Research Article

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Chemiluminescence determination of bromhexine hydrochloride with morin as chemiluminescent reagent

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ABSTRACT: A new chemiluminescence (CL) reaction was observed when cerium(IV) solution was injected into bromhexine hydrochloride–morin solution. Based on this, a flow-injection CL method for the determination of bromhexine hydrochloride was established. A possible mechanism of the CL reaction was proposed via the investigation of the CL kinetic characteristics, the CL spectrum and the fluorescence spectra of some related substances. Under optimum conditions, the CL signal was correlated linearly with concentration of bromhexine hydrochloride over the range 2.0×10^{-9}–2.0×10^{-7} g/mL, with a linear correlation of 0.9995. The detection limit was 9×10^{-10} g/mL bromhexine hydrochloride and the relative standard deviation was 1.0% ($c = 2.0 \times 10^{-8}$ g/mL bromhexine hydrochloride, $n = 11$). The method was applied to the determination of bromhexine hydrochloride in pharmaceutical preparations and human urine samples with satisfactory results. Copyright © 2008 John Wiley & Sons, Ltd.

Keywords: chemiluminescence; cerium(IV); morin; bromhexine hydrochloride

Introduction

Bromhexine hydrochloride (*N*-(2-amino-3, 5-dibromobenzyl)-*N*-methyl cychlohexanamine hydrochloride), a bronchial mucolytic drug, has an affinity for acinar cells and causes patients to secrete pancreatic juice of low viscosity. It may be a new drug for the morbidity of chronic pancreatitis, in which there is increased viscosity of the pancreatic juice and formation of a protein plug (1). Although some methods have been developed for the determination of bromhexine hydrochloride, such as spectrophotometry (2, 3), near-infrared spectroscopy (4) and chromatography (5–10), the limitation to these methods is low sensitivity or expensive instrumentation. To the best of our knowledge, a CL method has not yet been reported for the determination of bromhexine hydrochloride.

Morin, a common fluorescent reagent, has been widely applied in fluorometric analysis of metals (11, 12). Recently, it has been reported that CL can be produced in the reaction between morin and potassium permanganate (13). We found that morin can still react with many other oxidants and produce CL. In many cases, morin is able to be used as chemiluminescent reagent.

In this study, a CL reaction of cerium(IV)–morin–bromhexine hydrochloride was found. Based on this, a new, rapid, simple and sensitive method was established for the determination of bromhexine hydrochloride. A possible mechanism of the CL reaction was discussed, and the proposed method was applied to the determination of bromhexine hydrochloride in pharmaceutical preparations and human urine samples, with satisfactory results.

Experimental

Apparatus

An IFFM-D flow injection CL analyser (Xi'an Remex Electronic Instrument High-Tech Ltd, China), equipped with an automatic injection system and a detection system, was used. A schematic diagram of the CL flow system employed is shown in Figure 1. PTFE tube (0.8 mm i.d.) 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. The CL spectrum was measured using a BPCL ultra-weak luminescence

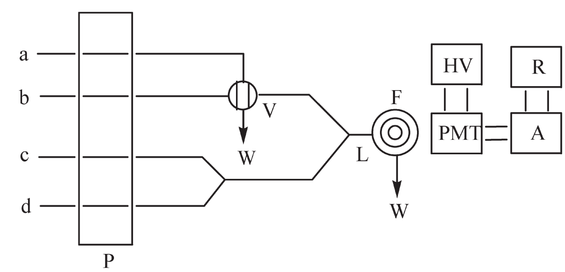

Figure 1. Schematic diagram of CL flow system; a, cerium(IV) solution; b, water; c, morin solution; d, bromhexine hydrochloride standard/sample solution; P, peristaltic pump; V, six-way valve; L, mixing tube; F, flow cell; PMT, photomultiplier tube; HV, high voltage; A, amplifier; R, recorder; W, waste.

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analyser (Institute of Biophysics, Chinese Academy of Sciences). Fluorescence spectra were obtained using a 970CRT spectrofluorometer (Shanghai Analysis Instrument Main Plant, China).

Reagents

Bromhexine hydrochloride was purchased from Fluka. $Ce(SO_4)_2 \cdot 2(NH_4)_2SO_4 \cdot 4H_2O$ was purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). Morin was obtained from Beijing Chemical Works (Beijing, China). Other chemicals were purchased from Xi'an Chemical Plant (Xi'an, China).

A standard solution of bromhexine hydrochloride (1.00×10^{-4} g/mL) was prepared by dissolving 0.0100 g bromhexine hydrochloride in water and diluting to 100 mL with water. The stock solution of morin (1.0×10^{-3} mol/L) was prepared by dissolving 0.085 g morin in 1.0 mL 0.2 mol/L NaOH and diluting to 250 mL with water, after which it was stored in a refrigerator and protected from light. A solution of cerium(IV) (1.0×10^{-3} mol/L) was prepared by dissolving 0.17 g $Ce(SO_4)_2 \cdot 2(NH_4)_2SO_4 \cdot 4H_2O$ in 2 mol/L sulphuric acid and diluting to 250 mL with water.

All reagents were of analytical reagent grade, except for bromhexine hydrochloride, which was a check sample, and morin, which was chemical purity grade. Double-distilled water was used throughout.

Procedure

As shown in Fig. 1, flow lines (a–d) were connected with cerium(IV) solution, water, morin solution, bromhexine hydrochloride standard/sample solution, respectively. Cerium(IV) solution was injected into the water stream via a six-way valve and then was merged with the mixture of morin and bromhexine hydrochloride standard/sample solution via a Y-piece, to produce CL. The concentration of bromhexine hydrochloride was quantified as ΔI_{CL} ($\Delta I_{CL} = I_{sample} - I_{blank}$).

Results and discussion

Kinetic characteristics of the CL reaction

The kinetic characteristics of the CL reaction were examined using the static measuring system of the IFFM-D multifunction CL analyser. The CL intensity–time curve is shown in Fig. 2. When cerium(IV) was injected into the mixture of morin and bromhexine hydrochloride, a strong CL reaction was initiated immediately and the maximum CL intensity was obtained within 2.0 s. This showed that the cerium(IV)–morin–bromhexine hydrochloride CL reaction was rapid.

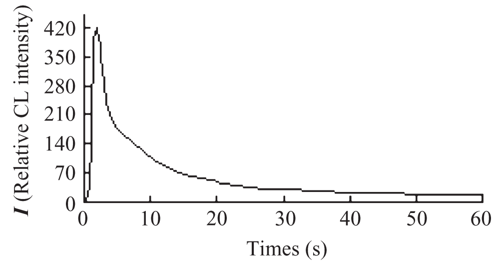

Figure 2. Kinetic curve of the CL reaction. Cerium(IV) solution, 1.0×10^{-3} mol/L (2.0 mL); morin solution, 5.0×10^{-6} mol/L (1.0 mL); bromhexine hydrochloride solution, 2.0×10^{-8} g/mL (1.0 mL).

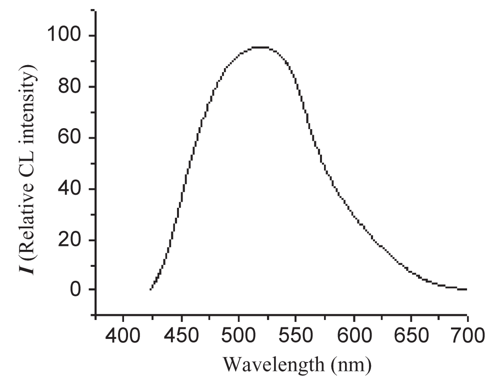

Figure 3. CL spectrum of the CL reaction. Cerium(IV), 1.0×10^{-3} mol/L; morin, 5.0×10^{-6} mol/L; bromhexine hydrochloride, 1.0×10^{-6} g/mL.

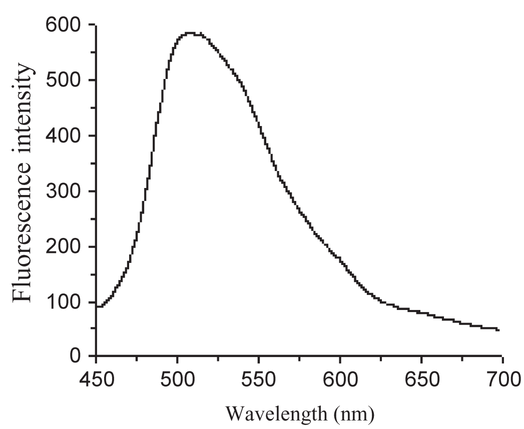

Figure 4. Fluorescence spectrum of morin (1.0×10^{-5} mol/L).

Possible reaction mechanism

An important process in studying the CL mechanism is to ascertain the luminant of the CL reaction. The CL spectrum of the cerium(IV)–morin–bromhexine hydrochloride reaction was drawn (Fig. 3) using the BPCL ultra-weak luminescence analyser. The fluorescence spectrum of morin was obtained (Fig. 4) using a 970 CRT fluorescence spectrophotometer. The CL spectrum showed that the maximal CL wavelength was 510 nm, which accorded with the maximal fluorescence wavelength of morin. Therefore, it was deemed that the luminant was morin.

Both morin and bromhexine hydrochloride can react with cerium(IV) to produce CL. When cerium(IV) reacted with the mixture of morin and bromhexine hydrochloride, the CL intensity was much higher. The kinetic curves of the CL reactions (Fig. 5) showed that the reaction of cerium(IV)–bromhexine hydrochloride was much faster than that of cerium(IV)–morin. When the CL signal of cerium(IV)–bromhexine hydrochloride reaction reached maximum, the cerium(IV)–morin reaction just started. Therefore, when cerium(IV) was added to the merged stream of morin and bromhexine hydrochloride, cerium(IV) reacted with bromhexine hydrochloride first and released energy. Fig. 6 shows that the fluorescence emission spectrum of cerium(IV)–bromhexine hydrochloride (a′) largely

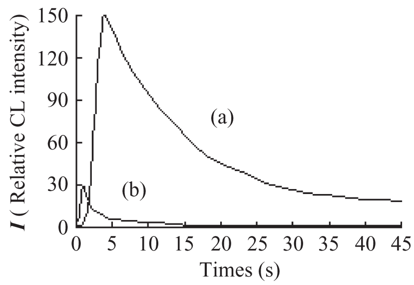

Figure 5. Kinetic curves of the CL reactions. (a) CL reaction of 1.0×10^{-3} mol/L cerium(IV) and 5.0×10^{-6} mol/L morin. (b) CL reaction of 1.0×10^{-3} mol/L cerium(IV) and 2.0×10^{-8} g/mL bromhexine hydrochloride.

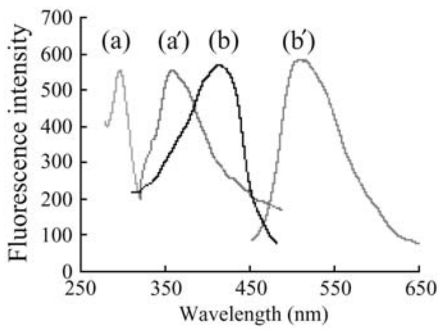

Figure 6. Fluorescence spectra of cerium(IV)–bromhexine hydrochloride and morin: a, a′, excitation and emission spectra of 1.0×10^{-4} mol/L cerium(IV) and 1.0×10^{-6} g/mL bromhexine hydrochloride; b, b′, excitation and emission spectra of 1.0×10^{-5} mol/L morin.

overlapped with the excitation spectrum of morin (b), which suggests that morin can absorb the energy from the reaction of cerium(IV) and bromhexine hydrochloride and is excited to the excited state. The morin at excited state returns to the ground state and brings CL ($\lambda_{max} = 510$ nm).

According to the discussions above, the possible mechanism of the CL reaction of bromhexine hydrochloride in cerium(IV)-morin system might be interpreted as follows. Cerium(IV) reacts with bromhexine hydrochloride in sulphuric acid medium and releases energy; morin absorbs the energy and is excited to the excited state, then morin in the excited state returns to the ground state and produces CL. The mechanism can be expressed simply as follows:

$$\text{cerium (IV)} + \text{bromhexine hydrochloride} \rightarrow \text{energy } (E) + \text{product}$$

$$E + \text{morin} \rightarrow \text{morin*}$$

$$\text{morin*} \rightarrow \text{morin} + h\nu$$

Optimization of experimental conditions

Flow system. Different flow systems were designed. When bromhexine hydrochloride solution was injected into the merged stream of morin and cerium(IV), or morin solution was injected into the merged stream of bromhexine hydrochloride and cerium(IV), no CL signal was obtained. However, a weak CL signal was observed when cerium(IV) solution was injected into the merged stream of morin and bromhexine hydrochloride. If the flow system shown in Fig. 1 was used, a strong CL signal with good repeatability was obtained. Therefore, the flow system shown in Fig. 1 was employed.

Flow rate. The kinetic curve of cerium(IV)–morin–bromhexine hydrochloride reaction showed that the reaction was a fast one. Therefore, flow rate is an important factor. To fix the length of mixing tube L at 4 cm, which is the minimum length to which this apparatus could be set, the effect of flow rate on the CL reaction in the range 0.9–3.0 mL/min was examined. Finally, a 2.7 mL/min flow rate was chosen for further experiments in consideration of the sensitivity, reproducibility and reagents consumption.

Concentration of cerium(IV) solution. Cerium(IV) is the oxidant in the cerium(IV)–morin–bromhexine hydrochloride system. The effect of cerium(IV) concentration was examined in the range 1.0×10^{-4}–1.0×10^{-2} mol/L. The maximum ΔI_{CL} was obtained when 1.0×10^{-3} mol/L cerium(IV) solution was used, therefore, 1.0×10^{-3} mol/L cerium(IV) was chosen for further experiments.

Acidity of cerium(IV) solution. Cerium(IV) is the oxidant in the system, and it has strong oxidant action only in acidic medium. In the experiments, the acidity of cerium(IV) solution was adjusted using sulphuric acid. The effect of sulphuric acid concentration in cerium(IV) solution was investigated in the range 0.1–3 mol/L. The results showed that 2 mol/L sulphuric acid gave the maximum ΔI_{CL}, therefore, 2 mol/L sulphuric acid was selected as optimum.

Concentration of morin solution. Morin is the CL reagent in the system, and its concentration had a great effect on the CL signal. The effect of morin concentration was studied in the range 1.0×10^{-6}–5.0×10^{-5} mol/L. The results indicated that ΔI_{CL} increased with increasing morin concentration until reaching a maximum ΔI_{CL} at 5.0×10^{-6} mol/L. Thus, 5.0×10^{-6} mol/L morin was chosen as optimum.

Analytical performance

Under the optimum conditions, the calibration graph was linear over the range 2.0×10^{-9}–2.0×10^{-7} g/mL (Fig. 7). The linear regression equation was $\Delta I_{CL} = 2.51C - 3.04$ ($C = 10^{-9}$ g/mL) and the correlation coefficient was 0.9995. The relative standard deviation for the determination of 2.0×10^{-8} g/mL bromhexine hydrochloride solution was 1.0% in 11 repeated measurements. The detection limit (3σ) was 9×10^{-10} g/mL bromhexine hydrochloride.

Interference

The interference of some common foreign species was investigated. A substance was considered non-interfering if it caused a relative error within ±5%. The tolerable concentration ratios of foreign species to 2.0×10^{-8} g/mL bromhexine hydrochloride were over 1000 for starch, carbamide, sucrose, Ca^{2+}, Cu^{2+}, Mg^{2+}, Zn^{2+}, Ni^{2+} and Mn^{2+}; 500 for NO_3^-, glucose, dextrin, Cd^{2+} and HCO_3^-; 100 for uric acid, stearic acid, $H_2PO_4^-$ and Cl^-; 50 for lactose, HPO_4^{2-} and Ba^{2+}; 10 for ascorbic acid, CO_3^{2-}, K^+ and Pb^{2+}; 1 for Co^{2+} Al^{3+}, Fe^{2+} and Fe^{3+}; and 0.1 for Cr^{3+}.

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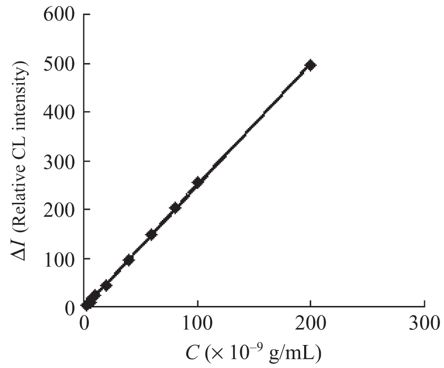

Figure 7. Calibration graph: cerium(IV) solution, 1.0×10^{-3} mol/L; sulphuric acid, 2 mol/L; morin solution, 5.0×10^{-6} mol/L; flow rate, 2.7 mL/min.

Sample analysis

Determination of bromhexine hydrochloride in pharmaceutical preparations. Two kinds of commercial bromhexine hydrochloride samples were analysed. 20 tablets of each kind were accurately weighed so as to obtain the average weight of a tablet, and ground to fine powder. A portion of the powder, equivalent to one tablet's weight, was weighed accurately, dissolved in water and diluted to 100 mL with water. The solution was then filtered and the filtrate was further diluted with water to the appropriate concentration, which was used as the sample solution and determined according to the method described above. Control experiments were also performed according to the method of the *Chinese Pharmacopoeia* (14). The results are shown in Table 1. The *t*-test assumed that there was no significant difference between the proposed CL method and the method of the *Chinese Pharmacopoeia* at a confidence level of 95%.

Table 1. Determination of bromhexine hydrochloride in pharmaceutical preparations

Sample	This method* (mg/tablet)	RSD (%)	*Pharmacopoeia* method (14) (mg/tablet)
1	7.5	2.1	7.7
2	7.9	2.5	7.6

*Average of three measurements.

Analysis of human urine samples. The proposed method was applied to the determination of bromhexine hydrochloride in human urine samples. The urine samples were collected from healthy volunteers. 5.0 mL 0.1 mol/L $ZnSO_4$ and 5.0 mL 0.1 mol/L $Ba(OH)_2$ were added to a 1.0 mL human urine sample, and each sample was pretreated according to methods described in the literature (15). The supernatant was then transferred into a 50 mL volumetric flask, and diluted to the mark with water, and the solution was used for sample analysis, following the procedure described above. Recovery tests were also made. The results are given in Table 2. The *t*-test assumed that there was no significant difference between the recovery efficiency and 100% at confidence level of 95%.

Conclusion

The CL reaction of cerium(IV)–morin–bromhexine hydrochloride was discovered. Based upon this, a new, simple and sensitive method was established for the determination of bromhexine hydrochloride and the possible mechanism of the CL reaction was discussed. In this study, morin, a common fluorescent reagent, was used as the CL reagent, which is helpful to study the CL characteristics of other fluorescent reagents and is useful to enrich the content of CL reactions and broaden the scope of CL analysis.

Table 2. Analytical results of bromhexine hydrochloride in human urine samples

Sample	Added (10^{-8} g/mL)	Found* ± SD (10^{-8} g/mL)	Recovery (%)
1	0.00	0.00	—
	2.00	2.02 ± 0.029	101.1
	4.00	3.93 ± 0.087	98.2
	6.00	5.78 ± 0.029	96.4
2	0.00	0.00	—
	2.00	1.97 ± 0.029	98.5
	4.00	4.03 ± 0.050	100.8
	6.00	5.84 ± 0.035	97.3
3	0.00	0.00	—
	2.00	2.06 ± 0.068	102.8
	4.00	3.91 ± 0.076	97.8
	6.00	5.78 ± 0.029	96.4

*Average of three measurements.

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