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Flow injection spectrophotometric determination of adrenaline in pharmaceutical formulations using a solid-phase reactor containing lead(IV) dioxide immobilized in a polyester resin

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Abstract

A flow injection spectrophotometric procedure is proposed for determining adrenaline in pharmaceutical formulations. In this work, the adrenaline in acetate buffer reacts with a solid-phase reactor containing lead(IV) dioxide immobilized in a polyester resin and the adrenochrome yielded was continuously monitored at 486 nm. The analytical curve was linear in the adrenaline concentration range from 0.1 to 0.8 mmol l^{-1} with a detection limit of 8×10^{-3} mmol l^{-1}. Recoveries of 96.5–105% and relative standard deviation of 0.2% for a solution containing 0.4 mmol l^{-1} adrenaline ($n = 10$) were obtained. The analytical frequency was 130 determinations per hour and the results obtained for adrenaline in pharmaceutical formulations using this procedure and those obtained using a pharmacopoeia procedure are in agreement at the 95% confidence level. © 2002 Published by Éditions scientifiques et médicales Elsevier SAS.

Keywords: Adrenaline; Pharmaceutical formulations; Flow injection analysis; Solid-phase reactor

1. Introduction

Adrenaline (4-[1-hydroxi-2-methylamine]) is an endogenous catecholamine drug widely used in the treatment of hypertension, bronchial asthma, cardiac arrest, myocardial infarction, cardiac surgery and glaucoma [1].

Several flow injection procedures have been proposed in the literature for the determination of adrenaline in pharmaceutical formulations using amperometric [2–4] and piezoelectric detectors [5,6]. Recently, Medina et al. [7] described the determination of adrenaline by flow injection using an optosensor as spectrophotometric detector in the UV region. The determination of catecholamine drugs by chemiluminescence reaction in flow injection analysis (FIA) has been widely explored [8–13]. However, those procedures show limited linear range, low selectivity and/or relatively expensive reagents were used.

Solich et al. [14] described the determination of adrenaline by spectrophotometry based in the ferrous complex formation with this drug in aminoacetic–carbonate buffer (pH 8.3). The analytical curve was linear in the adrenaline concentration range from 5 to 200 mg l^{-1} with an analytical frequency of 120 determinations per hour. Nevado et al. [15] used a metaperiodate solution and spectrophotometric detection for the determination of adrenaline and isoprenaline. In addition, the spectrophotometric determination of adrenaline and dopamine has been proposed in the visible region after reaction with sodium hydroxide [16].

Kojlo and Martínez-Calatayud impregnated a poly(vinyl chloride) coil with iodine solution [17] and applying it to the fluorimetric flow injection determination of adrenaline. The main drawback of this flow system is the irreproducible results obtained in the beginning of the adrenaline determinations, the low lifetime of the reactor containing I_2 and the need of periodic re-calibrations. The same authors developed a flow injection system containing manganese dioxide

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entrapped in a polymeric material in a solid-phase reactor at a temperature range of 20–80 °C, and the oxidized drug was monitored fluorimetrically at 540 nm ($\lambda_{exc} = 330$ nm) [18]. Nevertheless, this work temperature range and the high NaOH (1.5 mol l^{-1}) concentration employed can cause some adversities, such as air bubbles, high hydrodynamic impedance and chemical attack of fluorimetric cell.

Solid-phase reactor coupled in flow injection system is an interesting strategy because it is possible to use reagents that are not available in soluble form. Furthermore, the use of insoluble reagents can avoid the time-consuming steps of reagent solutions preparation. The immobilization procedure is fairly expeditious, simple and non-specific [19].

In this work, a flow injection spectrophotometric procedure is reported for determining adrenaline in pharmaceutical formulations using a solid-phase reactor containing PbO_2 immobilized in polyester resin. The method is based on the oxidation of adrenaline with PbO_2 producing adrenochrome [20], which is monitored at 486 nm.

2. Experimental

2.1. Apparatus

An eight-channel Ismatec (Zurich, Switzerland) model 7618-40 peristaltic pump supplied with Tygon pump tubing was used for the propulsion of the fluids. The manifold was constructed with polyethylene tube (0.8 mm id.). Sample and reference solutions were inserted in the flow system with the aid of a Micronal (São Paulo, Brazil) automatic proportional unit, model B 353) [21].

Flow injection spectrophotometric measurements were carried out using a Femto (São Paulo, Brazil) spectrophotometer, model 435, equipped with a glass flow-cell (optical path, 1.0 cm) and the signals were recorded on a Cole-Parmer (USA) recorder, model CR 53125.

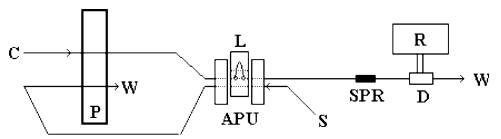

Fig. 1. Schematic diagram of the flow injection system used for spectrophotometric determination of adrenaline. APU, automatic proportional unit; P, peristaltic pump; S, sample or reference solutions; L, sample loop (375 μl); C, carrier solution (acetate buffer solution at pH 4.8 and flowing rate of 2.7 ml min^{-1}; SPR, solid phase reactor (70 mm long and 2 mm i.d.) containing immobilized PbO_2; D, spectrophotometer at 486 nm; R, recorder and W, waste.

2.2. Reagents and solutions

All solutions were prepared using a Millipore (USA) Milli-Q water. All chemicals were analytical-reagent grade and were used without further purification.

The acetate buffer solution (pH 4.8) was prepared by mixing appropriate volumes of 0.4 mol l^{-1} sodium acetate and 0.4 mol l^{-1} acetic acid (Merck).

A 1.0×10^{-3} mol l^{-1} adrenaline stock solution was prepared by dissolving 18.2 mg of adrenaline (Aldrich) in 100 ml of acetate buffer solution previously de-oxygenated with nitrogen.

The immobilization of PbO_2(s) (Merck) was made using a commercial polyester resin solution (Resapol T-208, Resana, SP, Brazil) and methylethyl ketone (Ibere, Ramires and Cia, Taboão da Serra, SP, Brazil) as catalyst.

2.3. Preparation of the solid-phase reactor containing PbO_2

The immobilization of PbO_2(s) was similar to that previously reported [22–24]. The solid-phase reactor was prepared by mixing lead(IV) dioxide and polyester resin in the following percentage compositions (m/m) such as: 33.3% PbO_2 and 66.7% polyester resin; 50% PbO_2 and 50% polyester resin; 66.7% PbO_2 and 33.3% polyester resin. After manual homogenization in each mixture, some drops of the catalyst (methylethyl ketone) were added and stirred until an increase of viscosity. After 3 h, a rigid solid was obtained, which was broken with a hammer and a Tecnal (Piracicaba, Brazil) multiuse mill, model TE 631/1 was used to obtain small particles. The particle size was selected by passing the particles in known mesh sieves.

The solid phase reactor (SPR) was prepared by packing a polyethylene tube (70 mm long and 2.0 mm i.d.) with one end plugged with glass-wool to prevent the packing material escaping from the reactor with 300 mg of PbO_2(s) immobilized in polyester beads (particle size 100–350 μm) with the aid of a syringe.

2.4. Flow injection system

The solid phase reactor (SPR) was inserted in the flow injection system between the automatic proportional unit (APU) and the spectrophotometric detector (D) as schematically shown in Fig. 1. The acetate buffer solution at pH 4.8 was used as the carrier solution (C) at a flow rate of 2.7 ml min^{-1}. Adrenaline sample or reference solution in the same acetate buffer solution containing in the sample loop (L, 375 μl) was inserted and transported by the carrier stream after the baseline had reached a steady-state value. The analytical length from the solid phase reactor to spectrophotometer was the minimum required (50 cm). When the solution of

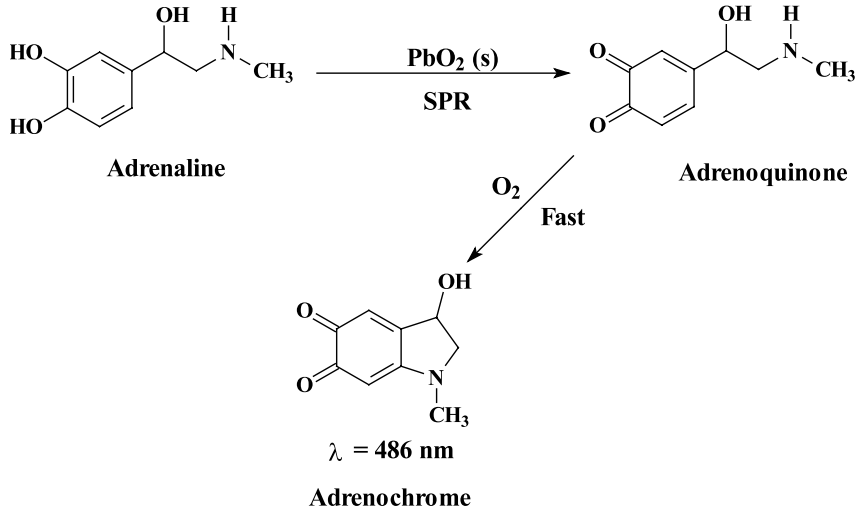

Fig. 2. Schematic representation of the oxidation of adrenaline at SPR containing PbO_2 yielding adrenochrome.

adrenaline is inserted this drug is oxidized by PbO_2 immobilized yielding adrenochrome, which was monitored at 486 nm. Fig. 2 shows a schematic representation of the reaction between adrenaline and lead dioxide.

All solid phase reactors tested were previously conditioned by passing the carrier solution for 10 min before starting the first injection in order to minimize the effect of compaction of the particles in the reactor on the analytical signal. At least ten injections were necessary to obtain reproducible absorbance signals after the system was started.

2.5. Preparation of pharmaceutical samples

Liquid formulations were appropriately diluted with acetate buffer solution (pH 4.8) to obtain concentrations in the range from 1.0×10^{-1} to 8.0×10^{-1} mmol l^{-1} of adrenaline. The percentage content of adrenaline in these samples was determined using the analytical curve method (see Fig. 3) and compared to the results obtained using an UV spectrophotometric procedure [25].

3. Results and discussion

3.1. Preliminary studies

Initially, an extensive study of adrenaline oxidation with immobilized PbO_2 (Fig. 2) in the solid-phase reactor using acetate buffer solutions at several pH (2.0–6.0) was investigated. It was observed that the analytical signal (absorbance) decreased gradually with the decrease of the pH solution from 4.8 to 2.0, which is due to the protonation of the NH_2 group of the adrenaline, influencing thus the kinetic of conversion of adrenaline to adrenochrome. For pH solutions higher than 4.8, the solubility of the adrenaline in the medium is prejudiced. Therefore, acetate buffer solution at pH 4.8 was selected for further investigations.

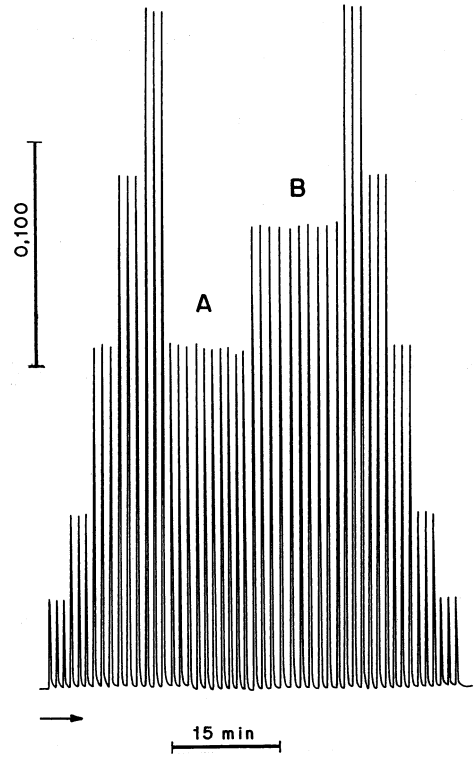

Fig. 3. Transient spectrophotometric signals in triplicate for reference adrenaline solutions (1.0×10^{-1}; 2.0×10^{-1}; 4.0×10^{-1}; 6.0×10^{-1} and 8.0×10^{-1} mmol l^{-1}), two samples (A, B) (10 transient signals each one) and reference solutions again in triplicate obtained using the flow injection system conditions specified in the Fig. 1 legend.

Table 1
Optimization of chemical and flow injection parameters

Parameter	Studied range	Selected value
Reactor composition	33.3–66.7% PbO_2	66.7% PbO_2
Particle size	100–500 μm	100–350 μm
Reactor length	5–11 cm	7 cm
Sample loop length	62.5–700 μl	375 μl
Flow rate	1.1–4.8 ml min^{-1}	2.7 ml min^{-1}

In addition, the adrenochrome yielded in the reactor was collected at flow cell point and the spectrum in the UV–visible region was obtained off-line. Adrenochrome showed a maximum absorbance at 486 nm at pH 4.8. Thus, in all spectrophotometric flow injection measurements at this wavelength was used.

3.2. Effect of composition, particle size and solid-phase reactor length

The effect of the reactor composition on the analytical signal was initially evaluated in ten injections of 6.0×10^{-1} mmol l^{-1} adrenaline acetate buffer solution (pH 4.8) at a carrier flow rate of 2.7 ml min^{-1}. The highest analytical signal was obtained for the 66.7% (m/m) PbO_2 and 33.3% (m/m) polyester composition.

The effect of particle size was studied in three size ranges (< 100, 100–350 and 350–500 μm). The analytical signal decreased with increasing particle size in the range studied, because with the increase of the particle size there was a decrease on the contact of the analyte in the carrier solution with the solid surface containing the reagent ($PbO_{2(s)}$), leading to a decrease in the oxidation reaction of adrenaline and consequently the formation of adrenochrome. Particle sizes less than 100 μm only permit modest carrier flow rates, then particle size of 100–350 mm was chosen for further experiments. These results were similar to that obtained in our previous works [22–24].

The influence of the reactor length on the absorbance was also studied in the 5–11 cm range at a carrier flow rate of 2.2 ml min^{-1} and 6.0×10^{-1} mmol l^{-1} adrenaline solution. The absorbance signals increased gradually with the reactor length. However, a reactor length larger than 7.0 cm showed low reproducibility of the analytical signals and baseline instability. Thus, a reactor length of 7.0 cm was used in further experiments.

3.3. Flow injection parameters

To determine the optimal conditions for the flow injection system performance, parameters such as sample loop length and carrier flow rate were initially investigated.

The effect of varying sample loop length from 12.5 to 350 cm (62.5–700 μl) on the analytical signal was evaluated by injection of 6.0×10^{-1} mmol l^{-1} adrenaline in acetate buffer solution (pH 4.8) at a carrier flow rate of 2.2 ml min^{-1}. The absorbance increased with the increase of sample volumes from 62.5 to 500 μl and it was maintained constant in sample volume higher than 500 μl. Therefore, a sample volume of 375 μl was selected for showing better engagement between sensibility and analytical frequency. The effect of flow rate from 1.1 to 4.8 ml min^{-1} over the analytical signal was studied and the optimal flow rate found was 2.7 ml min^{-1}. Table 1 presents the optimization of chemical flow injection parameters studied in this work.

3.4. Analytical characteristics

Recoveries of 96.5–105% of adrenaline from two pharmaceutical formulations ($n = 3$) were obtained using the flow-injection procedure. In this study, 2.0×10^{-1}; 4.0×10^{-1} and 6.0×10^{-1} mmol l^{-1} of adrenaline were added to each product. The recovery results obtained suggest an absence of the matrix effect in the determination of adrenaline in those samples.

At pH 4.8, the flow injection system shows an analytical curve for adrenaline in the concentration range from 1.0×10^{-1} to 8.0×10^{-1} mmol l^{-1} ($A = 0.00312 + 0.378C$; $r = 0.9999$, where A is the absorbance and C the concentration of adrenaline in mmol l^{-1}) (see Fig. 3). The quantification limit (10-fold blank standard deviation/slope) was 4.5×10^{-2} mmol l^{-1} and the detection limit (3-fold blank standard deviation/slope) was 8.0×10^{-3} mmol l^{-1} with relative standard deviation (RSD) of 0.2% for a solution containing 4.0×10^{-1} mmol l^{-1} of adrenaline ($n = 10$). The analytical frequency was 130 determinations per hour and a lifetime of 380–400 reproducible results can be obtained with a SPR. After a re-calibration of the flow injection system, about 300–350 determinations were obtained with the same SPR.

3.5. Determination of adrenaline in pharmaceutical formulations

Table 2 presents the results obtained using an official UV spectrophotometric procedure [25] and the proposed flow injection procedure. Applying paired t-test in the results obtained by either procedures, it was found that all results are in agreement at the 95% confidence level and within an acceptable range of error, confirming the accuracy of the FIA method using solid-phase reactor. The advantages of the proposed flow injection method over the reference USP method are the reducing of the reagents consumed and the higher analytical frequency.

Table 2
Determination of adrenaline in formulations by UV spectrophotometric [25] and proposed flow injection methods

Samples	Adrenaline (mg ml^{-1})			E_{r1}	E_{r2}
	Label value	Spectrophotometry	Flow injection		
A [a]	1.00	0.95 ± 0.01	0.96 ± 0.01	−5.0	+1.1
B [a]	1.00	1.00 ± 0.01	1.03 ± 0.02	+3.0	+3.0

E_{r1} = relative error = flow injection method versus label value. E_{r2} = relative error = flow injection method versus UV spectrophotometric method.
[a] Sample composition: A: adrenaline bitartrate; B: adrenaline chloridrate.

4. Conclusions

The solid-phase reactor containing PbO_2 immobilized in polyester resin which has been developed is easy to make, has a long lifetime (~ 750 reproducible results), and its larger sampling rate permits the determination of the adrenaline in pharmaceutical formulations with good accuracy and precision.

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References

[1] A.J. Pesce, L.A. Kaplan, S. Bircher, Methods in Clinical Chemistry, C.V. Mosby, St. Louis, 1987.
[2] A.M. Galvez, J.V.G. Mateo, J. Martínez-Calatayud, Study of various indicating redox systems on the indirect flow-injection biamperometric determination of pharmaceuticals, Anal. Chim. Acta 396 (1999) 161–170.
[3] J.V.G. Mateo, A. Kojlo, Flow-injection biamperometric determination of epinephrine, J. Pharm. Biomed. Anal. 15 (1997) 1821–1828.
[4] E.M. Garrido, J.L.F.C. Lima, C. Delerue-Matos, Flow-injection amperometric determination of L-dopa, epinephrine or dopamine in pharmaceutical preparations, J. Pharm. Biomed. Anal. 15 (1997) 845–849.
[5] Z.H. Mo, X.H. Long, M.J. Zhang, Piezoelectric detection of ion pairs between sulphonate and catecholamines for flow-injection analysis of pharmaceutical preparations, Talanta 48 (1999) 643–648.
[6] Z.H. Mo, M.J. Zhang, M.L. Li, Z.L. Xia, Flow-injection analysis of norepinephrine bitartrate by ion association with anionic surfactant using a piezoelectric detector, Anal. Lett. 30 (1997) 663–671.
[7] A.R. Medina, M.L.F. de Cordova, A.M. Diaz, Sensitive determination of adrenaline by means of a flow-through solid phase UV spectrophotometric sensing device, Mikrochim. Acta 134 (2000) 101.
[8] O. Nozaki, T. Iwaeda, H. Moriyama, Y. Kato, Chemiluminescent detection of catecholamines by generation of hydrogen peroxide with imidazole, Luminescense 14 (1999) 123–127.
[9] C.X. Zhang, J.C. Huang, Z.J. Zhang, M.S. Aizawa, Flow injection chemiluminescence determination of catecholamines with electrogenerated hypochloride, Anal. Chim. Acta 374 (1998) 105–110.
[10] N.T. Deftereos, A.C. Calokerinos, C.E. Efstathiou, Flow injection chemiluminometric determination of epinephrine, norepinephrine, dopamine and L-dopa, Analyst 118 (1993) 627–632.
[11] F. Yoshimura, T. Suzuki, M. Yamada, T. Hobo, Manganese(III)-tetrakis(4-sulfonatophenyl) porphirin immobilized on an anion-exchange resin as an indicator phase for chemiluminescence sensing for adrenaline, Bunseki Kagaku 41 (1992) 191.
[12] Y. Katsuoka, J. Hayashi, M. Yamada, T. Hobo, Flow chemiluminescent determination of adrenaline based of fenton oxidation, Bunseki Kagaku 40 (1991) 525–529.
[13] K. Matsue, T. Suzuki, M. Yamada, T. Hobo, Dioctadecyldimethylammonium chloride bilayer-membrane vesicle enhanced and manganese(II)-catalyzed chemiluminescence for determination of adrenaline by a flow-injection method, Anal. Lett. 22 (1989) 2445.
[14] P. Solich, C.K. Polydorou, M.A. Koupparis, C.E. Efstathiou, Automated flow-injection spectrophotometric determination of catecholamines (epinephrine and isoproterenol) in pharmaceutical formulations based on ferrous complex formation, J. Pharm. Biomed. Anal. 22 (2000) 781–789.
[15] J.J.B. Nevado, J.M.L. Gallego, P.B. Laguna, Spectrophotometric determination of catecholamines with metaperiodate by flow-injection analysis, Anal. Chim. Acta 300 (1995) 293–297.
[16] J.J.B. Nevado, J.M.L. Gallego, P.B. Laguna, Flow-injection spectrophotometric determination of adrenaline and dopamine with sodium hydroxide, J. Pharm. Biomed. Anal. 14 (1996) 571–577.
[17] A. Kojlo, J. Martínez-Calatayud, Spectrofluorometric flow-injection determination of adrenaline with an iodine solid-phase reactor, Anal. Chim. Acta 308 (1995) 334–338.
[18] A. Kojlo, J. Martínez-Calatayud, FIA-fluorometric determination of adrenaline by oxidation with a solid-phase reactor of manganese-dioxide incorporated in polyester resin beads, Anal. Lett. 28 (1995) 239–247.
[19] J. Martínez-Calatayud, J.V.G. Mateo, Bed reactors for use in unsegmented continuous-flow methodologies–analysis of inorganic species, Chem. Anal. 38 (1993) 1–24.
[20] S. Görög, Ultraviolet-visible Spectrophotometric in Pharmaceutical Analysis, CRC Press, Florida, 1995, pp. 246–253.
[21] M.F.S. Teixeira, O. Fatibello-Filho, C. Aniceto, C.O. Costa-Neto, Flow-injection potentiometric determination of iron (III) in vitamin formulations using a tubular ion-selective electrode in oxalic medium, Lab. Robot. Automation 11 (1999) 163–168.
[22] A.V. Pereira, O. Fatibello-Filho, Spectrophotometric flow injection determination of L-ascorbic acid with a packed reactor containing ferric hydroxide, Talanta 47 (1998) 11–18.
[23] O. Fatibello-Filho, L.H. Marcolino-Junior, A.V. Pereira, Solid-phase reactor with copper (II) phosphate for flow-injection spectrophotometric determination of aspartame in tabletop sweeteners, Anal. Chim. Acta 384 (1999) 167–174.
[24] L.H. Marcolino-Júnior, M.F.S. Teixeira, A.V. Pereira, O. Fatibello-Filho, Flow injection determination of levodopa in tablets using a solid-phase reactor containing lead(IV) dioxide immobilized, J. Pharm. Biomed. Anal. 25 (2001) 393–398.
[25] United States Pharmaopoeia, XXIII, US Pharmacopoeial Convention, Rockville, MD, 1994.