

Flow-injection chemiluminescence determination of isoniazid using on-line electrogenerated BrO^- as an oxidant

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By designing a novel electrolytic flow cell, the strong oxidant BrO^- was electrogenerated on-line in KBr solution and its concentration could be readily adjusted on-line with the flow-injection technique and an electrochemical method. The chemical reaction between BrO^- and luminol in alkaline medium is accompanied by weak emission of light due to chemiluminescence (CL). It was found that the isoniazid can greatly enhance this weak CL intensity when present in the luminol solution. Based on this observation, a new flow-injection CL method for the determination of isoniazid had been proposed in this paper. The detection limit was $7.0 \times 10^{-9} \text{ g ml}^{-1}$ isoniazid and the CL emission intensity was correlated with the isoniazid concentration in the range 2.0×10^{-8} – $1.0 \times 10^{-6} \text{ g ml}^{-1}$ with an RSD of $<5\%$ ($n = 7$). The system was applied successfully to the determination of isoniazid in pharmaceutical preparations and the possible CL mechanism is discussed.

Introduction

Isoniazid is an important drug for the chemotherapy of tuberculosis and many techniques have been proposed for its determination,^{1–17} including chemiluminescence (CL) with high sensitivity, a wide linear dynamic range and simple instrumentation.^{18,19} Halvatzis *et al.*¹⁸ reported a continuous-flow CL method for the determination of isoniazid by oxidation with *N*-bromosuccinimide, but this method suffers from instability of the oxidant *N*-bromosuccinimide for the detection of the CL signal and poor sensitivity. Zhao *et al.*¹⁹ proposed a flow-injection analysis (FIA) method for isoniazid based on a luminol– Mn(II) – KIO_4 CL system with isoniazid as a sensitizer. However, the method suffers from low sensitivity.

Although the idea of the CL reaction reagent being produced on-line by electrochemically oxidizing KBr with a flow-injection technique had been proposed for the determination of sulfide ion,²⁰ this method suffers from the limitation that in the flow cell the cathodic chamber is not separated from the anodic chamber, so it only provided a low concentration of CL reagent (BrO^-) since the product (H_2) of cathodic reduction is in the same stream as the BrO^- , and the higher the electrolytic current the greater are the concentrations of BrO^- and H_2 ; higher concentrations of H_2 produced the obvious H_2 bubbles in the stream of electrolyte, and these bubbles interfered with the detection of the CL signal.

In this work, a novel electrolytic flow cell was designed in which the cathodic chamber is separated from the anodic chamber with a glass frit. It has the advantage that it can be used to produce hypobromite ion, a highly active CL reaction reagent, in an FIA system by galvanostating the oxidation of KBr in alkaline medium and toxic Br_2 does not need to be prepared.²¹ Further, the BrO^- concentration can be readily adjusted on-line over a wide concentration range (1.0×10^{-2} – $1.0 \times 10^{-6} \text{ mol l}^{-1}$) with the flow-injection technique and changing the electrolytic current. In addition, the BrO^- standard solution which is prepared in the proposed method contain only two components (Br^- and BrO^-), and a chemical reaction method is not used (for example, using BrO_3^- oxidizing Br^-) to obtain BrO^- standard solution which contains other oxidants such as BrO_3^- in addition to BrO^- and Br^- , and the BrO_3^- can interfere with CL signal detection.

Based on the novel flow-through electrolytic cell, BrO^- can be used to perform the CL reaction in a simple manner. It was also found that the chemical reaction between BrO^- and luminol in alkaline medium (pH 10.40, Na_2CO_3 – NaHCO_3 buffer solution) is accompanied by the weak emission of light due to CL and the isoniazid had a strong enhancing effects on this weak CL intensity. Based on this enhancement effect, a new, rapid, sensitive and inexpensive FIA CL method for the determination of isoniazid was developed. Various experimental conditions such as the design of the electrolytic flow cell and effects of other conditions for the determination of isoniazid were optimized. This FIA CL system was also applied successfully to the determination of isoniazid in pharmaceutical preparations.

Experimental

Reagents

All solutions were prepared from analytical-reagent grade materials and distilled, de-ionised water.

Stock standard isoniazid solution, $100.0 \text{ } \mu\text{g ml}^{-1}$, was prepared by dissolving 0.1000 g of isoniazid (Shanghai Chemical Reagent Plant, Shanghai, China) in water, transferring the solution into a calibrated flask and diluting to 1 l with water. The solution was stable for at least 1 week. Carbonate buffer stock standard solution (pH 10.40) was prepared by dissolving 35.8 g of $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$ and 10.5 g of NaHCO_3 in water and diluting to 2.5 l. The stock standard solution was diluted as required. Potassium bromide buffer solution (pH 10.40, NaHCO_3 – Na_2CO_3 buffer solution) was prepared by dissolving 6.0 g of KBr in 100 ml of water, transferring the solution into a calibrated flask and diluting to 0.5 l with carbonate buffer solution (pH 10.40). The cathodic chamber was filled with saturated Na_2SO_4 solution.

Apparatus

The flow system is shown in Fig. 1. Two peristaltic pumps were used to deliver the carrier stream (H_2O) and 0.1 mol l^{-1} KBr

solution (pH 10.40, $\text{NaHCO}_3\text{--Na}_2\text{CO}_3$). The sample solution was injected using a six-port valve equipped with a 100 μl sample loop. The flow cell utilizes a conventional two-electrode set-up and was arranged as a flow-through system (Fig. 2). The flow cell was made of Plexiglas (length 3.0 cm, width 2.0 cm, height 2.5 cm). The working electrode was a piece of platinum (1.0 cm^2) and a similar piece of platinum was used as the counter electrode. The counter electrode chamber was separated from the chamber of the working electrode with a glass frit. The constant current applied for electrolysis was achieved with a JH2C potentiostat/galvanostat. The CL intensity was transformed into an electrical signal by an R456 photomultiplier (Hamamatsu, Tokyo, Japan) which was operated at -800 V and placed close to the CL flow cell. The signal was recorded with an XWT-204 recorder (Shanghai Dahua Instrumental Meter plant, Shanghai, China).

Procedures

In order to achieve good mechanical and thermal stability, the instruments were allowed to run for 10 min before the first measurement was made. The carrier stream, sample stream and electrolyte stream were all fed at a flow rate of 2.5 ml min^{-1} , and galvanostatic electrolysis with a constant current of $230\text{ }\mu\text{A}$ was performed using a JH2C potentiostat/galvanostat.

The blank solution which only contained $4.0 \times 10^{-6}\text{ mol l}^{-1}$ luminol was injected into the carrier stream by a six-port valve and a stable blank signal was recorded, then the sample or standard isoniazid solutions which contained not only $4.0 \times 10^{-6}\text{ mol l}^{-1}$ luminol but also an appropriate concentration of isoniazid was injected into the carrier stream and the CL signal was recorded. The concentration of isoniazid was quantified *via* the peak height of the relative CL emission intensity which was obtained by subtracting the blank CL intensity from that of the sample or standard isoniazid solution.

Results and discussion

Choice of electrogenerated oxidant for the CL reaction

In order to determine isoniazid using the proposed FIA CL system, different oxidants such as ClO^- , BrO^- and IO^- , which were obtained by electrochemically oxidizing Cl^- , Br^- and I^- ,

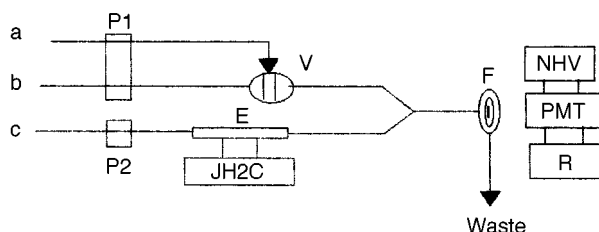


Fig. 1 Schematic diagram of FIA CL system. E, electrolytic flow cell; JH2C, potentiostat/galvanostat; P1, P2, peristaltic pumps; F, CL reaction flow cell; V, six-port valve; NHV, negative high-voltage supply; PMT, photomultiplier tube; R, recorder; a, sample solution; b, carrier stream; c, KBr electrolyte.

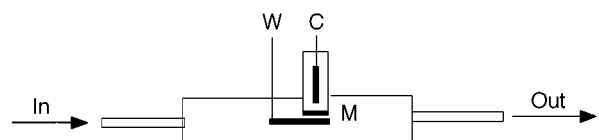


Fig. 2 Schematic diagram of the electrolytic flow cell. W, working electrode; C, counter electrode; M, glass frit.

respectively, were investigated. The results showed that although ClO^- can be used as the oxidant for the determination of isoniazid and possessed nearly the same sensitivity as BrO^- as the oxidant, the stability of the CL signal was very poor, especially when the electrolytic current which was used for ClO^- was above 0.5 mA . The reason is that when a high electrolytic current was applied to the electrolytic flow cell, the electrolytic efficiency for generation ClO^- was decreased²² and a few O_2 bubbles were produced by the electrochemical oxidation of H_2O in the ClO^- stream, and these bubbles interfered with the detection of the CL signal. IO^- cannot be used for the determination of isoniazid because the isoniazid did not enhance the CL reaction between IO^- and luminol satisfactorily. Of the three oxidants, BrO^- presented the best performance for the determination of isoniazid and was therefore selected for subsequent studies.

Design of the electrolytic flow cell

The structure of the electrolytic flow cell is a vital factor for the determination of isoniazid in this CL flow system. The ideal electrolytic flow cell could offer a high and stable electrolytic efficiency for electrogenerating a sufficiently high concentration of BrO^- for the CL reaction. The design of the electrolytic flow-through cell should consider the flow characteristics. First, in the flow cell, the electrochemical reduction product (H_2 bubbles) produced on the face of the counter electrode must be separated from the electrochemical oxidation product (BrO^-) generated on the face of working electrode in order to prevent the interference of the H_2 bubbles with the detection of the CL signal; at the same time, the electric resistance between the working electrode and the counter electrode should be the lowest that will permit sufficient electrolytic current to electrogenerate an effective amount of BrO^- for the CL reaction. We selected a glass frit as the separating material between the two electrode chambers. Second, the potentials dispersed on the face of working electrode should be sufficient to give a high and stable electrolytic efficiency for generating BrO^- . Based on consideration of the effects of the relative locations of the two electrodes in the electrolytic cell and the area of the counter electrode, the results showed that only when the two electrodes faced each other and the area of the working electrode was the same as that of the counter electrode, did the electrolytic cell satisfy our aims.

In addition to the points considered above, the volume of the electrolytic flow-through cell is also an important factor for using the cell in this CL flow system since it can affect the on-line concentration of BrO^- . To obtain a smaller volume of the cell and limited by the instruments which were used to make the cell, a 2.5 mm diameter of the chamber of the working electrode was adopted.

Considering all the points discussed above, the structure of the flow cell as shown in Fig. 2 is the optimum and it was used throughout subsequent experimental work.

Effect of pH in electrolyte on the relative CL intensity

The pH of the electrolyte is an important parameter which affects the relative CL intensity for the determination of isoniazid and it was investigated by varying the pH of the electrolyte with $0.10\text{ mol l}^{-1}\text{ NaOH}$ and $0.10\text{ mol l}^{-1}\text{ Na}_2\text{CO}_3$ solution. The results showed that when the pH was increased in the range 8.50–10.40 the relative CL intensity increased, the latter decreased above pH 10.40 since the blank CL intensity between BrO^- and luminol at higher pH was increased. Hence pH 10.40 was selected for subsequent studies.

Effect of luminol concentration in sample or standard isoniazid solution on the relative CL intensity

The concentration of luminol had a very important effect on the relative CL intensity for the determination of isoniazid. Initial tests showed that first, the CL signal between isoniazid and BrO^- is too weak to be used for the determination of isoniazid, and second, no appropriate concentration of luminol in isoniazid solution gave an enhanced CL signal of isoniazid. Based on these observations, the effect of luminol concentration on the relative CL intensity was investigated from 2.0×10^{-6} to $12.0 \times 10^{-6} \text{ mol l}^{-1}$ and the results are shown in Fig. 3. When the concentration of luminol was lower or higher than $4.0 \times 10^{-6} \text{ mol l}^{-1}$, the relative CL intensity decreased. Hence, the optimum concentration of luminol was $4.0 \times 10^{-6} \text{ mol l}^{-1}$ in the sample injection solution.

Effect of concentration of potassium bromite in the electrolyte

The concentration of KBr in the electrolyte not only strongly affected the concentration of the CL reaction species (BrO^-), but also affected the relative CL intensity. While other experimental conditions were maintained at the above optimum values, the study of the effect of KBr concentration on the relative CL intensity revealed that when the KBr concentration changed in the range $0.10\text{--}0.50 \text{ mol l}^{-1}$, the relative CL intensity was stable and possessed the maximum intensity. Hence 0.10 mol l^{-1} KBr was used for the determination of isoniazid in this CL system.

Effect of the electrolytic current on the relative CL intensity

Since the electrolytic current controlled the concentration of BrO^- , it should have a strong effect on the relative CL intensity. This effect was investigated and the results are shown in Fig. 4.

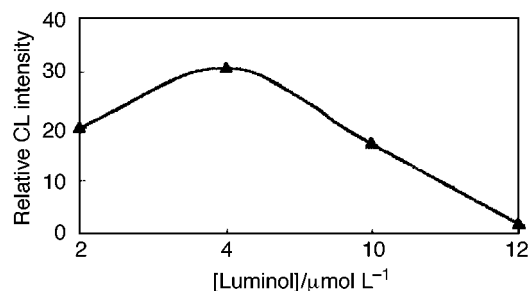


Fig. 3 Effect of luminol concentration on relative CL intensity. pH = 10.40; $\text{Na}_2\text{CO}_3\text{--NaHCO}_3$; $[\text{KBr}] = 0.10 \text{ mol l}^{-1}$; $[\text{isoniazid}] = 4.0 \times 10^{-8} \text{ g ml}^{-1}$; electrolytic current = $200 \mu\text{A}$; flow rates of carrier stream and electrolyte, 25 ml min^{-1} .

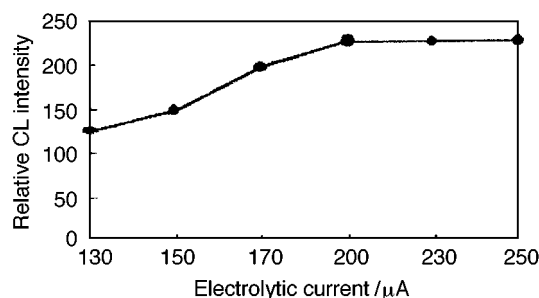


Fig. 4 Effect of electrolytic current on relative CL intensity. pH = 10.40; $\text{Na}_2\text{CO}_3\text{--NaHCO}_3$; $[\text{KBr}] = 0.10 \text{ mol l}^{-1}$; $[\text{isoniazid}] = 4.0 \times 10^{-8} \text{ g ml}^{-1}$; $[\text{luminol}] = 4.0 \times 10^{-6} \text{ mol l}^{-1}$; flow rates of carrier stream and electrolyte, 2.5 ml min^{-1} .

It can be seen that the highest relative CL intensity can be obtained when an electrolytic current of $230 \mu\text{A}$ for the electrolytic flow-cell is employed.

Discussion of the possible CL mechanism

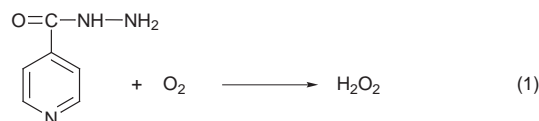
In order to explain the possible CL reaction mechanism and the possible CL emitter, a series of experiments were performed and the results were as the follows.

First, using the experimental conditions stated above, the CL emission spectrum was obtained using an RF-540 spectrofluorimeter and the results showed that the maximum CL emission wavelength is 425 nm, which suggested that the possible emission species is excited 3-aminophthalate.

Second, it was found that the weak CL intensity between BrO^- and luminol was also enhanced by H_2O_2 when present in the BrO^- stream.

Third, we further found that NH_2NH_2 has a similar enhancing ability as isoniazid for this weak CL intensity between BrO^- and luminol.

Based on the above experimental results and the properties of isoniazid, the dissolved O_2 (in isoniazid solution) is possibly reduced by the isoniazid and some H_2O_2 is possibly produced since the isoniazid has a similar reducing ability²³ as NH_2NH_2 ; hence when the isoniazid was injected into the BrO^- stream, active oxygen, a stronger oxidant species, is produced by the chemical reaction between BrO^- and H_2O_2 because the BrO^- has a similar oxidizing ability.²⁴ Then the active oxygen can oxidize luminol to produce a strong CL emission intensity. The possible CL reaction mechanism may be attributed to the following reactions:



Interference study

In order to assess the possible analytical applications of the proposed method, the effect of common excipients used in pharmaceutical preparations and some compounds were studied by analysing synthetic sample solutions contain $4.00 \times 10^{-7} \text{ g ml}^{-1}$ of isoniazid and various amount of each compound. The undissolved material, if any, was filtered before measurement. The recovery results are given in Table 1. The results showed that the proposed method has good selectivity; only riboflavin and ascorbic acid appeared to interfere with isoniazid. A possible reason is that riboflavin possesses similar properties to flavin adenine dinucleotide (FAD) and can catalyse the formation of H_2O_2 with O_2 in solution,²⁵ and hence can enhance the weak CL intensity, but ascorbic acid possesses the opposite properties to riboflavin, so it decreases the CL intensity.

Performance of the system for isoniazid measurement

Under the selected conditions given above, the calibration graph of emission intensity (I , mV) versus isoniazid concentration ($10^{-7} \text{ g ml}^{-1}$) was linear in the range $2.00 \times 10^{-8}\text{--}1.00 \times 10^{-6} \text{ g ml}^{-1}$. The correlation coefficient was 0.9987 ($n = 13$). The

detection limit was 7.0×10^{-9} g ml⁻¹ of isoniazid (S/N = 3). The regression equation was $I = 0.947 + 3.12C$ (where C is the concentration of isoniazid in 10^{-7} g ml⁻¹). A complete analysis, including sampling and washing, could be performed in 1 min with an RSD of <5% for 4×10^{-7} g ml⁻¹ of isoniazid ($n = 7$).

Application

Not less than 20 tablets were weighed, ground to fine powder and mixed. A sample equivalent to approximately 200 mg of isoniazid was weighed so the final solution was within the working range. The final solution should also contain 4.0×10^{-6} mol l⁻¹ luminol. After accurate transfer into a 1 l calibrated flask the solution was made up to volume with water. The mixture was sonicated for 10 min to aid dissolution and then filtered. An appropriate volume of the filtrate was diluted further with water so that the concentration of isoniazid was in the working range. The results are given in Table 2 and agree well with those obtained by an official method.²⁶

Conclusions

By designing a novel flow-through electrolytic cell and using flow-injection techniques, BrO⁻, a strong and unstable oxidant, was readily obtained on-line over a wide concentration range and was used for the determination of isoniazid with an FIA CL

Table 1 Recovery of isoniazid (4×10^{-7} g ml⁻¹) from solution containing various compounds or ions

Compound or ion	Concentration ratio (compound or ion to isoniazid)	Recovery (%) ($n = 3$)
Riboflavin	50	115.6
Nicotinic acid	2	101
Nicotinamide	100	99.9
Pyridoxine hydrochloride	50	107.1
Ascorbic acid	1	88.5
Thiamine hydrochloride	10	98.8
Calcium pantothenate	10	105.5
p-Aminosalicylic acid	5	97.8
Glucose	100	97.3
Sugar	1000	100.6
EDTA	100	98.7
Mg ²⁺	2500	100.2
Ca ²⁺	2500	99.6

Table 2 Results of determination of isoniazid in tablet samples

Sample No.	Official method (mg per tablet) ^a	Proposed method (mg per tablet) ^b	Recovery (%)
1	99.4	98.7 (± 4.4)	98
2	98.6	99.3 (± 4.6)	104
3	99.2	98.8 (± 3.8)	94
4	97.6	99.2 (± 3.4)	97

^a Average value of five determinations. ^b Mean of four replicates (\pm RSD, %).

method. Compared with other CL methods for the determination of isoniazid, the proposed method is sensitive, rapid, accurate and simple and is useful for the determination of isoniazid in pharmaceutical dosage forms. Consideration is being given to extrapolating the system to a liquid chromatographic set-up, so as to provide extra selectivity to the method. The results obtained with the proposed method showed that the electrogenerated unstable CL reaction reagent not only has a higher CL activity, but also extends the application field of CL analysis. These advantages provide encouragement for further work along these lines.

Acknowledgement

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