Short communication

Determination of metoprolol tartrate in tablets and human urine using flow-injection chemiluminescence method

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Abstract

In this paper, a simple, rapid and sensitive flow-injection chemiluminescence method has been developed for the determination of metoprolol tartrate, which acts as a kind of sensitizer in the chemiluminescence emission from the redox of SO_3^{2-} with Ce(IV) in acidic medium. Under the optimized conditions, the proposed method allows the measurement of metoprolol tartrate over the range of 1.5×10^{-8} to 7.3×10^{-6} mol/L with a detection limit of 4.7×10^{-9} mol/L (3σ), and the relative standard deviation for 7.3×10^{-7} mol/L metoprolol tartrate ($n = 11$) is 2.20%. The utility of this method was demonstrated by determining metoprolol tartrate in tablets and human urine sample.
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Keywords: Metoprolol tartrate; Urine; Flow-injection; Chemiluminescence; Ce(IV)

1. Introduction

Metoprolol tartrate, whose chemical formula is $(C_{15}H_{25}NO_3)_2C_4H_6O_6$, is a kind of β adrenaline receptor blocker. It is widely used for the treatment of hypertension, angina, miocardial infarction, arrhythmia, hyperthyroidism and other related diseases [1–3]. It is so sensitive that even a small oral dose of the drug gives sufficient blockade. Since the β-blockers are misused as doping agents in sports, these drugs have been added to the list of forbidden drugs by the International Olympic Committee [4].

Up to now, several analytical methods have been reported for the quantitative determination of metoprolol tartrate [5–16], which include gas chromatography [5,6], high-performance liquid chromatography (HPLC) with UV [7–9], fluorimetric [10–12], electrochemical [13–15] and MS detection [16]. Two HPLC methods with post-column chemiluminescence detection were also reported. One was based on $Ru(bpy)_3^{3+}$ electrogenerated chemiluminescence detection [17], the other was based on fluorogenic reagent labeling peroxyoxalate chemiluminescence detection [18]. Although these methods have been successfully employed, they require long and tedious steps for the sample pre-treatment. Therefore, a simple, rapid and sensitive determination of metoprolol tartrate in both tablets and real urine samples is of great importance.

Flow-injection chemiluminescence methods can provide a high versatility in the determination of a wide variety of species along with rapidity, simplicity, consecutive automatism, high sensitivity, wide linear range, and good reproducibility, which require only simple and low-cost-measuring devices [19–25].

In this paper, we present a simple flow-injection chemiluminescence system to determine metoprolol tartrate. We found that strong chemiluminescence can be generated after metoprolol tartrate was added to $Ce(IV)/SO_3^{2-}$ acidic solution. The relative chemiluminescence intensity was linear with metoprolol tartrate concentration in a wide range. Based on these observations, a simple, rapid and sensitive flow-injection chemiluminescence method has been developed for the determination of metoprolol tartrate. The proposed method was applied to determine metoprolol tartrate in tablets and human urine sample successfully.

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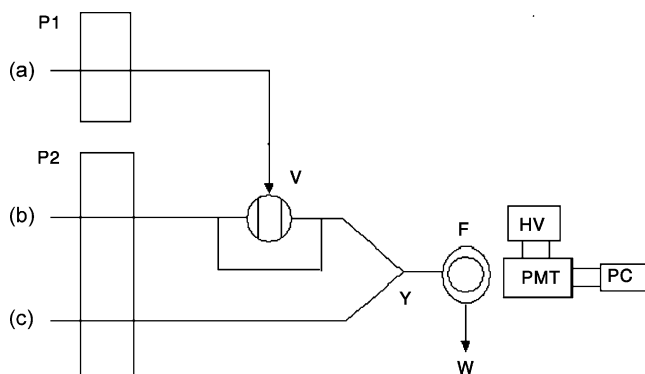yuzhi@online.sh.cn (Y. Fang).

Fig. 1. Schematic diagram of the flow-injection chemiluminescence manifold: a, sample solution; b, Na_2SO_3 carrier stream solution; c, $Ce(SO_4)_2$ acidic solution; P1 and P2, peristaltic pump; V, eight-way injection valve; Y, Y-shaped mixing element; F, chemiluminescence flow cell; PMT, photomultiplier tube; HV, negative high voltage supply; PC, computer; W, waste solution.

2. Experimental

2.1. Reagents and chemicals

All reagents were analytically pure unless otherwise stated and prepared in doubly distilled water. The standard product of metoprolol tartrate ($\geq 99.8\%$) was friendly provided by shanghai institute for drug control. A stock solution of metoprolol tartrate (1.000×10^{-3} mol/L), was prepared daily, stored in the refrigerator ($4\,^\circ$C) and diluted as required. A stock solution of Na_2SO_3 (0.1000 mol/L, Shanghai Chemical Reagent Company, China) was freshly prepared daily and diluted as required. A stock solution of $Ce(SO_4)_2$ (0.0250 mol/L, Shanghai Chemical Reagent Company, China) was prepared daily in 1.0 mol/L H_2SO_4 (Shanghai Chemical Reagent Company, China) solution.

2.2. Apparatus

The chemiluminescence emission was recorded with a set of flow-injection chemiluminescence analyzer (IFFL-D, Xi'an Ruike Electronic equipment Corporate, Xi'an, China). The schematic diagram of the flow-injection chemiluminescence analytical system is shown in Fig. 1. Two peristaltic pumps were used to deliver flow streams in this system. PTFE tubing (0.8 mm i.d.) was used as connection material in the flow system. Sample or standard solution of metoprolol tartrate was injected into Na_2SO_3 carrier stream using a eight-way injection valve equipped with a 100 μL sample loop, merged with $Ce(SO_4)_2$ solution stream just before a spiral flow cell, and then generated chemiluminescence emission in the flow cell. The chemiluminescence signal was detected with an R456 Photomultiplier tube (Hamamatsu) with no wavelength discrimination and recorded with computer employing an IFFL-D flow-injection chemiluminescence analysis system software.

The fluorescence and UV absorption spectra were monitored using a 850 fluorescence spectrophotometer (Hitachi, Japan) and a Cary 50 probe UV–vis spectrophotometer (varian, USA), respectively. The fluorescence spectrophotometer

was also adapted for the measurement of chemiluminescence spectrum.

2.3. Procedure for flow-injection analysis

In order to obtain good stability, the instruments were run for at least 10 min before the first measurement. Flow tubes were inserted into water, 5.0×10^{-3} mol/L of Na_2SO_3 (in 0.2 mol/L H_2SO_4) solution, and 3.0×10^{-4} mol/L of $Ce(SO_4)_2$ (in 0.7 mol/L H_2SO_4) solution, respectively. Flow rate was set at 3.5 mL/min for all lines. Pumps were started to wash the whole system until a stable blank signal was recorded. A 100 μL sample solution was injected into a carrier stream. This stream was merged with $Ce(SO_4)_2$ solution and then reached the flow cell. The concentration of sample was quantified by the relative chemiluminescence intensity.

2.4. Determination of tablets

The average tablet mass was calculated from the mass of 20 tablets of Betaloc (metoprolol tartrate tablet, which was composed of metoprolol tartrate and some common excipients, bought from local pharmaceutical shop, 50 mg per tablet). They were then finely ground, homogenized and a portion of the powder was weighed accurately, transferred into a 50 mL brown measuring flask and diluted to scale with water. The mixture was sonicated for at least 15 min to aid dissolution and then filtered. An appropriate volume of the filtrate was diluted further with water so that the concentration of metoprolol tartrate in the final solution was within the working range, and then analyzed according to the procedure described above.

2.5. Determination of metoprolol tartrate in human urine

Urine samples were collected from volunteers and a 1 mL of sample was mixed with 0.5 mL of acetonitrile and centrifuged for 5 min at 3000 rpm/min. Then the supernatant was fetched and the rest acetonitrile was blow-dried under a gentle stream of nitrogen gas. Finally, the prepared sample was diluted with distilled water directly or supplemented with metoprolol tartrate to test the recovery of the method.

3. Results and discussion

3.1. Kinetic characteristic of the chemiluminescence reaction

Fig. 2 shows the kinetic characteristic of the chemiluminescence reaction of metoprolol/SO_3^{2-}/Ce(IV) system in acidic medium, which was investigated with a static system. As shown in Fig. 2, the oxidation of sulphite with Ce(IV) in acidic medium produced a weak chemiluminescence emission (Fig. 2a), however, the chemiluminescence emission was greatly enhanced when metoprolol tartrate was present (Fig. 2b). Both reactions were very quickly.

SO_3^{2-} could act as the chemiluminescence emitter [26–28] or transfer the energy [29–32] to fluorescent compounds which
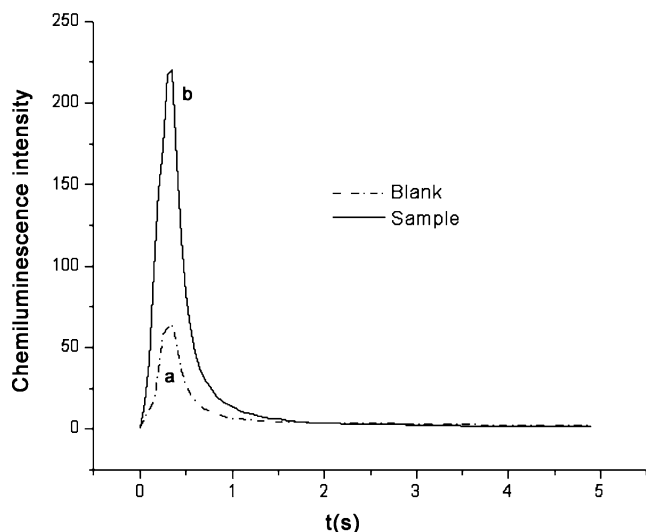
Fig. 2. The characteristics of flow-injection chemiluminescence reaction: (a) chemiluminescence intensity in the absence of metoprolol tartrate; (b) chemiluminescence intensity in the presence of metoprolol tartrate (2.4×10^{-6} mol/L).

acted as sensitizers in chemiluminescence reaction system. In order to investigate the reaction mechanism of chemiluminescence enhancement, we measured the UV spectra of metoprolol tartrate before and after the reaction, chemiluminescence and fluorescence spectra of metoprolol tartrate.

The UV spectra of metoprolol tartrate were very similar before and after the addition of chemiluminescence reaction reagent. The results indicated that the structure of metoprolol tartrate had not changed after the chemiluminescence reaction.

On the other hand, metoprolol tartrate is one kind of fluorescent compound and Fig. 3a is its fluorescence spectrum; therefore it can be excited by absorbing energy in a proper chemical reaction. The chemiluminescence spectrum of metoprolol tartrate/Ce^{4+}/SO_3^{2-} reaction system (Fig. 3b) was similar to the fluorescence spectrum of metoprolol tartrate. So we can postulate that the chemiluminescence has the same luminophor as
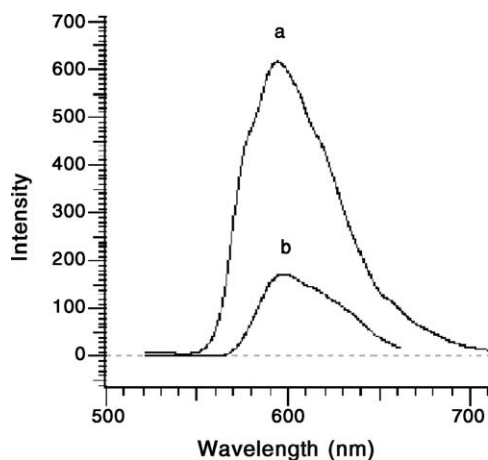

Fig. 3. The comparison of chemiluminescence spectrum and fluorescence spectrum: (a) chemiluminescence spectrum of metoprolol tartrate/Ce^{4+}/SO_3^{2-}; (b) fluorescence spectrum of metoprolol tartrate, Ex: 224.7 nm.

fluorescence of metoprolol which may be excited metoprolol tartrate.

As a result, the chemiluminescence reaction process is assumed as following:

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

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

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

$$SO_2{}^* + \text{metoprolol tartrate} \rightarrow \text{metoprolol tartrate}^* + SO_2$$

$$\text{metoprolol tartrate}^* \rightarrow \text{metoprolol tartrate} + h\nu$$
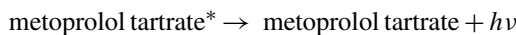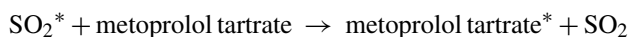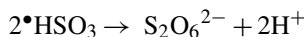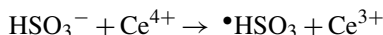
3.2. Optimization of the experimental conditions

To determine the reaction parameters that give the higher sensitivity and optimum ratio of signal to noise which can further be used in the determination of metoprolol tartrate in real sample, a series of univariate searches were performed on reagent concentration, conditions of reaction medium, reagent flow rate and injection sample volume.

3.2.1. Selection of acid medium

Ce(IV) is soluble in dilute acidic solution. The kinds and concentration of acid in the reaction system influence the chemiluminescence emission intensity. Therefore, four different acids including HCl, HNO_3, H_2SO_4, and H_3PO_4 of different concentration in the range of 0.05–2.40 mol/L were tested, respectively. The results showed the best signal was obtained in sulphuric acid medium, and the highest chemiluminescence intensity was produced between 0.6 and 0.8 mol/L sulphuric acid in $Ce(SO_4)_2$ solution. When 0.7 mol/L of sulphuric acid in $Ce(SO_4)_2$ solution were selected, the variation of sulphuric acid concentration in Na_2SO_3 solution had less influence between 0.01 and 0.3 mol/L. Therefore, 0.2 mol/L sulphuric acid in Na_2SO_3 solution and 0.7 mol/L of sulphuric acid in $Ce(SO_4)_2$ solution were selected, respectively for further research.

3.2.2. Effect of Na_2SO_3 solution concentration

The effect of Na_2SO_3 concentration on the chemiluminescence intensity in the presence and absence of metoprolol tartrate was studied. The result showed that the chemiluminescence intensity increased with the increase of the concentration of Na_2SO_3 when it was lower than 5.0×10^{-3} mol/L. While the intensity had little variation from 5.0×10^{-3} to 6.0×10^{-3} mol/L, and began to decrease when it was higher than 6.0×10^{-3} mol/L. Thus, 5.0×10^{-3} mol/L was selected as optimum concentration of Na_2SO_3.

3.2.3. Effect of $Ce(SO_4)_2$ solution concentration

The effect of cerium(IV) concentration upon the chemiluminescence intensity was examined in the range of 1.0×10^{-4} to 9.0×10^{-4} mol/L. The chemiluminescence intensity increased with the increase of cerium(IV) concentration lower than 3.0×10^{-4} mol/L. Higher than 3.0×10^{-4} mol/L of Ce(IV)

resulted in the decrease of chemiluminescence emission intensity, which may be explained the excess of Ce(IV) might absorb a significant amount of the emitted light [33]. Therefore, 3.0×10^{-4} mol/L Ce(IV) concentration was used for subsequent work.

3.2.4. Effect of flow rate

Because the proposed chemiluminescence reaction was very fast, the distance between Y-shaped mixing element and the flow cell was made to be as short as possible, and the flow rate of pump P2 was investigated from 1.0 to 4.0 mL/min in order to determine the maximum of the chemiluminescence signal. When the flow rate was 3.5 mL/min, the relative chemiluminescence intensity, reproducibility of signal, peak shape, and ratio of signal to noise was the best. So, 3.5 mL/min was chosen as the optimum flow rate throughout the experiment.

At a flow rate of 3.5 mL/min, the determination of metoprolol tartrate, including sampling and washing, could be performed in 20 s, giving a sample measurement frequency of about 180 samples h^{-1}. Considering the flow rate of 2.0 mL/min for P1 and 3.5 mL/min for P2, the reagent consumption per determination is less than 3.0 mL.

3.2.5. Effect of sample volume and negative high voltage of photomultiplier tube

The effect of sample volume on the chemiluminescence intensity was tested at 50, 75, 85, 100, and 125 μL. The biggest relative chemiluminescence intensity and best ratio of signal to noise was obtained when it was fixed between 85 and 100 μL.

The effect of negative high voltage of photomultiplier tube on the chemiluminescence intensity was examined between −600 and −800 V employing 85 and 100 μL sample volume, respectively. The biggest relative chemiluminescence intensity and best ratio of signal to noise was obtained when it was fixed at −725 V and 100 μL sample volume.

3.3. Analytical characteristics

Under the optimum conditions, the relative chemiluminescence intensity was linear with the concentration of metoprolol tartrate from 1.5×10^{-8} to 7.3×10^{-6} mol/L and three injec-

Table 1
Tolerable limit of some foreign substances on the determination of metoprolol tartrate

Substance	Concentration ratio to metoprolol tartrate
K^+, Na^+, Mg^{2+}, Ca^{2+}, Ni^{2+}, Cu^{2+}, SO_4^{2-}, NO_3^-, Cl^-, Co^{2+}, Pb^{2+}, Zn^{2+}, Al^{3+}, Mn^{2+}, NH_4^+, urine, tartaric acid	≥200
Dimethylamine	150
Uric acid	50
Starch	40
Polyvinyl alcohol	30
B-cyclodextrin, ascorbic acid	20
Sucrose, lactose, glucose	10
Glutin	5
Ethanol, maltose	2
Galactose	1

tions were performed for each standard solution. The regression equation was $\Delta I = 2.00 + 0.15 \times 10^8 C$ (C is the concentration of metoprolol tartrate, mol/L) with a correlation coefficient of 0.9997 ($n = 11$). The detection limit was 4.7×10^{-9} mol/L which was calculated according to IUPAC regulation that is three times of standard deviation of blank value. The relative standard deviation for 11 parallel measurement of 7.3×10^{-7} mol/L metoprolol tartrate was 2.20%.

3.4. Interferences experiments

Considering that the developed method would be applied to determine metoprolol tartrate in tablets and urine sample, the interference effect of common ions and several compounds commonly used as excipients and generally presented in urine sample was assessed. Samples containing metoprolol tartrate at a fixed concentration of 3.6×10^{-7} mol/L and increasing concentration of the interferences were analyzed by the method. The tolerable limit of a foreign species was taken if it caused a relative error of less than 5%.

The obtained results in Table 1 showed that under the optimized conditions, the common ions and the studied excipients at concentrations usually found in the tablets did not interfere the determination of metoprolol tartrate. So the content of metopro-

Table 2
Determination of metoprolol tartrate (MT) in tablets

Sample	Proposed method[a]					Pharmacopoeia method[a,b] (g/tablet)
	MT supplement (10^{-7} mol/L)	Found (10^{-7} mol/L)	Recovery (%)	R.S.D.%	Content (g/tablet)	
1	0	5.0		2.3	0.0511	0.0518
	5.0	10.1	102.0			
2	0	4.7		2.2	0.0501	0.0496
	5.0	9.7	100.0			
3	0	4.8		2.0	0.0489	0.0499
	5.0	9.7	98.0			

[a] Average of five measurements.
[b] Nonaqueous titration method.

Table 3
Recoveries of metoprolol tartrate in human urine samples

Human urine sample	Concentration[a] (10^{-7} mol/L)			Recovery (%)
	Content	Added	Found	
No. 1	5.1	5.0	9.9	96.0
No. 2	7.2	5.0	12.6	108.0

[a] Mean of five measurements.

lol tartrate in tablet could be determined after filtration without any pretreatment.

3.5. Determination of metoprolol tartrate in tablets

Following the procedure described above, the proposed method was applied to the determination of metoprolol tartrate in tablets. The results (Table 2) compared favourably with those obtained by pharmacopoeia method [3] and the recovery test was satisfactory.

3.6. Determination of metoprolol tartrate in human urine

The high sensitivity attained by the proposed method allows the determination of metoprolol tartrate in biological fluids. Considering that the β blockers are misused as doping agents in sports, the proposed chemiluminescence detection method was applied to the determination of metoprolol tartrate in human urine samples.

Two healthy male volunteers took 200 mg metoprolol tartrate tablets orally in morning with empty stomach. After that, urine samples were collected in glass beakers after 4 h. The sample treatment and determination procedure were immediately applied to the urine samples without any delay.

Table 3 showed the results of the recovery studies of metoprolol tartrate from human urine sample, which was satisfactory.

4. Conclusions

Based on the weak chemiluminescence reaction of Ce(IV) and sulphite in sulphuric acidic medium, sensitized greatly by metoprolol tartrate, a new flow-injection chemiluminescence system has been developed for the determination of metoprolol tartrate. The method is simple, rapid and sensitive, and has been applied to determine metoprolol tartrate in tablets and human urine sample. The results compared well with pharmacopoeia method.

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