Research Article

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Flow-injection analysis for meloxicam based on tris(2,2′-bipyridine) ruthenium(II)–Ce(IV) chemiluminescent system

Huazhen Ye[a,b], Bin Qiu[a], Jing Chen[a], Jinming Lin[a] and Guonan Chen[a]*

ABSTRACT: It was found that meloxicam could enhance the chemiluminescence (CL) of the tris(2,2′-bipyridine) ruthenium(II)–Ce(IV) system in the medium of sulfate acid. Based on this phenomenon a new flow-injection system with chemiluminescent detection has been proposed for determination of meloxicam. Under optimum conditions, meloxicam had a good linear relationship with the CL intensity in the concentration range of 6.0×10^{-4} to 1.0 μg/mL and the detection limit was 3.7×10^{-4} μg/mL. The proposed method was applied to detect meloxicam in tablets and a satisfactory recovery was obtained. The possible mechanism for this CL system is also discussed in this paper. Copyright © 2009 John Wiley & Sons, Ltd.

Keywords: meloxicam; tris(2,2′-bipyridine)ruthenium(II); flow-injection; chemiluminescence

Introduction

Meloxicam, an oxicam derivative, is a member of the enolic acid group of nonsteroidal antiinflammatory drugs (NSAIDs) (1), and its structural formula is shown in Fig. 1. Meloxicam was successfully developed by Boehringer Ingelheim company in 1996 and was variably claimed to be the first generation of selective cyclooxygenase-2 (COX-2) inhibitors. It is used to relieve pain, tenderness, swelling and stiffness caused by osteoarthritis (arthritis caused by a breakdown of the lining of the joints) and rheumatoid arthritis (arthritis caused by swelling of the lining of the joints). Prostaglandin is one of mediators of inflammation, and the inflammatory reaction is relieved when the synthesis of prostaglandin is decreased. The therapy mechanism of NSAIDs is to decrease the synthesis of phlogogenic prostaglandin (PGs) by the inhibitory action of cyclooxygenase (COX). COX has two isomerism bodies, COX-1 and COX-2. As the inhibitory action of meloxicam to COX-2 (related to the regulation of inflammatory reaction) is stronger than that to COX-1 (necessary for normal physiological process), meloxicam has fewer side effects, such as alimentary canal anabrosis, bleeding and kidney damage, than other NSAIDs.

Until now, the determination methods reported for the analysis of meloxicam were HPLC (2, 3), UV spectrophotometry (4), fluoro-metric (5), electrochemical (6, 7), capillary electrophoresis (8), videodensitometric determination (9), flow injection chemiluminescence (10), electrochemiluminescence (11), etc. The method of HPLC with UV detection is most commonly used for the determination of meloxicam in pharmaceuticals and body fluid. Spectrophotometric and electrochemical methods also play a significant role in the quantification of meloxicam. As far as we know, tris(2,2′-bipyridine)ruthenium(II) chemiluminescent system has not been reported to detect meloxicam.

In our experiments, we found that meloxicam could enhance the CL of $Ru(bpy)_3^{2+}$–Ce^{4+} system in the medium of sulfate acid. Based on this phenomenon, a new flow-injection system with CL detection was proposed for the determination of meloxicam. This method has higher sensitivity than previous methods, and the apparatus for analysis is simple and cheap. Satisfactory results were obtained when it was applied to the assay of meloxicam in tablets.

Experimental

Apparatus and reagents

An IFFM-E chemiluminescence analyzer (Xi'an Rumile Analysis Apparatus Ltd, China) was used for flow-injection analysis and record CL intensity. A BPCL ultra-weak luminescence analyzer (institute of biophysics chinese academy of sciences) was used to record static injection CL intensity.

Meloxicam standard solution (70 μg/mL) was prepared by dissolving 0.0070 g meloxicam (National Institute for the Control of Pharmaceutical and Biological Products, batch no.100679-200401) in 0.20 mol/L sodium hydroxide solution and diluted to 100 mL, and stored in the refrigerator. Tris(2,2′-bipyridyl)ruthenium(II) solution (1.0×10^{-3} mol/L) was prepared by dissolving 0.07486 g Tris(2,2′-bipyridyl)ruthenium(II) chloride hexahydrate (AR, Sigma company) with water and diluted to 100 mL. Ammonium Cerous Sulfate (AR, Sinopharm Group Chemical Reagent Co. Ltd), sulfuric acid (AR, Guangdong Xilong Chemical Co. Ltd),

1*Correspondence to: Guuonan Chen, Ministry of Education Key Laboratory of Analysis and Detection Technology for Food Safety (Fuzhou University), Department of Chemistry, Fuzhou University, Fuzhou, Fujan 350002, People's Republic of China. E-mail: gnchen@fzu.edu.cn

[a] Ministry of Education Key Laboratory of Analysis and Detection Technology for Food Safety (Fuzhou University), Department of Chemistry, Fuzhou University, Fuzhou, Fujan 350002, People's Republic of China.

[b] Fujian Health College, Fuzhou, Fujan 350101, People's Republic of China.

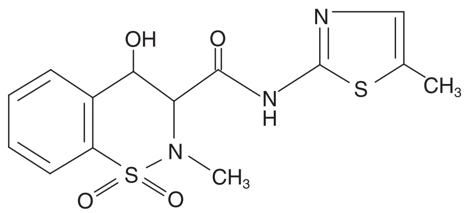

Figure 1. Structure of meloxicam.

meloxicam tablet (Nan'tong FeiMa Drug Ltd, batch no. 051 101). All other reagents were analytical grade or better and the water used in the experiment was double-distilled.

Treatment of tablets

Twenty meloxicam tablets were placed in a weighting bottle and weighed exactly; the average weight of one pill was 0.1395 g. The tablet powder which was porphyrizated in a mortar was moved to a weighing bottle and weighed satis quantum (containing 0.9 mg meloxicam or so) to a beaker by weight relief method. Meloxicam sample solution, which was dissolved with 0.22 mol/L NaOH and sonicated with oscillation for 10 min, was diluted to a 25 mL volumetric flask and filtered with 0.22 μm micropore film. A 0.5 mL aliquot of filtrate was diluted with 0.22 mol/L NaOH to a concentration in the linear range of the calibration graph.

General procedure

The flow-injection system used for the determination and CL detection of meloxicam is shown schematically in Fig. 2. Each stream was driven by peristaltic pump (P_1 or P_2) at a constant flow rate using PTFE tubing. Meloxicam solution was added to acidic ammonium ceric sulfate solution (which was used as the carrier stream) by a sample injection valve. The carrier stream that contained meloxicam was then combined with Tris(2,2′-bipyridyl)ruthenium(II) solution just before the quartz flow-through cell. The CL signal coming from the mixed solution in the flow-through cell was detected by a photomultiplier tube (PMT) and recorded using a computer. Meloxicam can be easily assayed based on the fact that the CL peak height difference value between sample solution and blank solution is linear with the concentration of meloxicam.

Results and discussion

CL kinetic characteristics

A static injection method was used to study the CL kinetic characteristics of $Ru(bpy)_3^{2+}$–Ce^{4+} system and $Ru(bpy)_3^{2+}$–Ce^{4+}-MLX system by BPCL ultra-weak luminescence analyzer, and the kinetic curves are shown in Fig. 3. Curve 1 is the CL kinetic curve obtained when 10 μL 1.0×10^{-3} mol/L $Ru(bpy)_3^{2+}$ solution was injected into 1 mL 1.0×10^{-3} mol/L $Ce(SO_4)_2{\cdot}2(NH_4)_2SO_4$ solution in a quartz glass. $Ru(bpy)_3^{2+}$–Ce^{4+} system had an ultraweak CL signal; the light emission was produced immediately after injection and was extinguished within 3 s. Curve 2 is the CL kinetic curve obtained when 10 μL 1.0×10^{-3} mol/L $Ru(bpy)_3^{2+}$ solution containing MLX was injected into $Ce(SO_4)_2{\cdot}2(NH_4)_2SO_4$ solution. The CL signal was extinguished within 5 s and was greatly enhanced. Therefore we drew a conclusion that the addition of MLX to the system can greatly enhance the CL intensity and prolong the lifetime of the CL signal.

Optimization of apparatus parameters

Flow rate is an important factor in a flow-injection system with CL detection. In this experiment, pump 1 was used to carry meloxicam solution and the quantitation polyethylene tube (6 cm) could be fully filled in 4 s under the pump running speed of 5.90 mL/min. Pump 2 was used to control the carrying speed of $Ru(bpy)_3^{2+}$ and Ce^{4+} solution, which determines the retention time of mixed solution in the flow-through cell. It was found that, when the pump run speed changed from 2.36 to 11.8 mL/min, the CL intensity was increased and the appearance time was shortened, and when the run speed was increased above 8.26 mL/min, the CL intensity only changed a little. Allowing for the consumption of sample and analysis time, 8.26 mL/min was selected as the optimum run speed of pump 2.

The voltage applied for the photomultiplier tube is closely connected with the sensitivity and noise of the detector. The effect of voltage on the CL intensity was studied at 600, 800, 900 and 1000 V, respectively. The results showed that the optimum voltage applied to photomultiplier tube was 1000 V.

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

The effect of concentration of $Ru(bpy)_3^{2+}$ on the CL intensity was investigated in the range 3.0×10^{-5} to 1.0×10^{-4} mol/L. The results [see Fig. 4(a)] showed that the CL intensity difference value (ΔINT)

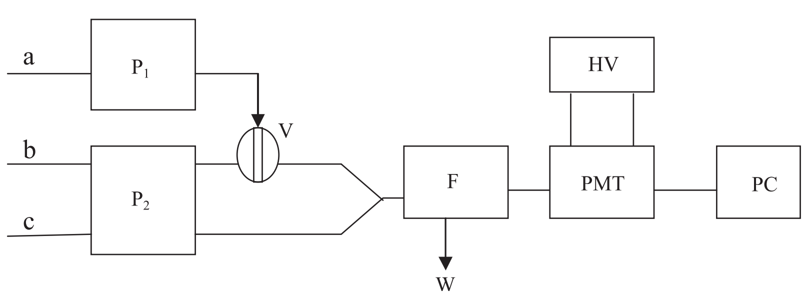

Figure 2. Schematic diagram of flow injection chemiluminescence analysis. (a) Meloxicam solution; (b) $Ce(SO_4)_2{\cdot}2(NH_4)_2SO_4$ solution; (c) $Ru(bpy)_3^{2+}$ solution; P, peristaltic pump; V, sample injection valve; F, flow-through cell; W, waste water; HV, high voltage; PMT, photomultiplier tube; PC, computer.

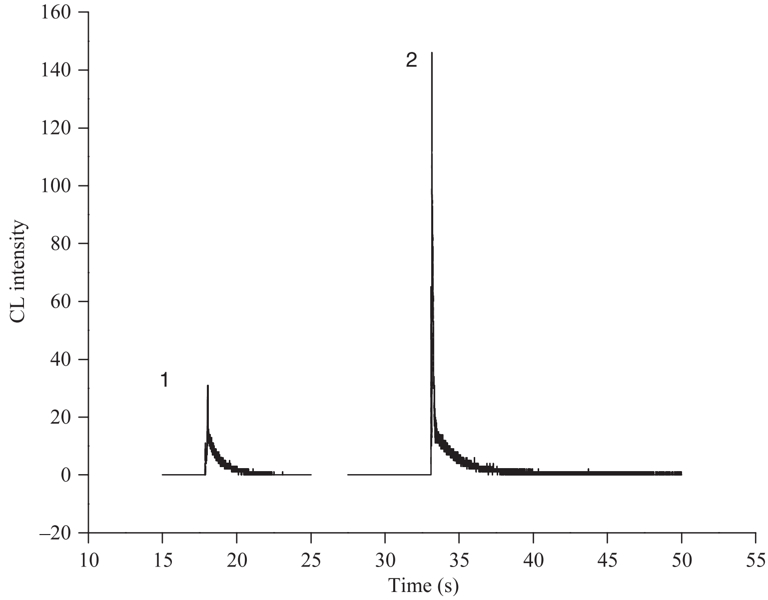

Figure 3. Kinetic curve of chemiluminescence system. 1, 1.0×10^{-3} mol/L Ce^{4+} (0.08 mol/L H_2SO_4) + 1.0×10^{-5} mol/L $Ru(bpy)_3^{2+}$; 2, 1.0×10^{-3} mol/L Ce^{4+} (0.08 mol/L H_2SO_4) + 1.0×10^{-5} mol/L $Ru(bpy)_3^{2+}$ + MLX (1.0 μg/mL).

of the blank system from the sample system was increased with $Ru(bpy)_3^{2+}$ concentration and the increasing degree was slowed down when the concentration of $Ru(bpy)_3^{2+}$ was higher than 8.0×10^{-5} mol/L. Considering the sensitivity and consumption of $Ru(bpy)_3^{2+}$, 9.0×10^{-5} mol/L was chosen as the optimum concentration of $Ru(bpy)_3^{2+}$ in this experiment.

Effect of NaOH concentration

As the solvent for meloxicam, sodium hydrate would affect the acidity of CL system and further affect the CL intensity. The effect of NaOH concentration on the CL intensity was examined in the range 0.12–0.30 mol/L. The result [see Fig. 4(b)] showed that the maximum CL intensity difference of blank from sample system could be obtained when NaOH concentration was 0.22 mol/L, so 0.22 mol/L was used in subsequent experiments.

Effect of Ce^{4+} concentration

Ce^{4+} was used as the oxidant in this CL system. The effect of Ce^{4+} concentration on CL intensity in the range 6.0×10^{-4} to 4.0×10^{-3} mol/L was examined under the selected optimum conditions. The result showed that the CL intensity difference (ΔINT) of blank system from sample system increased initially, then decreased with the concentration of Ce^{4+} [see Fig. 4(c)], and the maximum value was obtained at 1.0×10^{-3} mol/L of Ce^{4+}. The reason for this phenomenon may be the absorption of light emission by the colored Ce^{4+} solution and the scattering of light emission by the hydrolysis product of Ce^{4+} at the experimental acidity (12). In order to obtain the maximum CL intensity difference value, 1.0×10^{-3} mol/L Ce^{4+} solution was selected for following experiment.

Effect of sulfuric acid concentration

Sulphuric acid can inhibit hydrolysis of Ce^{4+} and affect the oxidation capability of Ce^{4+} (13). The effect of sulfuric acid concentration on CL instensity in the range 0.05 to 0.12 mol/L was tested. The results showed that the CL difference (ΔINT) of the blank system from the sample system was increased initially, reached a maximum at 0.08 mol/L, and then decreased [see Fig. 4(d)]. Therefore 0.08 mol/L of sulfuric acid was used in following experiment.

Calibration curve, precision and the detection limit

Under the optimum conditions, the CL intensity difference (ΔINT) of $Ru(bpy)_3^{2+}$–Ce^{4+} chemiluminescent system from the $Ru(bpy)_3^{2+}$–Ce^{4+}–MLX chemiluminescent system had good linear correlation with the meloxicam concentration (C) in the range 6.0×10^{-4} to 1.0 μg/mL. In order to enhance the determination accuracy rating, the calibration curve was drawn in segments. The linear regression equation is $\Delta INT = 1.4727C + 19.997$ and $r^2 = 0.9971$ in the range 6.0×10^{-4} to 2.0×10^{-2} μg/mL [see Fig. 5(a)]. The linear regression equation is $\Delta INT = 0.16084C + 274.76$ and $r^2 = 0.9915$ in the range 2.0×10^{-2} to 1.0 μg/mL [see Fig. 5(b)], where the unit of C is 10^{-4} μg/mL. The precision of the proposed method was evaluated by analyzing 0.10 μg/mL meloxicam standard solutions and the relative standard deviation was 2.1% for 11 parallel determinations. The detection limit, 3.7×10^{-4} μg/mL, was obtained by calculation from the formula $DL = 3S/K$, where S is standard deviation of analysing 11 blank solutions, and K is slope rate of the calibration graph.

Interferences

In order to investigate the interferences in meloxicam determination of some ions in humour and some adjuvant in meloxicam pharmaceutical preparations, an interference test for 0.10 μg/mL meloxicam was carried out under the optimum conditions. The results (see Table 1) show that 300-fold glucose, cane sugar, $FeCl_3$, NaCl, $ZnCl_2$ and $CaCl_2$, and 500-fold starch, KCl, $CuCl_2$ and $MgCl_2$ would not interfere with the determination of meloxicam in the range of relative error of ±10%.

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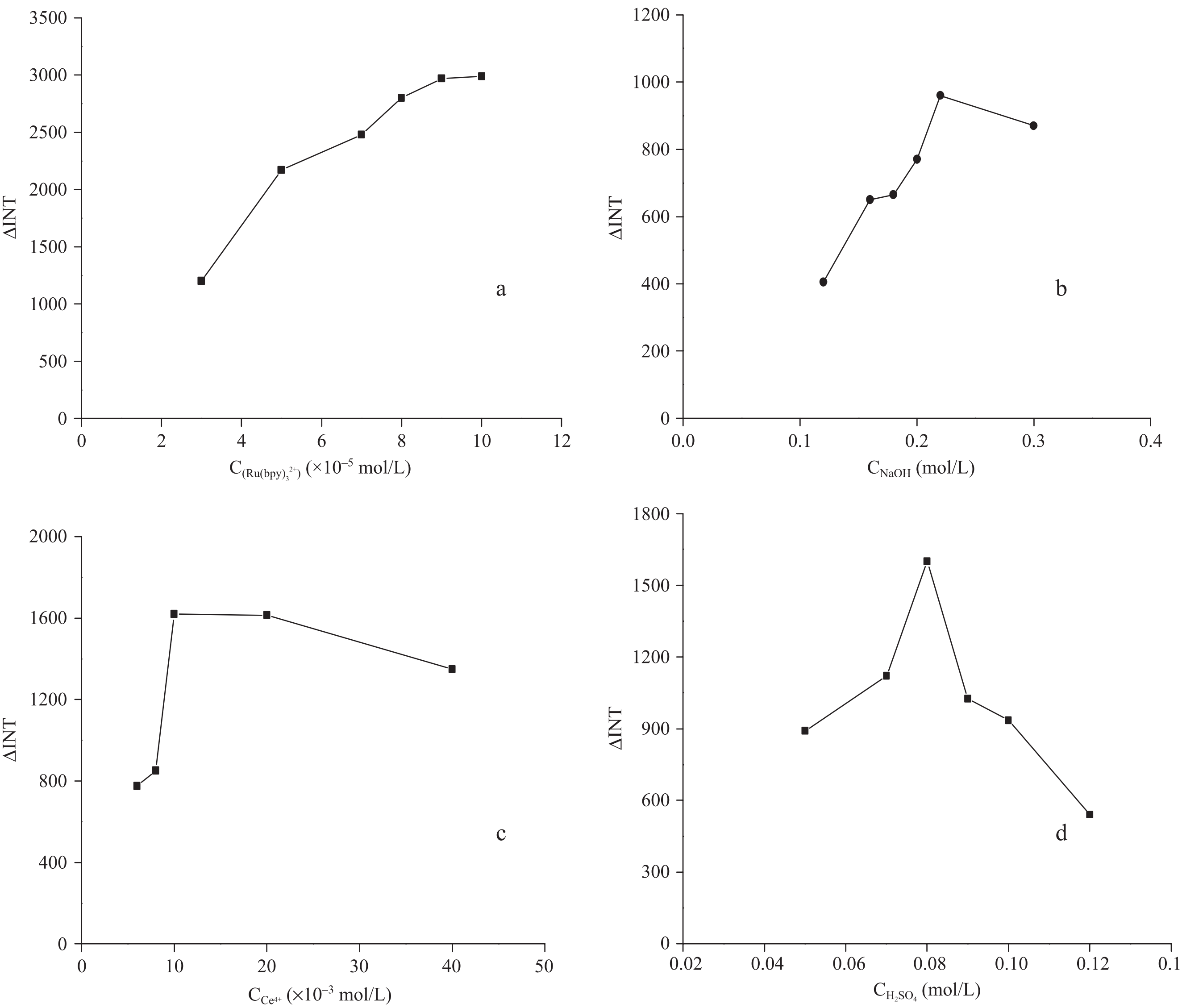

Figure 4. Effect of reactant concentration on CL intensity. (a) $Ru(bpy)_3^{2+}$ concentration; (b) NaOH concentration; (c) Ce^{4+} concentration; (d) H_2SO_4 concentration. Conditions: (a) $C_{Ce^{4+}} = 1.0 \times 10^{-3}$ mol/L (0.08 mol/L H_2SO_4), $C_{MLX} = 1.0$ µg/mL (0.22 mol/L NaOH); (b) $C_{Ru(bpy)_3^{2+}} = 5.0 \times 10^{-5}$ mol/L, $C_{Ce^{4+}} = 1.0 \times 10^{-3}$ mol/L (0.08 mol/L H_2SO_4), $C_{MLX} = 1.0$ µg/mL; (c) $C_{Ru(bpy)_3^{2+}} = 5.0 \times 10^{-5}$ mol/L, $C_{H_2SO_4} = 0.08$ mol/L, $C_{MLX} = 1.0$ µg/mL (0.22 mol/L NaOH); (d) $C_{Ru(bpy)_3^{2+}} = 5.0 \times 10^{-5}$ mol/L, $C_{Ce^{4+}} = 1.0 \times 10^{-3}$ mol/L, $C_{MLX} = 1.0$ µg/mL (0.22 mol/L NaOH).

Table 1. Influence of foreign substances (concentration of meloxicam was 0.10 µg/mL)

Foreign substance	Tolerance limit ($C_{species}/C_{MLX}$)	Relative error (%)
Starch	500	−2.8
Glucose	300	−4.5
Sucrose	300	−4.0
NaCl	300	4.4
$FeCl_3$	300	−4.7
$MgCl_2$	500	5.1
$CuCl_2$	500	1.4
KCl	500	5.7
$CaCl_2$	300	4.4
$ZnCl_2$	300	6.6

Assay of pharmaceutical preparations and recovery test

The proposed method was applied to determine the meloxicam tablet, which was pre-treated according to above procedure. The results of three parallel determinations (see Table 2) showed that the percentage of real content to declared content was in the range 95.0 to 105.0%. Therefore the meloxicam tablet is qualified according to drug quality specification. The recovery experiments were carried out by adding various amounts of meloxicam reference substance to the tablet powder. The obtained recovery rate values were in the range 91.4 to 101.5% (see Table 3), indicating that the proposed method was satisfactory.

Proposed CL mechanism

In the CL reaction system, the Ce^{4+} in sulfuric acid plays two roles: one is to oxidate $Ru(bpy)_3^{2+}$ to $Ru(bpy)_3^{3+}$, and the other is to oxidate meloxicam to an active intermediate (radical) (14, 15). The active intermediate can further react with $Ru(bpy)_3^{3+}$ to

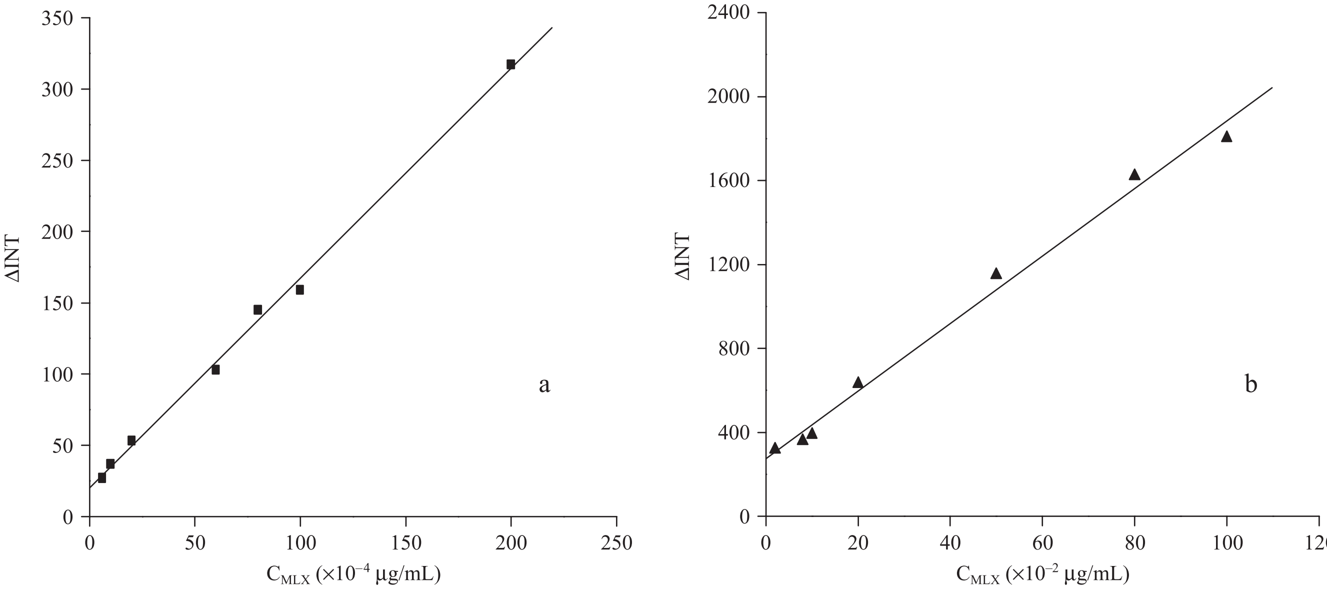

Figure 5. Calibration curve of MLX. (a) In the range of 6.0×10^{-4} to 2.0×10^{-2} μg /mL; (b) in the range of 2.0×10^{-2} to 1.0 μg /mL. Conditions: $C_{Ce^{4+}} = 1.0 \times 10^{-3}$ mol/L (0.08 mol/L H_2SO_4); $C_{Ru(bpy)_3^{2+}} = 9.0 \times 10^{-5}$ mol/L, $C_{NaOH} = 0.22$ mol/L.

Table 2. Assay of meloxicam in tablet

Batch number	Measured values (mg)	Content (%)	Average content (%)	Declared content (%)	Percentage (%)	RSD (%)
	0.894	5.50				
051101	0.896	5.40	5.28	5.38	98.1	5.4
	0.806	4.96				

Table 3. Results of recovery test($n = 9$)

Addition (mg)	Measured values (mg)	Recovery rate (%)	Average recovery (%)	RSD (%)
1.8	1.70, 1.77, 1.72	98.3, 94.4, 95.6	96.1	2.1
2.0	1.96, 2.03, 1.98	101.5, 98.0, 99.0	99.5	1.8
2.2	2.01, 2.11, 2.13	91.4, 95.9, 96.8	94.7	3.1

produce the excited state $[Ru(bpy)_3^{2+}]^*$ and lead to emission of light. By analogy to previously reported papers (14, 15), the CL reaction mechanism is proposed as follows:

$$Ru(bpy)_3^{2+} + Ce^{4+} \rightarrow Ru(bpy)_3^{3+} + Ce^{3+} \quad (1)$$

$$MLX + Ce^{4+} \rightarrow MLX^{+\bullet} + Ce^{3+} \quad (2)$$

$$MLX^{+\bullet} \rightarrow MLX^{\bullet} + H^{+} \quad (3)$$

$$Ru(bpy)_3^{3+} + MLX^{\bullet} + H_2O \rightarrow [Ru(bpy)_3^{2+}]^* + \text{other products} \quad (4)$$

$$[Ru(bpy)_3^{2+}]^* \rightarrow Ru(bpy)_3^{2+} + h\nu \quad (5)$$

Conclusions

It was found that meloxicam is able to enhance the CL of $Ru(bpy)_3^{2+}$–Ce^{4+} system in the medium of sulfate acid. Based on this, a new flow-injection system with CL detection was proposed for the determination of meloxicam. This method is simple, cheap and more sensitive than other methods reported before, and it has been successfully applied to assay meloxicam in a real sample. The mechanism for the enhancement of $Ru(bpy)_3^{2+}$–Ce^{4+} CL system is attributed to the oxidation of meloxicam to an active intermediate (free radical ions). The active intermediate can further react with $Ru(bpy)_3^{3+}$ to produce the excited state $Ru(bpy)_3^{2+*}$ and lead to light emission.

Acknowledgements

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