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Flow-Injection Chemiluminescence Determination of Epinephrine in Pharmaceutical Formulations using N-bromosuccinimide as Oxidant

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A novel flow-injection method for the determination of epinephrine (adrenaline) in pharmaceutical preparations is proposed. Detection of the drug is based on the measurement of the chemiluminescence produced by its direct oxidation with N-bromosuccinimide in alkaline medium. The detection limit of epinephrine in the optimized system is 6 mg L^{-1} and the calibration graph is linear over the range 6–200 mg L^{-1}. The interference of the different concomitant compounds usually present in pharmaceutical formulations was investigated. Among them, only strong reducing agents like ascorbic acid or formaldehyde can create problems when they are present in the sample at concentration higher than 10 mg L^{-1}. Using the developed method, 40 samples per hour can be determined.

Opisano nową wstrzykowo–przepływową metodę oznaczania epinefryny (adrenaliny) w preparatach farmaceutycznych. Detekcja w opisanej metodzie jest oparta na pomiarze chemiluminescencji powstającej w wyniku bezpośredniego utleniania leku za pomocą N-bromoimidu kwasu bursztynowego w środowisku alkalicznym. Granica wykrywalności epinefryny w zoptymalizowanym układzie wynosi 6 mg L^{-1}, a krzywa kalibracyjna jest liniowa w zakresie 6–200 mg L^{-1}. Zbadano zakłócenia wywołane obecnością substancji zazwyczaj towarzyszących epinefrynie w preparatach farmaceutycznych. Spośród zbadanych substancji jedynie silne reduktory, takie jak kwas askorbinowy czy formaldehyd mogą stanowić problem jeśli są obecne w stężeniu wyższym niż 10 mg L^{-1}. Przy użyciu opracowanej metody oznaczać można 40 próbek w ciągu godziny.

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Epinephrine is one of the catecholamines which play an important role as neurotransmitters and hormones. It is also used in medicine in the treatment of heart block, bronchial asthma and cardiac surgery. Several analytical methods have been proposed recently for the determination of epinephrine and other catecholamines in biological fluids and pharmaceutical preparations. The most suitable method for the determination of catecholamines in blood or serum seems to be liquid chromatography with electrochemical [1,2], fluorimetric [3] or chemiluminescence [4] detection. The importance of this drug prompted the development of an automated fast method for routine analysis and quality control of commercial formulations. In recent years several flow-injection (FI) methods with different detection techniques have been described for determination of epinephrine, most of which were based on its oxidation. Epinephrine is readily oxidized with various agents to adrenochrome, which in turn is converted by alkali into adrenolutine, a fluorescent substance [5]. Three spectrophotometric FI methods based on the oxidation of epinephrine to adrenochrome have been described in the literature. One of them consists in the use of the solid-phase reactor with microcrystalline manganese dioxide and absorbance measurement at 300 nm [6]. In the second method the oxidation of epinephrine is carried out by means of periodate and absorbance is measured at 491 nm [7]. The third of them is based on the hydrolysis of the drug in alkaline medium and its oxidation by atmospheric oxygen [8]. Another spectrophotometric procedure utilizes the complex-formation of epinephrine with Fe(II) [9]. The use of the spectrofluorimetric detection in the FI system was based on the oxidation of the drug in the solid-phase reactor of manganese dioxide incorporated in the polyester resin beds [10], and the solid phase reactor of iodine prepared by „impregnation" of the flexible pump tubing with the reagent [5]. Epinephrine was also determined by FI method with indirect biamperometric detection using Fe(III) as oxidant [11] and with chemiluminescence detection utilizing Fenton reagent [12] or permanganate in the presence of formaldehyde as a sensitizer [13].

A number of pharmaceuticals and other important analytes have been determined by the measurement of the chemiluminescence produced by their direct oxidation with N-bromosuccinimide (NBS). Oxidizing properties of NBS are attributed to hypobromite which is produced by its hydrolysis [14]. A great advantage of the use of NBS instead of hypobromite is relatively good stability of this reagent.

This paper describes simple and non-expensive method for determination of epinephrine in pharmaceutical preparations. The use of N-bromosuccinimide as a source of hypobromite creates an alternative for more complicated approaches like electrogeneration [15–17] of this reagent. Solution of 0.04 mol L^{-1} of this reagent is stable for at least 12 h.

EXPERIMENTAL

Reagents

All used reagents were of analytical grade and all solutions were prepared with double distilled water. Epinephrine and N-bromosuccinimide (NBS) were purchased from Sigma. All other reagents used were produced by POCh (Poland).

The stock solution of epinephrine of 1000 mg L^{-1} was prepared by dissolving 0.2500 g of this substance in a 0.25 L volumetric flask after adding few mL of 0.1 mol L^{-1} hydrochloric acid to the double distilled water. To prepare working solutions of epinephrine, appropriate volumes of the stock solution were dissolved in the phosphate buffer (0.1 mol L^{-1}) of pH 7.

NBS solution (0.01 mol L^{-1}) was prepared daily by dissolving 0.8896 g of this substance in a 0.5 L volumetric flask using double distilled water and ultrasonic bath to promote its solubility.

Apparatus

The flow-injection set-up, shown schematically in Figure 1, consisted of an Ismatec MS–Reglo peristaltic pump, a Model 5021 rotary injection valve (Rheodyne, Cotati, CA), and flow luminometer (KSP, Poland) with a coiled quartz glass tube of 2 mm I.D. (length of 15 cm in 3 windings). The photomultiplier was operated at 1200V, and the detector response was recorded on a 386–series personal computer with KSP software. The flow system was made of PTFE tubing of 0.8 mm I.D. The reagent and carrier streams were merged in a Perspex T piece.

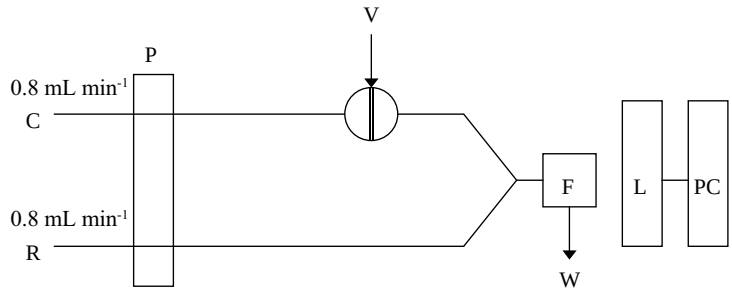

Figure 1. Schematic diagram of the flow-injection system used for determination of epinephrine: C — carrier stream (sodium hydroxide), R — reagent stream (NBS solution), V — injection valve, P — peristaltic pump, F— flow cell, L — luminometer, PC — computer, W — waste

RESULTS AND DISCUSSION

Optimization of the flow system for the determination of epinephrine

In order to optimize the proposed system, both chemical and hydrodynamic parameters were investigated. Concentration of NBS and sodium hydroxide, pumping velocity

and sample volume were the important parameters influencing the signal magnitude. The effect of the increased temperature was also tested. All these parameters were optimized for 10 mg L^{-1} of epinephrine with respect to the sensitivity and reproducibility, on the basis of the peak height.

The effect of the concentration of sodium hydroxide in the carrier stream on the chemiluminescence intensity was investigated in the range 1–5 mol L^{-1} (Fig. 2A). The highest signal intensity was observed by the concentration of 5 mol L^{-1} of NaOH but because of the high viscosity of the solution, reproducibility was not satisfactory and concentration of 4 mol L^{-1} was considered as the optimal in further experiments.

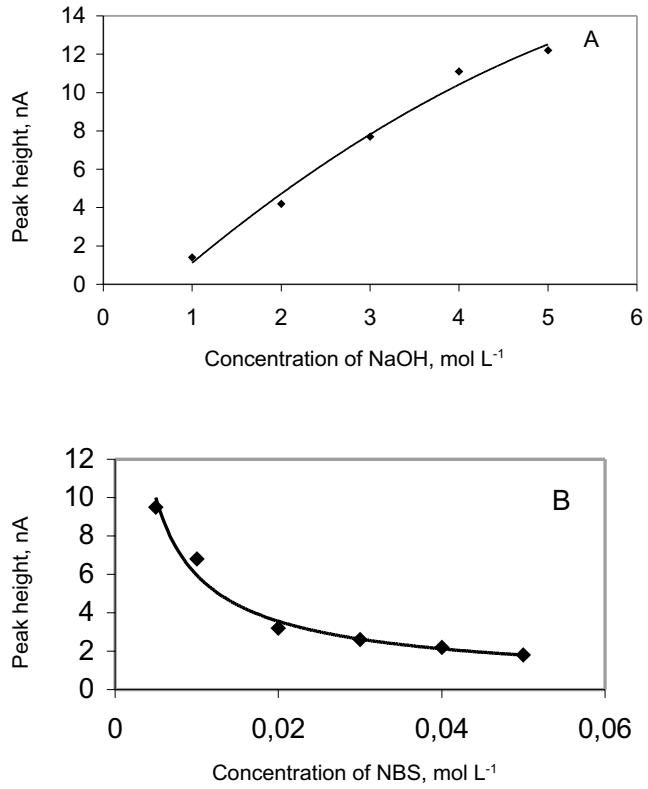

Figure 2. Optimization of the flow-injection system: A — concentration of sodium hydroxide in the carrier, B — concentration of NBS in the reagent stream (continuation on the next page)

The decrease of the signal magnitude with increasing NBS concentration in the reagent stream was observed until 0.05 mol L^{-1} (Fig. 2B), which is approximately the limit of solubility of this substance at the room temperature. At the concentration of NBS of 0.005 mol L^{-1} the highest signals were registered but reproducibility was ra-

ther poor. Concentration of the oxidant of 0.01 mol L^{-1} was chosen as optimal for the determination of epinephrine.

The chemical reaction producing chemiluminescence in the described experiments is not very fast. The optimal pumping speed of both, carrier and reagent stream was found to be 0.8 mL min^{-1}. Any change of the pumping velocity provided lower signals (Fig. 2C).

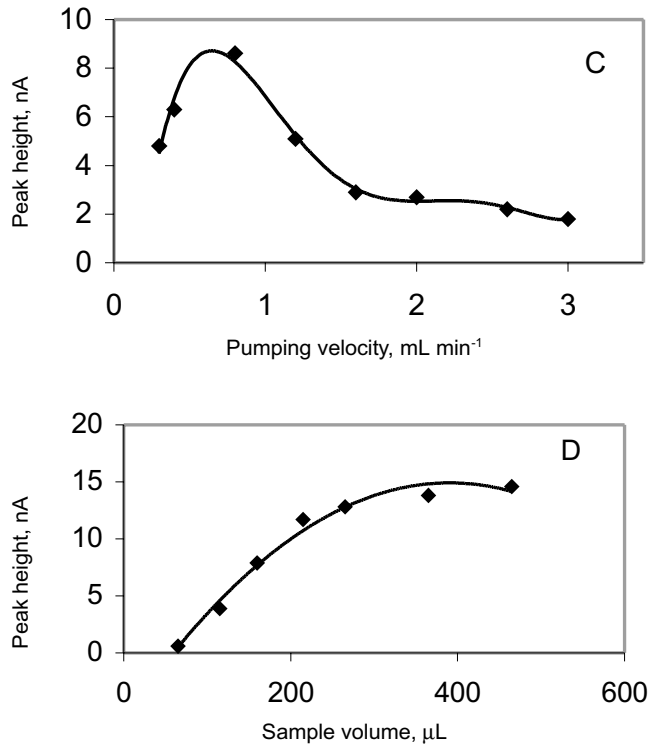

Figure 2. Optimization of the flow-injection system: C — effect of pumping velocity, D — effect of sample volume

The considerable signal increase was observed during increasing of the sample volume (Fig. 2D). For the sample volume higher than 265 µL double peaks were registered, so this value was chosen as optimal.

Increasing of the temperature of the flowing solutions resulted in only slightly increased signals which was connected with the considerable irreproducibility at temperatures higher than 30°C. The room temperature (18–20°C) was considered as optimal in all further experiments.

Few substances which are considered as potential sensitizers [20] were tested for their effect on the signal produced in the described system. For the compounds like dichlorofluorescein, rhodamine B, rhodamine 6G, calcein, ninhydrin, glycine or humic acid at concentrations in the range 10^{-4}–10^{-5} mol L^{-1}, only dichlorofluorescein (10^{-4} mol L^{-1}) caused increase of the signal magnitude of about 8%. Authors found these results not satisfactory and decided not to use dichlorofluorescein as sensitizer.

Analytical parameters of the optimised system

As a result of optimization procedure two linear ranges of a calibration graph were established: 6–20 mg L^{-1} with an equation $y = 1.834x + 0.376$ and correlation coefficient $r^2 = 0.9988$ and 20–200 mg L^{-1} with an equation $y = 1.241x + 1.275$ and correlation coefficient $r^2 = 0.9965$. In both equations y means the peak height in nA and x is the concentration of epinephrine in mg L^{-1}. The precision of the method was calculated for 11 injections of epinephrine at concentrations 10 and 100 mg L^{-1}. The relative standard deviation (RSD) values were 1.8 and 1.3%, respectively. The day-to-day reproducibility of the slope of the calibration graph for three succeeding days was 2.5%. The detection limit of the described method is 6 mg L^{-1} and the sample throughput 40 samples per hour.

Studies of interferences

The tolerance of the method to foreign compounds which can be found in a typical pharmaceutical samples containing epinephrine was investigated in mixed solutions containing 10 mg L^{-1} of epinephrine and different concentrations of the interferent. The resulting signals were compared to those obtained for epinephrine only at the same concentrations. Each substance was considered not to interfere if it caused a relative error less than 5%. The results are shown in Table 1. The most significant interferences were caused by norepinephrine, $NaHSO_3$, ascorbic acid and formaldehyde, however they do not occur at such high concentrations in commercial preparations.

Table 1. Tolerated concentration of foreign compounds with 10.0 mg L^{-1} of epinephrine

Substance	Tolerable concentration mg L^{-1} %	Relative error
Norepinephrine	5	–2.3
$NaHSO_3$	20	–3.8
Ascorbic acid	10	+2.1
Formaldehyde	10	–2.9
Glucose	6400	–3.1

Table 1 (continuation)

Lactose	1000	–4.3
EDTA	2000	+3.8
Citric acid	20 000	+2.2
Picric acid	100	–2.7
H_3BO_3	5000	+3.8
$Na_2B_4O_7 \cdot 10H_2O$	6000	+4.2
NaCl	28 000	+0.6
$NaHCO_3$	400	+0.1
$NaBO_2 \cdot 4H_2O$	20 000	+1.0
$ZnSO_4 \cdot 7H_2O$ 20	20	–2.8

Analysis of real samples

Two commercially available preparations were chosen for testing of the proposed method: Adrenalinum Solution 0.1% (Cefarm Gdańsk) and Injec. Adrenalini 0.1% (Polfa, Warszawa). In both cases 1 mL of the solution was transferred to the 10.0 mL volumetric flask and diluted to the mark with water. The obtained solution was then injected into the manifold. No or very little interference was found from the antioxidant ($NaHSO_3$) used by the manufacterer. The relative errors for Adrenalinum Solution and Injec. Adrenalini were –2.2 and +1.4%, respectively compared with values certified on the labels.

Discussion of the possible chemiluminescence mechanism

The particle of epinephrine consists of an aromatic ring with two hydroxyl groups and alkyl chain with one hydroxyl and one amino group: $(HO)_2C_6H_3$—CH(OH)—CH_2—NH—CH_3. It is well documented that one of the products of the decomposition of amino compounds with amino groups in an alkyl chain is ammonia or ammonium ion, when NBS is used as oxidant [19,20]. On the other hand ammonia undergoes further oxidation with NBS in an alkaline medium to produce nitrogen. This reaction is chemiluminescent and nitrogen produced in an excited state is supposed to be an emitting species [18,21]. It was apparent to the authors that one of the products of the reaction between epinephrine and NBS was a gas which bubbles were visible at the outlet of the detector cell. It is not clear, what are the non-gaseous products of the reaction between epinephrine and NBS. However, it seems not to be crucial from the point of view of the used detection method.

CONCLUSIONS

The proposed flow-injection chemiluminescence method for the determination of epinephrine in pharmaceutical preparations is fast, simple and easy to automation. Compared to the standard titrimetric method in non-aqueous solutions it is less time-consuming and its cost per assay is lower. When compared to the other flow-injection methods for catecholamines in pharmaceutical formulations it is one of the simplest in respect to used equipment and reagents, and has comparable parameters like detection limit, sample throughput and linear calibration range. One should realize that used detection method is not very selective against reducing agents present in many preparations containing epinephrine. The obtained results however demonstrate that determination of epinephrine in some preparations containing antioxidants is possible without any sample pretreatment.

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