

The interaction of Alizarin Red with Tryptophan by Voltammetry

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Abstract

The interaction of Alizarin Red (AR) with Tryptophan (Trp) was investigated by first order derivative linear sweep voltammetry. In pH5.2 NaAc-HAc buffer solution, AR had a well-defined voltammetric reductive peak at -0.392V(vs.SCE). After Trp addition, the peak current of AR decreased apparently with no change of the peak potential and no appearance of new peaks. The study about the effect of scan rate on peak current showed that a new electro-inactive complex was formed as a result of interaction of AR with Trp, which decreased the peak current. Based on the formation of AR-Trp complex, the conditions of interaction and the electrochemical detection were carefully investigated and the binding ratio was obtained by mole ratio method. Finally the character of the electrode process was discussed by the effect of scan rates on AR-Trp interaction system.

Keywords: Alizarin Red, Tryptophan, linear sweep voltammetry, interaction

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1. Introduction

Tryptophan, which is also called amino indole propionic acid, is a kind of natural amino acid and one of the human essential amino acids. It is the precursor of the key human neurotransmitter 5-hydroxytryptophane and has a certain sedative effect such as sleep inhibition, temperature regulation, pain sensitivity and aggressive behavior [1, 2], etc. Up to now, the study on the interaction of tryptophan and other substances has been proceeding mostly by ultraviolet spectroscopy or fluorescence spectroscopy [3-5], which is seldom proceeding by the electrochemical method. And electrochemically active dyes are used as probes not only to investigate the interaction mechanism between electro-active substances and amino acids by the electrochemical method but also to potentially develop a new electroanalytical method for amino acid detection [6, 7]. In this paper, an electro-active dye Alizarin Red (AR) was selected as a probe to investigate its interaction with Trp by linear sweep voltammetry. In the selected pH5.2 NaAc-HAc buffer solution, the AR molecules are negatively charged, while the Trp molecules (isoelectric point, pI=5.89) are mainly positively charged, so they can bind together by their electrostatic attraction. The study about the effect of scan rate on peak current indicated that the interaction of AR with Trp could form an electro-inactive complex, which decreased the reductive peak current. Based on the formation of the AR-Trp complex, the conditions of interaction and the electrochemical detection were carefully investigated and the binding ratio was obtained by mole ratio method. In addition, the character of the electrode process was discussed.

2. Materials and Methods

2.1. Reagents

Tryptophan (Trp, chem base) stock standard solution (0.204mg/ml) was prepared by dissolving 0.0408g Trp in 200ml water. The solution was diluted to convenient concentration with water properly in the experiment. Alizarin Red (AR, Sinopharm Chemical Reagent Co., Ltd) stock standard solution (0.3605mg/ml) was prepared by dissolving 0.0721g AR in 200ml water. 0.2 mol/l HAc-NaAc buffer solution was used to regulate pH value of the interaction medium. All

the chemicals used were of analytical reagent grade without further purification and doubly distilled water was used for making solutions [8].

2.2. Apparatus

A model JP-303 polarographic analyzer (Chengdu Apparatus Factory, China) was used in this work. Voltammetric curves were recorded in a three-electrode electrochemical cell at different temperature. A dropping mercury electrode (DME) as working electrode, a SCE reference electrode and a platinum-wire auxiliary electrode were used. All the values of pH were measured with a pHS-25 acidity meter (Shanghai Leici Instrument Factory, China)

2.3. Procedure

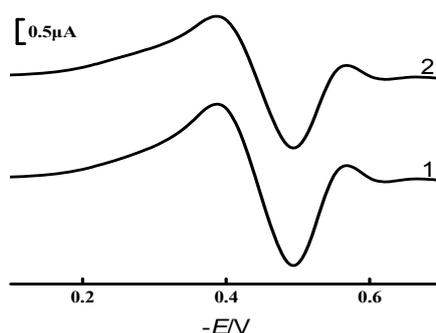
4.0ml of 0.2mol/l pH5.2 HAc-NaAc buffer solution, 1ml of 0.3605mg/ml AR and an appropriate amount of standard Trp solution were placed in a dry 10ml colorimetric tube in sequence. The mixtures were diluted to scale with water and shook well, then the first order derivative linear sweep voltammetric peak currents (I'_p) were recorded in the following conditions: potential scan rate, 900mv/s; potential range, -0.2~-0.7V(vs.SCE). A blank solution without Trp were measured in the same conditions and its reductive peak current (I'_{p0}) was obtained, and then the difference of peak current ($\Delta I'_p = I'_{p0} - I'_p$) was calculated.

3. Results and Discussions

3.1. Electrochemical Behaviors of AR in the Absence and Presence of Trp

At the optimized experimental conditions, the first order derivative linear sweep voltammograms of AR in the absence (curve 1) and presence (curve 2) of Trp were recorded and shown in Figure 1. After Trp was added, the reductive peak current of AR decreased apparently with no change of the reductive peak potential and no appearance of new peaks in the selected potential range. As for the reason, there maybe two possible different opinions: (1) The decrease of diffusion coefficient after the addition of Trp; (2) The formation of electro-inactive complex and no changes of diffusion coefficient. Then the diffusion coefficients of AR in the absence and presence of Trp were compared. From Figure 4, it could be seen that no matter whether Trp was present or not, I'_p increased linearly with $v^{1/2}$ increasing at higher scan rates. The equations were as follows:

$$I'_p = 12528v^{1/2} - 3953.0 (n=6, R^2=0.9995) \text{ and } I'_p = 11839v^{1/2} - 3623.8 (n=6, R^2=0.9997)$$



1) pH5.2HAc-NaAc +0.03605mg/ml AR, 2) 1+0.00204mg/ml Trp

Figure 1. First Order Derivative Linear Sweep Voltammograms of AR-Trp Interaction System

According to the general principal of electrochemical reaction, the slope item of the linear equation of $I'_p \sim v^{1/2}$ contains the diffusion coefficient factor, and the size of the slope is

related to the diffusion coefficient in the case of other experimental conditions unchanged [9-11]. By comparing the slopes of the linear equation in the absence and presence of Trp, so it could be found that the diffusion coefficient was essentially the same. According to the statement of Kelly [12], it might be that AR interacting with Trp formed a complex of large molecular weight, which couldn't be reduced on the Hg electrode surface, and the equilibrium concentration of free AR in solution decreased, so the peak current decreased obviously with the peak potential unchanged. In addition, the fact that the difference of peak current decreased linearly with the increase of Trp concentration also explained pretty well that the formed AR-Trp complex was electro-inactive.

3.2. Optimization of Interaction Conditions

3.2.1. Effects of Buffers and pH

In order to investigate the effect of various buffers on the interaction between AR and Trp, B-R, Tris-HCl, borax, $\text{NH}_3\text{-NH}_4\text{Cl}$, HAc-NaAc were tested respectively, and the results indicated that in HAc-NaAc buffer the peak shape was good and the value of $\Delta I'_P$ was the maximum. The experiments indicated that the buffer pH had great influence on the decrease of peak current. As shown in Figure 2, the maximum $\Delta I'_P$ appeared at pH 5.2, so pH 5.2 was selected as the optimal pH. In addition, with the increase of pH value, the reductive peak potential shifted negatively, which indicated that the hydrogen ions were involved in the electrode reaction. The optimal volume of HAc-NaAc buffer at pH 5.2 was estimated and 4.0 ml of buffer was used in the following experiments.

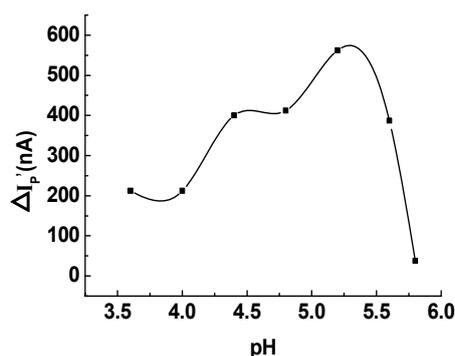


Figure 2. Dependences on the Decrease of Peak Current Against pH (0.03605mg/ml AR+0.00204mg/ml Trp)

3.2.2. Effect of AR Concentration

The effect of AR concentration was studied with 0.00204mg/ml Trp, and it could be found that the concentration of AR at 0.03605mg/ml gave the maximum $\Delta I'_P$. When AR concentration was more than 0.03605mg/ml, the $\Delta I'_P$ began to decrease. So 0.03605mg/ml of AR was recommended in subsequent studies.

3.2.3. Stability

The experiments showed that $\Delta I'_P$ reached the maximum immediately after mixture at room temperature and remained stable for at least one hour. The influence of temperature on the system was also estimated at 10°C, room temperature and 35°C, separately and no obvious difference was found. Different adding orders of AR, Trp and HAc-NaAc buffer were tested and the results showed that the best addition sequence was HAc-NaAc buffer, AR and Trp.

3.3. The Binding Ratio of AR and Trp

By keeping the Trp concentration of 1.0×10^{-4} mol/l constant and changing the AR concentration [13], the relationship between $\Delta I'_P$ and AR concentration was obtained and

shown in Figure 3. In the figure, ab and bc segments intersected in the b point, whose corresponding horizontal coordinate value was used to calculate the binding ratio, and the binding ratio of AR and Trp was 2:3.

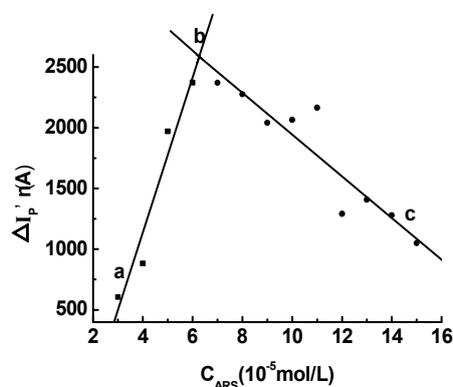
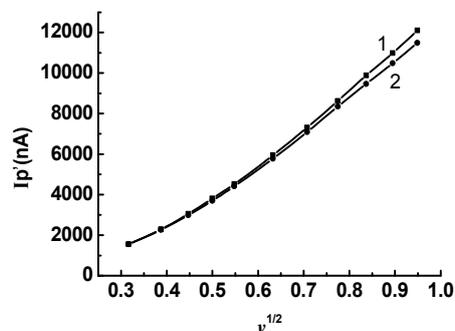


Figure 3. Relationship between AR and Trp

3.4. Effect of Scan Rates on the Electrode Process

The scan rates had important effect on the electrode process. The relationship between $v^{1/2}$ and I_p' of AR in the absence (curve 1) and presence (curve 2) of Trp were shown in Figure 4. It could be seen that no matter whether Trp was present or not, the relationship between I_p' and $v^{1/2}$ deviated from a straight line and curved upward at lower scan rates, which indicated that the electrode process was mainly controlled by adsorption; and when the scan rates were higher, I_p' was in linear relation with $v^{1/2}$, which showed that the electrode process was mainly driven by diffusion [14].



1) pH5.2HAc-NaAc +0.03605mg/ml AR, 2) 1+0.00204mg/ml Trp

Figure 4. Dependences of I_p' on Potential Scan Rates

The effect of scan rates on the peak potential of AR-Trp interaction system was investigated. As mentioned above, the reductive peak potential before and after the addition of Trp was the same in each of the selected scan rate. When the scan rates were lower, the peak potential basically had no changes, which was in good agreement with the character of the reversible reaction; and when the scan rates were higher, the reductive peak potential shifted negatively from -0.380 to -0.392V (vs.SCE) with the increase of scan rate in the scan rate range of 0.4~0.9V/s, which indicated that the electrode process was irreversible.

4. Conclusion

The investigation on the interaction of AR with Trp was described by voltammetry. Due to the almost unchanged diffusion coefficient after Trp addition, the binding of positively charged Trp with negatively charged AR resulted in the formation of a new electro-inactive complex, which decreased the reductive peak current. Based on the formation of the AR-Trp complex, the conditions of interaction and the electrochemical detection were carefully investigated and the binding ratio was obtained by mole ratio method. Finally, the character of the electrode process was discussed by the effect of scan rates on AR-Trp interaction system.

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