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Chemiluminescence flow-through sensor for pipemidic acid using solid sodium bismuthate as an oxidant

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Abstract

A novel chemiluminscence (CL) flow-through sensor for pipemidic acid is described. It was based on the sensitizing effect of pipemidic acid on the CL oxidation of sulfite by sodium bismuthate in H_2SO_4 media. The solid-phase sodium bismuthate was mechanicially immobilized on the sponge rubber inside of the CL flow cell as CL oxidant. The calibration graph is linear in the range 0.1–10 μg/ml with a detection limit of 6.2×10^{-8} g/ml (3σ). A complete analysis could be performed in 1 min with a relative standard deviation (R.S.D.) of 2.5% for 2 μg/ml pipemidic acid ($n = 8$). This method has been successfully applied to determine pipemidic acid in pharmaceutical preparation.

Keywords: Chemiluminscence; Flow-through sensor; Pipemidic acid; Sodium bismuthate; Solid-phase oxidant

1. Introduction

With appearance of the highly sensitive photomultipliers (PMT) in the 1950s, chemiluminescence (CL) became one of the most sensitive detection methods [1]. Due to the simplicity, low detection limit, large calibration ranges and rapid analysis speed, CL has been widely used in practical applications during the last 50 years [2,3]. However, the CL method often produces a problem: consuming reagents. Different from other detection methods (such as fluorometry, conductometry, UV–VIS spectrophotometry), the CL method based on the oxidation–reduction reactions is consumed. Apart from the analyte, the CL reaction needs at least another one reagent, which is used as the oxidant, reactant or catalyst. In order to obtain good replication, the CL method is often combined with flow injection analysis. In the CL flow system, the continuous delivery of reactants into reaction zone is required, and the continuous flows of the reactants cause reagent waste and environmental pollution, which limits the widespread application of CL.

In recent years, CL flow sensor systems with immobilized reagents have received much attention and many analytical applications have appeared in the literature [4–12]. In most of these systems, the reagents of CL reaction, including oxidant (such as IO_4^- [7], $Fe(CN)_6^{3-}$ [8]), catalyst (such as Co^{2+} [10], Cu^{2+} [11] and enzyme [9,10]) and luminescence reagent (such as luminol [8–11]) were immobilized on the suitable supporter (such as ion-exchange resin). Compared with the use of continuously delivered reagents in the conventional CL flow systems, these CL flow-through sensors are advantageous not only for operational convenience and instrumental simplification but also for cost, environment, and resource considerations.

Solid sodium bismuthate has a high redox potential (>1.8 V) in mineral acid solution [13]. As a stronger

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oxidant, sodium bismuthate can oxidize a large variety of organic and inorganic substances and is used in the spectrophotometrical analysis at present [14]. However, to the best of our knowledge, no CL reaction with sodium bismuthate has been reported in the open literature. The main reason is that sodium bismuthate is insoluble in water at room temperature.

In this paper, a new type of CL flow-through sensor for determination of pipemidic acid is proposed. It was based on the sensitizing effect of pipemidic acid on the CL oxidation of sulfite by sodium bismuthate in H_2SO_4 media. By a very simple mean, sodium bismuthate was immobilized inside of the CL flow cell as solid-phase CL oxidant. Compared to the reported homogeneous CL system for pipemidic acid [15], this flow sensor, which need not deliver oxidant to CL reaction zone, would cut the consumption of reagent, and would not require additional tubing, mixing chamber and pump. This method has been successfully applied to determine pipemidic acid in a pharmaceutical preparation.

2. Experimental

2.1. Reagents and solutions

All reagents were of analytical grade and the water used was deionized and double distilled.

A stock solution of pipemidic acid (Central Drug Check Shaanxi, China) (1×10^{-3} g/ml, in 0.8% HAc) was stored in the refrigerator (4 °C). Working standard solutions were prepared daily from the stock solution by appropriate dilution immediately before use. Sodium bismuthate was obtained from Beijing Chemical Reagents Plant (Beijing, China). Other reagents used were: sulfuric acid (Xi'an Reagents Plant, Xi'an, China), 0.1 and 1 mol/l, aqueous sodium sulfite solution (Yixing Xian Chemical Industry Reagent Plant, Wuxi, China), 1×10^{-3} mol/l.

Pipemidic acid tablets (Shaanxi Xi'an Pharmaceutical Plant, China) were purchased from the local market. The label value is 25 mg per tablet.

2.2. Apparatus

The schematic diagram of the flow system is shown in Fig. 1. A type-DDB320 peristaltic pump (Zhejiang Xiangshan Shipu Haitian Electronic Instrument Plant, China) was used to deliver all flow streams. PTFE tubing (0.8 mm i.d.) was used as connection material in the flow system. One hundred and ten microliter of the sodium sulfite solution was injected by a six-way injection valve into the carrier stream (H_2SO_4) and merged with the sample stream and then reached the solid-phase reactor, producing CL signal. The CL signal produced in the solid-phase reactor was detected and recorded with a computerized ultra-weak luminescence analyzer (type BPCL, manufactured at the Institute of Biophysics, Academia Sinica, Beijing, China). The solid-phase reactor was located directly facing the window of the CR-105 photomultiplier tube (operated at −800 V, Hammamatsu, Tokyo, Japan). Data acquisition and treatment were performed with BPCL software running under Windows 95.

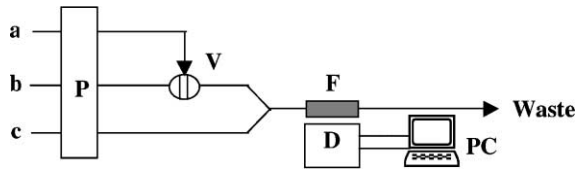

Fig. 1. Schematic diagram of CL flow-through sensor. (a) Na_2SO_3; (b) H_2SO_4; (c) sample; P: peristaltic pump; V: injection valve; F: flow cell immobilized $NaBiO_3$; D: detector; PC: personal computer.

2.3. Preparation of the solid-phase reactor

Kargosha et al. [14] used silica gel beads to immobilize $NaBiO_3$, and the silica beads were very small (mesh 230–400, 0.04–0.063 mm diameters). So, the solid-phase reactor in flow stream had very large resistance. In this flow system, the cheap and black sponge rubber was chosen for immobilization of yellow $NaBiO_3$.

About 1.5 g clean and CR-1211 sponge rubber (chloroprene, black) was cut into small pieces (about 2 mm × 2 mm × 2 mm); this then was stirred with about 1.5 g powder of $NaBiO_3$ for 30 min. The resulting sponge absorbed $NaBiO_3$ was packed into a glass column (70 mm × 5.5 mm i.d.) and some glass wool was inserted at both the ends to prevent loss of sponge. Each packed reactor had to be conditioned for at least 30 min before use. Conditioning involved pumping water through the reactor for 15 min, followed by pumping the carrier (H_2SO_4) for another 15 min at 2 ml/min.

2.4. Procedures

Flow lines were inserted into the sample, carrier (H_2SO_4) and Na_2SO_3 solution, respectively. The pumping was continued until a stable baseline was recorded. Then, a 110 μl volume of the sodium sulfite solution was injected into the carrier stream (H_2SO_4). This stream was merged with the sample stream and then reached the solid-phase reactor, producing CL emission. The concentration of the sample was quantified by the CL intensity.

3. Results and discussion

3.1. Mechanism of the CL system

The oxidation of SO_3^{2-} in acid solutions is a well known CL reaction and the analytical properties of the reaction have been thoroughly studied with MnO_4^- [16–18], Ce(IV) [19,20], Ag(II) [21] and Mn(III) [22] as the oxidants. According to different authors, the emission has been attributed to the formation of excited sulfur dioxide molecules (SO_2^*) which radiate during de-excitation [16–22]. Moreover, some fluorescent compounds (such as riboflavin [17,23], rhodamine 6G [24], qunine [22,25] and some benzamides [26]) could sensitize the CL oxidation reaction of SO_3^{2-} because the energy of the SO_2^* can easily be transferred to a fluorophore intentionally added to the system [17,22–26].

In this CL system, the CL emission is not observed when $NaBiO_3$ reacts with pipemidic acid in absence of sulfite and the reaction between sulfite and $NaBiO_3$ produces the weak CL. Pipemidic acid is fluorogenic compound and can sensitize this weak CL emission. It was found that the rate of this CL reaction in the solution was very fast: from the reagents mixing to the peak maximum, only 0.5 s was needed and it took about 5 s for the signal to reach zero again. We also examined the CL spectra by a modified RF-540 spectrofluorophotometer, which showed only one peak at about 430 nm (same as the maximum in the emission spectrum of pipemidic acid). Therefore, the possible CL mechanism of the reaction may be attributed to the following reactions in its simplest form:

$$NaBiO_3 + HSO_3^- \rightarrow BiO^+ + HSO_3^\bullet \quad (1)$$

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

$$S_2O_6^{2-} \rightarrow SO_4^{2-} + SO_2^* \quad (3)$$

$$SO_2^* + \text{pipemidic acid} \rightarrow SO_2 + \text{pipemidic acid}^* \quad (4)$$

$$\text{pipemidic acid}^* \rightarrow \text{pipemidic acid} + h\nu \quad (5)$$

3.2. Design of the flow system

In flow injection analysis, the sample solution is usually injected into carrier stream. In this flow system, the lifetime of the $NaBiO_3$ solid-phase reactor is a key factor. The experiment showed that if the sample solution was injected and the higher concentration Na_2SO_3 (1×10^{-3} g/ml) was continuously passed through the solid-phase reactor, the $NaBiO_3$ was quickly consumed due to the reaction of $NaBiO_3$ and Na_2SO_3. So, in order to prolong the lifetime of the $NaBiO_3$ reactor, the Na_2SO_3 solution was injected into the carrier in this flow system.

3.3. Effect of acidic media on the CL system

Because $NaBiO_3$ has a high redox potential only in acidic media, the acidic media was used as the CL reaction media. The influence of different acidic media was studied through the same flow manifold as above (Table 1). Sulfuric acid, like in vast majority of CL systems involving Ce(IV) or MnO_4^- [15–19,25], was then selected as the optimum. Furthermore, the effect of H_2SO_4 concentration was studied. The result is shown in Fig. 2. The CL intensity increased with increasing H_2SO_4 concentration in the range of 0.0–0.1 mol/l, probably because of the redox potential of $NaBiO_3$. However, above the concentration of 0.1 mol/l, the CL intensity declined probably because

Table 1
Effect of acidic media on the CL intensity

Acid (0.1 mol/l)	Relative CL intensity[a]
HCl	32
HAc	30
HNO_3	45
H_3PO_4	20
$H_3P_4O_{13}$	23
H_2SO_4	100

[a] Corresponding to the normalized maximum light intensity.

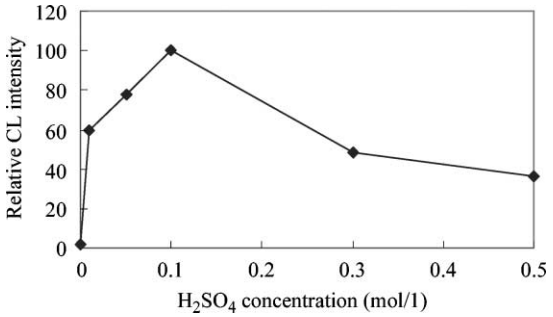

Fig. 2. Effect of H_2SO_4 concentration on the CL intensity. Pipemidic acid: 2×10^{-6} g/ml; Na_2SO_3: 5×10^{-4} g/ml; flow rate: 3.0 ml/min.

the fluorescence quantum yield of pipemidic acid decreased at higher $[H^+]$. (The experiment showed that the fluorescence intensity of pipemidic acid at the same concentration decreased with increasing H_2SO_4 concentration in the range of 0.05–0.25 mol/l). Then, the 0.1 mol/l H_2SO_4 was as optimum media.

3.4. Effect of sodium sulfite concentration

The effect of Na_2SO_3 concentration on the CL intensity is shown in Fig. 3. The CL intensity was increased as the Na_2SO_3 concentration increased from 0 to 1×10^{-3} g/ml, after which the CL intensity started decreasing. Therefore, the optimum Na_2SO_3 concentration was chosen to be 1×10^{-3} g/ml.

3.5. Effect of flow rate

In flow injection analysis, flow rate is an important factor. The effect of flow rate on the CL intensity was

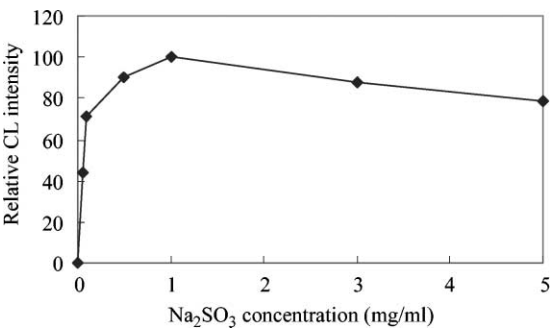

Fig. 3. Effect of Na_2SO_3 concentration on the CL intensity. Pipemidic acid: 2×10^{-6} g/ml; H_2SO_4: 0.1 mol/l; flow rate: 3.0 ml/min.

investigated. The result showed that the CL intensity increased with increasing the flow rate in the range of 0.5–3.5 ml/min. Based on the principle of flow injection analysis, it is certain that the high flow rate corresponds to a sharp peak signal. Flow rate >3.0 ml/min led to greater consumption of reagent, unacceptable reproducibility and higher pressure on the flow cell, which was fully packed with solid oxidant. Therefore, a flow rate of 3.0 ml/min for each reagent was chosen for further study.

3.6. The lifetime of the solid-phase reactor

The lifetime of each reactor was established by comparing the CL intensity of the same pipemidic acid concentration in different times. When the CL intensity started to decrease systematically and significantly, the reactor had to be replaced. In order to easily become conscious of the lifetime of the reactor, the black sponge was used to immobilize yellow $NaBiO_3$. So, another indication that the reactor was losing its oxidation capacity was the color of the packing itself. The color of the packing of a new reactor was yellow. After pumping several samples into the reactor, the color of the packing at the front-end of the reactor started to become brown and then black because bismuthate can dissolve in 0.1 mol/l sulphuric acid. So, the reactor became black after extended use. When the two-thirds of the reactor was black, it usually had to be replaced. The reactor could be reused about 400 times during a period of 50 h. Moreover, it was very easy to prepare and change the solid-phase reactor.

3.7. Performance of the system for pipemidic acid measurements

Under the optimum conditions described above, the calibration graph of emission intensity (I, mV) versus pipemidic acid concentration was linear in the range of 0.1–10 μg/ml and the detection limit was 6.2×10^{-8} g/ml (3σ). The regression equation was $I = 41 + 182.5C$ (C: pipemidic acid concentration, μg/ml) with a correlation coefficient of 0.9976 ($n = 9$). A complete analysis, including sampling and washing, could be performed in 1 min. The reproducibility of this sensor was studied by detecting 2×10^{-6} g/ml pipemidic acid eight times. Fig. 4 gives the result. The relative standard deviation (R.S.D.) was 2.5%.

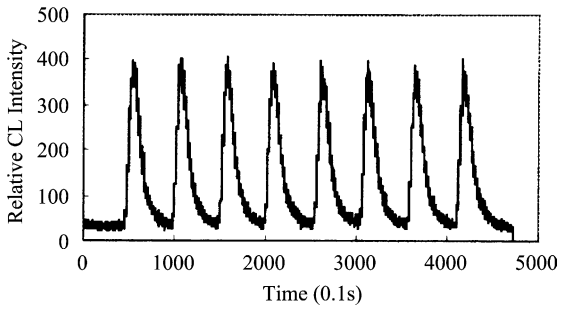

Fig. 4. Reproducibility of the CL flow-through sensor. The response signals for 2×10^{-6} g/ml pipemidic acid were obtained under the optimum experimental condition.

Although this CL flow sensor has less sensitivity and higher detection limit than the reported CL system for analysis of pipemidic acid (the detection limit was 3.0×10^{-9} g/ml) [14], this flow sensor is simple, and could cut the consumption of reagent, and could not require additional tubing, mixing chamber and pump.

3.8. Interference studied

The interference of common ions and excipients in pharmaceutical dosage was investigated in the determination of 6 μg/ml pipemidic acid. The tolerable concentration ratios for interference at 5% level were: >1000 for Na^+, Ca^{2+}, K^+, SO_4^{2-}, NO_3^- and Ac^-; 100 for starch, dextrin, glucose, sucrose, lactose and maltose; 50 for fructose, Cl^-, and Br^-; 5 for ascorbic acid and I^-, respectively. The results showed that the proposed method has the good selectivity, only ascorbic acid and I^- appeared negatively interference possibly because ascorbic acid and I^-, as strong reducers, can also react with $NaBiO_3$.

3.9. Application

Two commercial pipemidic acid tablets (Shaanxi Xi'an Pharmaceutical Plant, China) were ground to powder and a portion of the powder was weighted and dissolved in 100 ml 0.8% HAc. After filtering, the aliquots of the filtrate were further diluted with water in order that the concentration of pipemidic acid was in the working range. The results are given in Table 2 and agreed well with those obtained by the official method [27], in which pipemidic acid was detected spectrophotometrically at 333 nm in 0.04% NaOH media.

Table 2
Determination of pipemidic acid in pharmaceutical preparation

Sample no.	Proposed method (μg/ml)[a]	Official method (μg/ml)[a]	Relative error (μg/ml)
1	3.6 (±3.0)	3.4 (±2.4)	−0.2
2	4.1 (±2.5)	4.4 (±2.0)	0.3

[a] Average of three replicates (±R.S.D.).

4. Conclusions

This is a first report to use solid-phase $NaBiO_3$ as CL oxidant in the CL analysis. The $NaBiO_3$ was immobilized on the CL flow cell, which made the method very simple and reagent saving. Moreover, based on the sensitizing effect of pipemidic acid on the CL oxidation of sulfite by sodium bismuthate in H_2SO_4 media, a new type of CL flow-through sensor for determination of pipemidic acid is proposed. The sensor offers advantages of simplicity, rapidity, and has been applied successfully to pipemidic acid in pharmaceutical preparation.

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References

[1] J.-M. Lin, M. Yamada, Anal. Chem. 72 (2000) 1148.
[2] L.J. Blum, Bio- and Chem-Luminescent sensors, World Scientific, Singapore, 1997.
[3] L.P. Palilis, A.C. Calokerinos, Anal. Chim. Acta 413 (2000) 175.
[4] L.J. Blum, Enzyme Microb. Technol. 15 (1993) 407.
[5] N.M. Rao, K. Hool, T.A. Nieman, Anal. Chim. Acta 266 (1992) 279.
[6] W. Qin, Z. Zhang, H. Liu, Anal. Chem. 70 (2000) 3579.
[7] J.-M. Lin, K. Sato, M. Yamada, Microchem. J. 69 (2001) 73.
[8] Z. Zhang, W. Qin, Talanta 43 (1996) 119.
[9] B. Li, Z. Zhang, Y. Jin, Sens. Actuator B 72 (2001) 115.
[10] W. Qin, Z. Zhang, B. Li, S. Liu, Anal. Chim. Acta 372 (1998) 357.
[11] W. Qin, Z. Zhang, H. Chen, Int. J. Environ. Anal. Chem. 66 (1997) 191.

[12] S. Hanaoka, J.-M. Lin, M. Yamada, Anal. Chim. Acta 426 (2001) 57.
[13] Teaching and Research Group of Analytical Chemistry at Central South Polytechnical University, Handbook of Chemical Analysis, Science, Moscow, 1997, p. 592.
[14] K. Kargosha, M. Noroozifar, Anal. Chim. Acta 413 (2000) 57.
[15] B. Li, Z. Zhang, M. Wu, Mikrochim. Acta 434 (2000) 223.
[16] F. Mexiner, W. Jaeschke, Int. J. Environ. Anal. Chem. 10 (1981) 51.
[17] A.A. Al-Tamrah, A. Townshend, A.R. Wheatey, Analyst 112 (1987) 883.
[18] F. Mexiner, W. Jaeschke, Fresenius Anal. Chem. 317 (1984) 343.
[19] K. Takeuchi, T. Ibusuki, Anal. Chim. Acta 174 (1985) 359.
[20] M.C.S. Alonso, L.L. Zamora, J.M. Caltayud, Anal. Chim. Acta 437 (2001) 225.
[21] B. Li, Z. Zhang, M. Wu, Anal. Chim. Acta 432 (2001) 311.
[22] X. Zheng, Z. Zhang, Anal. Sci. 16 (2000) 1345.
[23] J.L. Burguera, M. Burguera, Anal. Chim. Acta 214 (1988) 429.
[24] Y. Huang, C. Zhang, X. Zhang, Z. Zhang, Anal. Chim. Acta 391 (1999) 95.
[25] I.I. Koukli, A.C. Calokerinos, Anal. Chim. Acta 236 (1990) 463.
[26] F.A. Aly, N.A. Alarfaj, A.A. Alwarthan, Talanta 54 (2001) 715.
[27] Pharmacopoeia Commission of the Ministry of Public Health, Chinese Pharmacopoeia, People's Republic of China, Health Press, Beijing, 1990, p. 221.