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Chemiluminescence method for the determination of piroxicam by the enhancement of the tris-(4,7-diphenyl-1,10-phenanthrolinedisulphonic acid) ruthenium(II) (RuBPS)–cerium(IV) system and its application

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ABSTRACT: A simple, rapid chemiluminescence (CL) method was described for the determination of piroxicam, a commonly used analgesic agent drug. A strong CL signal was detected when cerium(IV) sulphate was injected into tris-(4,7-diphenyl-1,10-phenanthrolinedisulphonic acid) ruthenium(II) (RuBPS)–piroxicam solution. The CL signal was proportional to the concentration of piroxicam in the range 2.8×10^{-8}–1.2×10^{-5} mol/L. The detection limit was 2×10^{-8} mol/L and the relative standard deviation (RSD) was 3.7% ($c = 7.0 \times 10^{-7}$ mol/L piroxicam; $n = 11$). The proposed method was applied to the determination of piroxicam in pharmaceutical preparations in capsules, spiked serum and urine samples with satisfactory results.

Keywords: chemiluminescence; tris-(4,7-diphenyl-1,10-phenanthrolinedisulphonic acid) ruthenium(II); cerium(IV); piroxicam

Introduction

Piroxicam,4-hydroxy-2-methyl-N-(2-pyridinyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide (Fig. 1), is a non-steroidal anti-inflammatory and analgesic agent drug belonging to a class of compounds called oxicams (1). Its efficacy has been demonstrated in humans for the treatment of various inflammatory diseases and arthropathies, such as rheumatoid arthritis and osteoarthritis. Piroxicam acts as an anti-inflammatory drug mainly by prostaglandin synthesis inhibition, as well as by leukocyte migration and phagocyte activity inhibition.

Although some methods have been developed for the determination of piroxicam, such as ultraviolet and visible spectrophotometry (UV/Vis) (2, 3), an electrochemical method (4), amperometry (5), spectrofluorimetry (6–10), liquid chromatography (LC) (11), high-performance liquid chromatography (HPLC) (12–14), high-performance liquid chromatography–ultraviolet and visible spectrophotometry (HPLC–UV) (15), liquid chromatography–tandem mass spectrometry (LC–MS/MS) (16), thin-layer chromatography (TLC)–matrix-assisted laser desorption (MALDI)–time-of-flight (TOF)–mass spectrometry (TLC–MALDI–TOF–MS) (17) and luminescence (18), the limitation to these methods is low sensitivity or expensive instrumentation. The flow injection–chemiluminescence (CL) method is known to be a powerful analytical technique that has a low detection limit, a wide linear dynamic range and relatively simple and inexpensive instrumentation. Based on the reaction of N-bromosuccinimide (NBS) and luminol, Bai *et al.* established a method for the determination of piroxicam in a flow-injection post-CL system (19). Wang *et al.* (20) described a flow injection quenching CL method for the determination of piroxicam, but the quenching CL had low sensitivity.

On the basis of studying the CL properties of $Ru(phen)_3^{2+}$, several novel ruthenium(II) complexes with diphenylsubstituted bipyridine and phenanthroline as ligands were synthesized and exhibited increased quantum efficiencies compared to $Ru(bipy)_3^{2+}$. The quantum yield of $Ru(dp\text{-}phen)_3^{2+}$ achieved 0.33

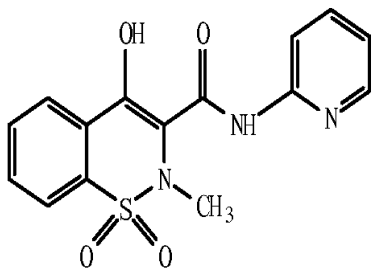

Figure 1. Structure of piroxicam.

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(21), but the hydrophobicity of this complex made its application limited (22). This problem was solved by deriving two sulphonate groups to each ligand of Ru(dp-phen)$_3^{2+}$ and it could be expected that the obtained complex, tris-(4,7-diphenyl-1,10-phenanthrolinedisulphonic acid) ruthenium(II) (RuBPS), would become a useful CL reagent showing advantageous characteristics (23, 24). In the present study it was observed that piroxicam could enhance the CL emission of the tris-(4,7-diphenyl-1,10-phenanthrolinedisulphonic acid) ruthenium(II) (RuBPS)–cerium(IV) system and the degree of enhancement was proportional to the concentration of piroxicam. Hence, a novel CL system was established to detect piroxicam. Under optimal experimental conditions, the CL signal was linear to the concentration of piroxicam in the range 2.8×10^{-8}–1.2×10^{-5} mol/L and the detection limit was 2×10^{-8} mol/L. The proposed method exhibits high precision and has been successfully applied to the determination of piroxicam in pharmaceutical formulations, spiked serum and urine samples.

Experimental

Apparatus

Chemiluminescence intensity was recorded by IFFM-D flow-injection luminometry (Remax Electron Technological Ltd, Xi'an, China). A schematic diagram of the CL flow system employed is shown in Fig. 2. PTFE tubing was used to connect all the components in the flow system. The flow cell was a coil of glass tubing that was positioned in front of the detection window of the PMT. The CL signal was treated using a personal computer.

Reagents

Piroxicam was obtained from the Institute of Medical Biotechnology (Beijing, China). RuBPS was prepared by the method used in (23). Sulphuric acid (No. 20061204) was purchased from Xinyang Chemical Reagents (Henan, China). $Ce(SO_4)_2{\cdot}4H_2O$ was purchased from Shanghai Chemical Co. of the Medicine Group of China (Shanghai, China). All other reagents were of analytical grade, and all water used was double-distilled in a fused silica apparatus.

Stock solution of piroxicam (1.0×10^{-3} mol/L) was prepared by dissolving 0.0331 g piroxicam with methanol solution and then diluted to 100 mL with methanol. Working piroxicam solution was prepared by diluting the stock solution with methanol daily. A stock solution of RuBPS (1.0×10^{-3} mol/L) was prepared by dissolving 0.1571 g RuBPS synthesized in the laboratory in 100 mL water. Working RuBPS solution was freshly prepared by diluting the stock solution. A 0.5 mol/L solution of H_2SO_4 was prepared by dissolving 6.90 mL concentrated sulphuric acid in 250 mL water. A 0.10 mol/L stock solution of cerium(IV) (0.5 mol/L·H_2SO_4) was prepared by dissolving 2.02 g $Ce(SO_4)_2{\cdot}4H_2O$ in a small amount of 0.5 mol/L H_2SO_4 solution and diluting to 50 mL. The working solutions used in experiments were prepared by diluting the stock standard solutions with water.

Procedure

0.40 mL RuBPS (1.25×10^{-4} mol/L) and 0.40 mL piroxicam (7.5×10^{-5} mol/L) were mixed in sample cuvettes and then the cuvettes were transferred into the measuring chamber of the luminometer. After the start button of the dispenser controller had been pushed, 0.20 mL cerium(IV) (6.25×10^{-3} mol/L) (0.20 mol/L H_2SO_4) was injected into sample cuvettes automatically by the dispenser and the CL signal produced was measured immediately. The peak CL intensity value was recorded for quantitative analysis.

Results and Discussion

Kinetic characteristics of the CL reaction

The CL intensities of the RuBPS–Ce(IV) system in the absence and presence of piroxicam were recorded continuously and the obtained CL kinetic curves are shown in Fig. 3. The experimental results indicated that a weak CL signal was recorded when RuBPS was mixed with cerium(IV) solutions (Fig. 3a). A strong CL signal was observed when RuBPS, cerium(IV) solutions and piroxicam were present in the reaction system (Fig. 3b).

Optimization of experimental conditions for piroxicam detection

A series of experimental conditions were investigated and optimized for maximum CL emission of the RuBPS–Cerium(IV)–piroxicam system.

Effect of RuBPS concentration

The influence of RuBPS concentration on sensitivity was studied with the optimum concentration of cerium(IV) in the range $3.0 \times$

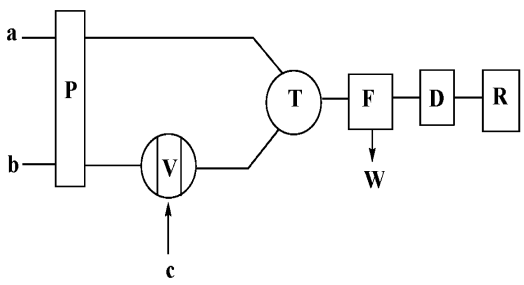

Figure 2. Schematic diagram of the flow injection system. a, Cerium(IV) (4.0×10^{-2} mol/L H_2SO_4) solution; b, H_2O; c, piroxicam + RuBPS solution; P, peristaltic pump; V, injection valve; T, T-piece; W, waste; F, flow-cell; D, detector; R, computer.

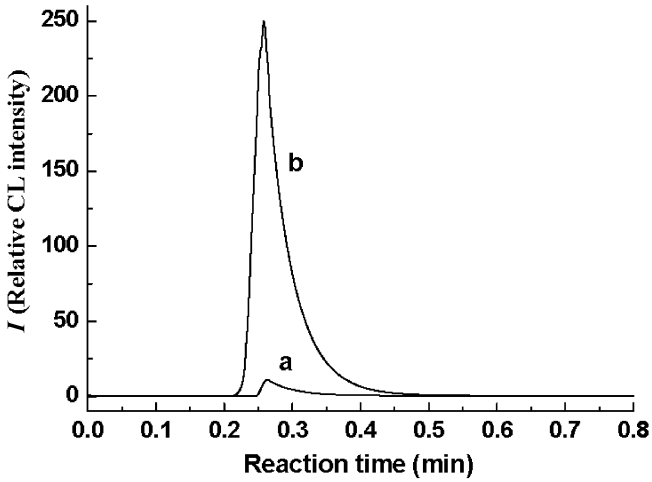

Figure 3. The chemiluminescence kinetic curves of the system in the absence (a) and in the presence (b) of piroxicam. Conditions: RuBPS 5.0×10^{-5} mol/L, cerium(IV) 1.25×10^{-3} mol/L, piroxicam 3.0×10^{-6} mol/L, H_2SO_4 4.0×10^{-2} mol/L.

Table 1. Comparison of analytical performances of the previously reported methods with the proposed method

Methods	Linear range (mol/L)	Detection limit (mol/L)	Reference
UV	1.51×10^{-7}–3.02×10^{-5}	3.02×10^{-7}	2
Electrochemical method	4.53×10^{-7}–1.51×10^{-5}	3.02×10^{-7}	3
CAM	1.0×10^{-5}–4.0×10^{-3}	1.0×10^{-5}	4
Spectrofluorimetry	6.04×10^{-7}–6.04×10^{-5} (sulphuric acid)	1.02×10^{-7}	5
	6.04×10^{-7}–3.62×10^{-6} (dioxane)	1.40×10^{-7}	
	3.02×10^{-6}–3.02×10^{-5}	2.72×10^{-7}	6
	9.06×10^{-8}–6.04×10^{-7}	3.02×10^{-8}	7
LC	3.02×10^{-7}–1.81×10^{-5}	6.04×10^{-8}	11
HPLC	1.51×10^{-7}–6.04×10^{-4}	3.02×10^{-8}	12
LC–S/MS	1.51×10^{-9}–6.04×10^{-7}	1.51×10^{-9}	16
Luminescence	3.0×10^{-7}–3.0×10^{-6}	8.8×10^{-8}	18
CL	2.98×10^{-7}–2.98×10^{-5}	1.2×10^{-7}	19
	2.8×10^{-8}–1.2×10^{-5}	2×10^{-8}	Proposed method

UV/Vis, ultraviolet and visible spectrophotometry; CAM, cmbining aperometry with mlticommutation; LC, liquid chromatography; HPLC, high-performance liquid chromatography; LC–MS/MS, liquid chromatography–tandem mass spectrometry method; CL, chemiluminescence.

10^{-6}–1.9×10^{-4} mol/L. The results show that the ΔI_{CL} signal increased with increasing RuBPS concentration until 5.0×10^{-5} mol/L and then remained stable. Therefore, 5.0×10^{-5} mol/L RuBPS was selected as optimum.

Effect of cerium(IV) concentration

As the oxidant, the concentration of cerium(IV) could affect the CL intensity of the systems (25) and the corresponding experiments were carried out under the fixed amount of RuBPS and H_2SO_4 and the variational concentration of cerium(IV) in the range 1.0×10^{-4}–3.0×10^{-3} mol/L. The experimental results indicated that when the concentration of cerium(IV) increased from 1.0×10^{-4} to 1.25×10^{-3} mol/L, ΔI_{CL} ($\Delta I_{CL} = I_{sample} - I_{blank}$; defined as the difference of CL intensity between piroxicam standard solution and the blank), which was used to evaluate the degree of enhancement of piroxicam to the CL emission of the RuBPS–Cerium(IV) system, increased, and after the concentration of cerium(IV) exceeded 1.25×10^{-3} mol/L, ΔI_{CL} decreased, which was perhaps due to the absorption of light emission by the coloured cerium(IV) solution and the scattering of light emission by the unsolvable hydrolysis product of cerium(IV) at the experimental acidity. In order to obtain the maximum ΔI_{CL}, 1.25×10^{-3} mol/L of cerium(IV) was chosen for the further experiments.

Effect of H_2SO_4 concentration

It has been confirmed that in the solution of H_2SO_4, the cation Ce^{4+} exists in the form of a series of SO_4^{2-} complexes and in all of the species, only Ce(IV), $Ce(SO_4)_2$ and $HCe(SO_4)^{3-}$ are active oxidants (26–28). So, the concentration of H_2SO_4 could affect the determination of the piroxicam and the corresponding experiments were carried out in the range 2.0×10^{-3}–8.0×10^{-2} mol/L H_2SO_4. Under the experimental conditions noted above, the concentration of H_2SO_4 was selected in the range 2.0×10^{-3}–8.0×10^{-2} mol/L, and when the concentration of H_2SO_4 was 4.0×10^{-2} mol/L, the maximum ΔI_{CL} was obtained. Thus, 4.0×10^{-2} mol/L H_2SO_4 was chosen as optimum.

Linear response range and detection limit

Under the optimum conditions described above, linearity for the determination of piroxicam was investigated in the range 2.8×10^{-8}–1.2×10^{-5} mol/L. The regression equation was $\Delta I_{CL} = 12.04 + 18.60 \times 10^{7}\, c$ (mol/L), R (correlation coefficient) = 0.9995 in the range 2.8×10^{-8}–9.0×10^{-7} mol/L, and $\Delta I_{CL} = 149.7 + 34.14 \times 10^{6}\, c$ (mol/L), R(correlation coefficient) = 0.9992 in the range 9.0×10^{-7}–1.2×10^{-5} mol/L. The detection limit (3σ) for the regression equation $\Delta I_{CL} = 12.04 + 18.60 \times 10^{7}\, c$ (mol/L) was 2×10^{-8} mol/L. RSD was 3.7% for the determination of 7.0×10^{-7} mol/L piroxicam ($n = 11$).

A comparison of the analytical performances of the previously reported methods and the proposed method for the determination of piroxicam is summarized in Table 1. Table 1 shows that the detection limit obtained by the proposed method was lower than the previously reported methods [2–7,11,12,18,19]. But higher than that obtained by LC–MS/MS (16).

Interferences

The effect of some common excipients in drugs, metal ions in the human body, and several organic compounds on CL intensity was investigated for the determination of 3.0×10^{-6} mol/L piroxicam. The CL emissions obtained using piroxicam solution alone and obtained using piroxicam solution with foreign species added were compared. Tolerance was defined as the amount of foreign species that produced a relative error (RE) not exceeding ±5% in the determination of piroxicam. As shown in Table 2, no significant interference could be observed for these foreign substances.

Application of the method

Analysis of pharmaceutical preparations. Three kinds of commercial piroxicam samples were analysed. The contents of 10 capsules or finely ground tablets were weighed and mixed. An amount of the tablet powder, or capsule powder equivalent to

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Table 2. Effect of various additives on CL emission intensity

Species added	Mole ratio ($C_{species}/C_{piroxicam}$)	Variation of CL peak height (%)
Starch	900	4.3
Dextrin	400	2.8
Glucose	500	2.9
Tenoxicam	750	3.4
Meloxicam	600	–2.5
Lactose	500	4.0
Sucrose	500	2.5
Maltose	800	–1.5
K^+	1000	2.3
Na^+	1000	2.8
Ca^{2+}	1000	–3.1
Co^{2+}	50	–3.8
Cu^{2+}	100	–2.2
Fe^{2+}	100	2.5
SO_4^{2-}	1000	2.3
NO_3^-	800	–3.5
EDTA	200	–1.4

Variation of the CL peak height (%): $(\Delta I_{species} - \Delta I_{piroxicam})/\Delta I_{piroxicam}$.

20 mg piroxicam, was weighed, dissolved in methanol and any remaining residue was removed by filtration. The clear solution was diluted to 250 mL with methanol in a 250 mL calibrated flask. Following the procedure detailed in the section, the proposed method was successfully applied to the determination of piroxcan samples. The results obtained are given in Table 3. There were no significant differences between the labelled contents and those obtained by the proposed method.

Analysis of spiked serum and urine samples. The proposed method was applied to the determination of piroxicam in human serum and urine samples. The serum and urine samples (20 mL, respectively) were collected from healthy volunteers. 2.0 mL 0.1 mol/L $ZnSO_4$ and 1.8 mL 0.1 mol/L $Ba(OH)_2$ were added to 1.0 mL human serum and urine samples, and each sample was pretreated according to methods described in the literature (29). To adjust the sample concentration of the drug to within the linear range of determination, after deproteinization and centrifugation of a serum sample the supernatant was used to investigate recovery. The urine samples were diluted appropriately with deionized water and analysed by the standard addition method. The results are given in Tables 4 and 5. Recovery was 92.0–103.3% for serum and 97.5–104.0% for urine.

Table 3. Results of the determination of piroxicam in tablets ($n = 6$)

Samples	Batch number	Labelled (g/tablet)	Proposed method (g/tablet)	RSD (%, $n = 6$)
Tablets	H41022631	0.020	0.0197	2.7
	H61021109	0.020	0.0215	3.1
	H36021370	0.010	0.0986	1.5

From Zhuzhou Yashen Pharmaceutical Co. Ltd, Zhuzhou, China (H41022631).
Xi'an No 4. Pharmaceutical Co. Ltd, Xi'an, China (H61021109).
Gannan Pharmaceutical Co. Ltd, Gannan, China (H36021370).

Table 4. Determination results of recovery of serum sample ($n = 6$)

Sample	Amount in sample[a] ($\times 10^{-7}$ mol/L)	Added ($\times 10^{-7}$ mol/L)	Found ($\times 10^{-7}$ mol/L)	Recovery (%)	RSD (%) ($n = 6$)
Serum	2.10 ± 0.16	1.0	3.02	92.0	2.5
		3.0	5.20	103.3	3.3
		5.0	7.01	98.2	1.1
		7.0	9.20	101.4	2.1

RSD, relative standard deviation.
[a]Mean ± SD.

Table 5. Determination results of recovery of urine sample ($n = 6$)

Human urine sample no.	Amount in sample ($\times 10^{-7}$ mol/L)	Added ($\times 10^{-7}$ mol/L)	Found ($\times 10^{-7}$ mol/L)	RSD (%)	Recovery (%)
1	0.95	2.0	3.03	2.2	104.0
2	0.92	2.0	2.98	1.1	103.0
3	1.16	2.0	3.11	4.2	97.5

RSD, relative standard deviation.

Conclusion

The enhancing effect of piroxicam on tris-(4,7-diphenyl-1,10-phlinedisulphonic acid)ruthenium(II) (RuBPS)–cerium(IV) CL reaction has been found. The experimental conditions affecting the CL reaction were optimized and the analytical characteristics for the determination of piroxicam are presented here. The proposed method was applied to the determination of piroxicam in pharmaceutical preparations in capsules, spiked serum and urine samples, with satisfactory results.

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