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Journal of Pharmaceutical and Biomedical Analysis 36 (2004) 219–223
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Short communication

Determination of dopamine by flow-injection analysis coupled with luminol-hexacyanoferrate(III) chemiluminescence detection

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Received 12 January 2004; received in revised form 30 April 2004; accepted 20 May 2004

Available online 28 July 2004

Abstract

A novel flow-injection method (FIA) for the determination of dopamine based on the inhibition of the intensity of chemiluminescence (CL) from luminol-hexacyanoferrate(III) system in basic medium is described. The present method allows the determination of dopamine over the range 30–100 $\mu g\,l^{-1}$ and 400–3000 $\mu g\,l^{-1}$. The relative standard deviation is 2.32% for 70 $\mu g\,l^{-1}$ dopamine and 1.22% for 1500 $\mu g\,l^{-1}$ dopamine ($n = 20$). The detection limit is 5 $\mu g\,l^{-1}$ with the sampling rate of 135 samples h^{-1}. This method has been applied for the determination of dopamine in commercial pharmaceutical injection samples. The results obtained by this method agreed with those by the official method.

Keywords: Flow injection; Chemiluminescence; Dopamine; Luminol; Pharmaceutical analysis

1. Introduction

Dopamine (3,4-dihydroxyphenylethylamine) is an important neurotransmitter in the central nervous system that provides a communication link between neurons [1]. It is derived from tyrosine and is the precursor to norepinephrine and epinephrine. Dopamine is used in the treatment of cardiogenic, septic shock and in chronic refractory congestive heart failure. The determination of dopamine has appeared of great importance both in biological fluids and pharmaceutical preparations. A variety of techniques have been utilized for the determination of dopamine, such as chromatography [2–5], capillary electrophoresis [6,7], spectrophotometry [8–10], fluorimetry [11,12], electrochemical [13–18] and chemiluminescence [19–21] detection.

A few sensitive methods based on the oxidation of luminol in an alkaline solution have been applied to the indirect determination of dopamine [22–24]. Zhang et al. [22] proposed a method based on the inhibition of the intensity of CL from the luminol-hypochlorite system by dopamine. The hypochlorite was electrogenerated on-line by electrolysis due to its instability. Zhu et al. [23] employed an inhibiting effect of dopamine on the electrochemiluminescent emission of luminol in aqueous alkaline solution for its quantification. Li et al. [24] combined on-line microdialysis sampling with the plant tissue-based chemiluminescence flow biosensor to monitor the variation of dopamine level in the blood of rabbit. In this method dopamine was oxidized by oxygen under the catalysis of polyphenol oxidase in the tissue column to produce hydrogen peroxide, which reacted with luminol in the presence of peroxidase of potato tissue and generated CL signal.

In the literature several analytical methods have been reported for determination of different drugs containing polyphenol group [25–27] which can enhance or inhibit strong CL produced while mixing a potassium hexacyanoferrate(III) solution with an alkaline luminol solution. We found that dopamine could strongly inhibit the CL reaction of luminol-potassium hexacyanoferrate(III) system in basic solution and the decrease of the CL intensity was dependent on the concentration of the studied drug. Based on this observations, a simple, sensitive and rapid new assay for dopamine has been developed using flow-injection CL technique. The proposed method was successfully applied

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doi:10.1016/j.jpba.2004.05.009

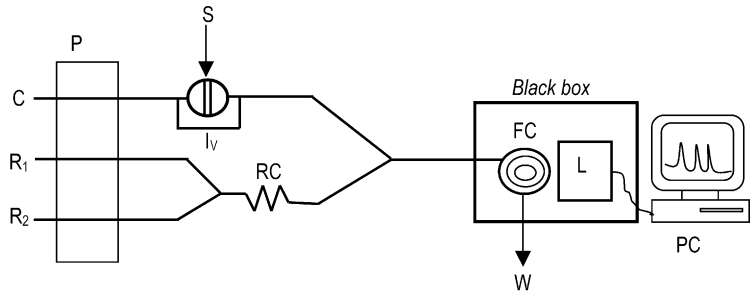

Fig. 1. Schematic diagram of the flow-injection system for dopamine determination. P: peristaltic pump; C: water carrier stream; R_1: 10^{-4} mol l^{-1} potassium hexacyanoferrate(III) solution; R_2: 1.25×10^{-3} mol l^{-1} luminol and 5×10^{-2} mol l^{-1} potassium hexacyanoferrate(II) in 2.0 mol l^{-1} sodium hydroxide solution; RC: mixing coil; S: sample; I_V: injection valve; L: luminometer; FC: flow cell; PC: computer; W: waste.

to the determination of dopamine in pharmaceutical preparation. Moreover, the mechanism of inhibition was briefly discussed.

2. Experimental

2.1. Reagents

Dopamine hydrochloride and luminol were purchased from Sigma (USA). The stock solutions of reagents were stored at 4 °C in refrigerator to avoid exposure to light and air. All other chemicals used were of analytical grade and were obtained from POCH (Poland). The stock solutions were prepared by dissolving an appropriate amounts of reagents in the calibration flasks. Working solutions were freshly prepared by successive dilutions of the standard solutions.

2.2. Apparatus and procedure

A schematic diagram of the flow-injection system used in this work is depicted in Fig. 1. An Ismatec MS-Reglo peristaltic pump was used to deliver all flow streams. All flow lines were made of Teflon tubing (0.8 mm i.d.). The sample solutions were injected into a carrier stream using a Model 5021 rotary injection valve (Rheodyne, Cotati, CA) with a 400 μl sample loop. A flow luminometer (KSP, Poland) equipped with a spiral flow-cell made from coiled PTFE tube of 1 mm i.d. (length of 25 cm in six windings) was used for measurement of CL intensities.

The absorption spectra were performed on a model 8452A diode array spectrophotometer (Hewlett-Packard, Germany).

3. Results and discussion

In order to establish the flow-injection parameters which gave the best analytical performance, a series of univariate searches were performed with the respect to the sensitivity and the reproducibility on the basis of the peak height and the ratio of the peak height to the noise. All these experimental parameters were optimised for four different concentrations of dopamine (100, 500, 750 and 1000 μg l^{-1}).

3.1. Effect of chemical variables

The influence of the concentration of luminol on the CL reaction was examined in the range of 5×10^{-4} to 3.5×10^{-3} mol l^{-1}. With the increase in the luminol concentration, both the negative signal and the background noise value increased. The signal to noise ratio (*S*/*N*) reached its maximum when 1.25×10^{-3} mol l^{-1} concentration was used. Therefore, 1.25×10^{-3} mol l^{-1} was selected as the concentration of luminol in CL reaction.

In aqueous alkaline solution luminol reacts with potassium hexacyanoferrate(III) to produce light emission. The influence of sodium hydroxide concentration on the CL reaction was examined over the range 1.0–3.5 mol l^{-1}. The negative peak height increased with increasing sodium hydroxide concentration and reached maximum at 2.0 mol l^{-1}. Thus, 2.0 mol l^{-1} sodium hydroxide was chosen as a medium in which luminol solution was prepared.

Potassium hexacyanoferrate(III) reacts with luminol to produce strong CL in alkaline solution. However, the background noises were very high and the base line was unstable. In the literature it has been reported that CL reaction of luminol with potassium hexacyanoferrate(III) could be inhibited by potassium hexacyanoferrate(II) [27]. So, if there was a suitable concentration of potassium hexacyanoferrate(II) the blank signal could be reduced efficiently by addition of potassium hexacyanoferrate(II) into the reaction system. Therefore, the effect of potassium hexacyanoferrate(III) and potassium hexacyanoferrate(II) was examined. The effect of potassium hexacyanoferrate(III) concentration on the CL intensity and the signal/noise ratio was studied in the range 10^{-5} to 2×10^{-4} mol l^{-1}. With increasing potassium hexacyanoferrate(III) concentration the negative peak height and base line noises increased continuously. The highest *S*/*N* ratio was obtained at 10^{-4} mol l^{-1} potassium hexacyanoferrate(III). Thus, this concentration was used in subsequent experiments. The effect of potassium hexacyanoferrate(II) concentration was investigated in the range 2.5×10^{-3} to 1.5×10^{-1} mol l^{-1}. Since the potassium hexacyanoferrate(II)

Table 1
Determination of dopamine in pharmaceutical injection samples by the proposed method and the official method

Sample	Labelled value (mg)	Found (mg)[a]		Relative error (%)	
		Official method	Proposed method	RE_1	RE_2
Dopaminum Hydrochloricum	200	202.47 ± 1.58	202.38 ± 1.30	1.19 ± 0.65	−0.04 ± 0.61

RE_1, proposed method vs. labelled value; RE_2, proposed method vs. official method.
[a] Mean of five determinations ± S.D.

concentration versus *S*/*N* ratio shows the best value for potassium hexacyanoferrate(II) levels around 5×10^{-2} mol l^{-1}, this concentration was chosen as the most suitable.

3.2. Effect of instrumental variables

The effect of the injected sample volume was examined over the range 200–1000 μl. The highest intensity of signal was obtained with 400 μl sample loop.

The influence of the flow rates of the carrier (*C*) and the reagent streams (R_1 and R_2) were examined in the range of 2.75–9.13 ml min^{-1} and 1.5–5.0 ml min^{-1}, respectively. The negative CL intensities become larger by increasing the flow rate up to 3.55 and 6.50 ml min^{-1} for the carrier stream and reagent streams, respectively. Beyond these values the negative peak height was almost constant, so 3.55 ml min^{-1} (carrier stream) and 6.5 ml min^{-1} (R_1 and R_2 streams) flow rate were selected considering low reagent consumption.

To improve the efficiency of the CL reaction between luminol and hexacyanoferrate(III) the mixing coil was used in the flow system. The influence of the length of the mixing coil (RC) was examined in the range 19−69 cm. With the increase in length of the mixing coil, the negative peak decreased remarkably. The most negative signal value was obtained when the distance to the detector was as short as possible. Therefore, 19 cm was selected as the best length of mixing coil.

3.3. Analytical application

3.3.1. Analytical characteristics

With the optimised experimental conditions as selected above the CL intensity (*I*, nA) was linearly proportional to the dopamine concentration (*C*, μg l^{-1}) in the range of 30–100 μg l^{-1} with the regression equation $I = -0.969C - 54.181$ ($r^2 = 0.9919$) and in the range 400–3000 μg l^{-1} with the regression equation $I = -0.101C - 292.410$ ($r^2 = 0.9929$). The theoretical detection limit defined as the analyte concentration, giving a signal equal to the blank signal plus three standard deviations of the blank [28], was 5 μg l^{-1} dopamine. The relative standard deviation (R.S.D.) was 2.32% for 20 determinations of 70 μg l^{-1} dopamine and 1.22% for 20 determinations of 1500 μg l^{-1} dopamine. In order to check day-to-day reproducibility, three calibration graphs were obtained in different days, the arithmetic mean of the slope was −1.101 with R.S.D. = 0.37%. The sampling rate was 135 samples h^{-1}.

3.3.2. Effect of interfering species

To evaluate the selectivity of the method developed the effect of various compounds usually present in the pharmaceutical preparation, common reducing agents and inorganic ions was studied. Additionally, the effect of other catecholamines was also investigated. The tolerable concentration ratio of the interfering substances was considered to be acceptable if the relative error was less than ± 5% for the peak height obtained for the standard solution of dopamine (1500 μg l^{-1}) containing no foreign substances. The tolerable concentration ratios were: higher than 1000 for $Na_2B_4O_7 \cdot 10H_2O$, EDTA, sodium citrate, $Na_2S_2O_3 \cdot 5H_2O$, NaCl, glucose, lactose; 267 for $CaCl_2 \cdot 6H_2O$; 40 for formaldehyde; 20 for synephrine, Na_2SO_3; 7 for $NaHSO_3$; 0.4 for adrenaline; 0.3 for methyl-L-alanine; 0.13 for L-dopa; 0.07 for noradrenaline. Adrenaline, noradrenaline, L-dopa and methyl-L-alanine significantly interfered with the determination of dopamine but usually only one catecholamine is present in pharmaceutical preparations.

3.3.3. Determination of dopamine in a pharmaceutical preparation

The proposed method was applied to the analysis of dopamine in a commercially available preparation *Dopaminum Hydrochloricum* (from POLFA, Warsaw). Each ampoule of the injection preparation with a certified amount of 200 mg of dopamine hydrochloride was diluted with water to fit the concentration of analyte within the range of calibration curve. The results obtained by the proposed method are in excellent agreement with the nominal contents (Table 1). They were also compared with the results obtained from the official method (HPLC) [29] by applying *F*- and *t*-tests [28]. The values obtained, Tables 1 and 2, show that the presented method is of comparable precision to that of the official method and there is no significant difference between the mean values obtained by

Table 2
Significance tests (*t*-test and *F*-test) for comparison between determinations of dopamine by two different methods

Method	Testing for significance[a]				
	n	*t* critical[b]	*t* calculated	*F* critical[b]	*F* calculated
Proposed method	5	2.31	0.10	9.61	1.47
Official method	5				

[a] Two-tailed tests were used.
[b] Tabulated 95.0% confidence limit.

Table 3
Recovery of dopamine from injection solution of dopamine

Sample number	Dopamine (mg per ampoule)		Recovery (%)
	Added	Found[a]	
1	200.00	200.22 ± 4.82	100.11 ± 2.41
2	300.00	302.23 ± 8.19	100.74 ± 2.73
3	400.00	393.52 ± 9.83	98.38 ± 2.73

[a] Mean of three determinations ± S.D.

both methods. In order to check accuracy of the proposed method the recovery study was carried out by adding the known amounts of dopamine standard solutions to the pharmaceutical preparation. In all the samples extremely good recoveries have been obtained (Table 3).

3.4. Discussion of the inhibition mechanism

In an alkaline solution, luminol was reduced by potassium hexacyanoferrate(III) to excited 3-aminophthalate which is luminophor of this system and the maximum emission wavelength was 425 nm. We found that the presence of dopamine causes an inhibition effect of the mentioned CL reaction. The negative peak height remarkably increased with increasing concentration of dopamine.

In order to explain the possible reaction mechanism, the UV–vis absorption spectra of reagents were made in a basic medium. Potassium hexacyanoferrate(III) has three absorption peaks at 224, 304 and 424 nm, and dopamine has two absorption peaks at 282 and 344 nm. Nevertheless, the maximum absorption peak of the mixed system of dopamine and potassium hexacyanoferrate(III) appeared at 228 nm, and the light absorption of the mixed system was not equal to the sum of the light absorption of the two individual systems, what suggests that dopamine has reacted with potassium hexacyanoferrate(III). The reaction between dopamine and potassium hexacyanoferrate(III) results in consuming the part of the oxidant. Therefore, the CL inhibition mechanism of dopamine is probably based on competition between dopamine and luminol for potassium hexacyanoferrate(III) what causes the decrease in the chemiluminescence intensity.

Fig. 2 shows the absorption spectra of dopamine (1), luminol-potassium hexacyanoferrate(III) (2), luminol-potassium hexacyanoferrate(III)-dopamine (3) and the algebraic sum of absorbance of dopamine and luminol-potassium hexacyanoferrate(III) (4) system in NaOH media. The experimental results indicated that the light absorption of the mixed system was not equal to the sum of two individual light absorption spectra of dopamine and luminol-potassium hexacyanoferrate(III) system. That indicates that dopamine is expected to be involved in the reaction between luminol and potassium hexacyanoferrate(III).

According to the experiments performed above the inhibitory effect of dopamine on a luminol-potassium hexacyanoferrate(III) chemiluminescence system was proposed.

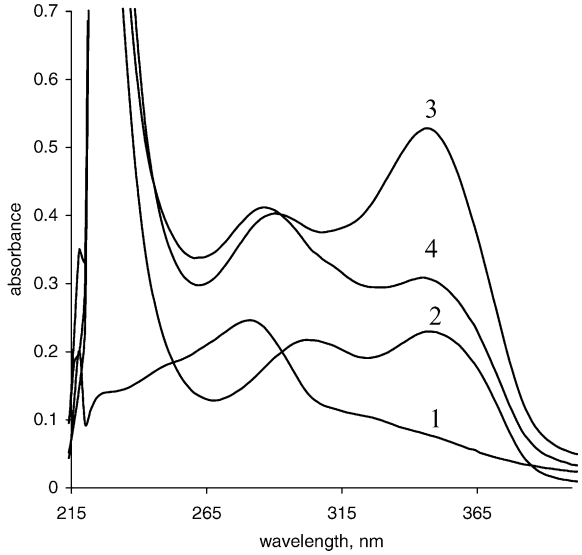

Fig. 2. UV–vis absorption spectra: (1) dopamine; (2) luminol-potassium hexacyanoferrate(III) system; (3) luminol-potassium hexacyanoferrate(III)-dopamine system; (4) 1 + 2. Luminol: 5×10^{-5} mol l^{-1}, potassium hexacyanoferrate(III): 2×10^{-5} mol l^{-1}, dopamine: 5 μg ml^{-1}, blank: sodium hydroxide 2 mol l^{-1}.

Dopamine could react with potassium hexacyanoferrate(III) consuming part of the oxidant. Therefore, the chemiluminescence intensity of the luminol-potassium hexacyanoferrate(III) system was decreased.

4. Conclusion

A flow-injection chemiluminescence inhibition method for the determination of dopamine has been well established based on the strong inhibition phenomenon of dopamine on the luminol-potassium hexacyanoferrate(III) system. The proposed procedure offers higher sample throughput, accuracy, reproducibility and precision compared to literature CL methods. This method is not very selective against other drugs containing polyphenol group but it does not interfere in determination of dopamine in commercial samples which contain only assayed drug. Therefore, it demonstrates the method is applicable to detection of dopamine without any sample pretreatment. The mechanism of the inhibition effect of dopamine may be that the investigated drug reacts with the part of potassium hexacyanoferrate(III), which is in competition with luminol reaction for the oxidant.

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