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Determination of Iproniazid by Flow Injection Analysis with Chemiluminescence Detection Based on the Oxidation of Luminol by Trichloroisocyanuric Acid and Sodium Dichloroisocyanurate

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ABSTRACT

A simple, rapid and accurate chemiluminescent method is described for the determination of iproniazid. The proposed method is based on the chemiluminescence (CL) generated during the oxidation of luminol by trichloroisocyanuric acid (TCCA) and sodium dichloroisocyanurate (SDCC) in alkaline medium. It was found that iproniazid could greatly enhance the CL intensity of the mentioned reactions. The detection limits (3σ) were 8.0 and 76 ppb of iproniazid for the TCCA-luminol and SDCC-luminol systems, respectively. The relative CL intensity was linear with the iproniazid concentration in the range of 50-1000 and 1000-2000 ppb for TCCA-luminol system, and 100-2000 ppb for SDCC-luminol system. The method was applied to the determination of iproniazid in a synthetic sample as well as in human serum with acceptable accuracy and precision.

Key words: iproniazid, chemiluminescence, flow injection analysis, trichloroisocyanuric acid, dichloroisocyanurate, luminol

INTRODUCTION

Iproniazid (N'-propan-2-ylpyridine-4-carbohydrazide) (Figure 1) and its phosphate salt, are white to slightly yellowish powders. Iproniazid is a monoamine oxidase inhibitor developed as the first anti-depressant(1). Some analytical methods have been reported in the literature for the determination of iproniazid such as fluorimetry(2), differential pulse polarography(3), spectrophotometry(4-7) and chromatography(8).

Because of the high sensitivity, simplicity, reproducibility and rapidity of flow injection chemiluminescence (FI-CL) analysis, there has been much interest in using this method in pharmaceutical analysis in recent years. To the best of our knowledge, there is only one report in the literature on using CL method for the determination of iproniazid(9). Sanfeliu Alonso *et al*. used direct chemiluminescence of iproniazid that is produced from its oxidation by cerium (IV), for the determination of iproniazid in synthetic samples and human urine. Thus, there is still great need for designing simple, sensitive and rapid chemiluminescent methods for the determination of iproniazid in pharmaceuticals and other complex samples such as human serum.

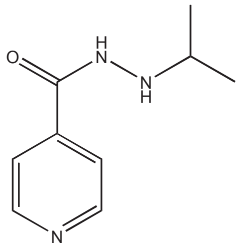

Figure 1. Chemical structure of iproniazid.

In this work, a new, simple and rapid FI-CL method for determination of iproniazid based on its enhancing effect on the CL reaction between luminol and isocyanuric acids (TCCA and SDCC) is proposed. This system was also applied successfully to the determination of iproniazid in human serum samples.

MATERIALS AND METHODS

I. *Reagents*

Analytical reagent-grade chemicals and triply distilled water were used throughout the experiments.

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Stock solutions of iproniazid (100.0 µg/mL, Merck), luminol (1.0×10^{-2} M, Merck), sodium dichloroisocyanurate (SDCC), (1.0×10^{-2} M, Merck) and trichloroisocyanuric acid (TCCA), (1.0×10^{-2} M, Fluka) were prepared by dissolving appropriate amounts of each compound in water. All other common laboratory chemicals were of the best grade available and were used without further purification.

II. *Apparatus*

A schematic of the flow-injection system used is shown in Figure 2. A peristaltic pump (Ismatec ISM 597) was used to deliver all solutions into a 150 µL flow cell. PTFE tubing (0.8 mm i.d.) was used between all components of the flow system. Sample solution (200 µL) was injected by a six-way injection valve (Rheodyne RH-5020) into the carrier stream (water).

The CL signal was transduced to an electrical signal by an R456 photomultiplier tube (Hamamatsu) placed close to the flow cell in a light tight box. The output signal was recorded with a computer via a laboratory-made analog to digital converter.

III. *Procedure*

As shown in Figure 2, flow lines were inserted into carrier stream (water), oxidant and luminol solutions. Flow rate at each line was 2.5 mL/min when TCCA was used as oxidant and 2.0 mL/min when SDCC was used. The blank solution was injected into the carrier stream with the aid of an injection valve with a 200 µL sample loop and a stable blank signal was recorded. Then, the sample or standard iproniazid solution was injected into the carrier stream and the CL signal was recorded. The concentration of iproniazid was quantified via the peak height of the relative CL intensity, which was obtained by subtracting CL intensity of the blank from that of the sample or standard iproniazid solutions.

RESULTS AND DISCUSSION

Different halogen-containing compounds such as N-bromosuccinimide (NBS), BrO^-, and ClO^-, have been used as oxidants in the CL reaction of luminol. Chloroisocyanurates are organic chloramines having one, two or three chlorine atoms attached to the nitrogen atoms of the symmetric triazine ring. Hypochlorite ions will generate in aqueous solution by dissolution of these compounds in water. Chloroisocyanurates are more stable than NBS, hypobromite, and hypochlorite. This stability has been attributed to the existence of isocyanuric acid which is in the equilibrium with the generated hypochlorite in aqueous solution(10).

It was reported previously that the oxidation of luminol by TCCA and SDCC in alkaline medium is chemiluminescent(11), and some compounds were determined using these oxidants by CL method(12,13). According to our preliminary experiments, it was found that iproniazid could greatly enhance these CL reactions. Based on this enhancing effect, a method for the determination of iproniazid is proposed.

I. *Optimization of Experimental Conditions*

Effects of oxidants, luminol and NaOH concentrations on the CL intensity of TCCA- and SDCC-luminol systems in the presence of iproniazid were investigated. As shown in Figure 3, the optimum concentrations of 6.0×10^{-5} M (TCCA) and 1.0×10^{-5} M (SDCC) were chosen for oxidants. The optimum concentrations of NaOH were 2.0×10^{-3} and 1.0×10^{-3} M for TCCA- and SDCC-luminol systems, respectively. Figure 4 shows the effect of luminal concentration on CL intensity for both systems and the optimum concentration for luminol at 1.4×10^{-4} M. The optimum values obtained for the flow rate of reactants were 2.5 and 2.0 mL/min for TCCA- and SDCC-luminol systems, respectively. According to the optimization studies on the sample volume, a 200 µL sample volume was chosen for further studies.

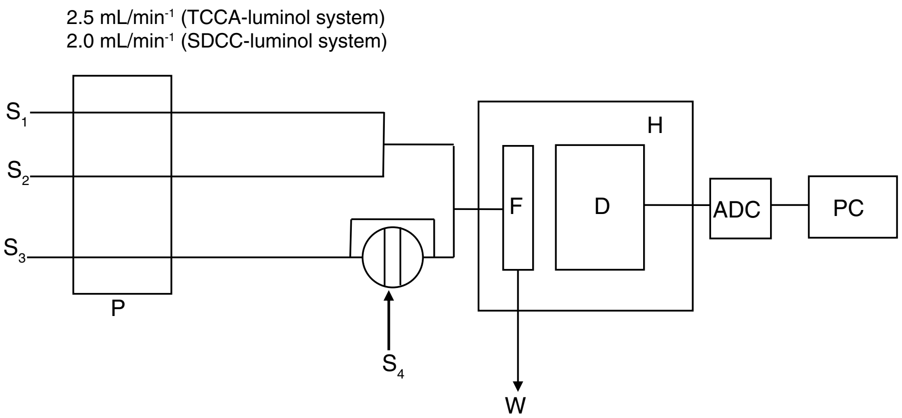

Figure 2. Schematic diagram of FIA setup. S1: oxidant solution (TCCA or SDCC), S_2: luminol solution, S_3: carrier solution (water), S_4: sample solution, P: peristaltic pump, H: sealed housing, F: flow cell, D: detector, ADC: analog to digital converter, PC: personal computer, W: waste.

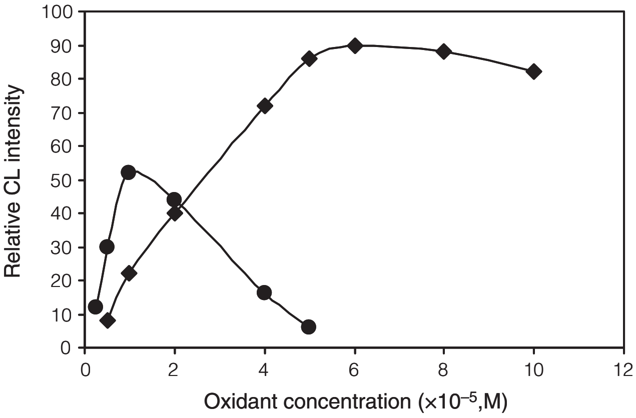

Figure 3. Effect of TCCA (♦) and SDCC (●) concentrations on the CL intensity. Experimental conditions for TCCA-luminol system: 1000 ppb iproniazid; 1.4×10^{-4} M luminol; 2.0×10^{-3} M NaOH; flow rate of 2.5 mL/min, and for SDCC-luminol system: 1.4×10^{-4} M luminol; 1.0×10^{-3} M NaOH; flow rate of 2 mL/min.

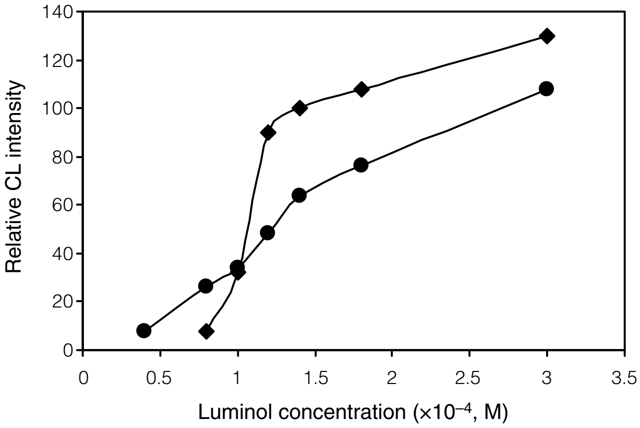

Figure 4. Effect of luminol concentration on the relative CL intensity. Experimental conditions for TCCA-luminol (♦) system: 1000 ppb iproniazid; 2.0×10^{-3} M NaOH; 6.0×10^{-5} M TCCA; flow rate of 2.5 mL/min, and for SDCC-luminol (●) system: 1.0×10^{-3} M NaOH; 1.0×10^{-5} M SDCC; flow rate of 2.0 mL/min.

II. *Possible CL Mechanism*

A series of experiments were conducted to explain the possible CL reaction mechanism of the proposed method. Under the selected conditions, no CL emission was observed from the reaction of iproniazid and TCCA or SDCC in the absence of luminol. Also, no CL emission was observed when iproniazid was injected into the basic solution of luminol in the absence of TCCA or SDCC. A strong CL emission occurred only when iproniazid was injected into the mixed solution of TCCA or SDCC with luminol. The CL spectrum of the iproniazid-luminol-oxidant system had a maximum at 425 nm, suggesting that the possible CL emitter is the excited state of 3-aminophthalate(14,15). It has been reported that the dissolved O_2 can be reduced to H_2O_2 by hydrazine(16). This might also be proposed for iproniazid which has a similar reducing functionality as hydrazine. The active oxygen can be produced by the chemical reaction between the produced H_2O_2 and an oxidizing agent such as TCCA or SDCC(12). Strong CL emission is produced when luminol reacts with active oxygen. To approve

Table 1. Recovery of iproniazid (400 ppb) from solutions containing various amounts of interferences

	Concentration ratio (interference/iproniazid)	Recovery (%) (n = 4)	
		with TCCA	with SDCC
Glucose	100	99.4	98.5
Lactose	100	98.1	99.7
Sodium citrate	10	100.1	100.8
Nicotinic acid	10	100.9	99.0
Ascorbic acid	1	95.3	96.4
Isoniazid	1	98.9	99.3
Starch	100	99.9	99.8
Polyethylene glycol 4000	10	98.8	98.0
Sorbitol	100	99.8	99.1
Talc	100	100.2	100.9
Calcium sulfate	100	99.1	100.9
Sodium carbonate	100	100.1	99.9
Urea	100	100.2	101.8
Sodium chloride	100	100.1	100.1

Table 2. Analytical figures of merit for the determination of iproniazid

Oxidant	Iproniazid concentration range (ppb)	Calibration equation Y = a + bX	Correlation coefficient (r)	RSD[a] (n = 6)	Limit of detection[b] (ppb)
TCCA	50 – 1000	Y = 5.0438 + 0.1176X	0.999	1.6[c]	8.0
	1000 – 2000	Y = -12.286 + 0.0589X	0.999	0.89[d]	
SDCC	100 – 2000	Y = 1.0844 + 0.0617X	0.999	3.1[c]	76

[a] Relative standard deviation.

[b] Theoretical detection limit.

[c] Corresponds to 250 ppb iproniazid.

[d] Corresponds to 1500 ppb iproniazid.

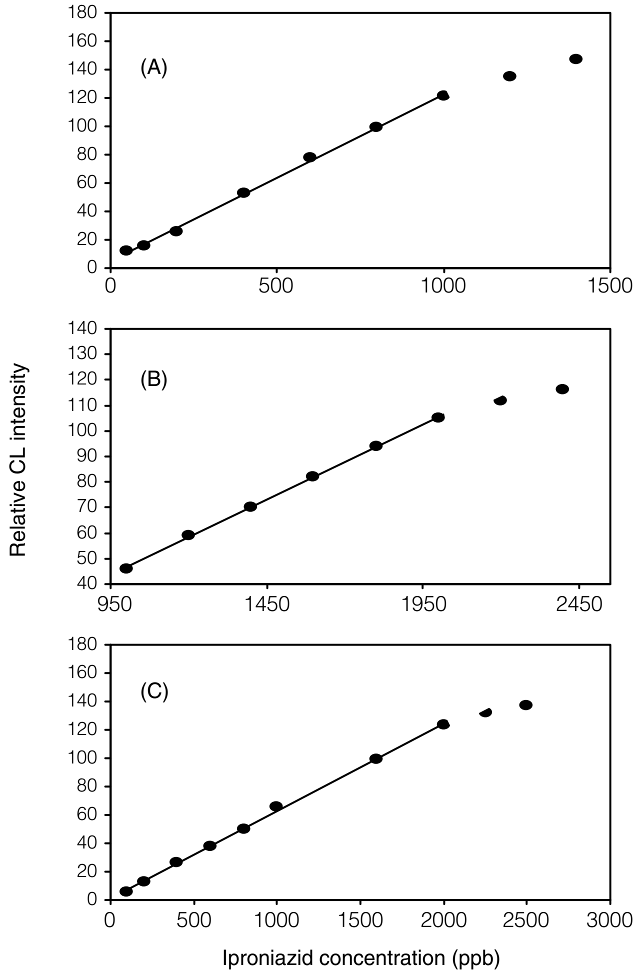

Figure 5. Analytical calibration graphs for the determination of iproniazid in the ranges of: (a), 50-1000 (b), 1000-2000 ppb (TCCA-luminol system) and (c), 100-2000 ppb (SDCC-luminol system).

Table 3. Results of determination of iproniazid in human serum samples

Sample	Amount added (ppb)	Amount found (ppb)	Recovery (%)	RSD (%) (n = 4)
1	0	ND	—	—
	200	190[a]	95.0	4.1
		188[b]	94.0	4.6
2	0	ND	—	—
	1000	973[a]	97.3	1.0
		977[b]	97.7	1.6

[a] and [b] correspond to TCCA and SDCC oxidizing agents, respectively.

ND: not detected.

the key role of the dissolved oxygen, all solutions in the proposed CL system were deaerated with pure argon gas. After deaeration, a substantial decrease in the CL emission intensity was observed. According to these results, the enhancing effect of iproniazid on the CL intensity of luminol could be attributed to the active oxygen produced by the reaction of iproniazid with dissolved oxygen in the presence of TCCA or SDCC. The possible reaction mechanism could be summarized as follows.

$$\text{TCCA or SDCC} \longrightarrow \text{HOCl}$$

$$\begin{aligned} &\text{dissolved } O_2 \xrightarrow{\text{Iproniazid}} H_2O_2 \\ &OCl^- + H_2O_2 \longrightarrow [O] \quad \text{(In alkaline media)} \\ &[O] + \text{luminol} \longrightarrow \text{Enhanced CL emission} \end{aligned}$$

III. *Interference Study*

The effect of possible interfering compounds was studied in analyzing synthetic sample solutions containing 400 ppb of iproniazid and various amounts of interfering compounds. For this purpose, the effect of each interferent was studied in the presence of 400 ppb of iproniazid. The maximum tolerance level was recorded as the concentration of the interferent which caused a variation in the analytical signal of iproniazid below 3%. The recovery results are summarized in Table 1.

As Table 1 shows, there is no significant interference from many of the tested compounds except ascorbic acid and isoniazid. Due to the rapid oxidation of ascorbic acid in alkaline media, the interference of ascorbic acid could be eliminated by measuring the samples in about one hour after sample preparation(17,18). Unfortunately isoniazid could interfere with the measurement of iproniazid. Thus, a separation step should be applied before using the proposed CL method to measure iproniazid in the presence of isoniazid.

IV. *Analytical Performance for Iproniazid Measurements*

Under the selected conditions given above, calibration graphs of relative signal intensity versus iproniazid concentration (ppb) were obtained for both CL systems. The linear calibration ranges and corresponding equations are shown in Table 2. Corresponding calibration graphs are depicted in Figure 5. The calibration curve obtained for TCCA-luminol system includes two separate parts with different slopes. One part could be used for lower concentration ranges and the other part for higher ranges. Calculated detection limits (3σ), were 8 and 76 ppb for TCCA and SDCC-luminol systems, respectively.

V. *Real Sample Analysis*

To evaluate the analytical applicability of the method, the recommended procedure was applied to the determination of iproniazid in human serum samples. After preparation of samples, various amounts of iproniazid were spiked into the samples. Then samples were diluted with distilled water (1:100 dilution). The results are given in Table 3, which shows acceptable recoveries and RSDs.

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