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Flow Injection With inhibited Chemiluminescence Method for the Determination of Adrenaline Hydrochloride

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ABSTRACT

A simple rapid and accurate flow injection inhibitory chemiluminescence method has been developed for the determination of adrenaline hydrochloride based on its inhibition of the chemiluminescence from the luminol – potassium hexacyanoferrate (III) system. The linear range of determination is 8.0 × 10-12 – 8.0 × 10-10 mgl-1 for adrenaline hydrochloride and the detection limit is 4.0 × 10-9 g ml-1. The method has been applied to determine the content of adrenaline in pharmaceutical preparation with satisfactory results.

Introduction

Adrenaline is an important neurotransmitter in the mammalian central nervous system. pharmaceutical preparations that include this compound have been available for many years. The methods for their analysis in dosage forms include titrimetry(1) spectrophotometry(2-4), chromatography (5) and voltametry(6). Deftereos et al. investigated the emission intensity from the chemiluminogenic oxidation of adrenaline by potassium permanganate(7) and the detection limit is 4.0 × 10-12 mg l-1 for adrenaline.

Luminol can be oxidized by potassium hexacyanoferrate (III) in alkaline medium. Producing strong chemiluminescence (CL). Recently, we found that adrenaline inhibited the chemiluminescence of the reation; the net chemiluminescence intensity was a linear function of the concentration of adrenaline hydrochloride. Thus: a flow injection system with inhibited CL determination of adrenaline hydrochloride was developed in the present paper. Compared with those methods mentioned above, this method has the merits of high sensitivity, wide linear range and good accuracy. The method is applied to the determination of adrenaline in pharmaceutical preparation, and the results are satisfactory.

Experimental:

Apparatus and reagents:

CL intensity was detected with a Model IFFL-D Flow Injection Chemiluminescence Instrument (Xi an, P.R. China).

Adrenaline hydrochloride stock solution (1× 10-3g ml-1): Prepared by dissolving 0.10 g adrenaline hydrochloride (ACROS USA) in 100 ml with redistilled water. Stock it in refrigerator.

Luminal stock solution (1 × 10-2 mol L-1): Prepared by dissolving 0.1772 g luminol (ACROS USA) in 0.10 mol L-1 potassium hydroxide solution.

Potassium hexacyanoferrate (III) stock solution (1.0 × 10-2 mol L-1) Prepared by dissolving the compound 0.32926 gm in 100 ml with redistilled water.

Analytical reagent – grade chemicals and redistilled water are used.

Procedure:

The FIA configuration used is outlined in Fig .1. Solutions with different concentrations of adrenaline hydrochloride and potassium hexacyanoferrate (III) solution were pumped at 4 ml min-1 and mixed with each other. The mixture described above was looked at as the carrier stream. The reagent solution (luminol–KOH) was also pumped at 4.0 ml min-1 and introduced with the aid of an injection value with a 100 µl sample loop. Then the solution merged with carrier stream in the flow cell in front of the photomultiplier tube (PMT). The signal from PMT was sent to a detector and then to a computing integrator.

The determination was based on the linear relationship between ΔI=Io – Is and adrenaline hydrochloride concentration. Where I_o and Is were luminescent signals in the absence and in the present of adrenaline hydrochloride, respectively.

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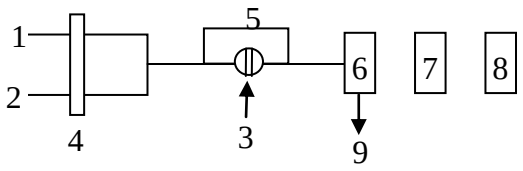

Fig.1. Chemical reaction manifold used for the flow injection chemiluminescence determination of adrenaline hydrochloride.

1- adrenaline hydrochloride; 2- hexacyanoferrate (III); 3- luminol, KOH; 4-Peristaltic Pump; 5- injection valve; 6- flow cell; 7- photomultiplier; 8- detector; 9-waste solution.

Results and Discussion

In the absence of adrenaline hydrochloride, the chemiluminescence reaction of the luminol Potassium hexacyanoferrate (III) system is strong. However, trace amounts of adrenaline hydrochloride inhibited the CL of the luminol – Potassium hexacyanoferrate (III) system.

At the same time we still found that the reverse flow injection system can reduce the waste of the reagents, and this kind of system had higher sensitivity and more steady base line so the reverse flow injection system was adopted in this paper. The kinetics curve of the CL reaction is shown in Fig.2.

Effect of reagent concentration:

The effect of luminol concentration on the net chemiluminescence intensity was studied (Fig-3). The ΔI increased with increasing the concentration of luminol up to 4.0×10^{-4} mol. l^{-1}. Above this, the ΔI decreased with increase of the concentration of luminol. So the concentration of 4.0×10^{-4} mol l^{-1} luminol was selected to be the optimum concentration for ΔI.

The effect of KOH concentration was investigated Fig-4- shows that the maximum response of ΔI was obtained at 1.4×10^{-2} mol l^{-1} KOH, this concentration was used in further studies.

Fig 5. shows that the optimum concentration of Potassium hexacyanoferrate (III) is 4.0×10^{-3} mol l^{-1}.

Effect of flow rate:

The effect of flow rate on the intensity of chemiluminescence was studied over the range of 0.3 – 10 ml/min in each stream. The CL intensity increased with the increase of total flow rate. However, flow rate of 4.0 ml/min are recommended for both carrier stream and the samples, because of good precision and lower reagent consumption.

Analytical parameters:

A calibration graph of relative CL intensity vs. the adrenaline hydrochloride concentration was established by applying the optimal conditions. The regression equation is:-

$\Delta I = 1.00 \times 10^{10} C + 1157.9$ r=0.9932. the linear range for the determination of adrenaline hydrochloride is $8.0 \times 10^{-12} - 8.0 \times 10^{-10}$ mgl^{-1}. The reproducibility of the method is satisfactory with a relative standard deviation of 0.99% (n=10) at 3.0×10^{-8} g ml^{-1} adrenaline hydrochloride. The detection limit calculated from three times the standard deviation of the blank is 4.0×10^{-9} g ml^{-1}.

Interference study:

The effect of various foreign species on the determination of 3.0×10^{-11} mg l^{-1} adrenaline hydrochloride were investigated. The results show that the tolerable concentration ratios with respect to 3.0×10^{-11} mg l^{-1} adrenaline hydrochloride were 200 for Na^{+}, K^{+}, Cl^{-}, SO_4^{-2}, NO_3^{-}, ethanol, glucose and citric acid. 100 for resorcinol. 3 for quinol, ascorbic acid. 2 for pb^{+2}, Ni^{+2}, Fe^{+3}, Al^{+3}, 0.5 for Cu^{+2}, Co^{+2}, Cd^{+2}, Mn^{+2}. Using 1×10^{4} mol ml^{-1} EDTA as a masking agent can eliminate the interference of Fe^{+3} up to 50 times of adrenaline and can remove the interfering effect of pb^{+2}, Ni^{+2}, Al^{+3}, Cu^{+2}, Co^{+2}, Cd^{+2}, Zn^{+2} up to 30 times of adrenaline.

Application

The proposed method was applied to the determination of adrenaline hydrochloride in pharmaceutical preparation. The measured adrenaline contents are listed in Table 1. This table also includes the values obtained during the analysis of the same samples by using the reference procedure described on P.R. China Pharmacopoeia [8]. The values presented on Table 1. reveal a good agreement between the proposed method and the reference one[8].

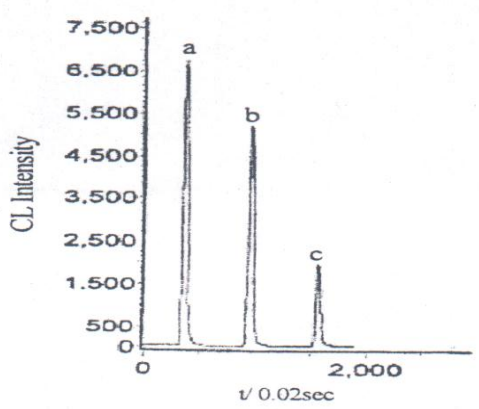

Fig. 2. signal profiles of the CL reaction.

a- $K_3Fe(CN)_6$ 1.0 × 10-^{3} mol ml^{-1} – luminol (4×10^{-4} mol ml^{-1}) – KOH 1.4×10^{-2} mol ml^{-1}. b- a+8×10^{-11} mg l^{-1} adrenaline hydrochloride. c. a+8×10^{-10} mgl^{-1} adrenaline hydrochloride.

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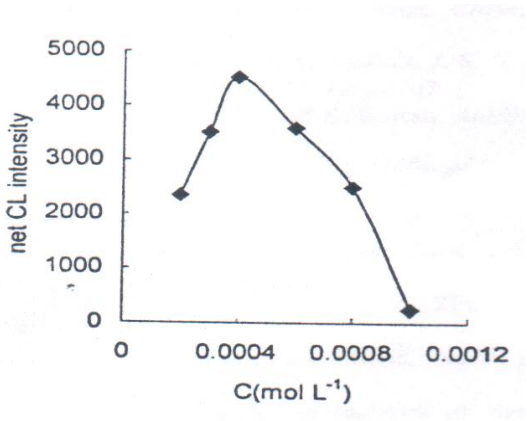

Fig. 3. The effect of luminol concentration

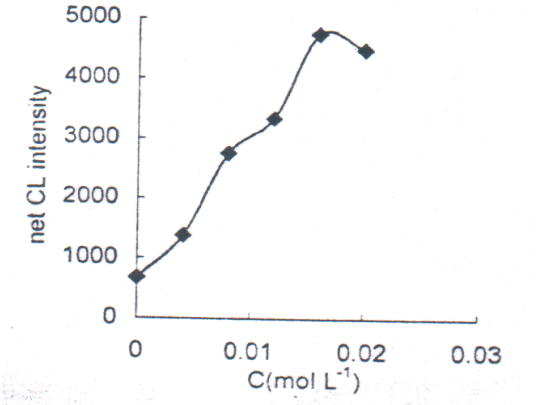

Fig. 4. The effect of KOH concentration.

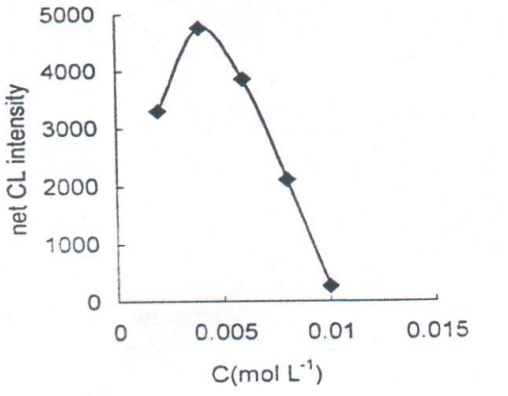

Fig. 5. The effect of $K_3Fe(CN)_6$ concentration

Table 1. Determination of adrenaline in injection of adrenaline hydrochloride (n=4)

Sample	Added mg ml-1	Present Method mg ml-3 x10	RDS %	Reco-very %	Method of pharmaco-peial mg ml-1-3 x10
Injec-tion	0	0.204	1.24	-	0.201
	0.60	0.81	2.01	101	0.83
	0.80	1.05	1.63	106	1.02

We first found the inhibiting effect of adrenaline on the CL reaction of the luminol – Potassium hexacyanoferrate (III) system. A flow injection – CL method for the determination of adrenaline has been developed. Compared. The method of chibese pharmacopia, the proposed method offers advantages simplicity,t rapidity, high sensitivity and low reagent consumption, and provides a wide linear range for the determination of adrenaline hydrochloride. The linear range of the method is $8.0 \times 10^{-12} - 8.0 \times 10^{-10}$ mgl^{-1} adrenaline hydrochloride. The reproducibility of the method is satisfactory with a relative standard deviation of 0.99% (n=10) at 8.0×10^{-10} mgl^{-1} adrenaline hydrochloride. The detection limit of adrenaline hydrochloride is 4.0×10^{-9} g ml^{-1}.

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الحقن الجرياني مع طريقة التثبيط بالتألق الكيميائي لتقدير هيدروكلوريد الادرنالين

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الخلاصة:

طريقة سهلة وسريعة ودقيقة للحقن الجرياني المثبط بالتألق الكيميائي تم تطويرها لتعيين هيدروكلوريد الادرينالين بالاعتماد على تثبيط التألق الكيمائي من نظام لومينال–بوتاسيوم هكسوسيانيد الحديد الثلاثي، المدى الخطي ٨.٠*١٠ $^{-٢}$ الى ٨.٠ * ١٠ $^{-١٠}$ ملغم/لتر لهيدروكلوريد الادرينالين وحد الكشف ٤ * ١٠ $^{-٩}$ مل /لتر، يمكن تطبيق الطريقة لتعدين الادرينالين في المستحضرات الصيدلانية بنتائج مقنعة.