Flow Injection Chemiluminescence Determination of Pipemidic Acid Using On-Line Electrogenerated Cobalt(III) as Oxidant

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Abstract. A novel flow injection chemiluminescence (CL) system for the determination of pipemidic acid is described. It is based on the direct CL reaction of pipemidic acid and Co(III) in acid medium. The unstable Co(III) was on-line electrogenerated by constant current electrolysis. The CL intensity was linear with pipemidic acid concentration in the range of $0.01\sim100\,\mu g/ml$, the determination limit was $3.3\times10^{-9}\,g/mL$. The whole process could be completed in 1 min with a relative standard deviation of 3.2%. The proposed method is suitable for automatic and continuous analysis and has been applied successfully to the analysis of pipemidic acid in a pharmaceutical preparation.

Key words: Flow injection; chemiluminescence; pipemidic acid; on-line electrogenerated Co(III); pharmaceutical analysis.

The application of strongly oxidizing agents in quantitative analysis has in general been restricted to those reagents that are not or only very slowly reduced by water, thus making possible the storage of standard solutions. Permanganate and cerium(IV) in acid solution, although thermodynamically unstable, fulfill this requirement. Stronger oxidants, such as cobalt(III), silver(II) and manganese(III), are quickly reduced by water, so their application in quantitative analysis under normal conditions is not possible.

The success of flow injection analysis (FIA) can be attributed to the reproducibility of the operations involved in the system, such as injection, pumping and timing, allowing quantitative analysis without the necessity of completeness of the chemical reaction [1]. As a complete chemical reaction is unnecessary, the idea arises that instability of the reagent also might be no problem as long as the reaction rate is reproducible. This would open possibilities for the application in quantitative analysis of these reagents, which are unstable under normal condition [2], by generating them in flow systems, starting from a stable oxidation state of the same element. An example is the application of the strongly oxidizing agent Co(III).

The Co^{3+}/Co^{2+} system has a high redox potential $(+1.81\,V)$ in mineral acid solution [3]. For this reason, a solution of Co(III) was studied to apply to analytical chemistry, such as titrimetric analysis [4, 5]. The most common procedure for the preparation of solutions of Co(III) is chemical oxidation of Co(II) to Co(III) by suitable oxidizing agents, such as ozone, hydrogen peroxide, sodium perborate, and chlorine [6]. These procedures are quite time-consuming. Several workers have studied the electrochemical oxidation of Co(II). Swann and Xanthalsos electrolyzed a saturated solution of $CoSO_4$ in sulfuric acid at temperatures below $10\,^\circ C$ [7]. Schothorst and den Boef electrochemically generated Co(III) in the flow system at a working electrode consisting of a packed bed of gold powder and applied Co(III) to spectrophotometric analysis [8]. Of these two methods, only the electrochemical generation of solutions of Co(III) is suitable for generation in flow system.

It is well known that Co(III), a stronger oxidant, can oxidize a large number of organic and inorganic substances. However, to the best of our knowledge,

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few CL reactions with Co(III) as oxidant have been reported in the literature. In this paper, a novel electrolytic flow-cell is designed, and Co(III) is electrogenerated on-line via. a positive Pt-electrode from $CoSO_4$ in H_2SO_4 medium by constant current electrolysis, resulting in the elimination of the inconvenience of its instability to water. Furthermore, we found that the reaction between Co(III) and pipemidic acid in H_2SO_4 medium produced strong CL. The purpose of the present paper is to describe a method for determining pipemidic acid that combines the detection of the CL signal and a flow injection system. Compared with other methods, the method offers potential advantages of simplicity, rapidity and high sensitivity for pipemidic acid determination. This method was successfully applied to the determination of pipemidic acid in a pharmaceutical preparation.

Experimental

Reagents

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. 0.03 mol/L $CoSO_4$ was prepared by dissolving 8.4 g of $CoSO_4 \cdot 7H_2O$ (Xi'an Chemical Regent Plant, China) in 1 L of 6.0 mol/L H_2SO_4 solution.

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

Apparatus

A schematic diagram of the CL-FIA system is shown in Fig. 1. A peristaltic pump was used to deliver all flow streams. PTFE tubing (0.8 mm i.d.) was used as connection material in the flow system. 90 μL of the sample solution was injected by a six-way injection valve into the carrier stream (H_2O) and then merged just prior to reaching a spiral flow-cell with the stream of Co(III) electro-generated from an electrolytic flow-cell. The CL signal produced
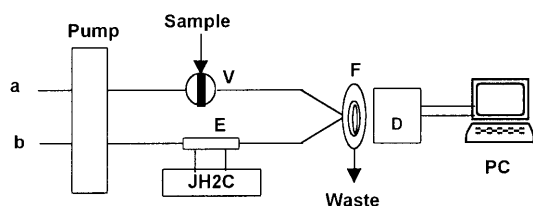

Fig. 1. Flow-injection manifold for pipemidic acid. (*a*) H_2O carrier stream, (*b*) $CoSO_4$ electrolyte. *JH2C* Potentiostat; *E* electrolysis flow-cell; *F* CL flow-cell; *V* injection valve; *Pump* peristaltic pump; *D* ultra-weak luminescence analyzer; *PC* personal computer
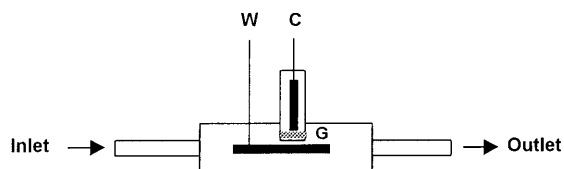

Fig. 2. The structure of electrolysis flow-cell. *W* Working electrode; *C* counter electrode; *G* glass frit

in the flow cell was detected and recorded with a computerized ultra-weak luminescence analyzer (type BPCL, manufactured at the Institute of Biophysics, Academia Sinica, China). The voltage in the photomultiplier tube (Hamamatsu, Japan) was kept at 820 V. The flow-cell is a spiral colorless glass tuber (i.d. 2 mm, length 15 cm).

Co(III) was generated on-line by galvanostating oxidation of $CoSO_4$ in H_2SO_4 medium. The electrolytic flow-cell (Fig. 2) was made of plexiglass (3.0 cm × 2.0 cm × 2.5 cm) in which cathodic and anodic chambers were separated from each other by a glass frit.

Two platinum foils (area 1.0 cm^2) were used as the working and counter electrodes, respectively. The constant current electrolysis was performed using a JH2C galvanostat (Shanghai Electric Instrument Plant, China)

Procedure

As shown in Fig. 1, flow lines were inserted into the $CoSO_4$ solution, water carrier and sample/standard solution, respectively. The pump was started to wash the whole flow system and electrolysis was started to generate Co(III) until a stable baseline was recorded. 90 μL of the sample solution was injected into the water carrier stream. This stream was merged with Co(III) in the flow-cell, producing CL emission. The concentration of sample was quantified employing the emitted CL.

Results and Discussion

CL Intensity-Time Profile

In order to measure the CL at maximum sensitivity, the CL time profile was examined by a two-channel stopped-flow technique. It was found that the rate of the reaction was very fast, the CL intensity reached a maximum 1.5 s after initiating the reaction, and then came to zero within 10 s. Since the lifetime of the CL is very short, the point of mixing was designed to be inside the CL cell. As shown in Table 1, if pipemidic acid and electrogenerated Co(III) were mixed at 2.0,

Table 1. Effect of the distance between mixing point of reagents and the CL cell on the CL intensity

Distance (cm)	3.0	2.5	2.0	0
Relative CL intensity	0	12	40	100

Distance: The distance between mixing point of reagents and the CL cell

2.5 and 3.0 cm before the entrance of the CL cell, the intensities declined rapidly.

Influence of the Structure of the Electrolytic Flow Cell

The structure of the electrolytic flow cell (EFC) was the key factor for the determination of pipemidic acid in the proposed CL flow system. The electrochemical reduction products in the auxiliary electrode chamber must be separated from the electrochemical oxidation products generated on the face of the working electrode because electrochemical reduction product (H_2) interfered with the detection of the CL signal. At the same time, in order to obtain a high and stable electrolytic efficiency, different structures of an electrolytic flow-cell were investigated. It was found that the structure of the electrolytic flow-cell as shown in Fig. 2 was to be preferred. In this electrolytic flow-cell, the electrochemical reduction product on counter electrode was separated from the electrochemical oxidation products on working electrode by a glass frit. Thus, this electrolytic flow-cell was used for throughout the experiments.

Effect of CoSO_4 Concentration

CoSO$_4$ was used as the precursor participating in the reaction needed for the CL production. In the absence of CoSO$_4$, no CL emission was observed. This suggests that dissolved oxygen from the oxidation of water cannot oxidize pipemidic acid to produce the light. The concentration of CoSO$_4$ was expected to affect the concentration of electrogenerated Co(III) which had an effect on the CL intensity. The result is shown in Fig. 3. The CL intensity increased with increasing the concentration of CoSO$_4$ in the range of

0∼0.03 mol/L probably because the concentration of electrogenerated Co(III) increased. Above a concentration of 0.03 mol/L, the CL intensity declined probably because higher concentration of colored CoSO$_4$ could absorb the CL. Then CoSO$_4$ concentration of 0.03 mol/L was chosen as optimum.

Effect of H_2SO_4 Concentration

Preliminary experiments showed that the oxidation of pipemidic acid by Co(III) was accompanied by CL emission only in H_2SO_4 medium. The possible reason is: (1) the electrochemical generation of Co(III) in sulfuric acid proceeds more easily than in perchloric or nitric acid [6]; (2) in sulfuric acid medium, oxidations with Co(III) are often more rapid than with hydrated Co(III) [9]. So H_2SO_4 was used as the medium of the CL reaction and supporting electrolyte. The effect of H_2SO_4 concentration in the range of 2.0∼8.0 mol/L was investigated (Fig. 4). The CL intensity increased with increasing H_2SO_4 concentration. The possible reason is that the reaction, $4Co^{3+} + 2H_2O \rightarrow 4Co^{2+} + 4H^+ + O_2$, can be suppressed by increasing the sulfuric acid concentration. However, because of the durability of the tube and the occurrence of leakage in the connection, the acid concentration should be as low as possible for practical application in a flow system. In the present system, 6.0 mol/L H_2SO_4 was chosen for further work.

Effect of Electrolysis Current

In the present flow system, the electrolysis current could control the concentration of electrogenerated Co(III) which affects the CL intensity. Hence the dependence of the CL intensity on the electrolysis
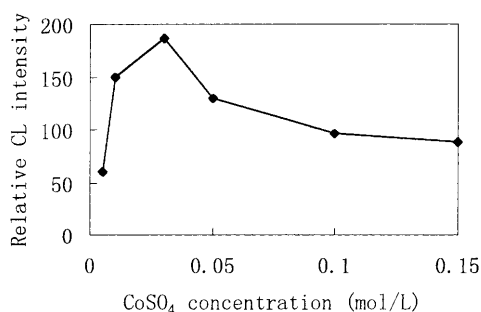

Fig. 3. Effect of CoSO$_4$ concentration. Pipemidic acid: 5 μg/ml, H$_2$SO$_4$: 6.0 mol/L, electrolysis current: 2 mA, Flow rate: 3.0 ml/min
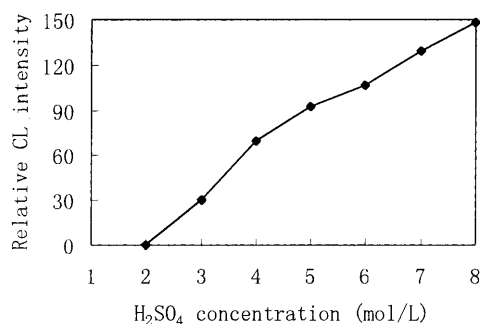

Fig. 4. Effect of H$_2$SO$_4$ concentration. Pipemidic acid: 5 μg/ml, CoSO$_4$: 0.03 mol/L, electrolysis current: 2 mA, Flow rate: 3.0 ml/min
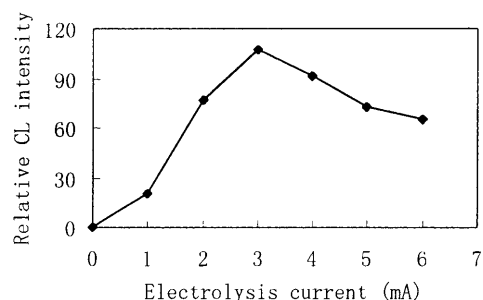
Fig. 5. Effect of electrolysis current. Pipemidic acid: 5 μg/ml, H_2SO_4: 6.0 mol/L, $CoSO_4$: 0.03 mol/L, flow rate: 3.0 ml/min

current was investigated. The result is shown (Fig. 5) that the optimal electrolysis current for the detection of pipemidic acid is 3 mA.

Effect of Flow Rate

The effect of the flow rate on the CL intensity was also investigated (Fig. 6). The CL intensity increased with increasing flow rate over the range of 0.5∼6.0 ml/min probably because the nascent Co(III) is very unstable, the half-life of Co(III) determined being about 60 s in the flow system; or the rise in the CL intensity with the flow rate may be due to efficient mixing. Lower flow rates (< 2.0 ml/min) resulted in lower CL emission, while flow rate higher than 4.0 ml/min led to greater consumption of reagents and unacceptable reproducibility. Therefore a flow rate of 4.0 ml/min for each of the reagents was chosen for further studies.

Performance of the System for Pipemidic Acid Measurements

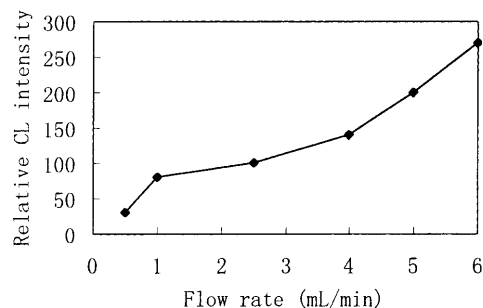Under the optimum conditions described above, the calibration graph of emission intensity (*I, mV*)

Fig. 6. Effect of flow rate. Pipemidic acid: 5 μg/ml, H_2SO_4: 6.0 mol/L, $CoSO_4$: 0.03 mol/L, electrolysis current: 3 mA

versus pipemidic acid concentration was linear in the range 0.01∼100 μg/ml and the detection limit was 3.0×10^{-9} g/ml (3σ). The regression equation was $I = 13.1 + 3.55 \times C$ (*C*: pipemidic acid concentration, μg/ml) with a correlation coefficient of 0.9991 (n = 7). A complete analysis, including sampling and washing, could be performed in 1 min with a relative standard deviation (RSD) of 3.2% for 6×10^{-6} g/ml pipemidic acid (n = 11).

Interferences 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 the 5% level were over 1000 for Na^+, Ca^{2+}, K^+, Cl^-, SO_4^{2-}, NO_3^-, Ac^-, 500 for starch, dextrin, glucose, sucrose, lactose and maltose, 100 for fructose, and 50 ascorbic acid, respectively. The results showed that the proposed method has good selectivity, only ascorbic acid are caused interference possibly because as a strong reducer ascorbic acid can also react with Co(III).

Application

Several commercial pipemidic acid tablets (Shaanxi Xi'an Pharmaceutical Plant, China) were grounded to powder and a portion of the powder was weighted and dissolved in 100 ml 0.8% HAc. After filtering, 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 agree well with those obtained by the official method [10], in which pipemidic acid was detected spectrophotometrically at 273 nm and 333 nm in 0.04% NaOH media.
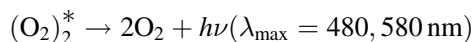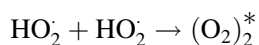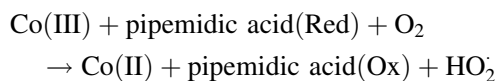
Possible CL Mechanism of the Reaction

In order to get an idea about the reaction product generating the CL, the CL spectrum of the reaction

Table 2. Determination of pipemedic acid in pharmaceutical preparation

Sample (catalogue number)	Proposed method (μg/ml)	RSD% (n = 4)	Official method (μg/ml)	Relative error
980716	7.42	2.0	7.20	−0.22
990520	7.68	2.3	7.79	0.11

was examined by a series of interference filters. The results indicated that there were two luminescence peaks, at 480 nm and at 580 nm. Furthermore, it was found that the reaction of Co(III) and quinine in H_2SO_4 medium could also generate CL emission. The structure of quinine is very different from that of pipemidic acid, but the CL spectrum of the reaction was the same as the CL spectrum of the reaction of Co(III) and pipemidic acid. When nitrogen or oxygen was bubbled through the solutions, the CL intensity decreased (about 50%) with nitrogen and increased with oxygen. The results showed that the reaction involved dissolved oxygen. The CL spectra coincide with the emitting wavelength (480 and 580 nm) of excited double molecular oxygen $(O_2)_2^*$ reported by Khan and Kasha [11]. Since these authors [12] reported early on the CL of hypochlorite-oxygen reaction due to liberated singlet oxygen, there have been many CL systems [13, 14, 15] dealing with $(O_2)_2^*$. These systems could be well explained by the formation of the $(O_2)_2^*$ specie. Hence, it is proposed that $(O_2)_2^*$ is the luminous species in this system. In this work, the formation of $(O_2)_2^*$ may occur two steps: one is generating HO_2^- free radicals, then HO_2^- can attack each other, giving a molecular pair $(O_2)_2^*$ as reported by Stauff [16].

Therefore, the CL mechanism of the reaction may be attributed to the following reactions in its simplest form:

$$Co(III) + \text{pipemidic acid(Red)} + O_2$$
$$\rightarrow Co(II) + \text{pipemidic acid(Ox)} + HO_2^-$$

$$HO_2^- + HO_2^- \rightarrow (O_2)_2^*$$

$$(O_2)_2^* \rightarrow 2O_2 + h\nu(\lambda_{max} = 480, 580\,nm)$$

Red: reduced form, Ox: oxidized form, $h\nu$: light emission.

Conclusion

The unstable reagents required for the CL reaction can be easily electrogenerated on-line and lead to the methods for many applications in analytical chemistry. In this flow system, the Co(III) solution was prepared from $CoSO_4$ in H_2SO_4 medium with constant current electrolysis. The proposed CL-FIA method for the determination of pipemidic acid is economical, simple, rapid and sensitive. These merits suggest its use in drug control laboratories.

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