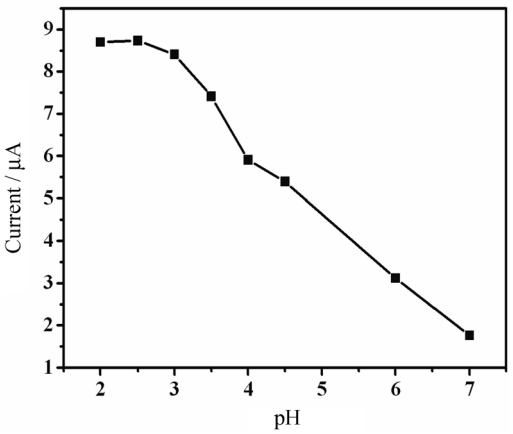

Figure 7. Effect of pH on the oxidation peak current of 2.0×10^{-6} mol L^{-1} Trp at the poly(*p*-ABSA) film electrode.

Trp inecreased with pH decrease. It may be the fact that the protonated degree of Trp increased with pH decreasing, hence the electrostatic interaction between Trp and poly(*p*-ABSA) film increased. However, when the pH was lower than 3.0, the poly(*p*-ABSA) film modified electrode exhibit a poor reproducibility. Therefore, a pH value 3.0 was adopted in the subsequent analytical experiments.

Effect of scan rate. Useful information involving electrochemical mechanism usually can be acquired from the relationship between peak current and scan rate. Therefore, effect of scan rate (ν) on the oxidation of Trp was investigated. Figure 8 shows the oxidation peak current of 2.0×10^{-6} mol L^{-1} Trp at the film electrode with different scan rate. From Figure 8, we can see that the peak current increased linearly with the scan rate in the range of 10-175 mV s^{-1}, and can be expressed as following: Ipa (μA) = 0.07166 ν (mV s^{-1}) + 0.6215, r = 0.9983. Thus, the electrochemical reaction is rather an adsorption-controlled step than a diffusion-controlled process.

Interferences and reproducibility. In order to assess the proposed method to the analysis of Trp in pharmaceutical dosage forms and biological samples, the interference of

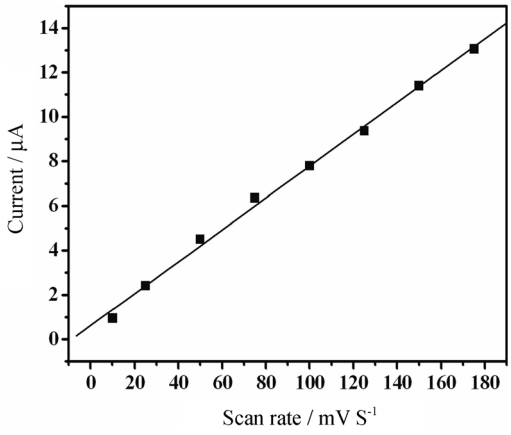

Figure 8. Effect of scan rate on the oxidation peak current of 2.0×10^{-6} mol L^{-1} Trp.

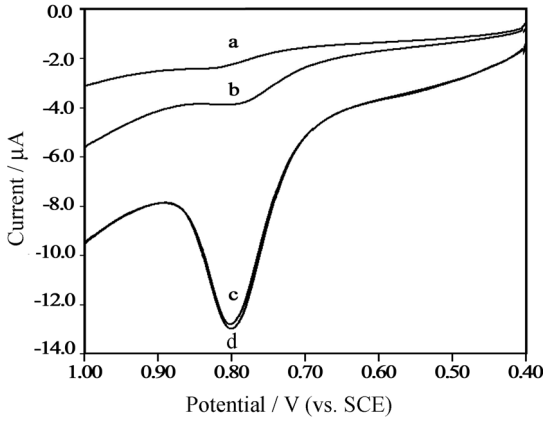

Figure 9. Linear sweep voltammograms of 2.0×10^{-6} mol L^{-1} Trp at the bare GCE (a) and the poly(*p*-ABSA) film electrode (c), 2.0×10^{-6} mol L^{-1} Trp + 2.0×10^{-5} mol L^{-1} Tyr at the bare GCE (b) and the poly(*p*-ABSA) film electrode (d).

other 17 basic amino acids and organic substances such as uric acid and vitamin C etc. were examined. Tyrosine (Tyr) often coexists with Trp in humans and herbivores bodies, moreover, the oxidation potential of them are almost the same at the bare GCE. Therefore, they were investigated in detail. As shown in Figure 9, the peak current increased with the addition of ten-folds of Tyr to the Trp solution at bare GCE (curve a and b), however the peak current of Trp at the Poly(*p*-ABSA) film electrode (curve c and d) in the absence and presence of Tyr was about the same, suggesting that Tyr did not influence the measurement of Trp with this method. Interferences of other amino acids and organic substances for the determination of Trp were shown in Table 1, the electrochemical signals of Trp changed slightly (RSD ≤ 5%) in the presence of ten-folds of these substances. These facts demonstrate that poly(*p*-ABSA) film electrode possesses high selectivity for the determination of Trp.

Stability of the poly(*p*-ABSA) film modified electrode for the determination of 2×10^{-6} mol L^{-1} Trp was investigated by linear sweep voltammetry (LSV). The film electrode

Table 1. Influence of some potential Interferents on the oxidation of Trp

Interferents	Concentration (mol L^{-})	Signal change	Interferents	Concentration (mol L^{-})	Signal change (%)
Tyrosine	2×10^{-5}	+4.0%	Leucine	2×10^{-5}	+2.1%
Valine	2×10^{-5}	+2.8%	Isoleucine	2×10^{-5}	+2.7%
Serine	2×10^{-5}	–2.4%	Glycine	2×10^{-5}	–3.6%
Arginine	2×10^{-5}	–3.7%	Glutamic acid	2×10^{-5}	–3.2%
Aspartic acid	2×10^{-5}	+2.6%	Proline	2×10^{-5}	+3.1%
Phenylalanine	2×10^{-5}	+4.3%	Lysine	2×10^{-5}	–2.1%
Histidine	2×10^{-5}	+2.7%	Threonine	2×10^{-5}	+3.4%
Cysteine	2×10^{-5}	+3.1%	Alanine	2×10^{-5}	+3.2%
Methionine	2×10^{-5}	+2.9%	VitaminC	2×10^{-5}	–3.8%
Dopamine	2×10^{-5}	–3.1%	Uric acid	2×10^{-5}	+3.4%

retained a response of 96.8% of the initial current after 1 week stored at room temperature. Relative standard deviation (RSD) of six successive determinations was 2.2% for 2.0×10^{-6} mol L^{-1} Trp. Reproducibility of six independently fabricated electrodes showed a satisfactory value of 2.4% (RSD).

Determination of Trp. Poly(*p*-ABSA) film modified electrode was employed for the determination of Trp with LSV. The results showed that the oxidation peak current (i_{pa}) was proportional to the concentration of Trp over two concentration intervals, viz. 1.0×10^{-7}-1.0×10^{-6} mol L^{-1} and 2.0×10^{-6}-1.0×10^{-5} mol L^{-1}. The linear regression equation can be expressed as following: ip (μA) = 0.6469 + 5.538 C (μmol L^{-1}) and ip (μA) = 7.073 + 0.648 C (μmol L^{-1}) with the correlation coefficient of 0.9978 and 0.9979. The detection limit was estimated to be 7.0×10^{-8} mol L^{-1} (S/N = 3). Trp is not encouraged to determine by this method when its concentration was in the range of 1.0×10^{-6}-2.0×10^{-6} mol L^{-1} in real samples, because we can not calculate its concentration accurately from either linear regression equation above.

Analytical application. In order to evaluate the validity of the Poly(*p*-ABSA) film electrode for the determination of Trp in pharmaceutical formulations, the content of Trp in a commercial amino acid oral solution (Jinri Co., Xiamen, China) was determined with the proposed method. For the amino acid liquid sample, an aliquot of 10 μL was directly introduced into 10 mL phosphate buffer solution (pH 3.0) and voltammetric measurement was performed. The recorded peak current was 8.842 μA and the calculated content of Trp in the analyzed sample was 0.558 g L^{-1}. The result obtained from voltammetric determination was consistent well with the certified value (0.540 g L^{-1}) of the analyzed pharmaceutical product, suggesting that the poly(*p*-ABSA) film electrode was very reliable, selective and sensitive enough for the determination of Trp in real samples.

Conclusion

A poly(*p*-ABSA) film modified glassy carbon electrode was constructed for the determination of tryptophan in an acid solution (pH 3.0). Influences of the film thickness, potential range for electropolymerization, accumulation conditions and pH value on the determination of Trp have been investigated carefully. The film electrode was found to be selective and sensitive for the determination of Trp even in a pharmaceutical sample without pretreatment.

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