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CHEMILUMINESCENCE

Flow-Injection Chemiluminescence Determination of Papaverine Using Cerium(IV)-Sulfite System

Sichun Zhang, Yafeng Zhuang, and Huangxian Ju*

Department of Chemistry, Institute of Analytical Science, State Key Laboratory of Coordination Chemistry, Nanjing University, Nanjing, P.R. China

ABSTRACT

A sensitizing effect of papaverine on the weak chemiluminescence (CL) reaction of sulfite with acidic cerium(IV) is studied. In papaverine and cerium(IV) solution, the increase in the fluorescent intensity of cerium(III) at 360 nm with an increasing time indicates a slow oxidation of papaverine by acidic cerium(IV). This reaction results in the formation of intermediate radical of papaverine, which enhanced the CL emission of sulfite–cerium(IV) system. Based on this finding, a flow injection (CL) method is proposed for the determination of papaverine. The CL intensity is proportional to the concentration of papaverine from 1.0×10^{-7} to 1.0×10^{-5} M with a correlation coefficient of 0.9991. The detection limit

*Correspondence: Huangxian Ju, Department of Chemistry, Institute of Analytical Science, State Key Laboratory of Coordination Chemistry, Nanjing University, Nanjing 210093, P.R. China; Fax: +86-25-83593593; E-mail: hxju@nju.edu.cn.

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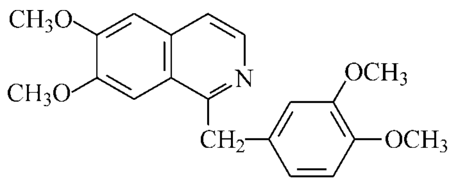
is 8.7×10^{-8} M (3σ). The relative standard deviation for seven independent determinations of 1.0×10^{-6} M papaverine is 2.0%. The proposed method has been satisfactorily used for the determination of papaverine in pharmaceutical preparations and biological fluids.

Key Words: Papaverine; Cerium(IV); Sulfite; Chemiluminescence; Flow injection analysis.

INTRODUCTION

Papaverine (6,7-dimethoxy-1-veratryl-isoquinoline) is a benzylisoquinoline alkaloid obtained from opium that possesses a wide range of biological effects and is commonly known as a cerebral vasodilator and smooth muscle relaxant. It is generally used in clinics as a cardiac vasodilator and has a nonspecific direct action upon coronary blood vessels with a reduction in muscular tonus. In addition, papaverine attracts more attention recently due to its interaction with cellular membranes and the effect on the affinity of hemoglobin to oxygen. Thus its determination is very important for the study of biological science. The determination of papaverine in the British Pharmacopoeia is carried out with potentiometric titration.[1] Several other methods have also been reported for its quantitative determination, including electrochemical,[2–4] spectrophotometric,[5] and fluorescent methods.[6]



Papaverine

Analytical procedures based on chemiluminescence (CL) have been frequently used for the analysis of pharmaceutical compounds due to their simplicity, rapidity and sensitivity.[7–9] However, the CL behavior of papaverine has been noted as either weak or not observed with *tris*(2,2′-bipuridyl)ruthenium(III) or other CL reagents during the study of CL property of principal alkaloids in opium.[10,11] To the best of our knowledge, no CL method for the determination of papaverine has been reported except a photostorage chemiluminescence method.[12] In this work papaverine is found to exhibit a significant sensitizing effect on the CL oxidation of sulfite by acidic cerium(IV), which have also been sensitized by fluorescein[13] and *N*-tetrahydrobenzothiazolyl imine.[14] Combining with flow injection technique, this effect provides a sensitive and convenient method for the determination of papaverine in injection, urine, and serum.

EXPERIMENTAL

Reagents

Papaverine hydrochloride was obtained from the College of Public Health, Southeast University (Nanjing, China). All other reagents were of analytical grade. All solutions were prepared with deionized water. Sodium sulfite (Xi'an Reagent Co., China) solution was prepared daily. A 0.01 M stock solution of cerium(IV) was prepared by dissolving 0.6683 g of $Ce(SO_4)_2 \cdot 2(NH_4)_2SO_4 \cdot 2H_2O$ (Peking Chemical Works, China) in 0.1 M sulfuric acid and diluting to 100 mL with the same acid.

Apparatus

Two pumps of IFFM-D Luminescence Analyzer (Remex Electronic Instrument Limited Co., China) propelled flow streams of sulfite and acidic cerium(IV) solutions at a total flow rate of 5 mL min^{-1}. PTFE tube (0.8 mm i.d.) was used to connect all components in the flow system. The sample was injected into the flow stream via the six-way injection valve. A Y-shaped mixing element, positioned just before the flow cell inlet, was used for mixing the two streams. The flow cell was a coil of glass tubing (i.d. 2 mm, total length 100 mm), positioned in front of the detection window of the photomultiplayer tube (PMT). For maximum light collection, the coil was backed with a mirror. The CL emission was converted by PMT to current signal and the output was fed to Luminescence Analyzer, recorded with a computer via an A/D convert card and special software.

The fluorescence spectra were recorded by an RF-5301 spectrofluorimeter (Shimadzu, Japan). The CL spectra, which were too weak to be recorded by a spectrofluorophotometer, were achieved with the IFFM-D Luminescence Analyzer and a set of 13 narrow band interference filters (400–745 nm).

Procedures

The CL reaction conditions were optimized with the following procedures. By keeping the valve in washing position, sulfite and acidic cerium(IV) solutions were continuously pumped into the manifold until the baseline was established on recorded signal. 30 μL Papaverine solutions were injected into the carrier stream (acidic cerium(IV)) with the sampling frequency of 90 $h^{-1}$and allowing 20 s for sampling and washing. The content of papaverine was determined with the calibration plot of CL emission intensity vs. papaverine concentration.

Chemiluminescence spectrum information was achieved with the interference filters, which were inserted between the cuvette and PMT. As the total light transmitted by difference filters was not the same, they were calibrated by the manufacturer. The recorded data were calibrated according to the manufacturer's instruction.

RESULTS AND DISCUSSION

Optimization of Experimental Conditions

Preliminary studies were carried out to screen the oxidant for the CL determination of papaverine. The results indicated that no significant CL emission could be observed when KIO_4, $K_2S_2O_8$, H_2O_2, $K_2Cr_2O_7$, $KMnO_4$, or $Ce(SO_4)_2$ were used as oxidants in either acidic or alkaline medium. However, when papaverine was used as an enhancer, the weak CL emission of cerium(IV) and sulfite was enhanced significantly (Fig. 1). Figure 1 shows a background emission, which results from the CL reaction of cerium(IV) and sulfite. However, the low background can be recorded as the baseline because a much stronger CL emission occurred when papaverine was injected into the mixing solution.

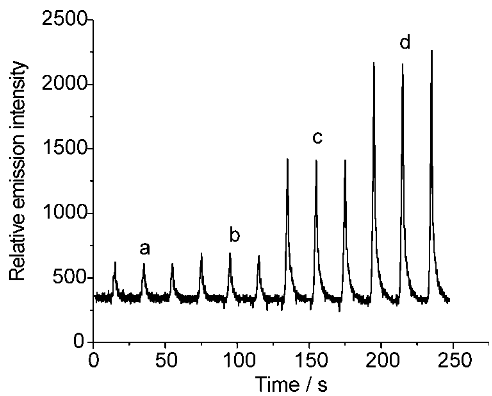


Figure 1. Typical CL signals of papaverine at different concentration. (a) Blank, (b) 1.0×10^{-7}, (c) 1.0×10^{-6}, and (d) 2.0×10^{-6} M papaverine.

The emission intensity was proportional to papaverine concentration. In an attempt to improve the S/N ratio, a series of optimization experiments were carried out by varying the concentration of cerium(IV), sulfite, sulfuric acid in cerium(IV) solution and flow rate whilst maintaining the concentration of papaverine at 1.0×10^{-6} M.

The solution of cerium(IV) over the range of 0.05–5.0 mM in 0.01 mM sulfuric acid was examined and the results were shown in Fig. 2. A 0.8 mM cerium(IV) solution was considered as the optimum concentration and was used for subsequent work.

The effect of sulfite concentration on CL emission was investigated over the range of 0.01–10.0 mM and the results were shown in Fig. 3. Maximum emission was observed with 1.0 mM sodium sulfite.

Cerium(IV) is a strong oxidant in acidic solution. In presence of perchloric acid it often undergoes a decomposition process. In sulfuric acid solution, however, cerium(IV) is highly stable and does not require any special precaution to prevent the decomposition. Therefore, sulfuric acid was used in the present work. The effect of sulfuric acid concentration on the CL reaction of cerium(IV)–sulfite was tested. As shown in Fig. 4, the emission intensity increased with a decreasing concentration of sulfuric acid. Considering the hydrolysis of cerium(IV) to form a white precipitate at sulfuric acid concentrations lower than 0.01 M, 0.01 M sulfuric acid solution was selected for our measurements.

The solutions of cerium(IV) and sulfite were introduced into the manifold at equal flow rate and the weak CL emission was continuously recorded as the baseline. The intensity of background emission was relevant to the flow rate. The signal intensity increased with the increasing flow rate, as it was expected

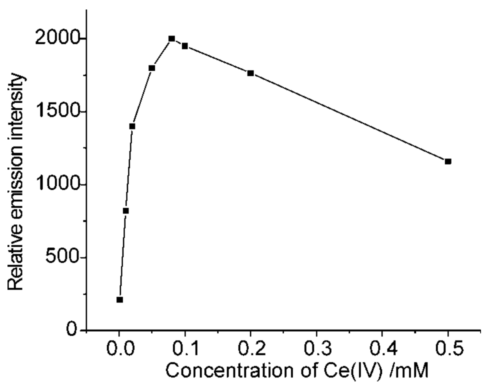


Figure 2. Effect of cerium(IV) concentration on the CL emission intensity. Sulfite, 1.0 mM; sulfuric acid, 0.05 M; and papaverine, 1.0×10^{-6} M.

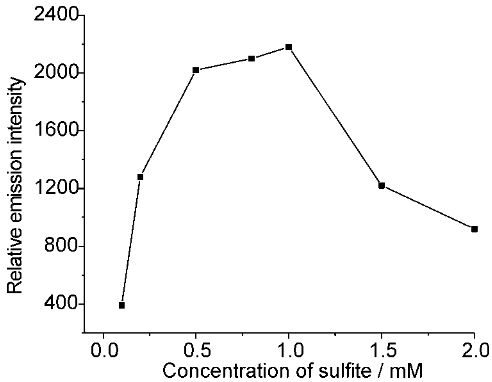


Figure 3. Dependence of the CL emission intensity on sulfite concentration. Cerium(IV), 0.8 mM; sulfuric acid, 0.05 M; and papaverine, 1.0×10^{-6} M.

from the increase of mixing rate. Higher flow rate led to more consumption of reagents and sample solution but less gain in CL intensity. Thus, it was decided to supply the cerium(IV) and sulfite solution at 2.5 mL min^{-1}, respectively.

Analytical Characteristics

Under the optimum conditions mentioned above, the calibration curve was obtained for papaverine determination by plotting the CL signal vs. papaverine concentration, which gave a linear range from 1.0×10^{-7} to

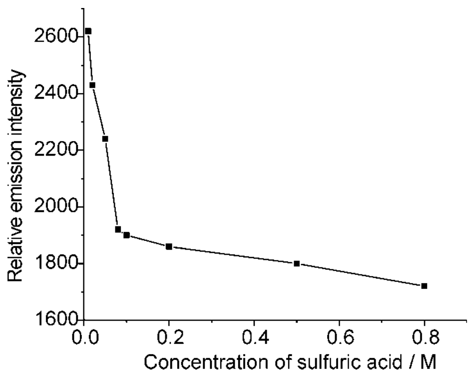


Figure 4. Effect of sulfuric acid concentration on the CL emission intensity. Cerium(IV), 0.8 mM; sulfite, 1.0 mM; and papaverine, 1.0×10^{-6} M.

1.0×10^{-5} M. The regression equation was $I = 14.4 + 851C$ with a correlation coefficient of 0.9991 ($n = 8$), where the unit of C was in 10^{-5} M. The detection limit was 8.7×10^{-8} M at 3σ. The relative standard deviation was 2.0% for seven repetitive measurements of papaverine at 1.0×10^{-6} M. The analytical figures of merit for papaverine have been compared to those from earlier investigations and summarized in Table 1.

Interference Studies

The influences of different metal ions and organic compounds on the CL intensity were investigated by determining the CL emission at 1.0×10^{-6} M papaverine. Morphine and codeine did not show any effect on the CL intensity up to the concentration of 5.0×10^{-6} M. Narcotine interfered the determination even at the concentration of 1.0×10^{-6} M. Considering that the concentration of morphine in opium is at 40 times more than that of papaverine and the concentration of narcotine in opium is at the same level as papaverine, the present method could not be used to determine papaverine in opium due to the severe interference. Metal ions, such as Cu(II), Cd(II), Pb(II), Zn(II), Ca(II), Ba(II), Co(II), Mo(II), Ni(II), Al(III), Cr(III), Mn(II), Fe(III), and Fe(II) at the concentration of 1.0×10^{-5} M did not interfere the determination. No obvious interference was observed in the presence of 1.0×10^{-5} g mL^{-1} starch, glucose, lactic acid, urea, and albumin. However, cysteine, ascorbic acid, and uric acid interfered the determination at the concentration of 1.0×10^{-6} M. Therefore, sample pretreatment was necessary when the proposed method was applied to the analysis of papaverine in biological samples.

Table 1. Comparison of the analytical characteristics of the proposed method with those of some previously reported techniques for the determination of papaverine

Method	LOD (M)	Linear range (M)	Reference
Spectrophotometry after derivatization (chromotrope 2R)	—	Up to 3.6×10^{-3}	[5]
Adsorptive stripping voltammetry at a Hg electrode	1.0×10^{-9}	8.0×10^{-9}–1.0×10^{-7}	[3]
Ion-selective electrode	—	1.0×10^{-5}–1.0×10^{-2}	[4]
Proposed method	8.7×10^{-8}	1.0×10^{-7}–1.0×10^{-5}	

Sample Analysis

In order to assess the validity of the proposed method, papaverine in injections was determined. A 1.0 mL volume of the injection solution was diluted with water so that the final concentration was in the linear range. The diluted solution was used directly for analysis without further treatment. No significant difference was observed between the results obtained by the proposed method and the values labeled (Table 2).

When the suggested method was used for the determination of papaverine in urine or serum sample, the sample was first extracted with 10 mL of chloroform after adjusting the pH to 9 with buffer. The organic extract was then evaporated to dryness to obtain a residue. The residue was dissolved in 100 mL water and used for the CL determination of papaverine. The recovery for papaverine was determined by comparing the CL intensity of the treated urine or serum samples with the CL intensity of the papaverine at the same concentration. The results are shown in Table 3.

Mechanism of the Chemiluminescence Reaction

The mixture of papaverine and acidic cerium(IV) does not give out detectable CL emission with either flow injection or static method. But the fluorescence intensity of acidic cerium(IV) solution after mixed with papaverine increases gradually (Fig. 5). The maximum of fluorescent emission spectrum is found at 360 nm, which is similar to that of the characteristic fluorescent spectrum of cerium(III).[15] The results indicated that papaverine can be oxidized by acidic cerium(IV).

The oxidation of sulfite by cerium(IV) in acidic medium exhibits a weak CL emission, which has been used in many analytical procedures based on CL emission.[14,16–21] The CL spectra of SO_3^{2-} oxidized by acidic cerium(IV)

Table 2. Results for the determination of papaverine in injections.[a]

Sample	Concentration (mM) Labeled value	Proposed method[b]	Added (mM)	Found (mM)	Recovery (%)
Injection 1	80.0	81.6 ± 3.8	30.0	112.6	102.4
Injection 2	80.0	78.3 ± 1.2	50.0	131.5	101.2

[a]Hengrui Medicine Ltd., China.
[b]Mean ± S.D ($n = 5$).

Table 3. Results of papaverine determinations in urine and serum

Sample	Concentration added (10^{-6} M)	Found (10^{-6} M)	Recovery (%)
Urine	20.0	20.6	103.0
	40.0	38.7	96.8
	60.0	59.2	98.7
	80.0	82.0	102.5
Mean ± RSD			100.2 ± 3.0
Serum	10.0	9.7	97.0
	20.0	20.3	101.5
	40.0	39.4	98.5
	60.0	58.0	97.8
	80.0	79.4	99.2
Mean ± RSD			98.8 ± 1.7

with and without the presence of papaverine are shown in Fig. 6. The CL spectrum extends from 400 to 680 nm with a maximum emission at 535 nm and a shoulder peak at 440 nm.

Papaverine does not show fluorescence in aqueous solution, thus the energy transfer from the oxidation process of SO_3^{2-} by cerium(IV) to

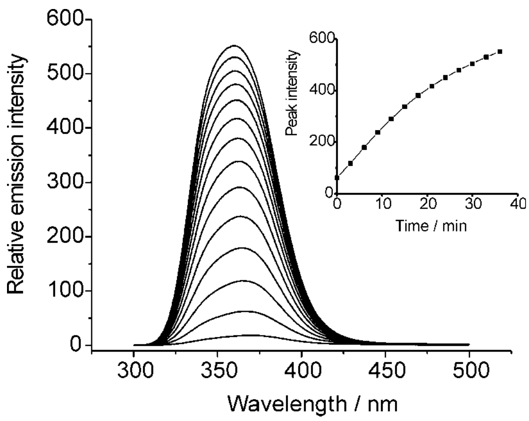


Figure 5. Time dependence of the fluorescence intensity of cerium(IV) in the presence of papaverine and sulfite. Cerium(IV), 0.1 mM; sulfuric acid, 0.01 M; and papaverine, 1.0×10^{-5} M; time: 0, 3, 6,9, 12,15, 18, 21, 24, and 27 min from bottom to up. Inset: plot of fluorescent peak intensity vs. time.

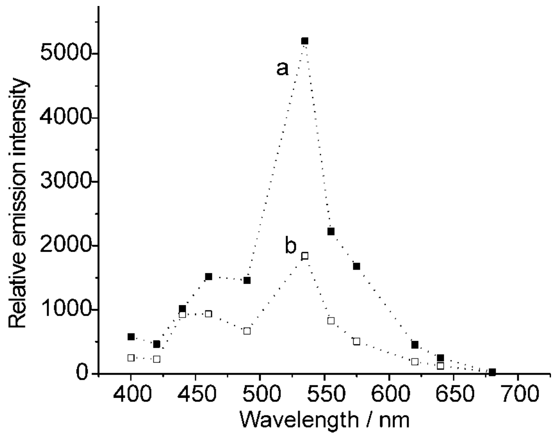
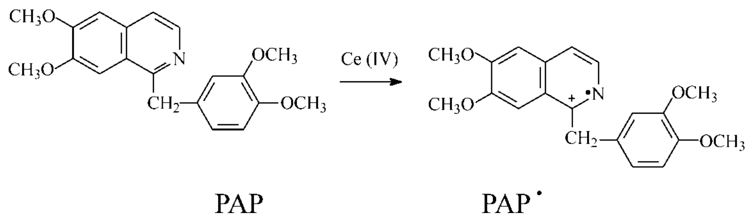


Figure 6. Chemiluminescence spectra of the papaverine sensitized CL reaction of cerium(IV)–sulfite reaction (a) and non sensitized CL reaction (b) cerium(IV), 0.1 mM; sulfite, 1.0 mM; sulfuric acid, 0.01 M; and papaverine, 1.0×10^{-5} M.

papaverine is not present during the sensitizing process. In the presence of papaverine, the range and profile of the CL spectrum is similar to that of SO_3^{2-} and cerium(IV) system, but the CL intensity has a significant increase.

The results indicate that the emitter of CL emission in the presence and absence of sensitizer is the same. In the CL system of cerium(IV)–SO_3^{2-}, SO_2^* has been extensively thought to be the emitter of CL emission.[22,23] Based on the CL spectrum information, it can be concluded that papaverine is not involved in the ultimate emission step in the CL process. Cerium(IV) is known as one-electron oxidant and can react with many organic compounds to form reactive intermediate radical.[24] Thus, it is possible that papaverine is oxidized by cerium(IV) to form an intermediate radical, which reacts with sulfite to initiate a free radical reaction.



It can be concluded from above discussions that the sensitizing effect of papaverine on the chemiluminescent oxidation of sulfite by cerium(IV) is due to the reaction of papaverine with cerium(IV), in which an intermediate radical is formed. The overall mechanism can be described as follows:

$$Ce^{4+} + HSO_3^- \rightarrow HSO_3^{\bullet} + Ce^{3+}$$

$$Ce^{4+} + PAP \rightarrow PAP^{\bullet} + Ce^{3+}$$

$$PAP^{\bullet} + HSO_3^- \rightarrow HSO_3^{\bullet} + PAP$$

$$2HSO_3^{\bullet} \rightarrow S_2O_6^{2-} + 2H^+$$

$$S_2O_6^{2-} \rightarrow SO_4^{2-} + SO_2^*$$

$$SO_2^* \rightarrow SO_2 + hv$$

CONCLUSIONS

A flow injection CL method has been proposed based on the sensitizing effect of papaverine on the weak chemiluminescent reaction of sulfite and cerium (IV). The formation of an intermediate radical of papaverine results in the increase of the emitter SO_2^*, which strengthens the CL emission intensity. This method can be used for the determination of papaverine in injection and biological fluids.

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