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Flow injection determination of isoniazid using *N*-bromosuccinimide- and *N*-chlorosuccinimide-luminol chemiluminescence systems

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Abstract

A chemiluminescent method for the determination of isoniazid is described. Method is based on the chemiluminescence (CL) generated during the oxidation of luminol by *N*-bromosuccinimide (NBS) and *N*-chlorosuccinimide (NCS) in alkaline medium. It was found that the isoniazid could greatly enhance this CL intensity when present in the luminol solution. Based on this observation, a new flow-injection CL method for the determination of isoniazid is proposed in this paper. The detection limits were 4 and 3 ng ml^{-1} isoniazid for the NBS- and NCS-luminol CL systems, respectively. The relative CL intensity was linear with the isoniazid concentration in the range of 8–600 and 600–5000 ng ml^{-1} for the NBS-luminol CL system, and 6–200 and 200–2000 ng ml^{-1} for the NCS-luminol CL system. The results obtained for the assay of pharmaceutical preparations compared well with those obtained by the official method and demonstrated good accuracy and precision.

Keywords: Chemiluminescence (CL); Isoniazid; Luminol; *N*-Bromosuccinimide (NBS); *N*-Chlorosuccinimide (NCS)

1. Introduction

Isoniazid (pyridine-4-carboxylic acid hydrazide) is a compound for the chemotherapy of tuberculosis. Several analytical techniques have been proposed for the determination of isoniazid including titration [1,2], spectrophotometry [3–11], fluorimetry [12], electroanalytical [13,14], atomic absorption spectrometry [15,16], chromatography [17] and capillary electrophoresis [18,19].

In recent years extremely sensitive analytical techniques based on CL systems have received considerable attention. Simplicity of detection, low detection limit, large calibration ranges and short analysis times are some of the characteristics that make the method attractive. CL has been successfully extended to reactions involving organic compounds of pharmaceutical or biological interest. Halavitzis et al. [20] reported a continuous-flow CL method for the determination of isoniazid by oxidation with NBS. However, the method

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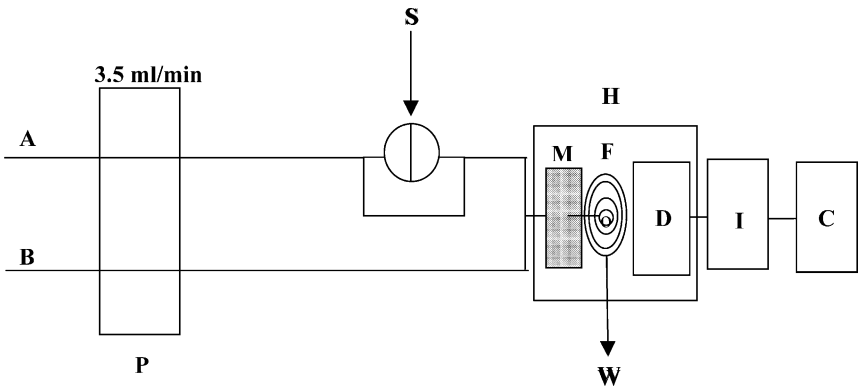

Fig. 1. Schematic diagram of FIA setup. A: Carrier solution (5×10^{-2} M NaOH); B: oxidant solution (NBS or NCS); P: peristaltic pump; S: sample solution; H: sealed housing; M: mirror; F: glass spiral flow cell; D: detector; I: computing integrator; C: computer; W: waste.

suffers from low sensitivity. Zhao et al. [21] proposed a flow-injection analysis (FIA) method for isoniazid based on a luminol–Mn(II)–KIO_4 CL system with isoniazid as a sensitizer. This method also suffers from poor sensitivity. Zhang et al. reported two electrogenerated CL (ECL) methods based on the chemical reaction of electrogenerated BrO^- [22] or ClO^- [23] with luminol and enhancing effect of isoniazid on the emission intensity generated by these CL systems. The design of flow cell is a vital factor for the determination of isoniazid in these CL flow systems. In the design of the electrolytic flow cell some characteristics, such as interference of the electrochemical reduction product (H_2 bubbles), selection of sufficient potential to give a high and stable electrolytic efficiency for generation of BrO^- and ClO^-, and even the volume of the electrolytic flow cell should be taken in to consideration. Recently, Li et al. [24] and Song et al. [25] reported CL sensors based on immobilizing both CL reagent (luminol) and oxidizing agent; either periodate [24] or ferricyanide [25], on an anion exchanger.

In this work, we show that both NBS-luminol and NCS-luminol CL systems can be employed for determination of isoniazid. Based on the strong enhancing effect of isoniazid on these CL systems, a new, rapid, simple, sensitive and inexpensive method is proposed for the determination of isoniazid. These FIA-CL systems were also applied successfully to the determination of isoniazid in pharmaceutical preparations.

2. Experimental

2.1. Reagents

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

Stock isoniazid solution, 100.0 μg ml^{-1}, was prepared by dissolving 0.1000 g of isoniazid (Merck) 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.

NBS and NCS solutions (5×10^{-2} M) were prepared daily by dissolving 2.225 g of NBS (Merck) and 1.703 g of NCS (Merck) in water, and diluting to 250 ml with water.

Luminol solution (1×10^{-2} M) was prepared by dissolving 0.177 g of luminol (Merck) in 1×10^{-1} M NaOH, and diluting to 100 ml with the same alkaline solution.

The minimum number of dilution steps possible was used for preparation of more dilute solutions.

All other common laboratory chemicals were of the best grade available and were used without further purification.

2.2. Instruments

A schematic diagram of the flow injection CL analyzer is shown in Fig. 1. The 12-channel peristaltic pump (Desaga PLG) was equipped with silicon rubber tubes (1 mm i.d.). The sample solution was injected with a Rheodyne sample injector, model 7125. The carrier stream merged with oxidant solution stream (NBS or NCS) in a spiral flow cell in front of a photomultiplier tube (PMT). The flow cell was a glass spiral (2 mm i.d, 600 μl internal volume) positioned in front of a mirror in a sealed housing. The signals from the PMT (RCA 931 VA) were sent to a computing integrator (Philips PU 4815) and then to an IBM-compatible computer (model 486 DX4) using an RS232 port.

A filter fluoremeter LS-2 B Perkin-Elmer was used for recording the CL spectra.

2.3. General procedure

The FIA configuration used is outlined in Fig. 1. In order to achieve good mechanical and thermal stability, the instruments were allowed to run for 10 min before the first measurement was made. A solution of 5×10^{-2} M oxidizing agent (NBS or NCS) and 5×10^{-2} M NaOH (carrier stream) were each pumped at 3.5 ml min^{-1}. The blank solution which only contained 5×10^{-7} M luminol was injected into the carrier stream with the aid of an injection valve with a 600 μl loop and a stable blank signal was recorded. Then the sample or standard isoniazid solutions which contained not only 5×10^{-7} M 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.

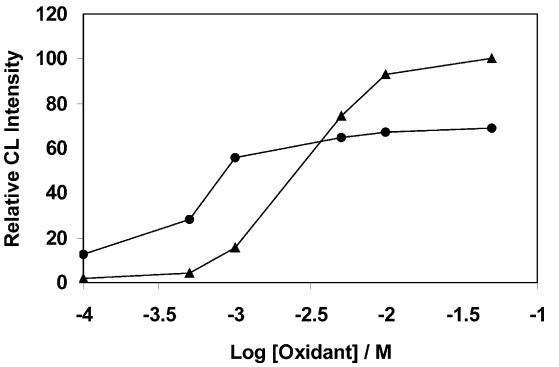

Fig. 2. Effect of NBS (●) and NCS (▲) concentrations on the CL intensity. Conditions: 5×10^{-7} M luminol; 1000 ng ml^{-1} isoniazid; 5×10^{-2} M NaOH; 3.5 ml min^{-1} flow rate.

2.4. Sample preparation

At least 20 isoniazid tablets (from Iran Daru Pakhsh) were weighed, ground to a fire powder and mixed. A sample equivalent to approximately 200 mg of isoniazid was weighed accurately, transferred into a 1 l calibrated flask and diluted 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 in the final solution was within the working range. The final solution should also contain 5×10^{-2} M sodium hydroxide and 5×10^{-7} M luminol.

3. Results and discussion

In the determination of isoniazid based on the CL methods, CL reactions that occurs by the action of oxidants containing positively charged chlorine and bromine atoms are restricted to hypobromite, hypochlorite and NBS [20,22,23]. Recently, we reported observation of CL phenomena accompanied with reaction between either NBS or NCS with sulfide in alkaline medium and proposed applying these CL systems to the determination of sulfide [26]. In addition to this, the reaction between either NBS or NCS as oxidant with luminol was chemiluminescent. These CL systems were applied to the determination of

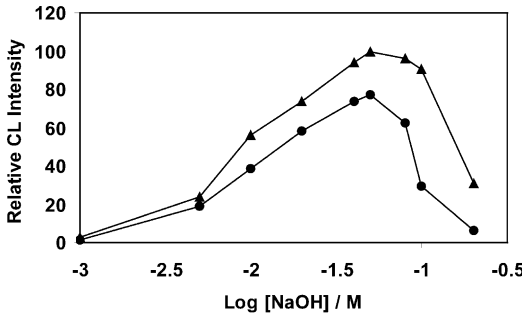

Fig. 3. Effect of NaOH concentration on the CL intensity of NBS-luminol (●) and NCS-luminol (▲) CL systems. Conditions: 5×10^{-7} M luminol; 1000 ng ml^{-1} isoniazid; 5×10^{-2} M NBS and NCS; 3.5 ml min^{-1} flow rate.

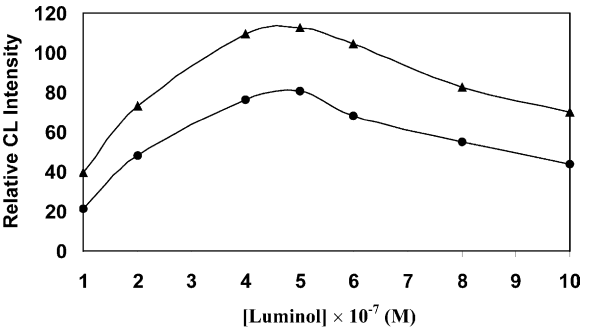

Fig. 4. Effect of luminol concentration on the relative CL intensity of NBS-luminol (●) and NCS-luminol (▲) CL systems. Conditions: 1000 ng ml^{-1} isoniazid; 5×10^{-2} M NBS and NCS; 5×10^{-2} M NaOH; 3.5 ml min^{-1} flow rate.

the above oxidants [27] and indirect determination of cationic surfactants [28]. In our preliminary experiments it was found that isoniazid greatly enhanced the CL due to both NBS-luminol and NCS-luminol reactions. Based on this strong enhancing effect a method was proposed for the determination of isoniazid.

A series of experiments were conducted to establish the optimum analytical conditions for the CL oxidation of luminol by NBS and NCS in the presence of isoniazid.

3.1. Effect of NBS and NCS concentrations

The effect of oxidant concentration on the CL intensity of luminol in the presence of isoniazid was investigated. The CL was found to increase with increasing the concentration of oxidizing agent (NBS or NCS) in the range of 1×10^{-4}–5×10^{-2} M. Further increase in oxidizing agent concentration was avoided due to the limited solubility of NBS and NCS in water. The maximum responses were obtained at 5×10^{-2} M for both oxidants (Fig. 2). Thus, this concentration was chosen as the most suitable for further studies. It should be noted that under similar conditions the stronger oxidizing agent, NCS, promotes higher CL intensity than NBS.

3.2. Effect of NaOH concentration

The effect of NaOH concentration on the CL intensity of luminol in the presence of isoniazid was investigated. The CL was observed only in an alkaline medium. The CL was found to increase with increasing the concentration of NaOH in the range of 1×10^{-3}–5×10^{-2} M. Further increase in NaOH concentration caused a decrease in emission intensity (Fig. 3). Thus, 5×10^{-2} M was chosen as the optimum concentration for both CL systems.

3.3. Effect of luminol concentration

The concentration of luminol had a very important effect on the relative CL intensity for the determination of isoniazid. Thus, the effect of luminol concentration on the CL intensity was investigated from 1×10^{-7} to 1×10^{-6} M, the results of which are shown in Fig. 4. The results show that 5×10^{-7} M luminol yielded the highest relative CL intensity for both CL systems, and when the concentration of luminol was lower or higher than this concentration, the relative CL intensity decreased. Thus, the optimum concentration 5×10^{-7} M of luminol was selected for further work.

3.4. Effect of flow rate

The effect of flow rate on the intensity of both CL systems was studied over the range 0.3–4.3 ml min^{-1} in each stream. A general improvement in the emission intensity was obtained with an increase in the flow rate in the range of 0.3–3.5

ml min^{-1}, but higher flow rates not only led to greater consumption of reagents but also caused irreproducibility. So, the chosen value for flow rate was 3.5 ml min^{-1} for both systems, at which samples could be analysed at a rate of about 65 samples h^{-1}, with relative error of about 2.8%.

3.5. Effect of sample volume

The variation of CL emission with the injected sample volume in the 50–700 μl range was studied. The results showed that higher CL intensity was obtained by increasing loop volumes up to 600 μl for both CL systems. Thus, in the present CL systems, a 600 μl loop was selected for subsequent investigations.

3.6. Suggestion of possible mechanism for the CL systems

For obtaining the possible CL reaction mechanism of this sensitizing kind of luminol-based CL reaction and the possible CL emitter, a series of experiments were performed and the results were as follows.

First, the CL spectra were taken using an LS-2 B filter fluoremeter. The results showed that for both CL systems the maximum CL emission wavelength was 425 nm. This showed that the possible CL emitter was the excited state of 3-aminophthalate [29–34].

Second, it was found that N_2H_4 has a similar enhancing effect as isoniazid for the CL intensity of NBS-luminol and NCS-luminol CL systems. This result showed that the hydrazine group in the isoniazid was the key enhancing CL active functional group.

Third, it was found that the CL emission intensity of both NBS- and NCS-luminol CL systems were also enhanced by H_2O_2 when present in the oxidants (NBS or NCS) stream.

Fourth, it was found that, when ascorbic acid as oxygen cleaning reagent was added to the luminol solution containing isoniazid, a decrease in CL signal was observed for both CL systems.

Fifth, it was found that, when all the solutions in these CL systems were deareated with pure argon gas, the enhancing function of isoniazid for CL signals nearly disappeared. This result showed that the dissolving oxygen in the solutions was another key species in the proposed CL systems.

Based on the above experimental results and the chemical property of isoniazid, it was suggested that the dissolved oxygen (in luminol solution containing isoniazid) is reduced by isoniazid and some H_2O_2 is possibly produced [35]. Thus, when isoniazid was merged with the oxidant solution (OBr^- and/or OCl^- produced from NBS and NCS hydrolysis, respectively), active oxygen (possibly the superoxygen anion radical) is produced by the chemical reaction between these oxidants and H_2O_2 [36]. Then, the active oxygen can oxidize luminol to produce a stronger CL emission intensity.

Table 1
Recoveries of 1000 ng ml^{-1} isoniazid from solutions with a 10-fold concentration of various additives used as excipients

Additive	Recovery, % ($n = 3$)	
	With NBS	With NCS
Glucose	98.1	97.7
Galactose	98.8	98.6
Lactose	99.5	99.5
Sorbitol	98.6	98.5
Sugar	100.5	99.1
Starch	99.6	99.4
EDTA†	98.9	98.2
Carbowax‡	100.8	101.0
Sodium lauryl sulfate	99.7	99.5
Talc	101.2	101.7
Calcium sulfate	100.4	99.4
Sodium citrate	100.9	100.7

† Ethylenediamino-*N*,*N*,*N*′,*N*′-tetraacetic acid.
‡ Polyethylene glycol 4000.

3.7. Interference study

In order to assess the possible analytical applications of the proposed methods, the effect of common excipients used in pharmaceutical preparations was studied by analysing synthetic sample solutions containing 1000 ng ml^{-1} of isoniazid and 10-fold concentration of each excipient. The undissolved material, if any, was filtered before measurement. The recovery results are given in Table 1. No interference was observed

Table 2
Recoveries of 1000 ng ml^{-1} isoniazid from solutions with some co-existing compounds

Compound	Concentration ratio (Compound to isoniazid)	Recovery, % ($n = 3$)	
		With NBS	With NCS
Ascorbic acid	1	86.2	89.0
Thiamine hydrochloride	10	106.3	104.5
Nicotinic acid	10	101.6	104.2
Nicotinamide	100	102.0	102.8
Isonicotinic acid	10	102.7	103.5
Riboflavin	1	148.5	157.4
Pyridoxine hydrochloride	10	101.3	102.6
p-Aminosalicylic acid	10	95.4	96.2
Streptomycin sulfate	100	103.3	103.8
Calcium pantothenate	10	100.7	101.0
Hydrazine sulfate	1	144.0	155.6

Table 3
Analytical figures of merit for the determination of isoniazid

Oxidant	Range of application (ng ml^{-1})	Calibration equation (RCI[†] vs. C[‡])	Detection limit* (ng ml^{-1})	RSD**
NBS	8–600	$RCI = 7.00(\pm1.26)^a + 0.137(\pm0.007)^b C$ ($r = 0.997$; $n = 10$)	4.0	2.3[c], 2.6[e]
	600–5000	$RCI = 18.67(\pm2.52)^a - 0.014(\pm0.002)^b C$ ($r = 0.996$; $n = 6$)		2.9[d], 2.4[f]
NCS	6–200	$RCI = 5.20(\pm1.05)^a - 0.440(\pm0.016)^b C$ ($r = 0.997$; $n = 9$)	3.0	2.4[c], 2.7[e]
	200–2000	$RCI = 24.02(\pm3.37)^a - 0.038(\pm0.005)^b C$ ($r = 0.997$; $n = 6$)		2.8[d], 3.2[f]

[a] and [b] correspond to intercept (±S.D.) and slope (±S.D.) of calibration equation, respectively. [c] and [d] correspond to repeatability (within-run precision) of 20 and 1000 ng ml^{-1} isoniazid, respectively. [e] and [f] correspond to reproducibility (analysing on five different days) of 20 and 1000 ng ml^{-1} isoniazid, respectively.

[†] RCI is the relative CL intensity.

[‡] C is isoniazid concentration (ng ml^{-1}).

* Theoretical detection limit (blank plus three times its S.D.) [40].

** RSD is the relative standard deviation.

from any of the excipients tested, which showed recoveries in the range 98.1–101.2 and 97.7–101.7% for NBS-luminol and NCS-luminol CL systems, respectively.

The effect of some common co-existing compounds on the recovery of 1000 ng ml^{-1} of isoniazid was studied by analysing synthetic samples, as for the excipient study, but with various amounts of each co-existing compound (Table 2). No interference was observed from most co-existing compound studied; only hydrazine, riboflavin and ascorbic acid appeared to interfere with isoniazid for both CL systems. As was to be expected, hydrazine has a similar enhancing effect as isoniazid for the CL intensity of both NBS-luminol and NCS-luminol CL systems. Riboflavin has also enhancing effect on the emission intensity of these CL systems. A possible reason is that riboflavin can catalyze the formation of H_2O_2 with O_2 in solution [37], and hence can enhance the CL emission intensity. Ascorbic acid decreases the CL intensity. The interference of ascorbic acid was eliminated when the synthetic sample solution was measured after ≥ 1 h, due to rapid oxidation of

Table 4
Results of determination of isoniazid in tablet samples

Sample*	Isoniazid found (mg per tablet)			Recovery (%)		Relative difference (CL–BP), %
	Claimed	CL†	BP‡	CL	BP	
1	100	(101.9±3.5)[a]	100.8	101.9[a]	100.8	+1.1[a]
		(102.4±4.2)[b]		102.4[b]		+1.6[b]
2	300	(311.6±4.4)[a]	307.5	103.9[a]	102.5	+1.4[a]
		(312.2±4.3)[b]		104.1[b]		+1.6[b]

[a] and [b] correspond to NBS and NCS oxidizing agents, respectively.
* Samples 1 and 2 were isoniazid tablet samples of Iran Daru Pakhsh.
† Mean of three replicates (±RSD, %).
‡ Average value of three determinations.

ascorbic acid by atmospheric oxygen in alkaline medium [38,39].

3.8. Analytical figures of merit

Under the selected conditions given above, calibration graphs of relative CL intensity versus isoniazid concentration (ng ml^{-1}) were obtained for each of the chemiluminescent systems described. Table 3 shows the results.

3.9. Application

Following the procedure described in Section 2.4, the proposed method was applied for the determination of isoniazid in pharmaceutical preparations. The results were compared with those obtained by the British Pharmacopoeia (BP) official method [1]. The results are given in Table 4 and agree well with those obtained by an official method.

4. Conclusions

The potential application of NBS and NCS as organic CL oxidizing agents has been demonstrated in this work. The main advantages of these oxidants over other common oxidants are that these oxidants are more stable, colorless, provides higher sensitivity and they have the ability of being dissolved in the aqueous and nonaqueous solvents.

This work has shown that the proposed FIA-CL method based on NBS and NCS as oxidants can be successfully used for the determination of isoniazid. NBS and NCS are more stable than oxidants such as hypobromite and hypochlorite that have been used as oxidants for detemination of isoniazid based on CL method [22,23]. Hypobromite and hypochlorite oxidants usually are prepared in situ since they disproportionate easily. On the other hand, the dynamic range and detection limits of the proposed method for isoniazid determination are better than the previously reported CL methods [20–23]. This method is very simple, rapid, sensitive, inexpensive and sufficiently accurate and precise and is useful for the determination of isoniazid in pharmaceutical dosage forms.

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