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Determination of tiopronin based on the enhancement of $Ru(bpy)_3^{2+}$ co-reactant electrochemiluminescence

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ABSTRACT

It was found that tiopronin could strongly enhance the electrochemiluminescence of tris(2,2'-bipyridine) ruthenium(II) ($Ru(bpy)_3^{2+}$) in alkaline solution on a bare Pt electrode, based on which a sensitive, simple and rapid method for the determination of tiopronin was established. Under the optimal conditions, the logarithm of ECL enhancement has a linear relationship with the logarithm of tiopronin concentration in the range from 2.0×10^{-7} to 2.0×10^{-4} mol L^{-1} with a detection limit of 1.0×10^{-8} mol L^{-1} ($S/N=3$), and the relative standard deviation of 1.6% ($n=7$, $c=5.0\times10^{-6}$ mol L^{-1}). The proposed method has been applied to the determination of tiopronin in pharmaceutical preparations and the results were satisfactory with recoveries of 91.7 ± 1.7%, 98.3 ± 1.0% and 100.8 ± 0.5%, respectively, for three different concentration levels (0.61 μmol L^{-1}, 6.1 μmol L^{-1} and 12.2 μmol L^{-1}). According to the study of electrochemical behavior, ECL behavior and ECL emission spectrum of $Ru(bpy)_3^{2+}$/tiopronin system, a possible ECL mechanism was proposed.

1. Introduction

Tiopronin (2-mercaptopropionylglycine), whose chemical structure is shown in Fig. 1, is a novel sulfhydryl glycin derivative which is widely used in the treatment of cystinuria, rheumatoid arthritis and hepatic disorders. It also has been employed as antidote to heavy metal poisoning and as a radioprotective agent [1,2]. Despite of its acceptability, the use of tiopronin in clinical treatments is associated with many complications, such as stomach upset and loss of taste, due to the uncertain dosage. Therefore, a simple, rapid, sensitive and reliable method for tiopronin determination is of great importance in order to address the potential prevention of adverse effects. Up to now, several methods have been used for tiopronin measurements, such as spectrophotometry [3], fluorescence [4–8], CE-UV [9], HPLC–UV [10–12], LC-ESI-MS [13–15], chemiluminescence [16,17] and amperometric flow injection analysis [18], etcetera. Spectrophotometric and chemiluminescent methods are simple but relatively insensitive [3,16,17]. The fluorometric methods are more sensitive, but complex chemical reactions are required in most cases [4–8]. Modern instrumental analytical techniques such as CE [9], HPLC [10–12], LC [13–15] methods are powerful separation methods that could be coupled with different detectors, but they involve relatively expensive instruments, complicate operations and are time-consuming. In addition, the detection linear ranges of some methods are relatively narrow [3,9,15,18].

In recent years, electrochemical methods have been proved to be an inexpensive and effectives way for the determination of various compounds because of their sensitivity, low cost and relatively short analysis time compared with other techniques [19–22]. Electrochemiluminescence (ECL), combining the analysis technology of electrochemistry and chemiluminescence, has been widely studied in recent years. It is a powerful tool that not only retains the simplicity of electrochemistry, but also possesses the inherent sensitivity and owns a wide dynamic concentration response range of chemiluminescence [23,24]. Among the many ECL systems, $Ru(bpy)_3^{2+}$ has received substantial attention because of its excellent stability and high ECL quantum yield [25], and has led to widespread application of ECL detection in biochemical and medical analytes, such as amino acid [26], protein [27], DNA [28] and pharmaceutical preparations [29] to date. To the best of our knowledge, no attention has been paid to the determination of tiopronin employing an ECL method. Recently, our group have found that some sulfhydryl compounds can generate strong

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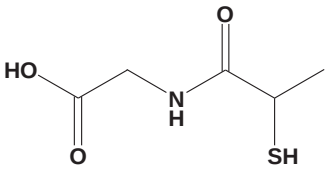

Fig. 1. Chemical structure of tiopronin.

reducing intermediates under the optimal potential and serve as co-reactants in the ECL process of $Ru(bpy)_3^{2+}$ [30,31]. Coincidentally, tiopronin is a sulfhydryl glycin derivative and the sulfhydryl can be easily oxidized [18]. These results enlightened us that tiopronin can be employed as a co-reactant in the ECL process of $Ru(bpy)_3^{2+}$.

In this work, the ECL behavior of $Ru(bpy)_3^{2+}$ in the presence of tiopronin was investigated. We found that tiopronin can strongly enhance the ECL of $Ru(bpy)_3^{2+}$ in alkaline media, on the basis of which a sensitive, simple and rapid ECL method for the determination of tiopronin was established. The developed method has been applied to the detection of tiopronin in pharmaceutical preparations and satisfactory results have been obtained. Based on the electrochemical, ECL and spectroscopic study, a possible mechanism for the ECL system has been proposed.

2. Experimental

2.1. Reagents and solutions

Tris(2,2-bipyridyl) dichlororuthenium(II) hexahydrate ($Ru(bpy)_3Cl_2 \cdot 6H_2O$, 99.95%) was purchased from Sigma (USA). Tiopronin was obtained from Chinese National Institutes for Food and Drug Control (Beijing, China). Tiopronin enteric-coated tables (each contains 100 mg tiopronin) were obtained from Henan Xinyi Pharmaceutical Co. Ltd (Henan, China). All other reagents were of analytical-reagent grade or better and were directly used in experiments without further purification. Double-distilled water was used throughout all experiments.

A 0.010 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 10 mL of double-distilled water and stored in a refrigerator.

A 0.010 mol L^{-1} stock solution of tiopronin was prepared by dissolving 0.0163 g of tiopronin in 10 mL of double-distilled water and stored in a refrigerator too.

BR buffer solutions (pH 7.0–12.5) were prepared by mixing 0.2 mol L^{-1} NaOH with mixture of 0.04 mol L^{-1} H_3PO_4, H_3BO_3 and CH_3COOH to obtain the appropriate pH and validated with a pH meter. Test solutions were prepared by diluting the stock solutions with appropriate buffer solutions before use.

2.2. Apparatus

ECL measurements and electrochemical test were carried out on a model MPI-E ECL analyzer system (Xi'an Remex Analyse Instrument Co. Ltd., Xi'an, China). A conventional three-electrode system was used for electrochemical and ECL measurements. It consisted of a platinum (2 mm diameter) working electrode, a platinum wire counter electrode and an Ag/AgCl (sat. KCl) reference electrode. A 5 mL cylindroid glass cell was used as an ECL cell, and it was placed directly above the photomultiplier tube (PMT). The working electrode was pretreated before use by polishing the surface with aqueous slurries of alumina powders (average particle diameters: 0.3 μm α-Al_2O_3) on the polishing microcloth and rinsed with water, and then sonicated in water for 1 min and thoroughly rinsed with water. Before ECL measurements, the pretreated electrode was immersed into the BR buffer solutions (pH 11.5) and scanned with cyclic voltammetry until the emergence of stable curves.

2.3. Procedures

5.0 mL pH 11.5 BR buffer solutions containing 1.0×10^{-4} mol L^{-1} $Ru(bpy)_3^{2+}$ were used as background solutions, 5.0 mL pH 11.5 BR buffer solutions containing 1.0×10^{-4} mol L^{-1} $Ru(bpy)_3^{2+}$ and certain amount of tiopronin were used as sample solutions. ECL was generated by cyclic voltammetry scanning from 0.2 to 1.3 V with a scan rate of 150 mV s^{-1}. The PMT was biased at −800 V, unless explicitly stated, the magnification factor was set at 3. The cyclic voltammograms and ECL curves were recorded simultaneously. All experiments were conducted at room temperature.

The quantitative analysis was carried out based on the ECL enhancement (ΔI), $\Delta I = I - I_0$, where I_0 was the ECL intensity of the $Ru(bpy)_3^{2+}$ background solution, and I was the ECL intensity of the sample solution. Hence, the enhanced ECL intensity reflected the concentration of the tiopronin in sample solution.

2.4. Analysis of tiopronin in pharmaceutical preparations (enteric-coated tables)

Five tablets were finely pulverized and homogenized, and then a proportion of this powder (equivalent to 100 mg of tiopronin) was accurately weighed and transferred into a beaker containing 100 mL double-distilled water, finally sonicated for 20 min in order to fully dissolve the tiopronin. Insoluble excipients were removed with centrifugation at 4000 rpm for 10 min and the supernatant was collected for the following analysis [8]. Certain amount of the above supernatant was transferred into the ECL cell that contains 5.0 mL BR buffer solution (pH 11.5) with 1.0×10^{-4} mol L^{-1} $Ru(bpy)_3^{2+}$. The sample solution was analyzed by the proposed ECL method.

3. Results and discussion

3.1. Electrochemistry behaviors

The cyclic voltammetry curves of tiopronin without and with $Ru(bpy)_3^{2+}$ at bare Pt electrode were shown in Fig. 2. Tiopronin solution gave an large irreversible anodic peak at approximately

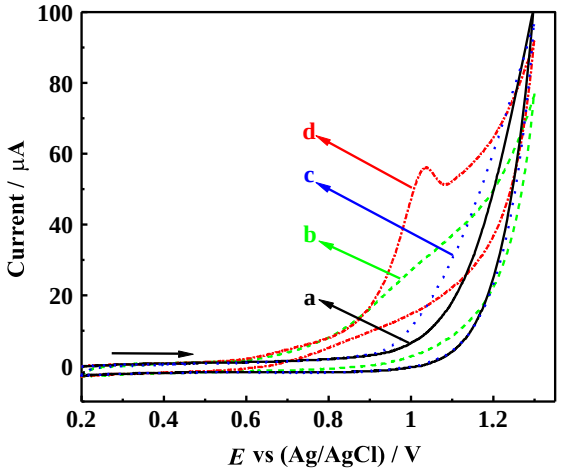

Fig. 2. The cyclic voltammetry curve of supporting electrolyte (a), 5.0×10^{-3} mol L^{-1} tiopronin (b), 1.0×10^{-4} mol L^{-1} $Ru(bpy)_3^{2+}$ (c) and 1.0×10^{-4} mol L^{-1} $Ru(bpy)_3^{2+}$ + 5.0×10^{-3} mol L^{-1} tiopronin (d) at Pt electrode in the potential range between 0.2 to 1.3 V. Buffer solution: pH 11.5 BR; Scan rate: 150 mV s^{-1};

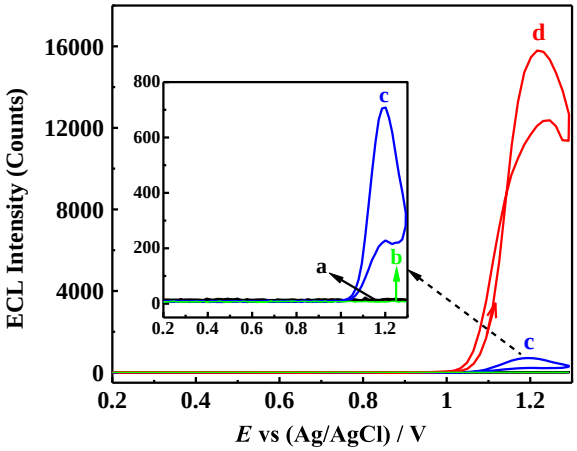

Fig. 3. The ECL intensity–potential curve of supporting electrolyte (a), 5.0×10^{-3} mol L^{-1} tiopronin (b), 1.0×10^{-4} mol L^{-1} $Ru(bpy)_3^{2+}$ (c) and 1.0×10^{-4} mol L^{-1} Ru $(bpy)_3^{2+}+5.0\times10^{-3}$ mol L^{-1} tiopronin (d) at Pt electrode in the potential range between 0.2 to 1.3 V. Buffer solution: pH 11.5 BR; Scan rate: 150 mV s^{-1}; PMT magnification factor was set at 2.

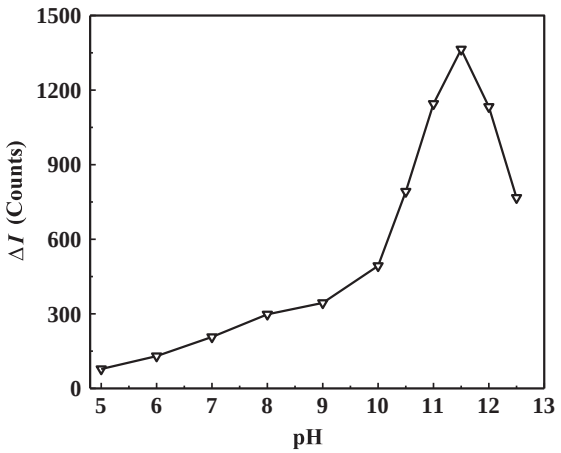

Fig. 4. Effect of pH in BR buffer solution on the ECL enhancement of 1.0×10^{-4} mol L^{-1} $Ru(bpy)_3^{2+}/1.0\times10^{-4}$ mol L^{-1} tiopronin system. Scan rate was 150 mV s^{-1}.

+0.95 V versus Ag/AgCl (curve (b) in Fig. 2), this may be ascribed to the irreversible oxidation of the thiol group of tiopronin [18]. Because the hydroxyl ions in alkaline media can react with Ru $(bpy)_3^{3+}$ and give rise to the decrease in redox reversibility of the $Ru(bpy)_3^{3+}$/ $Ru(bpy)_3^{2+}$ couple, only a small irreversible oxidation wave from the oxidation of $Ru(bpy)_3^{2+}$ into $Ru(bpy)_3^{3+}$ at potential higher than +1.0 V could be found (curve (c) in Fig. 2). A comparison between curves (c) and (d) in Fig. 2 demonstrated that $Ru(bpy)_3^{2+}$ can catalyze the oxidation of tiopronin since the anodic current of $Ru(bpy)_3^{2+}$ increased while the cathodic current of $Ru(bpy)_3^{2+}$ decreased in the presence of tiopronin, and the oxidation potential of $Ru(bpy)_3^{2+}$ (~+1.1 V) is larger than that of tiopronin (~+0.95 V) [32]. In this electro-catalytic reaction, Ru $(bpy)_3^{2+}$ served as the catalyst while the tiopronin or its oxidation intermediates acted as the highly reducing reagent.

3.2. ECL behavior of $Ru(bpy)_3^{2+}$ in the presence of tiopronin

Fig. 3 shows the ECL intensity–potential curves of 1.0×10^{-4} mol L^{-1} $Ru(bpy)_3^{2+}$ either with or without 5.0×10^{-3} mol L^{-1} tiopronin in a BR buffer solution (pH 11.5) at bare Pt electrode. No ECL emissions were found when $Ru(bpy)_3^{2+}$ was absent (curves (a) and (b) in Fig. 3). The solution containing 1.0×10^{-4} mol L^{-1} $Ru(bpy)_3^{2+}$ gave a weak ECL emission with an onset potential of ~+1.05 V and reached a maximum at ~+ 1.20 V (curve (c) in Fig. 3), which may be assigned to the reaction between electrogenerated $Ru(bpy)_3^{3+}$ and OH^- [33]. After 5.0×10^{-3} mol L^{-1} tiopronin was added into the above solution, the ECL intensity at ~+ 1.20 V increased about 20 folds (compare curves (c) and (d) in Fig. 3). On curve (d) in Fig. 3, we found that no ECL was observed when potential was lower than ~+1.05 V, although at this potential the thiol group of tiopronin was already oxidized, while the intense ECL emerged only when $Ru(bpy)_3^{3+}$ was produced. These results indicated that the oxidation of both tiopronin and $Ru(bpy)_3^{2+}$ was needed in the strong ECL process and the tiopronin was served as a strong co-reactant in the ECL process of $Ru(bpy)_3^{2+}$.

3.2.1. Effect of pH

The effect of pH on the ECL response of $Ru(bpy)_3^{2+}$/tiopronin system was studied when the pH of buffer solution changed from 5.0 to 12.5 and the ΔI-pH curve was shown in Fig. 4. It was found that pH has a significant influence on the ECL intensity of Ru $(bpy)_3^{2+}$/tiopronin system. The ΔI increased slowly with pH varying from 5.0 to 10.0, and then increased sharply when pH was higher than 10.0. This phenomenon may be ascribed to the fact that the sulfhydryl moiety is easy to accept proton when pH is lower than the dissociation constant of –SH of tiopronin (according to the previous report [34], the p*Ka* values of aliphatic thiols in water are generally in the range of 10~11), and thus it is difficult to be deprotonated to form the high-reducing free radical intermediate. The ΔI reached a maximum at pH 11.5 and then began to decrease if the pH was further increased. Therefore, pH of 11.5 BR buffer solution was chosen for the subsequent measurements.

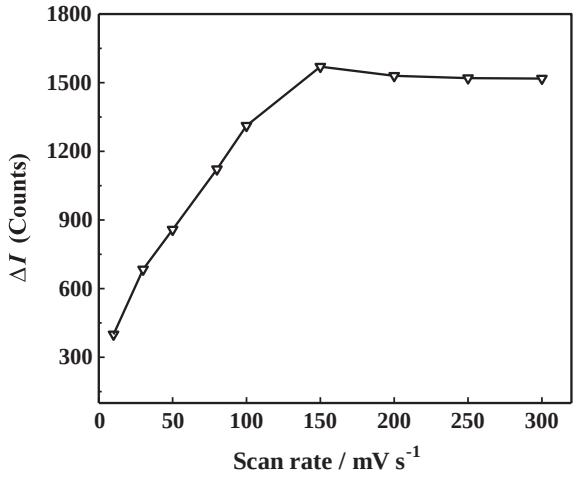

Fig. 5. Effect of potential scan rate on the ECL enhancement of 1.0×10^{-4} mol L^{-1} $Ru(bpy)_3^{2+}$ $/1.0\times10^{-4}$ mol L^{-1} tiopronin system in pH 11.5 BR buffer solution.

3.2.2. Effect of scan rate

As shown in Fig. 5, the scan rate can greatly influence the ECL intensity of $Ru(bpy)_3^{2+}$/tiopronin co-reaction system since the ECL efficiency significantly depended on the rate of generation/annihilation of the excited state $Ru(bpy)_3^{2+*}$ [35]. When the applied scan rate was changed from 10 to 150 mV s^{-1}, the ΔI increased gradually. However, if the scan rate was greater than 150 mV s^{-1}, the ΔI reached a plateau. Therefore, a scan rate of 150 mV s^{-1} was used in the following experiments as it gave the maximum ECL enhancement value.

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

The effect of the concentration of $Ru(bpy)_3^{2+}$ on ΔI was studied. Results indicated that ΔI increased rapidly with the increasing concentration of $Ru(bpy)_3^{2+}$ from 1.0×10^{-6} to 1.0×10^{-4} mol L^{-1} and the increment of ΔI was very small when the concentration was higher than 1.0×10^{-4} mol L^{-1}. Consequently, 1.0×10^{-4} mol L^{-1} was selected as the optimal concentration of $Ru(bpy)_3^{2+}$.

3.3. Linear response range, precision and detection limit

Under the optimal conditions, ECL intensity of $Ru(bpy)_3^{2+}$/tiopronin system increases with the increasing of tiopronin concentration (Fig. 6). The logarithm of the ECL intensity increases linearly with the logarithm of tiopronin concentrations over the range of 2.0×10^{-7}-2.0×10^{-4} mol L^{-1} (see inset of Fig. 6). The regression equation was $\lg\Delta I = 5.0244 + 0.4306\times\lg c_{\text{tiopronin}}$ (mol L^{-1}) with a correlation coefficient (r) of 0.9955. The relative standard deviation for 5×10^{-6} mol L^{-1} tiopronin determination was 1.6% ($n=7$). The detection limit for tiopronin by the proposed method is 1.0×10^{-8} mol L^{-1} ($S/N=3$). Table 1 shows the comparison between the analytical performance of the present method and previous literature methods for the determination of tiopronin. A survey of the data reveals that the obtained results from the present method are superior to most of the other methods for determination of tiopronin. The new ECL method possesses the merits of simplicity and rapidity, with a wide linear range and low detection limit.

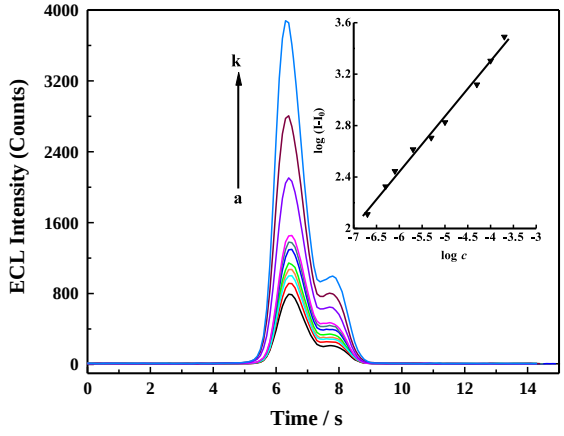

Fig. 6. ECL responses of 1.0×10^{-4} mol L^{-1} $Ru(bpy)_3^{2+}$ in the presence of various concentrations of tiopronin: (a) 0; (b) 2.0×10^{-7}; (c) 5.0×10^{-7}; (d) 8.0×10^{-7}; (e) 2.0×10^{-6}; (f) 5.0×10^{-6}; (g) 8.0×10^{-6}; (h) 1.0×10^{-5}; (i) 5.0×10^{-5}; (j) 1.0×10^{-4}; (k) 2.0×10^{-4} mol L^{-1}. Inset: linear calibration curve for tiopronin detection. Buffer solution: pH 11.5 BR; Scan rate: 150 mV s^{-1}.

3.4. Analytical application

The proposed method has been applied to evaluate tiopronin content in pharmaceutical preparation samples. After a simple pre-treatment as described in Section 2.4, 0.5 μL, 5 μL and 10 μL of the supernatant were transferred into 5 mL background solutions, respectively, to get sample solutions with three different concentration levels (0.61 μmol L^{-1}, 6.1 μmol L^{-1} and 12.2 μmol L^{-1}). The average results of seven replicate measurements expressed as confidence interval for 95% level of confidence obtained for each sample solution using the proposed method were summarized in Table 2. The values obtained by the calibration method were in excellent agreement with the reference values. The recovery study was also performed at the same time with standard addition

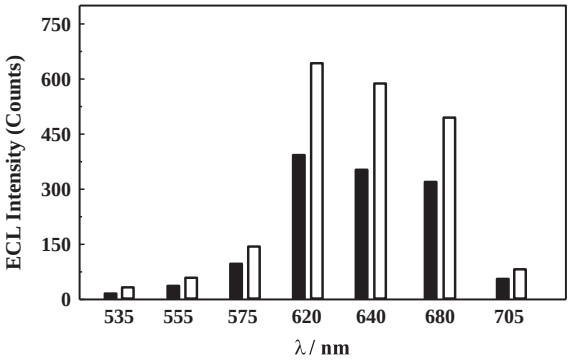

Fig. 7. ECL spectra obtained for $Ru(bpy)_3^{2+}$ ECL systems in the absence (black columns) and presence of (white columns) tiopronin. The concentration of Ru $(bpy)_3^{2+}$ and tiopronin were 5.0×10^{-4} and 1.0×10^{-4} mol L^{-1}, respectively. Other experimental conditions were the same as those in Fig. 6.

Table 1
Comparison between the previous literature methods for the determination of tiopronin with the proposed method.

Method	Linear dynamic range (μmol L^{-1})	Detection limit (μmol L^{-1})	Samples analyzed	Reference
Spectrophotometry	6–200	4	Pharmaceutical preparations	[3]
Fluorescence	3.7–340	0.005	Pharmaceutical preparations, human serum	[4]
Fluorescence	0.92–122.5	0.92	Pharmaceutical preparations	[8]
CE-UV	5–160	5	Urine	[9]
HPLC-UV	0.25–25	0.25	Human plasma	[11]
LC-ESI-MS	0.66–33	0.66	Human plasma	[15]
Chemiluminescence	0.5–3000	0.2	Pharmaceutical preparations	[16]
Amperometric flow injection analysis	0.5–50	0.01	Pharmaceutical preparations	[18]
ECL	0.2–200	0.01	Pharmaceutical preparations	This work

Table 2
Analysis of tiopronin in pharmaceutical formulations and recovery study of the proposed ECL method.

Concentration level (μmol L^{-1})	Detection Results[a] (μmol L^{-1})	R.S.D. (%)	Added (μmol L^{-1})	Found[a] (μmol L^{-1})	R.S.D. (%)	Recovery[a] (%)
0.61	0.58 ± 0.01	2.1	0.60	0.55 ± 0.01	1.6	91.7 ± 1.7
6.1	6.21 ± 0.06	1.4	6.0	5.9 ± 0.06	1.5	98.3 ± 1.0
12.2	12.40 ± 0.09	0.95	12.0	12.10 ± 0.06	0.64	100.8 ± 0.5

[a] The values of uncertainty have been estimated by using the expression $\pm t_{n-1}\,s/\sqrt{n}$: n is the number of replicate measurements, t_{n-1} is the statistic parameter often called Student's t (with $n=7$, at 95% level of confidence, $t=1.94$) and s is the standard deviation (S.D.).

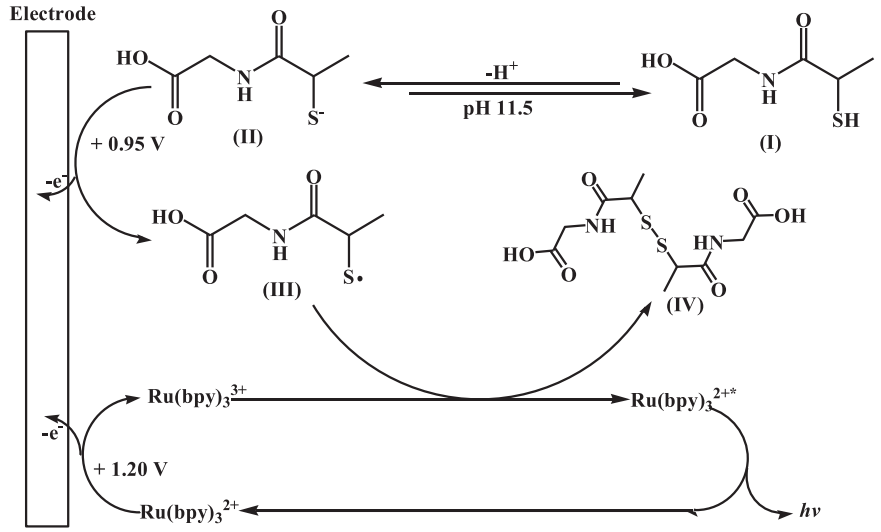

Fig. 8. The possible ECL mechanism proposed for $Ru(bpy)_3^{2+}$/tiopronin system.

method by adding a known amount of standard solution to the above sample solutions and the results were presented in Table 2 as well. The average recoveries and RSDs were 91.7 ± 1.7% and 1.6%, 98.3 ± 1.0% and 1.5%, 100.8 ± 0.5% and 0.64%, respectively, for three different concentration levels.

3.5. Possible mechanism for the enhanced ECL response of tiopronin

The ECL responses of many organic species in $Ru(bpy)_3^{2+}$ system have been well studied and they generally contain the following three processes. Firstly, electrochemical oxidation of the organic species and $Ru(bpy)_3^{2+}$ under an appropriate potential, producing strong reducing intermediates (usually neutral radical species) and strong oxidizing $Ru(bpy)_3^{3+}$; secondly, high-energy electron transfers between the strong reducing intermediates and $Ru(bpy)_3^{3+}$ to generate the excited-state luminophore, $Ru(bpy)_3^{2+}$*; Finally, the excited-state $Ru(bpy)_3^{2+}$* gives light emission when it returns to its ground state, $Ru(bpy)_3^{2+}$ [25,35]. In order to verify the luminophore which gives the light emission when $Ru(bpy)_3^{2+}$ and tiopronin were in the same solution, the ECL emission spectrum from 535 to 705 nm was studied using a series of filters. As shown in Fig. 7, the maximum ECL emission wavelength was 620 nm for Ru $(bpy)_3^{2+}$*. When tiopronin was added, the ECL intensity was enhanced obviously. However, the maximum emission wavelength was still at 620 nm, which indicated that the luminophore of Ru $(bpy)_3^{2+}$/tiopronin system was still $Ru(bpy)_3^{2+}$*.

It has been reported that many aliphatic amine compounds such as tripropylamine can be served as co-reactants in the ECL process of $Ru(bpy)_3^{2+}$ for the electrochemical generated amino-free radicals [25,35]. Tiopronin is a sulfhydryl glycin derivative containing both amine and thiol groups, but the amine exists in the form of amide. Paul A. Millner et al. have pointed out that acetylation of the amine group might completely abolish the activity of the amine group, thus the thiol group is easier to be oxidized than the amine group [34]. Therefore, according to the electrochemical behavior, ECL behavior and the ECL emission spectrum study, a possible mechanism for the enhanced ECL response of $Ru(bpy)_3^{2+}$ in the presence of tiopronin was proposed in Fig. 8.

When tiopronin **(I)** was in a alkaline buffer solution (pH 11.5), the proton on the thiol group is easily dissociated to produce the anion (**II**) which can be oxidized under an appropriate potential (~ +0.95 V) to generate the neutral active free radical intermediate (**III**). The strongly reducing free radical intermediate (**III**) will react with the electrogenerated $Ru(bpy)_3^{3+}$ at the presence of Ru $(bpy)_3^{2+}$ to produce the excited $Ru(bpy)_3^{2+}$* thus the ECL is greatly enhanced (compare Fig. 3 curves (c) and (d)). We can also find a support from our previous ECL observation on $Ru(bpy)_3^{2+}$/2-thiouracil [30] and $Ru(bpy)_3^{2+}$/ thiamazole [31] systems for the formation of the highly reducing free radical (**III**). Although further detailed work is needed to exactly clarify, the final oxidation product of neutral radical might be disulfides [36,37] (**IV**).

4. Conclusion

The electrochemical and ECL behavior of tiopronin, a novel sulfhydryl glycin derivative containing both amine and thiol groups, was investigated in the presence of $Ru(bpy)_3^{2+}$. It was found that tiopronin can strongly enhance the ECL intensity of Ru $(bpy)_3^{2+}$ according to the reaction between electrogenerated thiol free radical and $Ru(bpy)_3^{3+}$, based on which a ECL method was established for the determination of tiopronin. Compared with the previous methods for the determination of tiopronin [3–18], the method in this article is simple, rapid, with a wide linear range and low detection limit. The proposed method was applied to the determination of tiopronin in pharmaceutical preparations, and the results were satisfactory with recoveries of 91.7 ± 1.7%, 98.3 ± 1.0% and 100.8 ± 0.5%, respectively, for three different concentration levels (0.61 μmol L^{-1}, 6.1 μmol L^{-1} and 12.2 μmol L^{-1}). According to the study of electrochemical behavior, ECL behavior and ECL emission spectrum of $Ru(bpy)_3^{2+}$/tiopronin system, a possible ECL mechanism was proposed.

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