

## Study on Rapid Electrochemiluminescence Detection of Allura Red

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In this study, a fast and convenient method to immobilize Ru(bpy)<sub>3</sub><sup>2+</sup> on glassy carbon electrode (GCE) surface is developed. A Ru(bpy)<sub>3</sub><sup>2+</sup>/poly (sulfosalicylic acid) (PSA) modified glassy carbon electrode was prepared by electrochemical polymerization and used as the working electrode in the electrochemiluminescence (ECL) detection of Allura Red (AR). Under the optimal conditions, the linear range of the method was  $1.0 \times 10^{-7} \sim 1.0 \times 10^{-4}$  mol/L and the detection limit was  $6.0 \times 10^{-8}$  mol/L. The modified electrode shows good sensitivity and stability, which can be used in the determination of Allura Red in food samples.

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**Keywords:** Electrochemiluminescence; Ru(bpy)<sub>3</sub><sup>2+</sup>; electrochemical polymerization; Poly(sulfosalicylic acid); Allura Red

### 1. INTRODUCTION

Nowadays, synthetic food dyes have been widely used in food production in order to improve the appearance of the product as well as to have a greater appeal to consumers [1-2]. Allura Red (AR) is a kind of water-soluble synthetic dyes with bright crimson, strong stability [3-4], widely used in meat food, soft drinks, ice cream, jelly, puffed food and candy colored. However, AR belongs to azo dyes, whose functional groups and aromatic ring structures of azo dyes may cause harm to human health because they may be reduced to form aromatic amines [5]. In addition, many food contain excessive amount of colorants can cause a variety of symptoms of attention deficit hyperactivity disorder (ADHD) in children, such as over-active and inattentive [6-7]. Due to their younger age, liver

detoxification function is weak, it is likely to cause chronic poisoning and even affect the physical and mental development [8]. Therefore, the acceptable daily intake (ADI) is maximum at 7 mg/kg per day [9].

At present, the main methods to detect AR are high performance liquid chromatography (HPLC) and UV-visible spectrophotometry [10-11]. These methods generally have the disadvantages of large interference, low sensitivity, expensive instrument, complicated operation and time-consuming. Therefore, it is of great importance to develop an effective, high sensitivity and low cost method for the determination of AR. Electrochemiluminescence (ECL) is a novel technology which combines electrochemistry with chemiluminescence. Moreover, it has the advantages of high sensitivity, good reproducibility, selectivity, controllability, wide linear range and low detection limit [12].  $\text{Ru}(\text{bpy})_3^{2+}$  has the characteristics of stable chemical properties, good solubility and high luminous efficiency, which has become a hot spot for ECL researchers. Nevertheless,  $\text{Ru}(\text{bpy})_3^{2+}$  reaction consumes more reagents, which causes environmental pollution and high cost of analysis. Compared with the solution-phase  $\text{Ru}(\text{bpy})_3^{2+}$  ECL system, the ECL sensor was prepared by immobilizing  $\text{Ru}(\text{bpy})_3^{2+}$  on the electrode by electrochemical polymerization has more advantages [13]. This could greatly save the reagents and overcome the above problems. In this study, a novel ECL sensor was developed for the determination of AR by  $\text{Ru}(\text{bpy})_3^{2+}$ /poly (sulfosalicylic acid) (PSA) modified glassy carbon electrode (GCE). The modified electrode has good sensitivity, selectivity and stability, which can be used in the determination of AR in food samples.

## 2. EXPERIMENTAL

### 2.1 Materials

Allura Red, Tris(2,2'-bipyridyl) ruthenium(II) chloride hexahydrate ( $\text{Ru}(\text{bpy})_3\text{Cl}_2 \cdot 6\text{H}_2\text{O}$ ) and sulfosalicylic acid were purchased from Aladdin Industrial Corporation (China). All other chemicals were of analytical grade and were used directly without any purification. All the water used in the work was deionized water. The working solutions were obtained by diluting the stock solution with phosphate buffer solution (PBS) and water. 0.1 M PBS consisting of  $\text{NaH}_2\text{PO}_4$  and  $\text{Na}_2\text{HPO}_4$  was used as the supporting electrolyte.

### 2.2 Apparatus

CHI 660D Electrochemical Workstation (Shanghai CH instrument Co., Ltd., China) was used for electrochemical measurements. MPI-B multi-parameter chemiluminescence analysis test system (Xi'an Remex Analysis Instrument co., LTD, China) was used for ECL test. A conventional three-electrode system was used with a GCE (with a diameter of 3 mm) as the working electrode, a platinum wire as the counter electrode and an Ag/AgCl electrode (saturated KCl aqueous solution) as the reference electrode. Scanning electron micrographs of the electrode surfaces were obtained using a JEOL (JSM-6360LA, Japan) Scanning Electron Microscope (SEM).

### 2.3 Preparation of $\text{Ru}(\text{bpy})_3^{2+}/\text{PSA}/\text{GCE}$

The bare glassy carbon electrode (GCE) was polished with 0.5  $\mu\text{m}$ , 0.1  $\mu\text{m}$  and 0.05  $\mu\text{m}$  neutral alumina powder as mirror surface, followed by ultrasonic cleaning with absolute ethanol and deionized water (1 min/time). The pretreated electrode was placed in an aqueous solution containing 0.01 mol/L sulfosalicylic acid and  $1.0 \times 10^{-4}$  mol/L  $\text{Ru}(\text{bpy})_3^{2+}$  and the electrode potential was scanned reversibly between -1.5 V and +2.0 V for 30 cycles at 0.1 V/s. Afterwards, The  $\text{Ru}(\text{bpy})_3^{2+}/\text{PSA}/\text{GCE}$  was prepared and dried at room temperature.

### 2.4 Methods

0.1 mol/L PBS buffer (pH=10.0) and a certain volume of the AR standard solution were added to the 50 mL cell, respectively, at 20 mL. The  $\text{Ru}(\text{bpy})_3^{2+}/\text{PSA}/\text{GCE}$  was used as the working electrode, an Ag/AgCl electrode as the reference electrode and a platinum wire as the counter electrode. The ECL intensity was recorded by setting the electrochemical parameters. With different concentrations of AR, compared with the ECL intensity of the blank solution, AR has a significant quenching effect on ECL, with the increase of the amount of AR, the ECL intensity quenching value ( $\Delta\text{ECL}$ ) is larger.

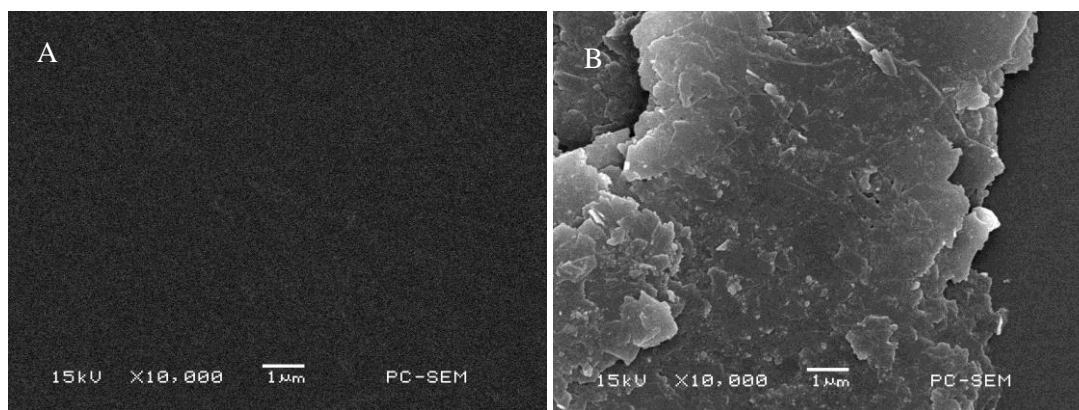
## 3. RESULTS AND DISCUSSION

### 3.1 Characterization of electrode

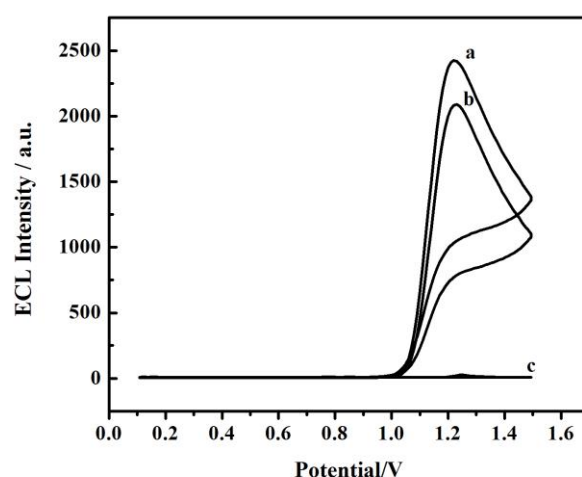
The surface morphology of GCE (Figure 1A) and  $\text{Ru}(\text{bpy})_3^{2+}/\text{PSA}/\text{GCE}$  (Figure 1B) were characterized by scanning electron microscopy (SEM). Figure 1A shows the GCE surface is smooth; Figure 1B shows the surface of electrode modified by poly(sulfosalicylic acid) has obvious lamellar structure, indicating that poly(sulfosalicylic acid) by electrochemical polymerization method was successfully modified onto the surface of GCE.

### 3.2 ECL behavior of AR on $\text{Ru}(\text{bpy})_3^{2+}/\text{PSA}/\text{GCE}$

Study on the ECL behavior of  $\text{Ru}(\text{bpy})_3^{2+}/\text{PSA}/\text{GCE}$  in PBS buffer and AR in PBS buffer was also investigated. As it is shown in figure 2, the ECL intensity is obviously decreased when a certain concentration of AR is added, indicating that  $\text{Ru}(\text{bpy})_3^{2+}/\text{PSA}/\text{GCE}$  has good electrochemical luminescence response function. If the electrode is not modified with PSA, it is found that the ECL intensity is almost 0 as shown in figure 2c, indicating that the PSA film plays an important role in the adsorption of ruthenium.



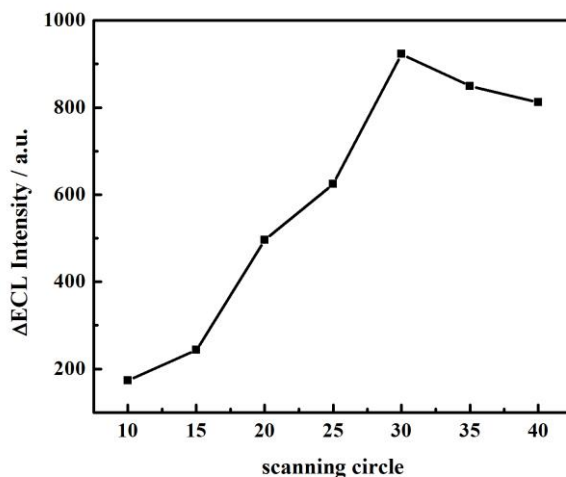
**Figure 1.** SEM image of bare GCE(A) and  $\text{Ru}(\text{bpy})_3^{2+}/\text{PSA}/\text{GCE}$  (B)



**Figure 2.** ECL of  $\text{Ru}(\text{bpy})_3^{2+}/\text{PSA}/\text{GCE}$  in 0.1 mol/L PBS buffer (pH = 10.0) with different concentrations of AR (a) 0 mmol/L and (b) 0.01 mmol/L at the scanning rate of 0.1 V/s from 0.1 V to 1.5 V and ECL of (c) modified electrode without PSA.

### 3.3 Influence of electrochemical polymerization scanning segment on $\Delta\text{ECL}$

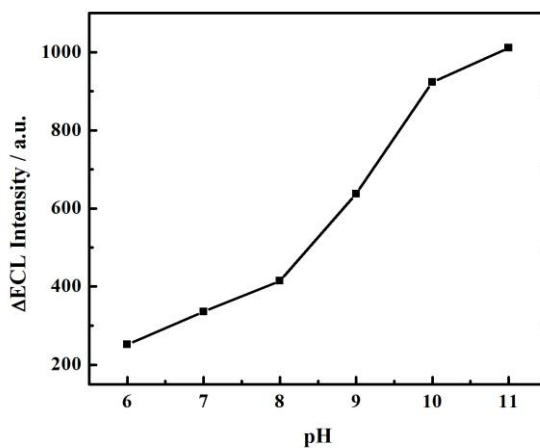
The scanning segments of electrochemical polymerization affects the ECL intensity of  $\text{Ru}(\text{bpy})_3^{2+}/\text{PSA}/\text{GCE}$ . The modified electrode was placed at 0.1 mol/L PBS buffer solution (pH = 10.0), using a cyclic voltammogram from 0.1 V to 1.5 V, sweeping 0.1 V/s, the degree of electrochemical polymerization can be changed by changing the scanning segments, so as to obtain different  $\Delta\text{ECL}$  in 0.1 mol/L PBS buffer solution (pH = 10.0) (Fig. 3). The thickness of the PSA film was affected by the electrochemical polymerization scanning segments,  $\Delta\text{ECL}$  gradually increases as the scanning segments increases from 10 to 30. The amount of  $\text{Ru}(\text{bpy})_3^{2+}$  on the surface of the modified electrode stabilized and the change of  $\Delta\text{ECL}$  was not obvious and the stable ECL intensity data could not be obtained when the scanning segment of electrochemical polymerization was more than 30, so 30 was chosen as the optimal electrochemical polymerization scanning segment.



**Figure 3.**  $\Delta$ ECL of  $\text{Ru}(\text{bpy})_3^{2+}/\text{PSA}/\text{GCE}$  with different electrochemical polymerization scanning segments in 0.1 mol/L PBS buffer (pH = 10.0) at the scanning rate of 0.1 V/s from 0.1 V to 1.5 V, AR (0.01 mmol/L)

### 3.4 Effect of pH on $\Delta$ ECL

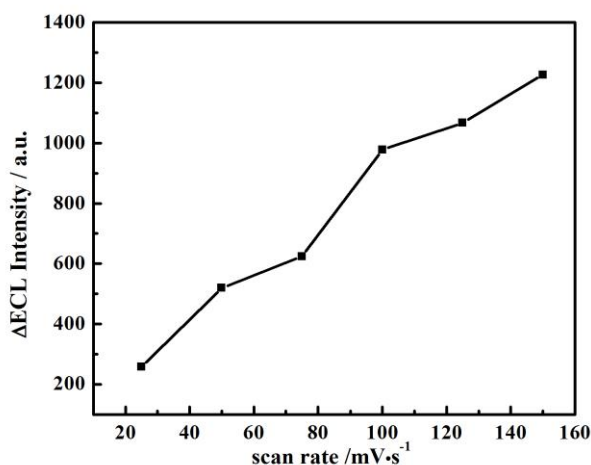
The ECL process is deeply correlated with the pH of the solution to be measured [14]. The ECL reaction was carried out in phosphate buffered solution at a certain concentration, the pH value of the buffer solution greatly influenced the ECL intensity of the buffer solution, which affected the electrochemical luminescence of  $\text{Ru}(\text{bpy})_3^{2+}$  directly. The results showed that  $\Delta$ ECL increased gradually with the increase of pH in 0.1 mol/L phosphate buffer solution. The  $\Delta$ ECL intensity increased gradually with the increase of pH because  $\text{Ru}(\text{bpy})_3^{2+}$  has good luminescence properties under the alkaline condition [15]. At pH = 10.0,  $\Delta$ ECL reached the maximum, and at pH = 11.0,  $\Delta$ ECL showed a rising trend but the measured luminous intensity is not stable, so the optimal pH of the buffer solution is 10.0 (Fig. 4).



**Figure 4.**  $\Delta$ ECL of  $\text{Ru}(\text{bpy})_3^{2+}/\text{PSA}/\text{GCE}$  with different pH at the scanning rate of 0.1 V/s from 0.1 V to 1.5 V in 0.1 mol/L PBS buffer, AR (0.01 mmol/L)

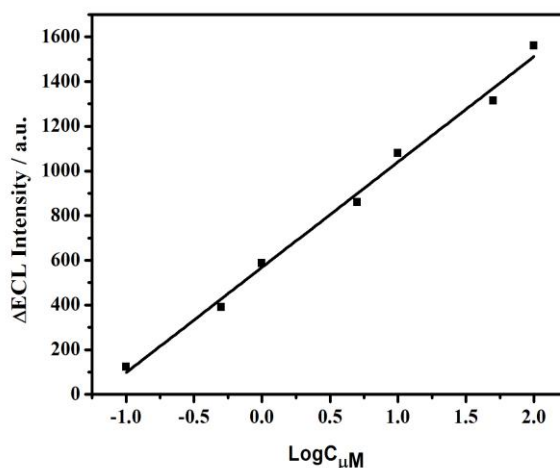
### 3.5 Effect of scanning rate on $\Delta ECL$

The ECL efficiency depends on the rate of generation/annihilation of the excited state  $Ru(bpy)_3^{2+*}$  [16]. The influence of different scanning rate (0.025 to 0.15 V/s) on the ECL intensity of AR was studied by using  $Ru(bpy)_3^{2+}/PSA/GCE$ , as given in Fig. 5. With 0.1 mol/L phosphate buffer solution at pH=10.0, the rate of  $\Delta ECL$  increased rapidly with the increase of scanning rate in the range of 0.025 to 0.1 V/s; The scanning rate from 0.1 to 0.15 V/s,  $\Delta ECL$  increases slowly. However, with the increase of scanning speed,  $\Delta ECL$  intensity fluctuates greatly and the stability was poor, and the reproducibility and stability are very good at 0.1 V/s. Therefore, 0.1 V/s is selected as the optimal scanning rate.



**Figure 5.**  $\Delta ECL$  of  $Ru(bpy)_3^{2+}/PSA/GCE$  with different scanning rate from 0.1 V to 1.5 V in 0.1 mol/L PBS buffer (pH = 10.0), AR (0.01 mmol/L)

### 3.6 Linear response range and detection limit



**Figure 6.**  $\Delta ECL$  of  $Ru(bpy)_3^{2+}/PSA/GCE$  with different concentrations of AR in 0.1 mol/L PBS buffer (pH = 10.0) at the scanning rate of 0.1 V/s from 0.1 V to 1.5 V

Under the above optimal conditions, with different concentrations of AR to observe the effects of  $\Delta$ ECL, the high voltage of the photomultiplier tube was 800 V and the scanning voltage was 0.1 to 1.5 V. When the concentration of AR was in the range of  $1.0 \times 10^{-7}$  to  $1.0 \times 10^{-4}$  mol/L,  $\Delta$ ECL showed a good linear relationship with the concentration of AR. The results were shown in Fig. 6. The regression equation was  $\Delta$ ECL = 568.926 + 471.576 logC ( $\mu$ mol/L) (R = 0.992). The limit of detection of the method (LOD) was  $6.0 \times 10^{-8}$  mol/L.

### 3.7 Analytical Application

A commercially available watermelon beverage sample was selected and placed in a 100 mL volumetric flask with 0.1 mol/L PBS (pH=10.0) buffer solution and detected the concentration of the AR. The analytical results are shown in Table 2. The relative standard deviation of each sample was less than 5%, which showed that the method had a good precision and the recovery rate was 99% to 101%. Determination of AR in beverage samples was also investigated by means of High Performance Liquid Chromatography [17]. In contrast, the method of ECL detection in this experiment showed a better linear range and lower detection limit. This method can accurately and quantitatively analyze the AR in the samples.

**Table 1.** Determination of AR in watermelon beverage samples

Sample	Added ( $\mu$ mol/L)	Detected ( $\mu$ mol/L)	Recovery (%)	RSD (%)	Average	
Watermelon beverage	1	0.98	101.1	3.64	0.99	
		0.96				
	1.5	1.03	99.3	2.03	1.50	
		1.53				
		1.51				
		1.93				
	2	1.93	101.3	3.15	1.98	
		2.05				
			1.96			

### 3.8 Analytical performance for detection of AR of the proposed method

Many analytical techniques for the detection of AR have been proposed in beverages and other products. The proposed sensor in this paper was compared with other methods and the results are showed in Table 2. The linear range of this experiment is wider than that of references [21-23], and the LOD is slightly lower than that of references [18-20], which is sensitive enough to detect AR.

Moreover, the method developed in this work with Ru(bpy)<sub>3</sub><sup>2+</sup>/PSA/GCE detection of AR is easier and more convenient compared with other methods.

**Table 2.** Comparison of different methods for the detection of AR

Methods	Linear range(μg/L)	LOD (μg/L)	RSD (%)	Ref.
HPLC	100-10000	100	4.2	[18]
	50-20,000	32	6.0	[19]
SIA Voltammetry	496-2482	149	2.6	[20]
Differential pulse polarography	0-4500	48	1.1	[21]
Electrochemical method	50-600	25	<5.0	[22]
LC-MS method	10-70	5	<20.0	[23]
Ru(bpy) <sub>3</sub> <sup>2+</sup> /Poly (sulfosalicylic acid)	50-4965	30	2.9	This work

#### 4. CONCLUSION

A simple and fast luminescence sensor was successfully developed for the determination of the AR by Ru(bpy)<sub>3</sub><sup>2+</sup>/PSA/GCE. Under the optimal experimental conditions, the sensor has a wide linear range of  $1.0 \times 10^{-7}$  to  $1.0 \times 10^{-4}$  mol/L, with a detection limit of  $6.0 \times 10^{-8}$  mol/L. On the basis of the above conditions, the modified electrode has good sensitivity, selectivity and stability, which provides a new way for detecting the AR in food samples.

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