Determination of Isoprenaline with Lucigenin Chemiluminescence Using Flow Injection Analysis

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A procedure is presented for determining isoprenaline (10^{-7} – 10^{-4} M) in a flow injection system using chemiluminescence detection resulting from the reaction of isoprenaline with 10,10'-dimethyl-9,9'-biacridinium dinitrate (lucigenin) in aqueous solution of potassium hydroxide as a carrier stream. The logarithmic calibration curve was linear over the range from 1×10^{-7} M to 1×10^{-4} M. The effects of some analytical conditions on the chemiluminescence yield were investigated. The RSD at 1×10^{-4} M isoprenaline is 0.9% (10 replicates). The method is directly applicable to aqueous solutions of isoprenaline injection (1 mg/5 ml).

Keywords Isoprenaline, flow injection analysis, lucigenin chemiluminescence, pharmaceutical preparation

Isoprenaline belongs to an important class of clinical drugs, the catecholamines. Many papers have been published on the determination of catecholamines using batch techniques, such as nonaqueous titrimetry^{1,2}, high-performance liquid chromatography (HPLC) with electrochemical³ or fluorometric detection^{4,5}, radio-immunoassay⁶, spectrophotometry⁷⁻¹⁰, and chemiluminescence.¹¹ Flow injection chemiluminescence methods have been shown to be reliable for the determination of trace metal ions and organic compounds with good reproducibility, selectivity and sensitivity.¹²⁻¹⁴

This paper describes a selective and sensitive method for the determination of isoprenaline utilizing lucigenin chemiluminescence (CL) detection. Applications of the method to the determination of the drug in some pharmaceutical preparations are also proposed.

Experimental

Reagents and chemicals

Distilled deionized water was used throughout. 10,10'-Dimethyl-9,9'-biacridinium dinitrate (lucigenin) was purchased from Aldrich, isoprenaline sulfate from Sigma and AnalaR potassium hydroxide from BDH. All chemicals were used as received with no further purification. A stock solution (1×10^{-2} M) of isoprenaline sulfate was prepared daily by dissolving 0.2783 g of pure substance in 50 ml of distilled water. This stock solution was stored in amber-colored bottles in a refrigerator. The lucigenin stock solution (1×10^{-2} M) was prepared by dissolving 0.5105 g of the chemical in 100 ml of distilled water. The base solutions were prepared from potassium hydroxide. Working

standard solutions were prepared by appropriate dilution of the stock solutions.

Apparatus

All (CL) measurements were made on an apparatus which was described previously.¹⁴

Flow system for the determination of isoprenaline by CL

A schematic diagram of the manifold is shown in Fig. 1. An aqueous solution of potassium hydroxide acting as a carrier stream for isoprenaline was supplied through R₁ and the lucigenin solution through R₂. The drug sample was injected into the alkaline stream from a 50- μ l Teflon rotary valve injector (Rheodyne RH-5020). The streams were propelled by a four-channel peristaltic pump (Gilson Minipuls 3MP4) and mixed at a PTFE T-piece before passing to a coiled glass flow cell in front of a photomultiplier tube in the usual way. Both streams

Fig. 1 Manifold used for chemiluminescent determination of isoprenaline: R₁, carrier stream; R₂, chemiluminogenic reagent solution; P, pump; S, injection valve; W, waste; T, perspex T-piece; F, coiled flow cell; D, detector; R, chart recorder; L, light-tight box.

Fig. 2 Variation of chemiluminescence intensity: A, with KOH concentration in presence of 1×10^{-4} M isoprenaline and 1×10^{-3} M lucigenin; B, with lucigenin concentration in presence of 1×10^{-4} M isoprenaline and 0.1 M KOH.

were pumped at the same flow rate *via* the Gilson pump (3.9 ml min^{-1} in each channel). PTFE tubing (0.8 mm i.d.) was used throughout the remainder of the manifold. The light emission was detected by a photomultiplier without wavelength discrimination. The resultant photocurrent was converted to a voltage that was displayed on a chart recorder (Yokogawa Model 3021) and the peak heights were measured manually. All results are the mean of four injections of the sample, unless stated otherwise.

Optimization of the CL flow system

The optimum reaction conditions for isoprenaline determination were investigated by injecting $50 \mu\text{l}$ of the drug (1×10^{-4} M) into the carrier stream with flow rates of 3.9 ml min^{-1} in each channel. The potassium hydroxide and lucigenin concentrations, the total flow rate and sample volume, all of which affect the intensity of the CL signal, were studied.

Effect of potassium hydroxide concentration

The dependence of the CL signal upon KOH concentration was examined in the range 0.01–0.5 M. The results of the KOH optimization study is shown in Fig. 2A. The chemiluminescent intensity exhibited a maximum at 0.1 M, which was selected for the analysis.

Effect of lucigenin concentration

The parameter next optimized was the lucigenin reagent. The optimization curve for lucigenin con-

Fig. 3 Effect of total flow rate on chemiluminescence intensity from 1×10^{-4} M isoprenaline, 1×10^{-3} M lucigenin and 0.1 M KOH solution.

centrations is shown in Fig. 2B. The CL intensity of isoprenaline increased with an increase in the lucigenin concentration. Above 5×10^{-3} M the CL intensity decreased; this might be due to an increase in intensity of the colored lucigenin solutions which would reduce the emission intensity due to the quenching effect. Thus, the optimum lucigenin concentration for isoprenaline was found to be 1×10^{-3} M.

Effect of flow injection variables

Volume of injected isoprenaline solution. Using a 1×10^{-4} M isoprenaline solution and the optimized reagent concentration, the volume of injected isoprenaline was evaluated. The obtained results showed an increase in the emission intensity up to $50 \mu\text{l}$, after which no significant increase was observed. It was therefore decided to continue the experimental optimization using this volume.

Effect of flow rates. The effect of the flow rate on the CL signal was studied. The results obtained are shown in Fig. 3, and indicate that a sufficiently high flow rate should be used to enable the excited product to reach the detector in a minimum time; hence maximum collection of the emitted light can be achieved. In this instance, the intensity increased with increasing flow rate. As the total flow rate was increased from 3.4 to 11.5 ml min^{-1} , the emission intensity also increased. At flow rates higher than 7.8 ml min^{-1} , no significant increase in the emission intensity was obtained and therefore a flow rate of 7.8 ml min^{-1} was adopted for isoprenaline determination.

Results and Discussion

Analytical performance

Under the above-mentioned optimized experimental conditions, a log-log calibration graph for isoprenaline determination was constructed over the range 1×10^{-7} – 1×10^{-2} M. The linear dynamic range was over three orders of magnitude between 1×10^{-7} M and 1×10^{-4} M.

Table 1 Effect of potential interferences (all 1×10^{-3} M) on the signal for 1×10^{-4} M isoprenaline

Interferent	Signal height	
	mV ^a	% ^b
—	20800	100
Ca(NO ₃) ₂ ·4H ₂ O	14300	68.80
CdCl ₂ ·H ₂ O	11300	54.33
Co(NO ₃) ₂ ·6H ₂ O	330	1.60
Mg(NO ₃) ₂ ·6H ₂ O	18300	87.98
Ni(NO ₃) ₂ ·6H ₂ O	8600	41.35
Pb(NO ₃) ₂	5100	24.52
NH ₄ VO ₃	6200	29.81
ZnCl ₂	13500	64.90
HgCl ₂	5500	26.44
CuCl ₂	7	0.03
Fructose	19400	93.27
Glucose	19200	92.31
Nicotinamide	19300	92.79
Carbowax ^c	19300	92.79
Riboflavin	16300	78.37
Mg stearate ^d	10500	50.50

a. Results based on four injections per sample.

b. Relative to isoprenaline solution=100%.

c. [Carbowax]= 1×10^{-4} M; [isoprenaline]= 1×10^{-5} M.

d. [Mg stearate]= 5×10^{-4} M; [isoprenaline]= 5×10^{-5} M.

The linear range allows the determination of 1×10^{-7} M levels of isoprenaline as a limit of detection ($2 \times$ blank standard deviation). The relative standard deviation (RSD) is 0.9% for 1×10^{-4} M isoprenaline solution (10 replicate injections).

Interferences

The chemiluminescent reaction apparent from the isoprenaline-lucigenin system is certainly of analytical value. Therefore, the specificity of the method for isoprenaline in the presence of frequently encountered excipients and some inorganic ions was studied. The results obtained are summarized in Table 1.

Among the potential interferents, Ca²⁺, Cd²⁺, Co²⁺, Ni²⁺, Pb²⁺, V⁵⁺, Zn²⁺, Hg²⁺, Cu²⁺, Mg²⁺, riboflavin and magnesium stearate interfered severely in the determination by decreasing the emission intensity. Nicotinamide and carbowax also affected the signal response, but their effects were mild compared with those above-mentioned interferents. Fructose and glucose did

Table 2 Determination of isoprenaline in a pharmaceutical preparation by the proposed method

Preparation	Amount of isoprenaline/M		Recovery, %
	Claimed	Found ^a	
Isoprenaline injection (1 mg/5 ml) (Elkins-Sinn)			
Sample 1	1 ×10 ⁻⁶	1 ×10 ⁻⁶	100
Sample 2	1.5×10 ⁻⁶	1.5 ×10 ⁻⁶	100
Sample 3	2 ×10 ⁻⁶	2.05×10 ⁻⁶	102.5

a. Average of four determinations per sample.

interfere, as was confirmed by other workers.¹⁵⁻¹⁷ These results suggested the possibility of using lucigenin reaction to develop CL methods of analysis for such compounds. However, the interference from these species, namely, the inorganic ions can be obviated by using 1×10^{-2} M EDTA as a masking agent.

Since the reaction does not differentiate between these compounds, the analysis would be most direct if the analytes were separated from each other. Thus, a major strength of this CL reaction could lie in its application as a detector for HPLC.

Analysis of pharmaceutical preparations

The method was evaluated by determining isoprenaline in a sample of isoprenaline injection (Elkins-Sinn, NJ), which contained *ca.* 1 mg of isoprenaline hydrochloride per 5 ml. The results obtained are summarized in Table 2; most of them agreed with reported values.

It is considered of interest to compare the figures of merit for this proposed procedure with those of another spectrophotometric assay for catecholamines, reported by Salem.¹⁰ Salem's method involves reacting the catecholamine drug (dissolved in acetic acid) with *o*-phenylenediamine dihydrochloride reagent in phosphate buffer medium. The absorbance of the orange color produced was measured at 490 nm within 20 min against a blank prepared similarly, but omitting the active ingredients.

Statistical analysis of the results reveals that there is no significant difference between them, as shown in Table 3.

In conclusion, the best sensitivity of the CL reaction of lucigenin with isoprenaline is achieved by using an alkaline medium of potassium hydroxide without any

Table 3 Determination of isoprenaline in a commercial formulation as an injection by the CL and Salem¹⁰ methods

Formulation	Amount of isoprenaline/ $\mu\text{g ml}^{-1}$			Recovery $\pm$ S.D., %	
	Claimed	CL ^a	Salem ^a	CL	Salem
Isoprenaline injection (1 mg/5 ml) (Elkins-Sinn)					
Sample 1	1.4	1.4	1.4	100 $\pm$ 0.35	100 $\pm$ 0.77
Sample 2	3.45	3.42	3.38	99.1 $\pm$ 0.90	98.0 $\pm$ 0.86
Sample 3	1.11	1.14	1.16	102.7 $\pm$ 0.15	104.5 $\pm$ 0.45

a. Average of five determinations per sample.

oxidizing agent (*i.e.* H_2O_2 , KMnO_4 , OCl^- , BrO_3^- ...etc.). The results obtained are sufficiently sensitive to be used for postcolumn detection of isoprenaline after HPLC separation and could thus be applied to the detection of traces of isoprenaline in body fluids.

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