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Electrochemiluminescent behavior of allopurinol in the presence of $Ru(bpy)_3^{2+}$

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Abstract

The electrochemiluminescent (ECL) response of allopurinol was studied in aqueous media over a wide pH range (pH 2–13) using flow injection (FI) analysis. It was revealed that allopurinol itself had no ECL activity, but could greatly enhance the ECL of $Ru(bpy)_3^{2+}$ in alkaline media giving rise to a sensitive FI-ECL response. The effects of experimental conditions including the mode of applied voltage signal, the potential of working electrode, pH value, the flow rate of carrier solution, and the concentration of $Ru(bpy)_3^{2+}$ and allopurinol on the ECL intensity were investigated in detail. The most sensitive FI-ECL response of allopurinol was found at pH 12.0, where the FIA-ECL intensity showed a linear relationship with concentration of allopurinol in the range 1×10^{-8} mol L^{-1} to 5×10^{-7} mol L^{-1}, and the detection limit was 5×10^{-9} mol L^{-1}.

Keywords: Allopurinol; Electrochemiluminescence; Flow injection analysis; $Ru(bpy)_3^{2+}$

1. Introduction

Allopurinol (mainly presents in the lactam form [1], see Scheme 1) is a well-known inhibitor of xanthine oxidase (XO). It has been widely used in the therapy of some diseases such as hyperuricemia, gout, and nonbacterial prostatitis [2–4]. Allopurinol may also block the production of oxygen free radicals caused by XO and act as a scavenger of the highly reactive hydroxyl radicals [5,6], and is thus envisioned to become a novel therapeutic strategy for the treatment of human heart failure [6]. However, it has been found that the treatments with allopurinol induced serious and sometimes fatal hypersensitivity among a few individual patients [7,8].

A sensitive method to determine allopurinol at low level is of considerable interest for pharmacokinetic and clinical studies. Since allopurinol has ultraviolet (UV) absorption activity and can be electrochemically oxidized, it is commonly detected by either UV [9–14] or electrochemical methods [15–17], combining with appropriate separation units, such as high-performance liquid chromatography (HPLC) [9–12,16,17] and capillary electrophoresis (CE) [13–15]. To our knowledge, no attention has been paid to the electrochemiluminescent (ECL) activity of allopurinol and its analytical applications. As ECL has been proved to be a powerful analytical method with high sensitivity and wide linear response range [18–22], herein, the ECL behavior of allopurinol itself and its ECL behavior in the presence of tris(2,2′-bipyridine) ruthenium(II) ($Ru(bpy)_3^{2+}$), were observed by a flow injection electrochemiluminescent (FI-ECL) system. On this basis, an ECL method for detection of allopurinol was evaluated.

2. Experimental

2.1. Chemicals and solutions

Tris(2,2′-bipyridine)ruthenium(II) chloride hexahydrate ($Ru(bpy)_3Cl_2{\cdot}6H_2O$) was obtained from Aldrich. Allopurinol was purchase from *Sigma*. Other chemicals were analytical grade or better. Double-distilled water was used to prepare sample solutions.

A 1×10^{-3} mol L^{-1} stock solution of $Ru(bpy)_3Cl_2$ was prepared by dissolving 0.0749 g $Ru(bpy)_3Cl_2{\cdot}6H_2O$ with 100 ml of double-distilled water and stored under refrigeration. A 1×10^{-2} mol L^{-1} stock solution of allopurinol was prepared by adding 0.0136 g sample in 10 ml of water and also stored in the refrigerator.

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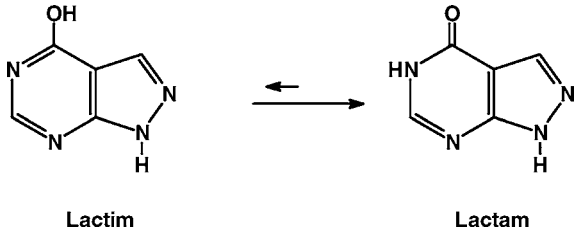


Scheme 1.

Carrier solution was prepared by diluting appropriate volume of above $Ru(bpy)_3Cl_2$ stock solution with phosphate buffer solution. Sample solution was prepared by diluting required volume of the allopurinol stock solution with the carrier solution. The phosphate buffer solutions (pH 2–13) were prepared by titrating 0.1 mol L^{-1} phosphoric acid solutions with sodium hydroxide to the required pH value.

2.2. Apparatus

The electrochemiluminescent experiments were performed on a homemade system (see Fig. 1), consisting of a flow injection unit and an ECL detection unit. The flow injection unit was a LZ-2000 flow injection processor, the details of which have been described previously [23]. The ECL detection unit included following sections: a GD-1 chemiluminescent detector (Ruike Electronic Instrument Ltd. Co., China), a potentiostat, a HW chromatography station (Qianpu Ltd. Co., China) and an ECL flow cell. The potentiostat was a Model 400 electrochemical detector (EG&G), which could perform dc, linear and differential pulse sweeps. The HW chromatography station was used for transferring the analogue ECL signals from the output of the GD-1 chemiluminescent detector into the digital ECL signals recorded by a computer. The ECL flow cell consisted of a piece of Teflon block, Teflon membrane and Perspex block tightly fixed together by the screws. The Teflon block was mounted with a working electrode (a glassy carbon disk with the area of 22.1 mm^2). The Teflon membrane (50 μm in thickness) with a rectangle hole in the center was sandwiched between the Teflon block and the Perspex blocked to keep ca. 2.5 μl of thin layer solution. The Perspex block (1 cm in thickness) not only provided an optical window with c. a. 75% transmittance in the wavelength range of 400–800 nm, but also accommodated a reference electrode and a counter electrode. Advantages of using the Perspex window include: good optical transparence; high stability in the aqueous media, especially in the strongly alkaline solutions, where glass and quartz windows might be etched; and easy fabrication and installation. The reference electrode was Ag/AgCl, and the counter electrode was a stainless steel pipe located at the solution outlet of the cell.

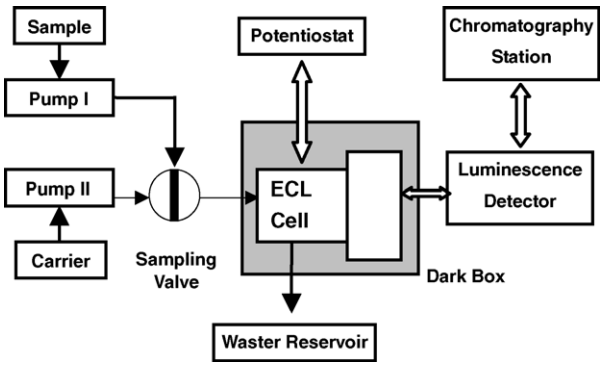


Fig. 1. Block diagram of the components needed for the FIA-ECL detection.

2.3. ECL measurement

The FIA-ECL manifold is shown in Fig. 1. The ECL measurement was carried out in two steps. Step 1: The sample solution was pumped into the sampling valve to fill the loop (100 μl) for 15 s by Pump I. Simultaneously, the carrier solution was pumped into the ECL detector by Pump II. Step 2: Pump I was stopped, and the sampling valve was turned to injection position, the carrier solution was pumped to the loop by Pump II to take the sample solution to ECL detector for measurement. The rates for both Pumps I and II were set at same value ranging from 0.5 to 2.0 ml min^{-1}. It should be noted here that the working electrode was well polished with fine aluminum powder and then cleaned with water before use.

3. Results and discussion

3.1. ECL response of allopurinol in the presence of $Ru(bpy)_3^{2+}$

The primary experiments showed that allopurinol itself did not have ECL activity in various pH conditions (pH 2–13) in the absence of $Ru(bpy)_3^{2+}$, however, it could inhibit or enhance the ECL emission of $Ru(bpy)_3^{2+}$ at the GCE. The inhibited or enhanced portion of ECL intensity (ΔI) was obtained by subtracting the ECL background of $Ru(bpy)_3^{2+}$ (in the absence of allopurinol) from the total ECL (in the presence of both $Ru(bpy)_3^{2+}$ and allopurinol). In the FIA-ECL measurements, when $Ru(bpy)_3^{2+}$ solution was used as the carrier solution, and allopurinol diluted with the $Ru(bpy)_3^{2+}$ carrier solution was used as the sample solution, the FI-ECL intensity obtained was actually the relative intensity, ΔI. It was apparent that, under this FI-ECL format, the enhanced ECL had a positive FI-ECL peak whereas the inhibited ECL had a negative FI-ECL peak.

3.1.1. Effect of applied potential

A constant potential (dc) and a double step pulse voltage scanning were used to examine the ECL response of allopurinol in the presence of $Ru(bpy)_3^{2+}$. The results showed that allopurinol had more sensitive ECL response under the constant potential mode than the double step pulse voltage scanning. Thus, the constant potential mode was selected for further ECL investigation.

The ECL response of allopurinol was strongly dependent on applied potential at GCE working electrode. As shown in Fig. 2, there was no FIA-ECL peak of allopurinol at <+1300 mV (see Fig. 2a), however, the ECL response was observed when the potential reached +1300 mV (see Fig. 2b), and increased apparently with the increasing of potential over the range of +1300 mV to +1500 mV (see Fig. 2b–d). Finally, the ECL peak height

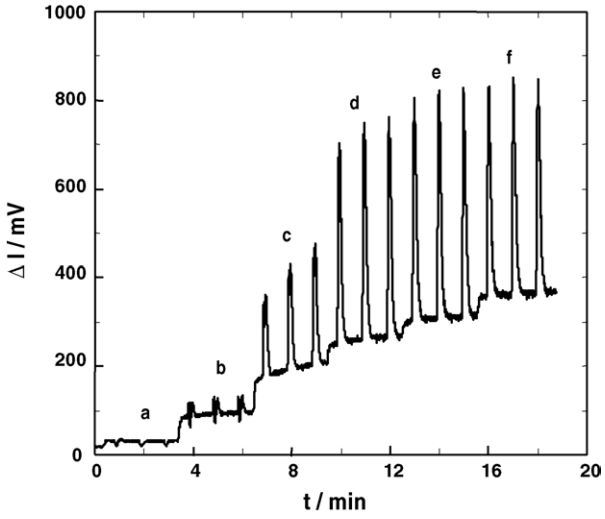


Fig. 2. The effect of the applied potential on the ECL response of allopurinol: (a) 1200 mV, (b) 1300 mV, (c) 1400 mV, (d) 1500 mV, (e) 1600 mV and (f) 1700 mV. Sample: 1.0×10^{-4} mol L^{-1} allopurinol + 1.0×10^{-4} mol L^{-1} $Ru(bpy)_3^{2+}$ (pH 12); carrier solution: 1.0×10^{-4} mol L^{-1} $Ru(bpy)_3^{2+}$ (pH 12); flow rate 1 ml min^{-1}.

tended to become constant after reaching +1500 mV. Further increasing the potential of the GCE working electrode did not result in higher ECL peak height but led to bigger background (see Fig. 2e and f). This might be due to the formation and aggregation of oxygen bubbles on the GCE electrode surface at >+1500 mV, causing the obvious ECL noise. It can be known from Fig. 2, the highest ratio of signal-to-noise is obtained at +1500 mV.

3.1.2. *Effect of pH*

The ECL response of allopurinol was investigated over a wide pH range (pH 2.0–13.0). In the most pH range (pH 2.0–11.0), allopurinol inhibited the ECL emission of $Ru(bpy)_3^{2+}$, giving rise to the negative FI-ECL peaks (see Fig. 3A). However, when the pH value of the testing solution was higher than pH 11.0, allopurinol exhibited to enhance the ECL of $Ru(bpy)_3^{2+}$, resulting in the observation of the positive FI-ECL peaks (see Fig. 3B–F). The plot of ECL intensity (ΔI) versus pH showed that the ECL response of allopurinol was increased with the increasing of pH. Although, more sensitive ECL response of allopurinol could be obtained by increasing the pH value (also see Fig. 3B–F), the background from ECL of $Ru(bpy)_3^{2+}$ was

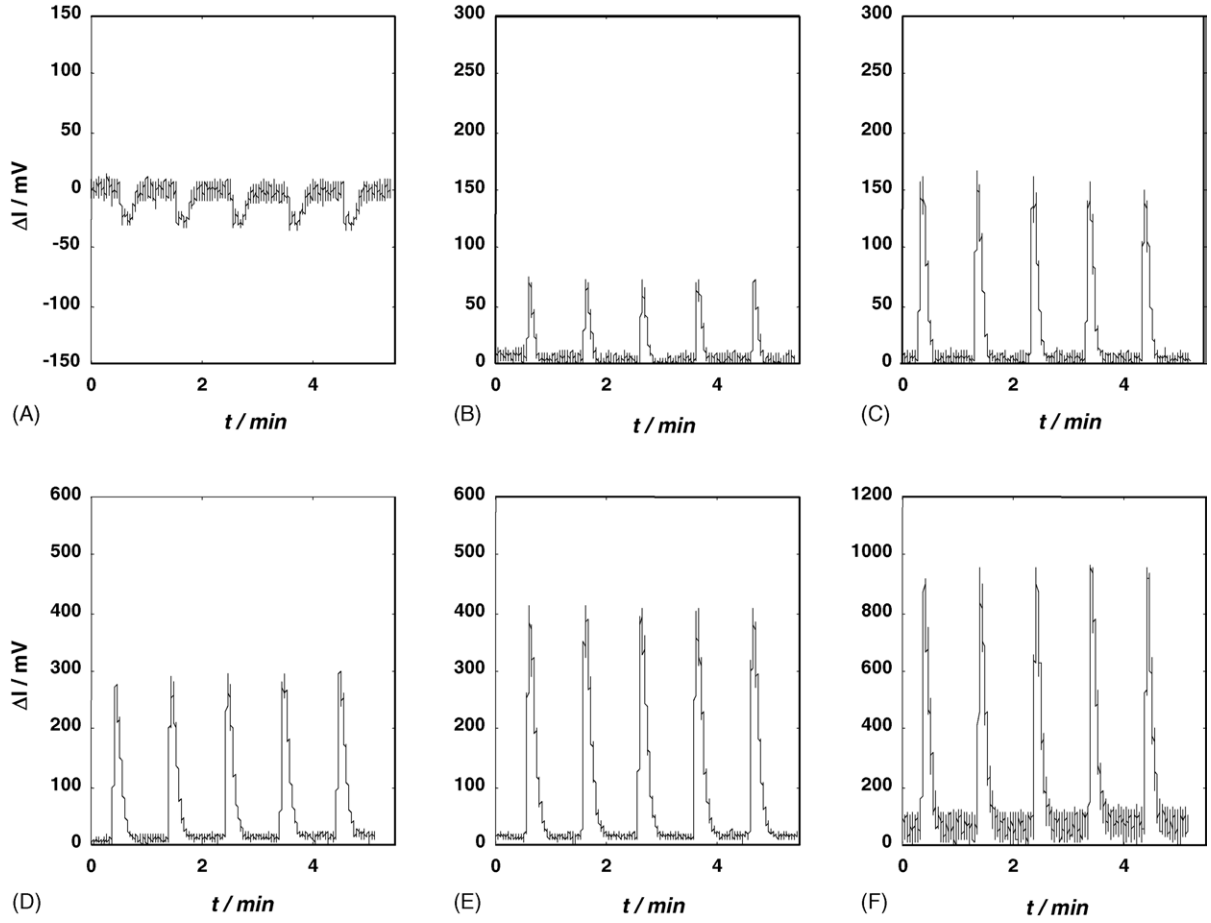


Fig. 3. The ECL response of allopurinol at various pH: (A) pH 11.0, (B) pH 11.25, (C) pH 11.50, (D) pH 11.75, (E) pH 12.0, and (F) pH 13.0. Sample: 1.0×10^{-4} mol L^{-1} allopurinol + 1.0×10^{-4} mol L^{-1} $Ru(bpy)_3^{2+}$; carrier solution: 1.0×10^{-4} mol L^{-1} $Ru(bpy)_3^{2+}$; flow rate: 1 ml min^{-1}; applied potential: +1500 mV.

increased obviously in the strongly alkaline range (pH > 12). For example, the enhance ECL intensity, ΔI, at pH 13.0 (see Fig. 3F) was about twice of that at pH 12.0 (see Fig. 3E), but the ECL background (baseline) at pH 13.0 was found to be about five times of that at pH 12.0. This indicated that the ratio of signal-to-noise was decreased at >pH 12.0. The ECL background is the ECL of $Ru(bpy)_3^{2+}$, which probably results from the chemiluminescent reaction of $Ru(bpy)_3^{3+}$ with the high energy intermediate, $HO_2^{\bullet}$ [24], electrogenerated in the alkaline solution. The most satisfied ratio of signal-to-noise was found at pH 12.0. Accordingly, pH 12.0 PBS was chosen for further ECL measurements.

3.1.3. Effect of flow rate

The ECL response of allopurinol was affected by flow rate. The ECL responses at some flow rates (0.5, 1.0 and 2.0 ml min^{-1}) are shown in Fig. 4. It was found that a higher flow rate produced narrower FI-ECL peaks (compare Fig. 4A with Fig. 4C). This can be explained by that a shorter time is consumed for a certain volume (100 μl) sample to go through the ECL cell at a higher flow rate, thus resulting in the observation of the narrower peaks. When the flow rate was changed from 0.5 to 2.0 ml min^{-1}, the ECL peak height was decreased gradually. At the same time, the ECL background was also affected by the flow rate. First, the ECL background decreased obviously in the low flow rate range (0.5–1.0 ml min^{-1}), but tended to reach a constant level when the flow rate was higher than 1.0 ml min^{-1}. The relatively lower ECL background at higher flow rate resulted from the fact that the peristaltic pump produced a relatively lower flow pulse at higher flow rate. The optimum ratio of signal-to-noise was obtained at the flow rate of 1.0 ml min^{-1}.

Table 1
Optimum conditions for FI-ECL response of allopurinol

Experimental items	Optimum conditions
Mode of applied voltage signal	dc mode
Applied potential	+1500 mV
Buffer	pH 12.0 PBS
Flow rate	1.0 ml min^{-1}
Concentration of $Ru(bpy)_3^{2+}$	1.0×10^{-4} mol L^{-1}

3.1.4. Effect of $Ru(bpy)_3^{2+}$ concentration

The experimental results showed that the ECL response of allopurinol was enlarged when the concentration of $Ru(bpy)_3^{2+}$ was increased. At the meantime, the ECL background from $Ru(bpy)_3^{2+}$ was also enhanced. The ratio of signal-to-noise was found to be significantly increased with the increasing of $Ru(bpy)_3^{2+}$ concentration in the concentration range of 5.0×10^{-6} to 1.0×10^{-4} mol L^{-1}, whereas be improved slightly in the $Ru(bpy)_3^{2+}$ concentration range of 1.0×10^{-4} to 1.0×10^{-3} mol L^{-1}. To obtain a relatively higher ratio of signal-to-noise while reducing the consumption of $Ru(bpy)_3^{2+}$, 1.0×10^{-4} mol L^{-1} $Ru(bpy)_3^{2+}$ concentration was selected for the later ECL measurements.

In summary, the optimum conditions for the ECL measurement of allopurinol are shown in Table 1.

3.1.5. Effect of allopurinol concentration and linear response range

Under the optimum conditions listed in Table 1, the effect of allopurinol concentration on its ECL response was measured. It was observed that the enhanced ECL intensity (ΔI)

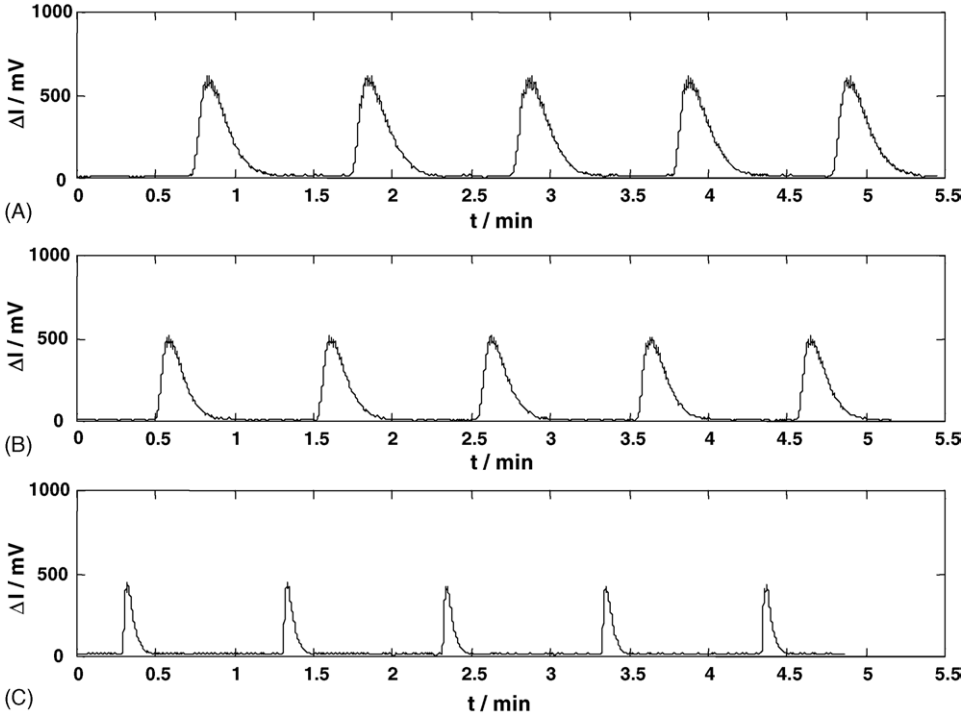


Fig. 4. The ECL response of allopurinol at various flow rate: (A) 0.5 ml min^{-1}, (B) 1.0 ml min^{-1}, and (C) 2.0 ml min^{-1}. Sample: 1.0×10^{-4} mol L^{-1} allopurinol + 1.0×10^{-4} mol L^{-1} $Ru(bpy)_3^{2+}$ (pH 12); carrier solution: 1.0×10^{-4} mol L^{-1} $Ru(bpy)_3^{2+}$ (pH 12); applied potential: +1500 mV.

was increased *linearly* with the increasing of allopurinol concentration in the *lower concentration* range of 1.0×10^{-8} to 5.0×10^{-7} mol L^{-1}, but the increase of ECL intensity was slowed downed when the concentration of allopurinol was higher than 5.0×10^{-7} mol L^{-1}. The linear response of ECL intensity with the concentration of allopurinol in the range of 1.0×10^{-8} to 5.0×10^{-7} mol L^{-1} can be used for quantitative analysis of allopurinol. The regression equation is shown in Eq. (1) with a correlation coefficient, $r = 0.998$ ($n = 6$).

$$\Delta I\,(\mathrm{mV}) = -1.11 + 19.91C\,(\times 10^{-7}\,\mathrm{mol\,L^{-1}}) \quad (1)$$

where ΔI is the enhanced ECL intensity, C the concentration of allopurinol. The detection limit of allopurinol was found to be 5.0×10^{-9} mol L^{-1} when the signal-to-noise was 3.

3.2. Possible mechanism for the enhanced ECL behavior of allopurinol

It is quite well known that many aliphatic amines [18,20,25–27] and alcohols [28–30] have well ECL responses in the presence of $Ru(bpy)_3^{2+}$. These ECL responses result from that the electrochemical oxidation of amines or alcohols produces strong reducing intermediates, usually, neutral radical species, which react with $Ru(bpy)_3^{3+}$ to generate excited state $Ru(bpy)_3^{2+*}$ and lead to light emission [28–31]. This kind of ECL response was also observed for some non-aliphatic amines, such as melatonin and its derivatives [32].

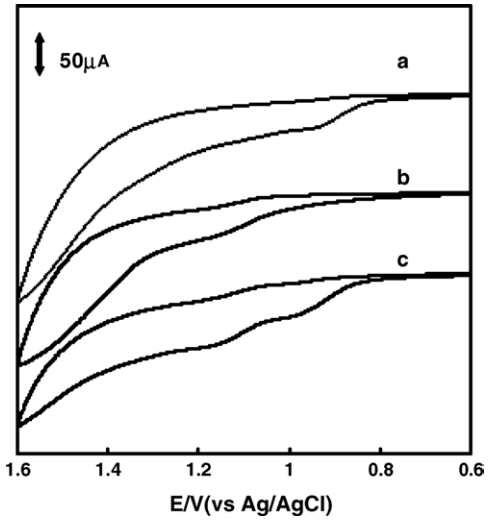
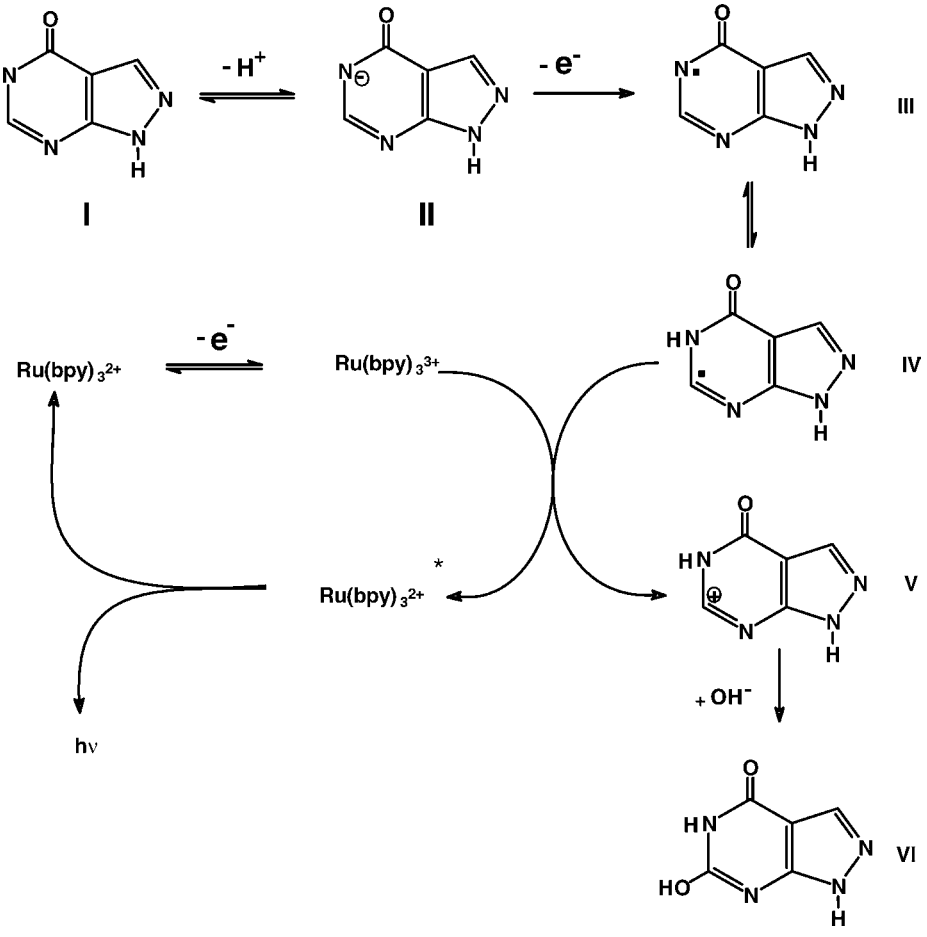


Fig. 5. Cyclic voltammograms obtained for: (a) 1.0×10^{-3} mol L^{-1} allopurinol; (b) 1.0×10^{-3} mol L^{-1} $Ru(bpy)_3^{2+}$; (c) 1.0×10^{-3} mol L^{-1} allopurinol + 1.0×10^{-3} mol L^{-1} $Ru(bpy)_3^{2+}$ at GCE. PBS: pH 12.0; potential scan rate: 200 mV s^{-1}.

In present study, allopurinol includes several amine moieties and one possible hydroxyl moiety (for the tautomeric equilib-



Scheme 2.

rium in Scheme 1), each of which is possible to take part in the ECL reaction. To reveal the enhanced ECL mechanism in present allopurinol/$Ru(bpy)_3^{2+}$ system, the cyclic voltammetric measurements were carried out. Fig. 5 shows that the oxidation peaks of allopurinol and $Ru(bpy)_3^{2+}$ are at +900 mV and +1200 mV, respectively (see Fig. 5a and b), and it is obvious that allopurinol is more readily oxidized than $Ru(bpy)_3^{2+}$ in the mixed solution (see Fig. 5c). It can be concluded that, under the optimum potential condition (+1500 mV), the oxidation product of allopurinol rather than allopurinol itself is involved in the ECL reaction. Although no detailed mechanism has been reported for the electrochemical oxidation of allopurinol, it seems reasonable to suggest that electrochemical oxidation occurs at the pyrimidine moiety rather than pyrazole moiety of allopurinol (see **I** and **II** in Scheme 2) by the well known fact that the chemical oxidation of allopurinol occurs at the pyrimidine and produces oxypurinol (see **VI** in Scheme 2). One-electron oxidation of allopurinol probably produces cathodic radical **II**. A lower pH medium (pH < 11.0) results in difficult deprotonation of the cathodic radical to neutral radicals (**III** and **IV**), which are essential to the ECL of allopurinol. Moreover, the connection of an oxygen or hydroxyl group to the conjunctive rings in allopurinol (see Scheme 1) might lead to the inhibition of the ECL of $Ru(bpy)_3^{2+}$ in lower pH range [33]. In contrast, a strongly alkaline solution (pH > 11) favors the further deprotonation of the cathodic radical (**II**) to the neutral radicals (**III** and **IV**). The neutral radicals react with electrogenerated $Ru(bpy)_3^{3+}$ to produce the excited state $Ru(bpy)_3^{2+*}$ and oxypurinol (**VI**). Finally when the excited state $Ru(bpy)_3^{2+*}$ returns back to its ground state $Ru(bpy)_3^{2+}$, light emission occurs.

4. Conclusion

This study shows that allopurinol itself has no ECL activity in the PBS solutions over the pH range of 2.0–13.0, however, it can enhance the ECL emission of $Ru(bpy)_3^{2+}$ in the alkaline media (pH 11.5–13.0). The ECL response of allopurinol is influenced by applied potential of working electrode, pH value and flow rate of solution, and concentration of $Ru(bpy)_3^{2+}$ and allopurinol. After optimizing these experimental conditions, we can observe a sensitive FIA-ECL response of allopurinol in the presence of $Ru(bpy)_3^{2+}$. On this basis, a sensitive FIA-ECL *method* for detection of allopurinol has been established. Investigations are going on to improve the selectivity of ECL detection of allopurinol. It is envisioned that this ECL method can be further used to detect allopurinol in complicated biological samples after combining with an appropriate separation unit, such as HPLC or CE.

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