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Flow-injection inhibition chemiluminescence determination of indapamide based on luminol–ferricyanide reaction

Zhouping Wang, Zhujun Zhang*, Xiao Zhang, Zhifeng Fu

Department of Chemistry, Analytical Science Institute, Southwest China Normal University, Chongqing 400715, China

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Abstract

A novel and sensitive chemiluminescence (CL) method for the determination of indapamide coupled with flow-injection analysis (FIA) technique is developed in this paper. It is based on the inhibition effect of the studied drug on the chemiluminescence emission of luminol–potassium ferricyanide system. Under the optimum conditions, the decreased CL intensity is proportional with the concentration of indapamide in the range of 1×10^{-8} to 1.0×10^{-6} g ml^{-1}. The detection limit is 3.4×10^{-9} g ml^{-1} (3σ). A complete analysis could be performed in 45 s including washing and sampling, giving a throughout of about 90 h^{-1}. The relative standard deviation (R.S.D.) for 11 parallel measurements of 1.0×10^{-7} g ml^{-1} indapamide is 3.0%. The proposed method has been applied for the determination of indapamide in its pharmaceutical formulations. The results obtained compared well with those by an official method. The possible inhibition mechanism of indapamide on luminol–potassium ferricyanide CL system was discussed briefly.

Keywords: Chemiluminescence; Flow-injection; Luminol–potassium ferricyanide; Indapamide

1. Introduction

Indapamide, 3-(aminosulfonyl)-4-chloro-*N*-(2,3-dihydro-2-methyl-1*H*-indol-1-yl)-benzamide (Fig. 1), is an oral antihypertensive diuretic agent indicated for the treatment of hypertensive and edema [1].

Some methods have been developed for the determination of indapamide in different matrixes. For biological fluids analysis, high performance liquid chromatography (HPLC) and CE methods were often proposed for the determination of indapamide [2–10]. An adsorptive stripping method at carbon paste electrode modified with castor oil for trace determination of indapamide in spiked serum was also described [11]. For the detection of indapamide in pharmaceutical preparations, a liquid chromatographic assay and a spectrophotometry have been proposed as a standard method in UPS XXIV and Chinese pharmacopoeia [12,13]. Two new colorimetric methods based on the oxidation of indapamide with iron(III) in acid medium have been used for direct determination of indapamide in pharmaceutical preparations [14]. İüslü and Altınöz [15] have developed two derivatives spectrophotometrically for indapamide determination, that carried out using the first-derivative values measured

* Corresponding author. Tel.: +86-23-6825-3863; fax: +86-23-6825-3863.
E-mail addresses: zzj18@hotmail.com, wangzp1974@hotmail.com (Z. Zhang).

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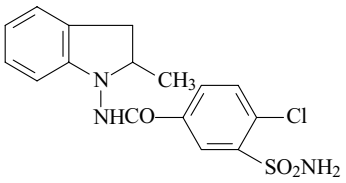

Fig. 1. Molecule structure of indapamide.

at 252.8 nm ($N = 6$) and the second-derivative values measured at 260.4 nm ($N = 9$). A first-derivative spectrophotometry with a zero-crossing technique of measurement and a ratio derivative spectrophotometry were proposed for indapamide determination in pharmaceutical formulations, in the presence of perindopril by Erk [16]. A reversed-phase HPLC based on a reversed-phase column using a mobile phase of phosphate buffer pH 2.4 and acetonitrile (7:3 v/v) was also described by the same author. Fluorimetry [17], gas chromatography–mass spectrophotometry [18–20], and thin layer chromatography [21] have been used for the determination of indapamide. However, these methods are often costly, tedious, time consuming and/or requiring prior separation procedure, or suffer from the disadvantages of low sensitivity and the use of volatile organic solvents. As an alternative to it, establishment of simple, rapid and sensitive analytical methods are necessary.

Chemiluminescence (CL) analysis offers high sensitivity, wide linear range and simple instrument. When coupled with flow-injection analysis (FIA), the CL-based FIA methods provide cheap, rapid, simple and reproducible means of detection, and therefore, have been successfully applied to many drugs detection [22–26]. To the best of our knowledge, however, there is a lack of information concerning CL assay of indapamide in literature. The aim of this work was to investigate the utility of CL in the detection of indapamide in pharmaceutical preparations without the necessity of sample pre-treatment.

In the present work, it was found that potassium ferricyanide could oxidize luminol to produce strong CL radiation in alkaline medium and the CL intensity could greatly be inhibited by the presence of indapamide. The decreased CL intensity is proportional with the concentrations of indapamide in certain range. Based on the observations, coupled with flow-injection analysis, a novel and sensitive inhibition CL method was developed for the rapid determination of trace amount of indapamide. Compared to other methods proposed for indapamide detection, this method is simple, rapid, inexpensive, and do not use volatile organic solvents and has been applied to the content detection of indapamide in its pharmaceutical preparations. The results obtained, compared well with those by an official method [13]. The possible inhibition mechanism of indapamide on luminol–potassium ferricyanide system was discussed briefly.

2. Experimental

2.1. Reagents

Analytical reagent-grade chemicals and deionized, doubly distilled water were used throughout. Stock solutions of indapamide (1×10^{-5} g ml^{-1}) were prepared by dissolving 10 mg indapamide standard substance (Drug and Biological Products Examination Institute of China, Beijing, China) daily, with 10 ml of 0.1 mol l^{-1} Na_2CO_3 solution and adding 200 ml water, surging with an ultrasonic cleaning machine for 20 min and further diluted to 1000 ml with water, then stored in the refrigerator (4 °C). Working standard solutions were prepared from the stock solution by appropriate dilution with water before use. Potassium ferricyanide stock solution (0.01 mol l^{-1}) was prepared daily, by dissolving 0.8231 g of potassium ferricyanide (Shanghai Chemical Reagent Company, China) in water, and diluting to 250 ml in an amber-colored measuring flask. Luminol stock solution (0.01 mol l^{-1}) was prepared by dissolving 1.7720 g of luminol (synthetized by Department of Chemistry, Shaanxi Normal University, China) in 0.05 mol l^{-1} NaOH solution, and diluting to 1 l with same NaOH solution in an amber-colored measuring flask. Stock NaOH solution (1.0 mol l^{-1}) was prepared daily, by dissolving 4.0 g sodium hydroxide (Chongqing Chemical Reagent Plant, China) in water, and diluting to 100 ml with water.

The possible minimum number of dilution steps was used to prepare more dilute solutions. All other chemicals were of the best grade available and were used as received.

Indapamide tablets (1) from Jinan Gaohua Pharmaceutical Factory, Shandong, China; (2) from Tianjin

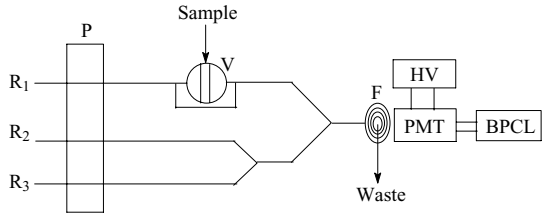

Fig. 2. Schematic diagram of the FIA–CL manifold employed for the determination of indapamide. R_1, water carrier; R_2, luminol solution; R_3, potassium ferricyanide solution; P, peristaltic pump; V, injection valve; F, CL flow cell; PMT, photomultiplier tube; HV, negative high-voltage supply; BPCL, luminescence analyzer controlled by personal computer.

Lisheng Pharmaceutical Stock Co. Ltd, Tianjin, China) were obtained from local hospital.

2.2. Apparatus

The schematic diagram of the flow system employed in this work is shown in Fig. 2. A peristaltic pump (type HL-2, manufactured at Huxi Instrument and Meter Plant, Qingpu, Shanghai, China) was used to deliver flow streams in this system. PTFE tubing (0.8 mm i.d.) was used as connection material in the flow system. Sample solution (50 μl) was injected into the carrier stream (water) using a eight-way injection valve equipped with a 50 μl sample loop, merged with the mixture of luminol (in 0.1 mol l^{-1} NaOH) and potassium ferricyanide and then reached the flow cell, decreasing CL emission. The change of CL signal 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, Beijing, China). The glass flow cell was located directly facing the window of the CR-105 photomultiplier tube (Hammamatsu, Tokyo, Japan). Data acquisition and treatment were performed with BPCL software running under Windows 98. CL spectra of the proposed system was investigated using a Model RF-540 Fluorospectrophotometer (Shimadzu, Japan). UV-Vis absorption spectra was achieved with a Model U-2001 Spectrophotometer (Hitachi, Japan).

2.3. Procedure for calibration

In order to obtain good mechanical and thermal stability, the instruments were run for 10 min before the first measurement. Flow lines were inserted with luminol solution, potassium ferricyanide solution, water carrier and sample solution. The flow rate was fed at 2.0 ml min^{-1} for all lines. The pumps were started to wash the whole system until a stable baseline was recorded. The 50 μl of sample solution was injected into the carrier stream. This stream was merged with the mixture of potassium ferricyanide and luminol solution and then reached the flow cell, accompanying the change of CL emission. The concentration of sample was quantified by the relative decreased CL intensity.

2.4. Procedure for pharmaceutical preparations

At least 20 of indapamide tablets (claimed contrast 2.5 mg per tablet) were weighed to obtain the mean tablet weight and ground to homogenized powder; a portion of the powder corresponding to 0.01 g was weighed and dissolved with 10 ml of 0.1 mol l^{-1} Na_2CO_3 solution and added 200 ml water, surged with an ultrasonic cleaning machine for 20 min and further diluted to 1000 ml with water. After filtering, aliquots of the filter were further diluted with water so that the concentration of indapamide was in the working range of determination of indapamide. Repeat the procedure described as above. The concentration of indapamide was quantified by the relative decreased CL intensity.

According to Chinese Pharmacopoeia (2000), spectrophotometry was used as an official method for contrast determination of indapamide in its commercial tablets [13]. Precisely weigh certain indapamide standard substance, add ethanol and make it to be 7.5 μg ml^{-1} solution. Take 20 pieces of indapamide tablets and precisely quantify. After porphyrize, measure appropriate quantity (corresponds to 7.5 mg indapamide) and transfer into a 100 ml measuring flask, add proper ethanol, shake adequately to make indapamide dissolve. Then add ethanol to the mark-up to the line, shake up and filter out. Precisely measure off 5 ml continuous filtrate, transfer into 50 ml measuring flask, add ethanol to the mark line and shake up. Finally, measure off appropriate standard and sample solution, according to spectrophotometry, measure the absorbance at 242 nm wavelengths and calculate the content of indapamide.

3. Results and discussion

3.1. Condition optimization of the CL system

3.1.1. Selection of oxidant and the effect of its concentration on the CL intensity

Redox reaction is often the basis of CL reaction. In the molecule structure of indapamide, there is indol group and oxidizable amide group, which make the redox reaction between indapamide and some typical oxidants used in CL reactions take place possibly. Therefore, various oxidants, including ferricyanide, permanganate, periodate, hydrogen peroxide, Ce(IV), and *N*-bromosuccinimide, were used for the CL reaction of indapamide. The results show that no CL signal was recorded when these oxidants were used alone. However, in the presence of luminol, the most significantly decreased CL signal was recorded when potassium ferricyanide was used as oxidant in basic medium. Therefore, a procedure based on the inhibition effect of indapamide on luminol–potassium ferricyanide CL reaction was proposed. Potassium ferricyanide was chosen as optimum and the effect of its concentration on the decreased CL intensity was further examined from 1×10^{-6} to 5×10^{-4} mol l^{-1}. The results show that at the concentration higher and lower than 1×10^{-5} mol l^{-1} there was a decrease in the decreased CL intensity. Thus, 1×10^{-5} mol l^{-1} was selected as optimum concentration of potassium ferricyanide throughout the research.

3.1.2. Effect of luminol concentration on the CL intensity

The effect of the concentration of luminol on the decreased CL intensity was investigated over the range of 1×10^{-6} to 7×10^{-5} mol l^{-1}. It was found (Fig. 3) that decreased CL intensity reached a maximum value when luminol concentration was 1×10^{-5} mol l^{-1}. Thus, the luminol concentration of 1×10^{-5} mol l^{-1} was chosen for consequent research work.

3.1.3. Effect of NaOH concentration on the CL intensity

The effect of NaOH concentration on the decreased CL intensity was examined from 0.01 to 0.3 mol l^{-1} with 1×10^{-7} g ml^{-1} indapamide (Fig. 4). Maximum decreased CL intensity was obtained when the concentration of NaOH was fed at 0.1 mol l^{-1}.

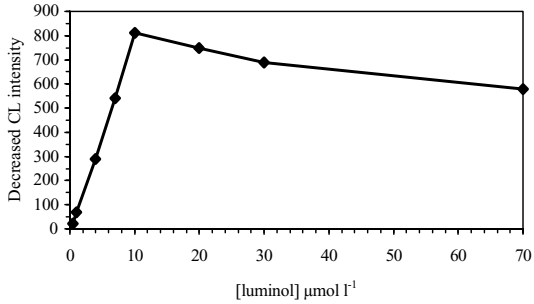

Fig. 3. Effect of concentration of luminol on the CL intensity of the system. Potassium ferricyanide, 1×10^{-5} mol l^{-1}; NaOH, 0.2 mol l^{-1}; indapamide 1×10^{-7} g ml^{-1}; flow rate, 2.0 ml min^{-1}.

Thus, 0.1 mol l^{-1} NaOH was chosen as optimum for consequent experiments.

3.1.4. Effect of flow rate on the CL intensity

The flow rate is an important factor in flow-injection CL system. An optimum flow rate of delivering the rejected product is necessary for the maximum collection of the emitted light in the flow cell. The effect of flow rate on the CL emission was tested in the range of 0.1–4.0 ml min^{-1}. The result showed that the highest decreased CL intensity was achieved when the flow rate of each line was fed at 2.0 ml min^{-1}. So, 2.0 ml min^{-1} was used as the optimum flow rate throughout the experiment. At the flow rate, a complete analysis could be performed in 45 s including washing and sampling, giving a throughput of about 90 h^{-1}.

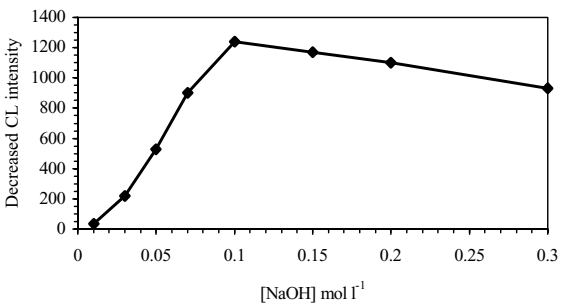

Fig. 4. Effect of concentration of NaOH on the CL intensity of the system. Potassium ferricyanide, 1×10^{-5} mol l^{-1}; luminol, 1×10^{-5} mol l^{-1}; indapamide, 1×10^{-7} g ml^{-1}; flow rate, 2.0 ml min^{-1}.

3.1.5. Effect of Na_2CO_3 on the CL emission

Since Na_2CO_3 was introduced into the standard and sample solution of indapamide, its effect on the system response must be examined carefully. In the experiment, the influence of each Na_2CO_3 concentration corresponding to its concentration in each indapamide solution on the CL emission was used as contrast and compared with that of corresponding indapamide standard solution. It was found that the influence from Na_2CO_3 could be neglected compared with the contribution of indapamide to the decreased CL emission.

3.2. Performance of the proposed method

As a result of the optimization procedure, the decreased CL intensity was proportional with the concentration of indapamide over 1×10^{-8}to1×10^{-6} g ml^{-1}. In order to improve the precision of detection, the calibration curve was drawn at different concentration ranges. The regression equations of calibration curve for indapamide are $\Delta I = 150 + 109.8$[indapamide] $\times 10^8$ (g ml^{-1}) (1×10^{-8} to 1×10^{-7} g ml^{-1}, $r^2 = 0.9982$, $n = 5$) and $\Delta I = 663 + 585$[indapamide] $\times 10^7$ (g ml^{-1}) (1×10^{-7} to 1×10^{-6} g ml^{-1}, $r^2 = 0.9987$, $n = 5$). The calibration of the measurement was performed with concentration of indapamide at 0.01, 0.03, 0.05, 0.07, 0.1, 0.3, 0.5, 0.7, 1.0 μg ml^{-1}. The detection limit is 3.4×10^{-9} g ml^{-1} (3σ). The relative standard deviation (R.S.D.) for 11 parallel measurements of 1.0×10^{-7} g ml^{-1} indapamide is 3.0%. A typical recording output of the proposed CL system for the measurements of different concentrations of indapamide is shown in Fig. 5.

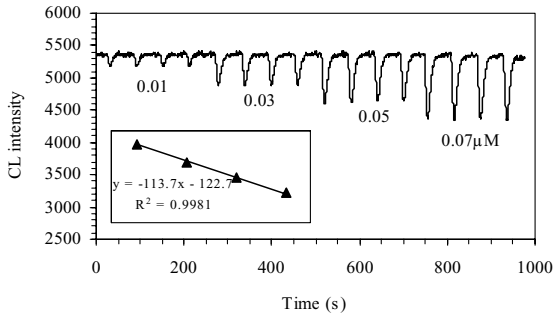

Fig. 5. A typical recording output of the proposed CL system for the measurements of different concentrations of indapamide. Potassium ferricyanide, 1×10^{-5} mol l^{-1}; luminol, 1×10^{-5} mol l^{-1}; NaOH, 0.1 mol l^{-1}; flow rate, 2.0 ml min^{-1}; high voltage, −700 V

3.3. Interference study

In order to determine indapamide in its pharmaceutical preparations, the interference by common ions and excipients in commercial forms of indapamide was investigated using 1×10^{-7} g ml^{-1} indapamide. The results of the tolerable concentration for interference at 5% level were listed in Table 1. Main interferences are from ascorbic acid and heavy metal ions, and the effects of the latter can be eliminated by using EDTA as a masking reagent.

Besides indapamide, main coexisting substances and excipients in tablet form of indapamide are starch, glucose, sucrose, β-cyclodextrin, magnesium stearate etc. It can be seen that the common ions and excipients in tablet form of indapamide showed no influence on the detection of indapamide (Table 1). Therefore, the proposed method could be used directly to determine indapamide in its pharmaceutical preparations.

Table 1
Tolerable concentration level of interferents to 1×10^{-7} g ml^{-1} indapamide

Interferants	Tolerable level (μg ml^{-1})
Starch, glucose, EDTA, Na^+, K^+, Mg^{2+}, Cl^-, NO_3^-	>100
CO_3^{2-}, PO_4^{3-}, SO_4^{2-}	50
Urea, HCO_3^-, NH_4^+	25
Oxalate, lactate, lactose, sucrose, β-cyclodextrin, $H_2PO_4^-$, Ca^{2+}	5
Uric acid, citric acid, magnesium stearate, Al^{3+}	2.5
S^{2-}, HSO_3^-, SO_3^{2-}	0.5
Ascorbic acid, Cd^{2+}, Cr^{3+}, Co^{2+}, Fe^{2+}, Fe^{3+}, Mn^{2+}, Ni^{2+}, Cu^{2+}	0.1

Table 2
Determination of indapamide in pharmaceuticals with the proposed CL procedure and official method

Sample		Concentration (mg) ± S.D. (%)[a]			Relative error
		Labeled	Proposed method	Official method	
Indapamide tablets	No. 1	2.5	2.493 ± 0.28	2.490 ± 0.13	0.003
	No. 2	2.5	2.498 ± 0.57	2.502 ± 0.33	−0.004

[a] Average of five determinations.

3.4. Applications

According to the procedure detailed in Section 2, the proposed method was applied to the determination of commercial preparations of indapamide and the results showed in Table 2. It agreed well with those obtained by an official method [13]. The recovery tests of standard addition were also carried out on the samples and the obtained recoveries were satisfactory (Table 3).

3.5. Possible mechanism of the CL system

It is well known that 3-aminophthalate ion, an oxidized product of luminol, is the emitter of luminol–potassium ferricyanide CL system. The CL emission spectra of the reaction between luminol and potassium ferricyanide is similar to those reported previously for luminol oxidation and showed a maximum at 425 nm [27].

In the present work, in order to get an idea about the reaction product generating the CL, the emission spectra of potassium ferricyanide–luminol CL reaction system in the absence and presence of indapamide was examined by a modified RF-540 fluorospectrophotometer (turn off the light source). The results showed that the maximum emission appeared at 425 nm for the two reactions, and the relative CL intensity was lower when indapamide was presented. It indicated that the CL spectra is independent of indapamide, and revealed that the luminophor of luminol–potassium ferricyanide–indapamide system is still 3-aminophthalate, which is the oxidation product of luminol.

Table 3
The recoveries of standard indapamide addition in pharmaceutical preparations

Sample	Concentration ($\mu g\,ml^{-1}$) ± S.D. (%)[a]			Recovery (%)
	Initially presented	Added	Found	
Indapamide tablet	0.108	0.1	0.205 ± 0.36	98.56
		0.3	0.414 ± 0.94	101.47
		0.5	0.607 ± 1.22	99.84

[a] Mean of five measurements.

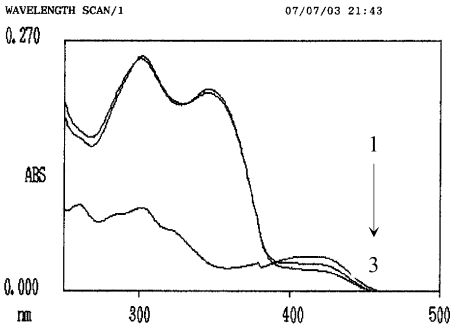

Fig. 6. UV-Vis absorption spectra. (1) Potassium ferricyanide in NaOH solution; (2) luminol–potassium ferricyanide in NaOH solution; (3) luminol–potassium ferricyanide–indapamide in NaOH solution. Luminol, $1 \times 10^{-4}\,mol\,l^{-1}$; potassium ferricyanide, $1 \times 10^{-4}\,mol\,l^{-1}$; indapamide, $5 \times 10^{-6}\,g\,ml^{-1}$; NaOH, $0.1\,mol\,l^{-1}$. Blank water.

There is oxidizable amide group in the molecule structure of indapamide, which make the redox between potassium ferricyanide and the studied drug occurred easily. The consumption of potassium ferricyanide, the oxidant of luminol–potassium ferricyanide system, led to the decrement of the CL intensity. A UV-Vis spectrum was obtained to demonstrate the consumption of potassium ferricyanide in the CL reaction (Fig. 6).

4. Conclusion

The proposed method is simple, sensitive, rapid, suitable for automatic and continuous analysis, and

can be applied to the determination of indapamide in pharmaceutical preparations with satisfactory results. If the system was coupled with some separation procedures, such as HPLC and CE, and used as a post-column detection system, the performance of the proposed system would be improved greatly.

Acknowledgements

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