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# Determination of L-tryptophan in the presence of ascorbic acid and dopamine using poly (sulfosalicylic acid) modified glassy carbon electrode

*Xi Ba, Liqiang Luo, Yaping Ding*, Xiao Liu*
*Department of Chemistry, Shanghai University, Shanghai 200444, P R. China*
*Corresponding e-mail address: wdingyp@sina.com*

**Abstract:**
In this work, a glassy carbon electrode modified with poly-sulfosalicylic acid (PSA/GCE) was prepared by electropolymerization and applied for the determination of L-Tryptophan (L-Trp) in the presence of ascorbic acid and dopamine. The morphologies and interface properties of PSA film were examined by scanning electron microscopy and electrochemical impedance spectroscopy.The electrocatalytic oxidation of L-Trp was investigated on the PSA/GCE using cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The proposed method exhibited wide linear response of $5\times10^{-8}$ to $4\times10^{-4}$ M with low detection limit and high selectivity, making it suitable for the analytical purpose.

**Keywords:** Poly-sulfosalicylic acid, L-Tryptophan, Electropolymerization, Differential pulse voltammetry

## Introduction

L-Tryptophan (L-Trp) is one of essential amino acids to human and a vital constituent of protein biosynthesis of living organisms [1]. As a biochemical precursor for neurotransmitter serotonin and neurohormone melatonin [2], it is very essential for people with sleep deprivation, anxiety and mood enhancement [3]. Due to the scarce presence of tryptophan in vegetables, L-Trp has been commonly added to dietary, food products as food fortifier or pharmaceutical formulations. When improperly metabolized, a waste product will be created in the brain to cause hallucinations and delusions [4]. So, it is necessary to establish a simple, accurate, rapid and inexpensive method for the determination of L-Trp in food, pharmaceutical products and biological fluids.

Some methods have been developed for the determination of L-Trp, such as spectroscopy [5], high-performance liquid chromatography [6], fluorometric methods [7], capillary electrophoresis [8] and electroanalysis [9]. Among them, electrochemical techniques have gained much more attention for its high sensitivity, high accuracy, simple operation mode and low cost. However, the voltammetric response of L-Trp at bare electrode is not optimal because of sluggish electron transfer processes and high overpotential [10]. Hence, many efforts have been devoted to promoting the electron transfer and reducing the overpotential for the electrochemical oxidation of L-Trp [11-16].

Recently, various modified electrodes have been reported for the determination of L-Trp, such as poly 4-aminobenzoic acid [11], poly glutamic acid [12], 1-[4-(ferrocenyl ethynyl) phenyl]-1-ethanone [13], Ni (II)/ACDA–AuNP–Au [14], gold nanoparticles [15] and nano-$TiO_2$/ferrocence carboxylic acid [16]. Among these electrodes, polymer film modified electrodes show unique properties and an enhanced response for the application in samples [17]. Since the thickness, permeation and charge transport characteristics of the polymeric films can be adjusted by the potential and current applied, the fabrication of conducting polymer film is flexible and controlled [18].

In this work, a novel sensor of L-Trp based on poly (sulfosalicylic acid) film (PSA) modified glassy carbon electrode by electrochemical polymerization (PAS/GCE) was developed. Because of high electron density of carbonyl and sulfonic groups in sulfosalicylic acid molecule ($COO^-$ and $SO_3^{2-}$), the PSA film has high concentrations of negatively-charged surface-functional groups. The modified electrode showed excellent electrocatalytic properties with obvious reduction of overpotential and enhancement of oxidation current and was applied for the detection of L-Trp coexisting with some possible interfering substances.

## Experiment

Electrochemical measurements were carried out on a CHI 660C electrochemical workstation (Shanghai Chenhua Co., Ltd., China) with a conventional three-electrode system. A clean GCE was immersed in 0.1 M PBS (pH 5.5) containing 10mM sulfosalicylic acid using cyclic voltammetry from -1.0 to 2.0 V at 100mv $s^{-1}$ for 30 cycles and washed with double distilled water for use.

## Characterization

To investigate the morphology of the modified electrode, we performed scanning electron microscope (SEM). Fig. 1 shows the typical morphology of PSA film, indicating that the film has a fine cluster-like structure. SEM image of PSA film was very uniform, which verified that the PSA film was successfully polymerized on the electrode surface and the structure of PSA film could improve the effective electrode surface.

Electrochemical impedance spectroscopy (EIS) was used as a powerful technique to study the interface properties of the electrode surfaces. Fig. 2 shows the typical Nyquist diagrams of the EIS in 5.0 mM $[Fe(CN)_6]^{3-/4-}$ solution at the bare GCE (a), PSA/GCE (b). Compared with the bare GCE (curve a), the electron-transfer resistance ($R_{et}$) for PSA/GCE was larger (curve b).This may be ascribed to the electrostatic repulsion force between the negatively charged $[Fe(CN)_6]^{3-/4-}$ and poly (sulfosalicylic acid) film [19]. The change of the $R_{et}$ value suggests that the PSA film is assembled onto the surface of GCE.

## Electrochemical behavior of L-Trp at PSA/GCE

The electrocatalytic activity of PSA/GCE was demonstrated by a comparison of the differential pulse voltammograms (DPVs) of different electrodes in 0.1 M PBS (pH 3.5). Fig. 3 shows DPVs of $1\times10^{-4}$ M L-Trp at the bare GCE (b), PSA/GCE(c). It can be seen that the oxidation peak current of L-Trp at PSA/GCE is enhanced and the peak is sharper than the bare GCE (curve b). The increase of peak current maybe attributed to the electrostatic interaction between the negatively charged PSA and the positively charged L-Trp in 0.1 M PBS (pH 3.5) [12]. Meanwhile, the peak potential shifted negatively from 0.91V to 0.79V, indicating that the PSA film has good electro-catalytic activity towards L-Trp.

## Calibration curve and interferences

Under the optimum conditions, the electrochemical behaviors of different concentrations of L-Trp were studied. From Fig. 4(A), the change of DPVs indicates that the oxidative peak current ($I_p$) has linear relationship with the concentration ($c$) of L-Trp. In the range from $5.0\times10^{-8}$ to $4.0\times10^{-4}$ M, two linear regression equations $I_p$ (μA) = 0.2524 + 0.3922 $c$ (μm), $I_p$ (μA) = 4.7947 + 0.0369 $c$ (μm) were obtained with the correlation coefficients of 0.9963 and 0.9943 consequently.

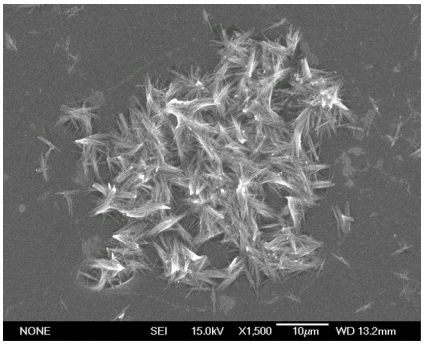


*Fig. 1. Scanning electron microscope of the PSA film.*

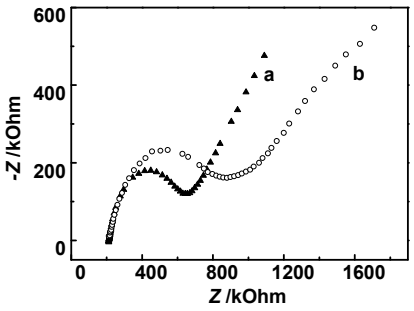


*Fig. 2. Electrochemical impedance spectra of bare GCE (a), PSA/GCE (b).*

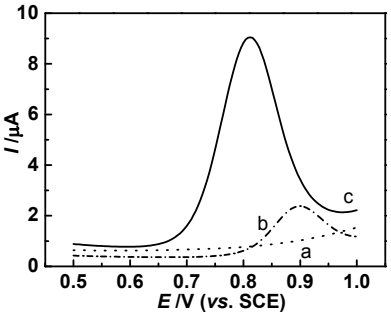


*Fig. 3. DPVs of $1\times10^{-4}$ M L-Trp in 0.1 M PBS (pH 3.5) at the bare GCE (b), PSA/GCE(c) and the bare GCE in the absence of L-Trp (a).*

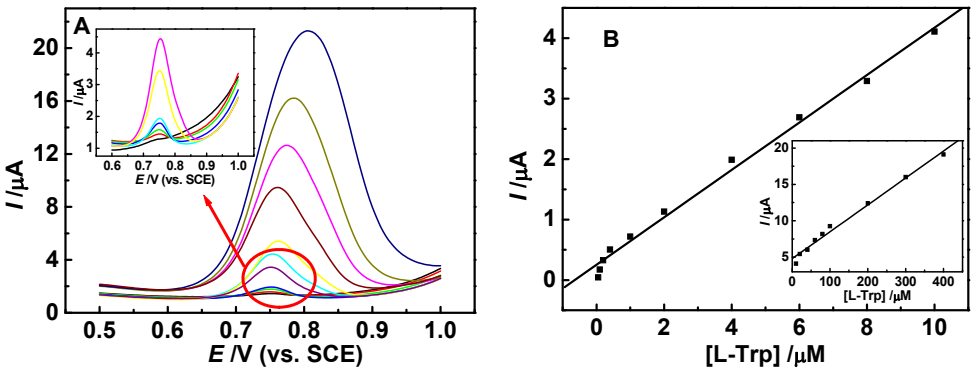


*Fig. 4. (A) DPVs of the PSA/GCE at different L-Trp concentrations from $5\times10^{-8}$ M to $4\times10^{-4}$ M. The above inset is the amplifying DPV of L-Trp from $5\times10^{-8}$ M to $1\times10^{-5}$ M. (B) the calibration curve for the determination of L-Trp. Insert: the plot of the peak current vs. L-Trp concentration*

The detection limit (S/N=3) and the sensitivity was calculated to be $6.8\times10^{-9}$ M and 1913.63 $\mu A\cdot mM^{-1}\cdot cm^{-2}$ respectively.

To evaluate the influences of some potential interference on the determination of L-Trp, various foreign species were added into 0.1 M PBS containing L-Trp ($1\times10^{-6}$ M), such as cysteine, alanine, phenylalanine, glutamic. The results indicate the interference effects of the above analytes toward response of L-Trp are negligible when the concentration is more than 100-times. Furthermore, there are some important biological substances like AA and DA often coexisting with L-Trp in biological samples. Fig.5 describes the DPVs of different concentrations of L-Trp at PSA/GCE in the presence of AA and DA. It can be seen that three well-separated peaks presented at the detached potentials, indicating that AA and DA had no interference for the detection of L-Trp. In addition, the peak currents of three compounds increased synchronously with the increasing of concentrations of L-Trp, AA and DA, implying that PSA/GCE can be also applied for the simultaneous determination of L-Trp, AA and DA.

Table 1 displays analytical results of the proposed method and were compared with other electrochemical methods reported previously for detecting L-Trp. It can be seen that the electrochemical performance of PSA/GCE here is favorable and the electrochemical sensor would be very suitable for practical L-Trp detection.

## Conclusions

A novel sensor of L-Trp was prepared with poly-sulfosalicylic acid modified glassy carbon electrode by electropolymerization in this work. The modified electrode showed wide linear concentration range, low detection limit and high selectivity. In addition, the method can be applied for the measurement of L-Trp in the presence of AA and DA. The results showed that PSA/GCE had good analytical performance, which can be used for routine analysis of L-Trp in biological samples.

*Tab. 1: Comparisons of the proposed PSA/GCE performance with those previously reported*

| Method | Dynamic ranges(μM) | Detection limits(μM) |
|---|---|---|
| 4-ABA/GCE[11] | 1–100 | 0.2 |
| PGA/CNTPE[12] | 0.05–100 | 0.01 |
| 4-FEPE/CPE[13] | 0.85–63.4 | 0.56 |
| AuNP-CNT/GCE[14] | 0.03–2.5 | 0.01 |
| Au-NPs/GCE[15] | 0.09-500 | 0.08 |
| Present work | 0.05-400 | 0.0068 |

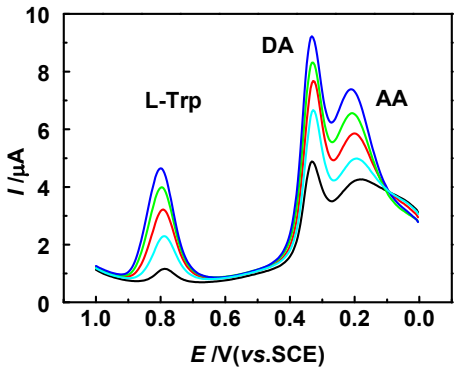


*Fig. 5. Differential pulse voltammograms of L-Trp (1-20 μM) in 0.1 M PBS (pH 3.5) in the presence of ascorbic acid (20-200 μM) and dopamine (1-20 μM).*

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