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Short communication

A novel chemiluminescence method for determination of terbutaline sulfate based on potassium ferricyanide oxidation sensitized by rhodamine 6G

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Abstract

This work reports a novel flow injection–chemiluminescence (FI–CL) system for determination of terbutaline sulfate, a drug for treatment of asthma and chronic obstructive pulmonary disease (COPD). It is based on the reaction of potassium ferricyanide with terbutaline sulfate in sodium hydroxide medium sensitized by the fluorescent dye rhodamine 6G. With the peak height as a quantitative parameter applying optimum working conditions, terbutaline sulfate is determined over the range of 0.01–1.2 μg ml^{-1} with a detection limit of 6.7×10^{-3} μg ml^{-1}. The relative standard deviation (R.S.D.) is 3.7% for 0.1 μg ml^{-1} terbutaline sulfate ($n = 11$). The proposed method is sensitive, simple, rapid, and was applied to determination of terbutaline sulfate in pharmaceutical preparations. The possible chemiluminescence (CL) reaction mechanism was also discussed briefly.

Keywords: Flow injection analysis; Chemiluminescence; Terbutaline sulfate; Ferricyanide; Rhodamine 6G

1. Introduction

Terbutaline sulfate (Fig. 1), 2-tert-butylamino-1-(3,5-dihydroxyphenyl) ethanol hemisulfate, is a selective β_2-receptor agonist widely used in the treatment of asthma and chronic obstructive pulmonary disease (COPD) [1,2]. The determination of such drug is important for quality assurance in pharmaceutical preparations. Various methods have been developed to determination of terbutaline sulfate, including colorimetry [3], spectrophotometry [2,4], electrophoresis [5–7], gas chromatography [8] and HPLC [9,10]. However, batch methods are time consuming and laborious; on the other hand, chromatography and electrophoresis methods are slow and require expensive instrumentation. Compared with these techniques, flow injection–chemiluminescence (FI–CL) has the advantage of simple instrumentations with high sensitivity, and has been used for the analysis of pharmaceutical compounds [11–15]. To the best of our knowledge, no chemiluminescence (CL)

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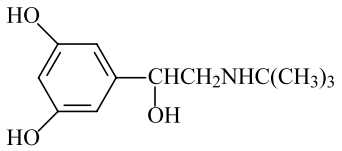

Fig. 1. Molecular structure of terbutaline.

method coupled with flow injection analysis (FIA) has been previously reported for the measurement of terbutaline sulfate in the open literature.

In this paper, we found that terbutaline sulfate can react with ferricyanide to produce weak CL in alkaline medium, and this CL reaction can be effectively sensitized by rhodamine 6G. Based on these observations, a novel flow injection CL method is developed for the determination of the terbutaline sulfate. The CL spectrum was measured; it showed that the rhodamine 6G was the possible CL emitter.

2. Experimental

2.1. Reagents and chemicals

All the reagents were of analytical grade, and doubly distilled water was used for the preparation of solutions. The Rhodamine 6G and potassium ferricyanide were obtained from Acros and Chongqing Chemical Regent Factory, respectively. Terbutaline sulfate was obtained from the Institute of Pharmaceutical and Biomaterial Authentication (China). The stock standard solutions of terbutaline sulfate (100 μg ml^{-1}) were prepared by dissolving 0.01 g terbutaline sulfate in water and diluting with water to 100 ml. The stock standard solution was stored in the refrigerator (4 °C). Working standard solutions were prepared by appropriate dilution of the stock standard solution with water. Terbutaline sulfate tablets in China were purchased from the local hospital.

2.2. Apparatus

The FI–CL system employed in this work is shown in Fig. 2. A peristaltic pump was used to deliver all flow streams. PTFE tubing (0.8 mm i.d.) was used as connection material in the system. Injection was made using a six-way injection valve (Wenzhou, China) equipping a sample loop of 80 μl. A homemade colorless glass coil (i.d. 2.0 mm, o.d. 3.5 mm, length 15 cm) was placed in front of the PMT (CC122, Hamamatsu, Japan). The intensity of CL signal was measured and recorded by the BPCL Ultra Weak CL Analyzer (Institute of Biophysics, Chinese Academy of Science, Beijing), which equipped with HP (Vectra VL4) computer. Data acquisition and treatment were performed with BPCL software running under WINDOWS 98.

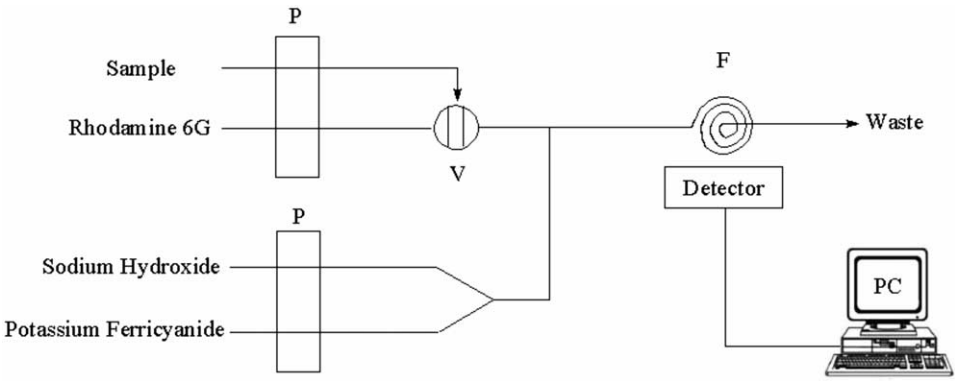

Fig. 2. The schematic diagram of flow injection system for determination of terbutaline sulfate. P, peristaltic pump; V, six-way injection valve; F, flow cell; PC, personal computer.

Table 1
Effect of some fluorohpores on the CL intensity

Fluorophore	Optimum concentration (mmol l^{-1})	Relative CL intensity
None	–	1.0
Riboflavin	–	Suppression
8-Hydroxyl quinoline-5-sulfuric acid	0.3	1.0
Fluorescein	0.1	1.0
Dichlorofluorescein	0.5	6.0
Rhodamine 6B	0.07	37.8
Rhodamine 6G	0.1	69.1

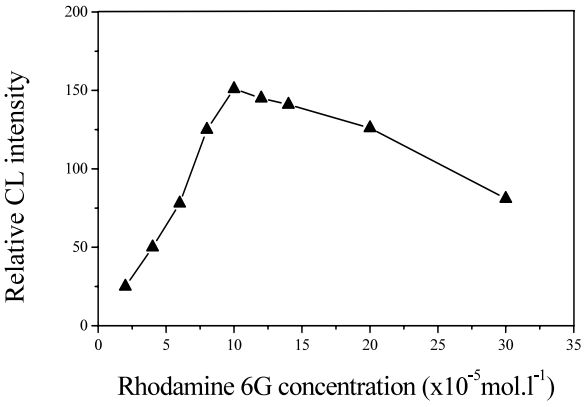

Fig. 3. Effect of rhodamine 6G concentration. Potassium ferricyanide 1.0×10^{-4} mol l^{-1}; sodium hydroxide 0.5 mol l^{-1}; terbutaline sulfate 0.1 μg ml^{-1}.

CL spectrum was examined by a modified RF-540 Fluorimetry (Shimadzu, Japan).

2.3. Procedures

As shown in Fig. 2, flow lines were inserted into the potassium ferricyanide solution, sodium hydroxide solution, carrier (rhodamine 6G) and standard solution, respectively. The pumps were started to wash the whole system, then 80 μl standard solution or sample solution was injected into the carrier stream. This stream was merged with potassium ferricyanide in flow cell, producing CL emission. The concentration of terbutaline sulfate was quantified by the CL intensity.

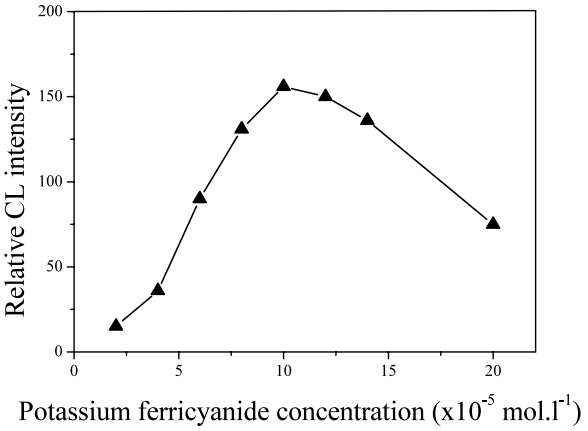

Fig. 4. Effect of ferricyanide concentration. Rhodamine 6G 1.0×10^{-4} mol l^{-1}; sodium hydroxide 0.5 mol l^{-1}; terbutaline sulfate 0.1 μg ml^{-1}.

3. Results and discussion

3.1. Effect of rhodamine 6G concentration

The preliminary experiment showed that the terbutaline sulfate could react with ferricyanide to produce a weak CL emission, and this CL reaction could be sensitized by some fluorescing compounds. So, the fluorophores including riboflavin, 8-hydroxyl quinoline-5-sulfuric acid, fluorescein, dichlorofluorescein, rhodamine B and rhodamine 6G were tested. As can be seen from Table 1, the rhodamine 6G proved to be the best fluorophore for the CL reaction. The effect of rhodamine 6G was further examined. The result (Fig. 3) showed that the maximum CL intensity was obtained when the concentration of rhodamine 6G was 1.0×10^{-4} mol l^{-1}, then 1.0×10^{-4} mol l^{-1} rhodamine 6G was chosen for the subsequent study.

3.2. Effect of potassium ferricyanide concentration

The effect of potassium ferricyanide concentration on the CL intensity was examined over the range of 2.0×10^{-5}–2.0×10^{-4} mol l^{-1}. The results are shown in Fig. 4. The CL intensity increased along with the increase of potassium ferricyanide concentration. Above 1.0×10^{-4} mol l^{-1}, the CL intensity decreased probably because

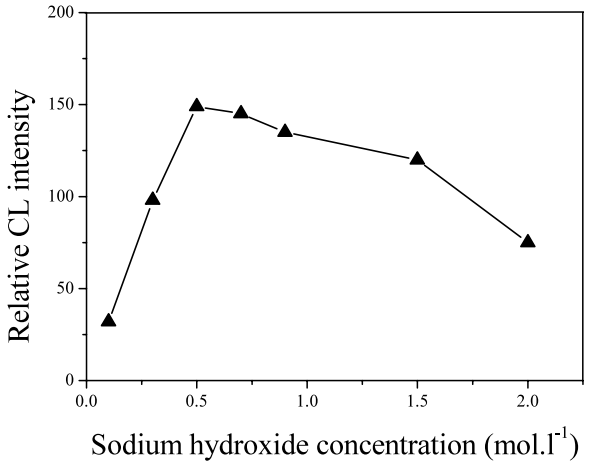

Fig. 5. Effect of sodium hydroxide concentration. Potassium ferricyanide 1.0×10^{-4} mol l^{-1}; rhodamine 6G 1.0×10^{-4} mol l^{-1}; terbutaline sulfate 0.1 μg ml^{-1}.

higher concentrations caused self-absorption of ferricyanide. So, the optimum concentration for ferricyanide is 1.0×10^{-4} mol l^{-1}.

3.3. Effect of sodium hydroxide

The influence of sodium hydroxide concentration on the CL intensity was investigated at different concentrations from 0.1 to 2.0 mol l^{-1}. The Fig. 5 illustrated the effect of sodium hydroxide. As can be seen, maximum CL intensity could be obtained when using a concentration of 0.5 mol l^{-1} sodium hydroxide. Therefore, 0.5 mol l^{-1} sodium hydroxide was selected for the present work.

3.4. Effect of surfactants

The surfactants were important reagents, which often had an effect on CL reaction and sometimes could enhance the CL intensity [16]. So, the common surfactants, including Triton-X100, Tween-20, Tween-80, cetyltrimethylammonium bromide, sodium lauryl sulfonate, tetrabutylammonium bromide, β-cyclodextrin were also investigated (Fig. 6). The results showed that no significant enhancement of surfactants above mentioned on CL was observed.

3.5. Effect of flow rate

The flow rate is an important parameter in the FIA–CL system because the time taken to transfer the excited product into the flow cell is critical for maximum collection of the emitted light, too low or too high flow rates resulting in the absence of

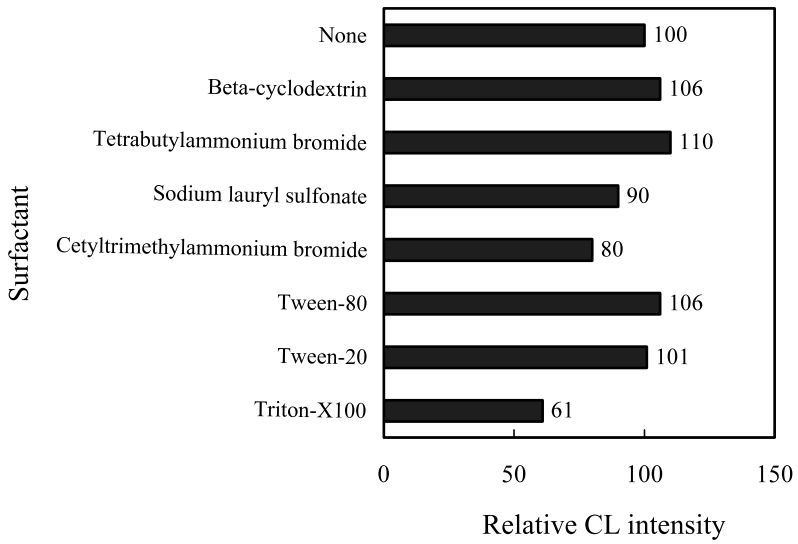

Fig. 6. Effect of surfactant. Ferricyanide, 1.0×10^{-4} mol l^{-1}; terbutaline sulfate, 0.1 μg ml^{-1}; rhodamine 6G, 1.0×10^{-4} mol l^{-1} (in surfactant); Tween-80, Tween-20 and Triton X-100 concentration are 10% (v/v), others are 1.0×10^{-3} mol l^{-1}.

Table 2
Determination of terbutaline sulfate in a pharmaceutical preparation applying the proposed FI–CL method

Sample	Detected (mg)[a]	Added (mg)	Found (mg)	Recovery (%)
No. 1	2.59 (±1.2%)	2.0	1.94	97.0
No. 2	2.57 (±2.3%)	2.0	1.97	98.5

[a] Mean of three measurements ±R.S.D.

Table 3
Determination of terbutaline sulfate in pharmaceuticals with the proposed CL method and the official method

Sample	Proposed method (mg)[a]	Official method (mg)[a]	Relative error
No. 1	25.1	25.3	0.02
No. 2	24.9	25.0	0.01

[a] Average of three measurements.

CL in the flow cell [17]. Thus various flow rates were investigated from 0.5 to 3.5 ml min^{-1}. The results showed that the optimum flow rates were 1.5, 2.0 and 2.0 ml min^{-1} for rhodamine 6G, ferricyanide and sodium hydroxide, respectively.

3.6. Interference studies

The influences of foreign species were investigated by analyzing a standard solution of 0.1 μg ml^{-1} terbutaline sulfate to which increasing amounts of interfering species were added. The tolerable concentration ratios for interference at 5% level were over 1000 for K^+, Na^+, Ca^{2+}, NO_3^-, lactose, galactose, starch, glucose, 200 for Zn^{2+}, Al^{3+}, Mg^{2+}, Cl^-, PO_4^{3-}, SO_4^{2-}, Br^-, and 10 for ascorbic acid, Fe^{2+}, Fe^{3+}. The results showed that the proposed method has good selectivity.

3.7. Performance of the system for terbutaline sulfate measurements

Under the optimum conditions as described above, the calibration graph of emission intensity versus terbutaline sulfate concentration was linear in the range of 0.01–1.2 μg ml^{-1}. The regression equation was $I = 351.7C + 110.44$ (C being the terbutaline sulfate concentration, μg ml^{-1}) with a correlation coefficient of 0.9994 ($n = 7$). The detection limit was 6.7×10^{-3} μg ml^{-1} (3S/N). And the relative standard deviation (R.S.D.) ($n = 11$) for 0.1 μg ml^{-1} terbutaline sulfate was 3.7%.

3.8. Application of the method

In order to evaluate the validity of the proposed procedures, the proposed method was applied to the determination of terbutaline sulfate in pharmaceutical preparation. Terbutaline sulfate tablets, each with a nominal content of 2.5 mg of terbutaline sulfate per tablet, were purchased from the local hospital. The average tablet weight was calculated from the weight of 20 tablets. They were ground to a fine powder using a pestle and a mortar. A portion of the powder, equivalent to about 2.5 mg of terbutaline sulfate was weighted and dissolving in 100 ml water. An appropriate volume of the resulting solution was diluted further with water so that the concentration of terbutaline sulfate was in the working range. Recovery studies were carried out on samples to which known amounts of terbutaline sulfate standards were added. The results are shown in Table 2. Furthermore, the comparison between the proposed CL method and the official method [2] was also carried out. As can be seen from Table 3, there was good agreement between the two methods.

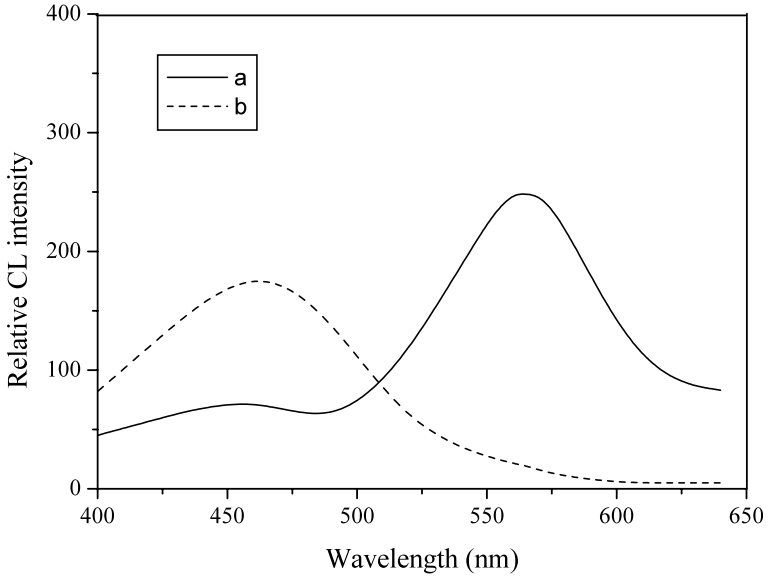

Fig. 7. The CL spectrum of ferricyanide–terbutaline–rhodamine 6G (a) and ferricyanide–terbutaline (b). Potassium ferricyanide 1.0×10^{-4} mol l^{-1}; rhodamine 6G 1.0×10^{-4} mol l^{-1}; terbutaline sulfate 0.1 μg ml^{-1}.

3.9. Possible mechanism of potassium ferricyanide–rhodamine 6G–terbutaline

In order to get an idea about the reaction CL reaction mechanism, we examined the CL spectrum of the reaction of potassium ferricyanide and terbutaline with and without rhodamine 6G by a modified RF-540 Fluorimetry. The results are shown in Fig. 7. It was found that only one peak at about 460 nm for potassium ferricyanide–terbutaline reaction without rhodamine 6G. However, when the rhodamine 6G was present, the maximum wavelength of CL spectrum was at 560 nm (same as the maximum emission spectrum of rhodamine 6G). It suggested that the possible emission species be excited rhodamine 6G. So, the possible CL reaction mechanism may be expressed as:

$$\text{Ferricyanide} + \text{Terbutaline} \rightarrow \text{Ferrocyanide} + \text{Terbutaline (ox)}^*$$

$$\text{Terbutaline (ox)}^* + \text{Rhodamine6G} \rightarrow \text{Terbutaline (ox)} + \text{Rhodamine6G}^*$$

$$\text{Rhodamine6G}^* \rightarrow \text{Rhodamine6G} + h\nu\ (560\ \text{nm})$$

4. Conclusions

In sodium hydroxide media, terbutaline sulfate, a drug often used to treat the asthma and COPD, can be oxidized by potassium ferricyanide, and accompanied with intensive CL in the present of rhodamine 6G (used as sensitizer). The method has been successfully applied to determination of terbutaline sulfate in pharmaceutical preparations.

Acknowledgements

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