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## Flow injection determination of papaverine based on its sensitizing effect on the chemiluminescence reaction of permanganate-sulfite

Received: 9 July 2002 / Revised: 16 October 2002 / Accepted: 16 October 2002 / Published online: 26 November 2002  
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**Abstract** A novel chemiluminescence (CL) method for the determination of papaverine (PAP) has been developed by combining the flow injection technique and its sensitizing effect on the weak CL reaction between sulfite and acidic permanganate. A mechanism for the CL reaction has been proposed on the basis of fluorescent and chemiluminescence spectra. The CL response is proportional to the concentration of PAP over the range 0.2–10  $\mu\text{mol L}^{-1}$ . The detection limit of PAP is 0.10  $\mu\text{mol L}^{-1}$  (3 s) with a relative standard deviation (RSD) of 2.0% for 10 repetitive determinations of 1.0  $\mu\text{mol L}^{-1}$  PAP. Interferences from other alkaloids in the opium, such as morphine and codeine, are negligible except that of narcotine. The method has been satisfactorily used for the determination of PAP in injections and compound liquorice tablets.

**Keywords** Chemiluminescence · Papaverine · Permanganate · Sulfite · Flow injection analysis

### Introduction

Analytical methods combining chemiluminescence (CL) with flow injection techniques have the advantages of simplicity and rapidity, and these have found extensive application in many areas. A variety of organic and inorganic compounds, such as bile acids [1], riboflavin [2, 3], morphine [4, 5, 6, 7, 8], ascorbic acid [9, 10], and RNA [11] have been determined by using  $\text{KMnO}_4$  as a reagent to generate CL. Recently, Hindson and Barnett reported a comprehensive and critical review on the wide range of analytical applications of  $\text{KMnO}_4$  in CL reactions [12]. The nature of these reactions has been postulated to involve the following excited state intermediates: manganese (II) or a

complex [4, 13, 14, 15], singlet oxygen [16, 17], sulfur dioxide [1, 18], molecular nitrogen [19, 20], and fluorescent oxidation products of the analyte [21]. Meixner and Jaeschke proposed the following mechanism to explain the CL reaction of sulfite with permanganate [22]:



In this mechanism sulfite acts as a reductant to produce an excited molecule of sulfur dioxide, which emits radiation in the range of 300–550 nm [1]. The energy of the excited molecule can be easily transferred to a fluorescent molecule intentionally added into the system [23]. Since the fluorophore has a higher quantum efficiency, its application can produce stronger photon emission, thereby facilitating the measurement [23]. Another way to enhance the CL emission is the application of intriguing examples of sensitizers. These compounds do not fluoresce but are capable of amplifying the CL emission intensity produced from the oxidation of sulfite. For example, although 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS) is not a fluorophore, it can enhance the CL emission intensity from the oxidation of sulfite by  $\text{KMnO}_4$ . The sensitizing effect of CAPS is attributed to the presence of the cyclohexyl ring [24].

Papaverine (6,7-dimethoxy-1-veratryl-isoquinoline, PAP) is a benzyloisoquinoline alkaloid obtained from opium. It has a direct spasmolytic effect on the smooth muscles of the bronchi, gastrointestinal tracts, ureters, and biliary system, and a pronounced relaxant effect on blood vessels including coronary, cerebral, pulmonary, and peripheral arteries [25]. Several methods have been reported for the quantitative determination of PAP, including electrochemical [26, 27, 28], spectrophotometric [29, 30, 31] and fluorescent methods [32, 33], thin-layer and liquid chromatography [34, 35], and capillary electrophoresis [36, 37]. Most of the reported methods suffer from disadvantages such as complicated procedure, long response time, requirement of expensive instruments, or low detection

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ability. Although the CL method possesses many advantages and the CL behaviors of other main alkaloids in opium have been studied extensively [5, 13, 38], to the best of our knowledge no work on the CL behavior of PAP has been reported except a study with the photostoragechemiluminescence method [39].

In order to develop a simple, rapid, and sensitive method for the determination of PAP in both pure form and its pharmaceutical preparations, this work studies the effect of PAP on the CL intensity emitted from the reaction of sulfite with acidic  $\text{KMnO}_4$ . A method for PAP detection is proposed on the basis on this sensitizing effect. The method has superior selectivity, since no serious interference is found with other opium alkaloids such as morphine or codeine. This method is simple and less expensive than the above-mentioned techniques and at the same time offers good accuracy and precision. It has also been used to determine PAP in pharmaceutical preparations.

## Experimental

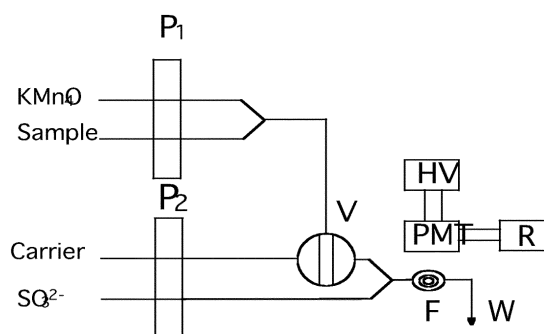
### Reagents

Analytical grade reagents and doubly distilled water were used to prepare all solutions.

A standard solution of PAP ( $1.0 \text{ mmol L}^{-1}$ ) was prepared by dissolving 18.8 mg of PAP (obtained from the College of Public Health, Southeast University, China) in water and diluting to 50 mL. Working standard solutions were prepared daily by appropriate dilution of the standard solution.  $\text{KMnO}_4$  (Jintan, China) was used as received to prepare a  $0.01 \text{ mol L}^{-1} \text{ KMnO}_4$ . The working solution of  $0.1 \text{ mmol L}^{-1} \text{ KMnO}_4$  was prepared daily by diluting the stock solution with  $0.4 \text{ mol L}^{-1}$  sulfuric acid. The solution of  $0.01 \text{ mol L}^{-1}$  sodium sulfite was prepared daily.

### Apparatus

The flow injection analysis (FIA)-CL system used in this work is shown in Fig. 1. Two pumps of Luminescence Analyzer (IFFM-D, Remex Electronic Instrument Limited Co., Xi'an, China) were used to deliver flow streams. Polytetrafluoroethylene (PTFE) tubing (0.8 mm i.d.) was used to connect all components in the flow system. The solutions of  $\text{KMnO}_4$  and PAP were mixed with a



**Fig. 1** Schematic diagram of the CL-FLA system for the determination of papaverine:  $P_1$  and  $P_2$ , peristaltic pump;  $V$ , six-way injection valve;  $F$ , flow cell;  $W$ , waste solution;  $HV$ , high voltage;  $PMT$ , photomultiplier tube;  $R$ , recorder

Y-shaped element and were allowed to react within a time controlled by the length of tube between the Y-shaped element and injection valve. The flow cell was a 10-cm long spiral glass tubing (2.0 mm i.d.) and the distance between injection valve and flow cell was about 10 cm. The CL signal was detected by the photomultiplier tube (PMT) placed near the flow cell and was recorded with a computer equipped with an A/D card.

Fluorescence spectra were recorded on a RF-5301 spectrofluorimeter (Shimadzu, Japan).

### Procedures

PAP and acidic  $\text{KMnO}_4$  solutions were mixed via a Y-shaped element and injected into the carrier stream (water) through a six-way injection valve. Sulfite solution was mixed with the carrier stream via another Y-shaped element in front of the flow cell. The CL emission signal was detected by PMT and recorded by special software. The procedure to obtain the CL spectrum information was achieved with a series interference filters by the static method. The filters were inserted between the sample cuvette and the PMT.

## Results and discussion

### Optimization of experimental variables

The direct oxidation of PAP by acidic  $\text{KMnO}_4$  gave out a very weak CL emission, which could not be used for analytical purpose. However, when PAP was used as an enhancer, the weak CL emission of sulfite and acidic  $\text{KMnO}_4$  increased significantly. The emission intensity was proportional to the concentration of PAP. The CL intensity was related to the concentrations of  $\text{KMnO}_4$  and sulfite, and the value of solution pH.

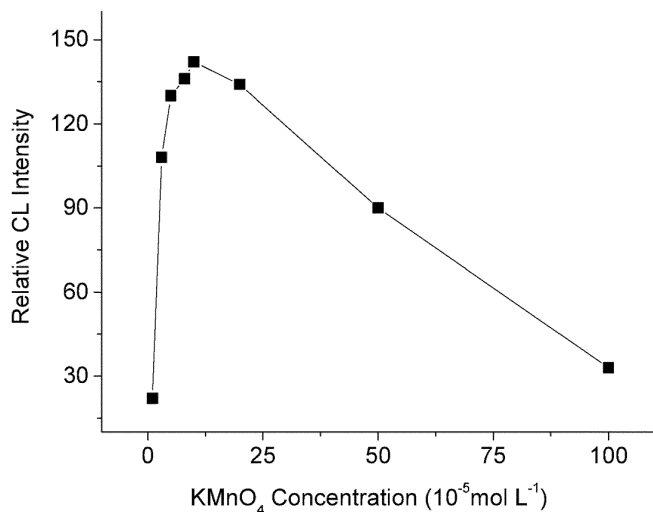
The effect of acid contained in the solution on the CL emission was initially examined. The CL emission intensity of the  $20 \mu\text{mol L}^{-1}$  PAP– $0.1 \text{ mmol L}^{-1} \text{ KMnO}_4$ – $1.0 \text{ mmol L}^{-1} \text{ Na}_2\text{SO}_3$  system in the presence of  $0.5 \text{ mol L}^{-1}$  HCl,  $\text{HNO}_3$ ,  $\text{CH}_3\text{COOH}$ ,  $\text{H}_6\text{P}_4\text{O}_{13}$ , or  $\text{H}_2\text{SO}_4$  is shown in Table 1. The strongest emission was observed in the system containing  $\text{H}_2\text{SO}_4$ . Thus,  $\text{H}_2\text{SO}_4$  was finally chosen as the acidic medium for the permanganate reduction.

The concentration of  $\text{H}_2\text{SO}_4$  in  $\text{KMnO}_4$  solution was subsequently optimized and  $0.4 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$  in aqueous  $\text{KMnO}_4$  was considered as the optimum concentration and was used for further experiments.

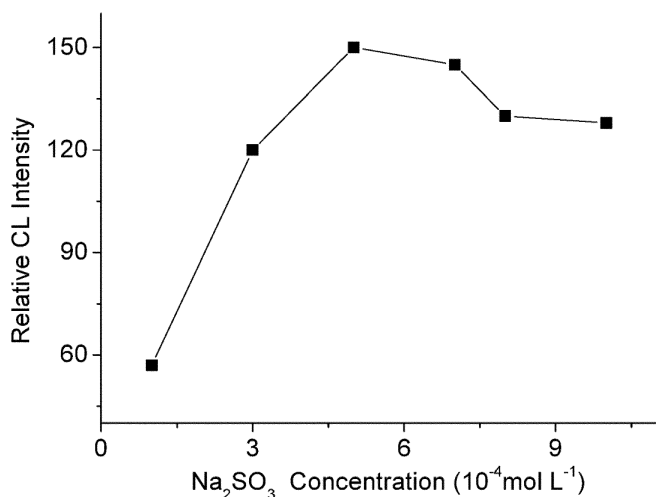
The CL intensity increased with increasing  $\text{KMnO}_4$  concentration and reached a maximum value at  $0.1 \text{ mmol L}^{-1} \text{ KMnO}_4$ . Larger concentrations resulted in a decrease of the emission intensity (Fig. 2). Therefore,  $0.1 \text{ mmol L}^{-1} \text{ KMnO}_4$  was used for subsequent work.

**Table 1** Effect of acids on the CL emission intensity of PAP– $\text{KMnO}_4$ – $\text{Na}_2\text{SO}_3$

Acid	CL emission intensity
HCl	273
$\text{HNO}_3$	254
$\text{CH}_3\text{COOH}$	509
$\text{H}_6\text{P}_4\text{O}_{13}$	1015
$\text{H}_2\text{SO}_4$	1034



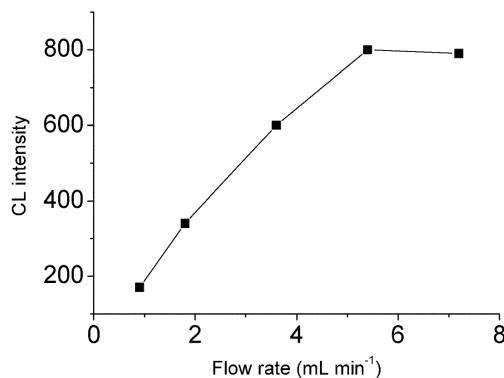
**Fig. 2** Effect of  $\text{KMnO}_4$  concentration on CL intensity of  $0.4 \text{ mol L}^{-1} \text{H}_2\text{SO}_4 + 2.0 \text{ } \mu\text{mol L}^{-1} \text{PAP} + 1.0 \text{ mmol L}^{-1} \text{Na}_2\text{SO}_3$



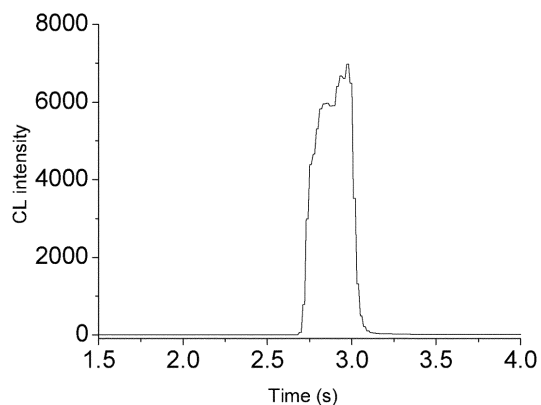
**Fig. 3** Dependence CL intensity on the concentration of  $\text{Na}_2\text{SO}_3$  in  $0.4 \text{ mol L}^{-1} \text{H}_2\text{SO}_4 + 2.0 \text{ } \mu\text{mol L}^{-1} \text{PAP} + 0.1 \text{ mmol L}^{-1} \text{KMnO}_4$

Figure 3 shows the effect of  $\text{Na}_2\text{SO}_3$  concentration on the CL emission intensity of the  $\text{PAP-MnO}_4^- - \text{SO}_3^{2-}$  system. The strongest emission occurred at a concentration of  $0.5 \text{ mmol L}^{-1}$ . This value was therefore chosen for the present work.

The total flow rate of the reagent stream is an important parameter affecting the CL emission. Figure 4 shows the effect of total flow rate on CL intensity. The flow rates of  $\text{Na}_2\text{SO}_3$  solution and the carrier are set at the same value and are twice those of both  $\text{KMnO}_4$  and the sample solution. The greatest emission occurs at a total flow rate of  $5.4 \text{ mL min}^{-1}$ . Thus, a flow rate of  $0.9 \text{ mL min}^{-1}$  is set for both acidic  $\text{KMnO}_4$  and PAP solutions. The flow rates of  $\text{Na}_2\text{SO}_3$  solution and the carrier are set at  $1.8 \text{ mL min}^{-1}$ . These parameters realized good analytical response for PAP and were maintained throughout the current investigation.



**Fig. 4** Plot of CL intensity versus the total flow rate of reagent stream



**Fig. 5** Time course of CL emission of  $10 \text{ } \mu\text{mol L}^{-1} \text{PAP} + 0.1 \text{ mmol L}^{-1} \text{KMnO}_4 + 0.4 \text{ mol L}^{-1} \text{H}_2\text{SO}_4$  upon addition of  $0.5 \text{ mmol L}^{-1} \text{Na}_2\text{SO}_3$

#### Kinetic characteristics of the CL reaction

The rate of CL reaction plays an important role in the design of a flow CL system. The kinetic curve of the CL reaction of  $\text{PAP-MnO}_4^- - \text{SO}_3^{2-}$  was studied with a static method. As shown in Fig. 5, the CL reaction occurs immediately after the addition  $0.5 \text{ mmol L}^{-1} \text{Na}_2\text{SO}_3$  into the solution containing  $0.1 \text{ mmol L}^{-1} \text{KMnO}_4$  and  $0.01 \text{ mmol L}^{-1} \text{PAP}$  and reaches a maximum within  $0.28 \text{ s}$ . The CL reaction is complete within  $0.4 \text{ s}$  after the reaction starts and is therefore very rapid. It is a flash-type emission and is apparently controlled by the mixing speed.

#### Determination of PAP

Under the optional conditions, the relationship between the CL emission intensity and PAP concentration has a linear calibration graph in the concentration range  $0.20\text{--}10 \text{ } \mu\text{mol L}^{-1}$  with a correlation coefficient of  $0.9986$ . The detection limit ( $3 \text{ s}$ ) was  $0.1 \text{ } \mu\text{mol L}^{-1}$ . The RSD for  $10$  repetitions determinations of  $1.0 \text{ } \mu\text{mol L}^{-1} \text{PAP}$  was  $2.0\%$ .

**Table 2** Tolerance to different substances in the determination of PAP

Species added	Maximum tolerable mole ratio <sup>a</sup>
Morphine, codeine.	100
Pb <sup>2+</sup> , Sn <sup>4+</sup> , La <sup>2+</sup> , Ca <sup>2+</sup>	20
Zn <sup>2+</sup> , Sn <sup>2+</sup> , Cd <sup>2+</sup> , Ni <sup>2+</sup> , Cr <sup>3+</sup> , Co <sup>2+</sup>	10
Glucose, fructose, sucrose	10

<sup>a</sup>100 is the largest ratio tested

**Table 3** Determination of PAP in injections

Sample	Labeled value	Proposed method <sup>a</sup>
Injection 1	30 mg mL <sup>-1</sup>	30.4±0.6 mg mL <sup>-1</sup>
Injection 2	30 mg mL <sup>-1</sup>	30.9±0.3 mg mL <sup>-1</sup>

<sup>a</sup>Average of five determinations

### Interferences

In order to assess the selectivity of the proposed method, the influences of common foreign species were examined by preparing the solutions containing 1.0 μmol L<sup>-1</sup> PAP and foreign species with continuously increasing concentration up to 100 μmol L<sup>-1</sup>. Each foreign species was considered not to interfere with the determination of PAP when the effect on the peak height was less than 5.0%. Most anions have no interference because the tolerable ratios were in this case far higher than those normally encountered in real samples. No interference could be found when 100-fold excess concentration of morphine or codeine coexisted in the solution. Although the reaction of morphine with permanganate in the presence of tetraphosphoric acid gave a strong chemiluminescence [4], morphine does not give any response in the present procedure, indicating that the oxidation product could not react with HSO<sub>3</sub><sup>-</sup>; thus, it does not have any contribution to the formation of the emitter or to the sensitizing effect. However, equal amount of narcotine increased the CL signal. No obvious interference was observed in the presence of common excipients in drugs. The results obtained are summarized in Table 2. Therefore, this method can be suggested for the determination of PAP in pharmaceutical preparations or biological fluids in cases in which the coexisting drugs do not interfere the determination.

**Table 4** Analytical results of PAP in liquorice tablets

Sample	Found <sup>a</sup> (μmol L <sup>-1</sup> )	RSD (%)	Added (μmol L <sup>-1</sup> )	Recovered (μmol L <sup>-1</sup> )	Recovery (%)
Tablet sample 1	37.0	5.6	10.0	46.8	98.0
			30.0	67.4	100.1
			50.0	84.3	94.6
Tablet sample 2	24.5	3.8	10.0	35.0	105.0
			30.0	53.2	95.7
			50.0	54.9	101.3

<sup>a</sup>Average of five determinations

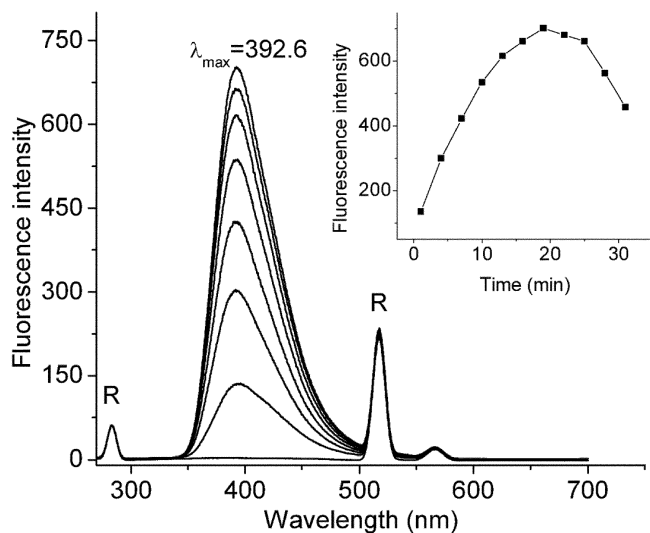
### Analysis of real samples

The concentrations of PAP in injections as a pharmaceutical preparation were determined with the proposed method. The sample was diluted appropriately with water prior to measurement so that the concentration of PAP was in its linear response range. As seen from Table 3, there are no significant difference between the labeled value and the measurement results. Table 4 shows the analytical results of PAP in compound liquorice tablets. The RSD for five determinations of two samples are 5.6% and 3.8% respectively. Recovery studies were performed on each of the analyzed samples with the recommended treatment by adding known amounts of PAP in the samples solutions. The results in Tables 3 and 4 suggest that the proposed method can be satisfactorily used for the determination of PAP in real samples.

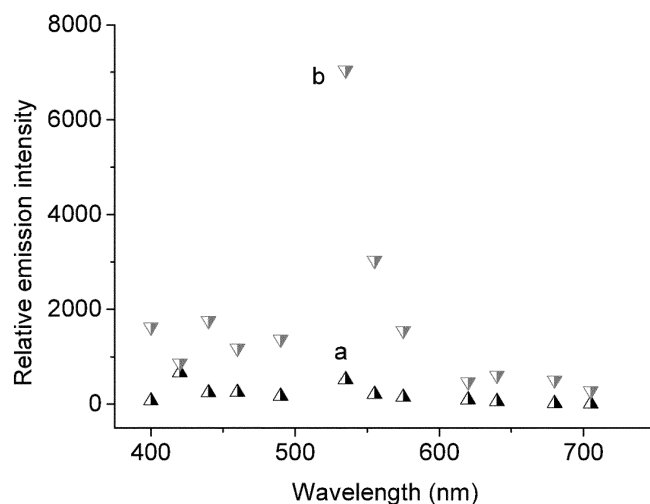
### Mechanistic studies of the CL reaction

Since PAP does not exhibit fluorescence in acidic medium and does not have a cyclohexyl structure, the mechanism of sensitizing effect may differ from those of fluorescent compounds and CAPS. The fluorescent spectra of PAP+KMnO<sub>4</sub> reveal that PAP can be oxidized by KMnO<sub>4</sub> slowly because the fluorescent intensity of the system increases gradually (Fig. 6). The maximum fluorescent emission occurs at 390 nm, which is different from that of the characteristic fluorescent spectrum of Mn<sup>2+</sup>, which shows a maximum emission at 330 nm in sulfuric acid [4]. The similar fluorescent spectrum is also observed during the oxidation of PAP by hydrogen peroxide. Thus, it can be concluded that the fluorescent substance is the oxidation product of PAP.

The CL spectra of SO<sub>3</sub><sup>2-</sup> oxidized by acidic KMnO<sub>4</sub> with and without PAP are shown in Fig. 7. The CL spectrum of sulfite-acidic KMnO<sub>4</sub> system extends from 400 to 680 nm with a maximum emission intensity at 535 nm. Although the CL intensity of the PAP-KMnO<sub>4</sub>-Na<sub>2</sub>SO<sub>3</sub> system has a significant increase, its spectrum shows a similar range and profile; thus, PAP is not involved in the ultimate emission step of the CL reaction process. The CL spectrum of the PAP-KMnO<sub>4</sub>-Na<sub>2</sub>SO<sub>3</sub> system is obviously different from its fluorescent spectrum, indicating that the energy transfer is not responsible for the sensitizing effect. In view of the CL spectra and the analysis reported by Meixner and Jaeschke [22], the emitter of



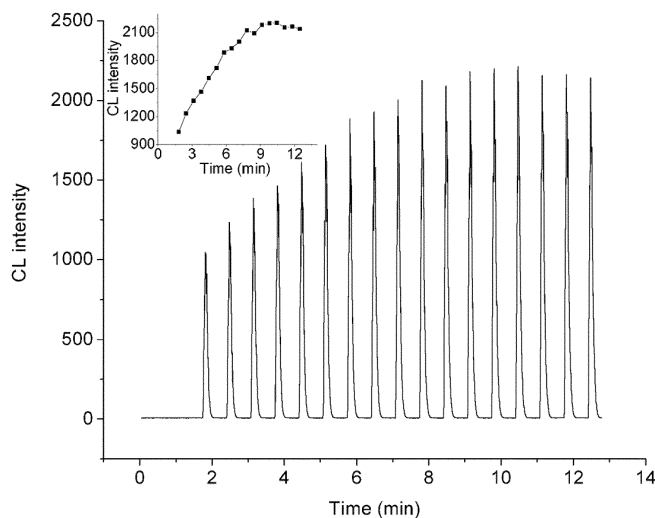
**Fig. 6** Effect of the reaction time on the fluorescent intensity of  $0.1 \text{ mmol L}^{-1} \text{ KMnO}_4 + 0.5 \text{ } \mu\text{mol L}^{-1} \text{ PAP} + 0.4 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$ ; 0, 4, 7, 11, 14, and 17 min from *bottom to top*; R, Rayleigh scattering. *Inset*: plot of the fluorescence intensity versus reaction time



**Fig. 7** CL spectra of  $\text{PAP-KMnO}_4\text{-Na}_2\text{SO}_3$  (b) and  $\text{KMnO}_4\text{-Na}_2\text{SO}_3$  (a)

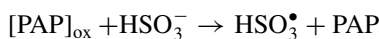
the present CL reaction is the excited sulfur dioxide, which gives out an emission when it returns to its ground state.

The dependence of CL emission intensity of the  $\text{PAP-KMnO}_4\text{-Na}_2\text{SO}_3$  system on the addition time of  $\text{Na}_2\text{SO}_3$  in the mixture of PAP and acidic  $\text{KMnO}_4$  is shown in Fig. 8. Obviously, the CL emission is related to the reaction time between  $\text{KMnO}_4$  and PAP. It coincides with the variety of the fluorescent intensity of  $\text{PAP-KMnO}_4$  (Fig. 6). The difference in the time reaching maximum intensity results from the different concentrations of reactants. Thus, the CL emission of  $\text{PAP-KMnO}_4\text{-Na}_2\text{SO}_3$  involves the oxidation product of PAP by  $\text{MnO}_4^-$ , which acts as an active intermediate that reacts with sulfite, leading to a free radical reaction of sulfite. The CL emission attributes to the for-



**Fig. 8** Effect of the reaction time of acidic  $\text{KMnO}_4$  with PAP on the CL intensity of the  $\text{PAP-KMnO}_4\text{-Na}_2\text{SO}_3$  system. The final concentrations of PAP,  $\text{KMnO}_4$ ,  $\text{H}_2\text{SO}_4$ , and  $\text{Na}_2\text{SO}_3$  are 20, 100, 400, and  $500 \text{ } \mu\text{mol L}^{-1}$ , respectively

mation of excited sulfur dioxide molecules. The mechanism can be expressed as follows:



## Conclusions

The weak CL reaction of sulfite and acidic  $\text{KMnO}_4$  can be enhanced significantly in the presence of PAP. The mechanistic studies show that the oxidation product of PAP by  $\text{MnO}_4^-$  is involved in the CL emission of the  $\text{PAP-KMnO}_4\text{-Na}_2\text{SO}_3$  system. The product acts as an active intermediate which oxidizes  $\text{HSO}_3^-$  leading to the formation of the excited sulfur dioxide. The sensitizing effect of PAP on the CL reaction of sulfite-acidic  $\text{KMnO}_4$  provides an analytically useful, simple, rapid, and sensitive method for the determination of PAP in pharmaceutical preparations.

**Acknowledgements** This project was supported by the National Natural Science Foundation of China (No 29975013 and 9835110), the Science Foundation of Jiangsu (No BS2001063) and the Doctoral Fund of the Education Ministry of China (No 20000284033).

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