Flow-injection spectrophotometric determination of adrenaline and dopamine with sodium hydroxide

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Abstract

A new, rapid and economical flow-injection method for determining adrenaline and dopamine is proposed on the basis of the hydrolysis of these compounds in alkaline medium. The method was optimized by using a spectrophotometer operating at $\lambda = 390$ nm as detector. Calibration graphs were linear up to 2×10^{-4} M with quantification limits of 2.5×10^{-6} M and 3.3×10^{-6} M for dopamine and adrenaline respectively. Flow-injection allows the measurement of 130 samples per hour. The method was successfully applied for the determination of catecholamines in pharmaceuticals.

Keywords: FIA; Spectrophotometry; Catecholamines; Aminochrome

1. Introduction

Dopamine and adrenaline are catecholamines widely used in the treatment of shock and heart failure respectively. Pharmaceutical preparations containing these catecholamines have been available for many years and several analytical procedures have been proposed for their control. The current USP XX method for catecholamine determination is a very long procedure. It is based on the polarimetric determination of triacetyl-derivatives. For similar compounds, several spectrophotometric methods have been applied, as cited in the literature, including derivatization reactions with organic (ninhydrin [1], p-benzoquinone [2,3], thiosemicarbazide [4,5], chloranil [6,7]) and inorganic (iodine [8], ammonium metavanadate [9], metaperiodate [10,12]) reagents. Some of these methods have been adapted to the stopped-flow [13–16] technique and to flow-injection analysis (FIA) [17–19].

Automated methods for a simple, fast, inexpensive and accurate determination of dopamine and adrenaline are of great interest to quality control laboratories. The purpose of the present work was to develop a new flow-injection method for determining dopamine and adrenaline on the basis of their behaviour in strongly alkaline solutions. Only sodium hydroxide solution is used as a

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chromogenic reagent and therefore this is a very economical method for determining dopamine and adrenaline in different pharmaceuticals.

2. Experimental

2.1. Reagents

All solutions were prepared from analytical-reagent grade materials with MilliQ-water. Dopamine and adrenaline stock solutions (1×10^{-3} M) were prepared from Sigma products in 0.05 M AcH/Ac buffer solution (pH 4.8), and stored at 4°C in a dark bottle before use. Working solutions were prepared daily from these solutions by diluting with Milli-Q water. The sodium hydroxide solution (0.4 M) from Panreac product was prepared by dissolving in Milli-Q water.

2.2. Apparatus

A Beckman Instruments DU-70 spectrophometer connected to an IBM PS/2 fitted with Beckman Data Leader software [20] was used for all measurements and treatment of data.

A schematic diagram of the semiautomatic flow-injection analyzer used is shown in Fig. 1.

The flow manifold included a peristaltic pump (Minipuls 3 from Gilson) which pumped the reagent solution (sodium hydroxide and the carrier (Milli-Q water) at the same flow rate through polyethylene flow tubes (0.5 mm i.d.). The sample was injected into the carrier stream by an injection valve (Omnifit n 1106) provided with a 350 μl loop. The absorbance peak of the resulting aminochrome is followed using the spectrophoto-

Fig. 1. Schematic diagram of the flow-injection manifold used for the determination of cateholamines.

meter of the flow-injection analyzer, equipped with an 18 μl flow-cell (Hellma). Data were stored in the computer.

2.3. Procedure

2.3.1. Continuous-flow procedure

Sample solution (350 μl) containing catecholamine was injected into Milli-Q water (temperature 65°C) and then sodium hydroxide solution (0.4 M) was added. The solution passed through a reactor (2.7 m length, 0.5 mm i.d.) The solution was carried to the flow cell, and the absorbance was recorded.

The variation of absorbance was monitored at 390 nm throughout the reaction. Each solution was assayed in triplicate. Sixty points per minute were measured. The peaks were stored in the computer, smoothed by Savizky–Golay filtering [21] using seven experimental points, the baseline was obtained with DATA LEADER Software, and for every peak the greatest height was obtained.

2.3.2. Determination of catecholamines in pharmaceuticals

An appropiate dilution with buffer solution (0.05 M AcH/Ac pH 4.8) of the pharmaceutical formulation was all that was required, after which the procedure described above was carried out.

3. Results and discussion

Dopamine and adrenaline solutions in alkaline medium are hydrolyzed and oxidized by atmospheric oxygen giving yellow–brownish colorations due to aminochrome derivatives [22,23]. These compounds are very unstable [24–27]. Some spectrophotometric methods use the high reactivity of the aminochrome in order to give coupling reactions with another reagent (vanillin [28], p-anisaldehyde[29], p-tolualdehyde [29], benzaldehyde [29], 1,2-dinitroanthracene [30], 2-aminophenol [30], thiosemicarbazide [31]. However, kinetic automated methods, such as FIA, can be used to avoid the instability problem and reproducible spectrophotometric measurements of the aminochrome in alkaline medium can be achieved.
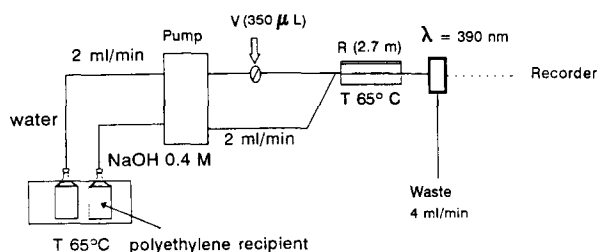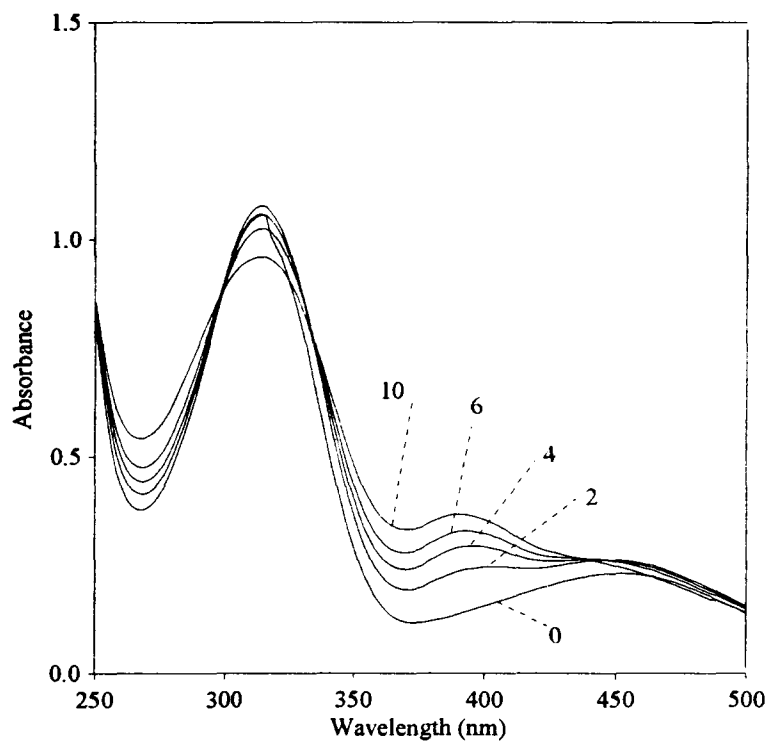
Fig. 2. Absorption spectra of dopamine (2×10^{-4} M) with NaOH up to pH 12.6 at different times (numbers above spectra as times in seconds).
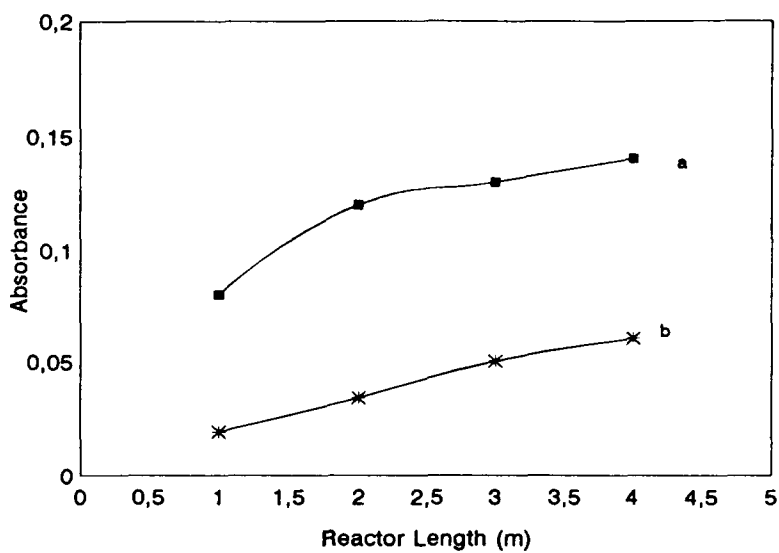

Fig. 3. Absorbance vs. length of reactor run at 390 nm. Catecholamine concentration = 2×10^{-4} M: (a) dopamine; (b) adrenaline. Volume injected = 350 μl; temperature = 65°C; NaOH concentration = 0.4 N; flow rate = 2.0 ml min^{-1}.
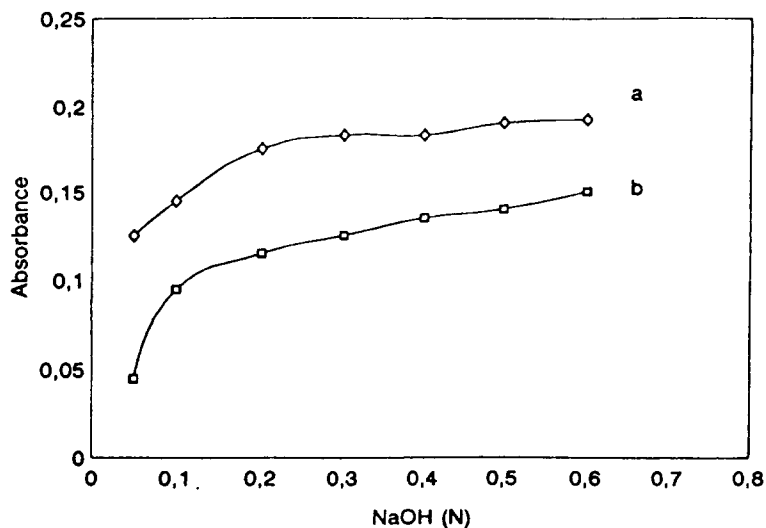
Fig. 4. Influence of sodium hydroxide concentration. $\lambda = 390$ nm. Catecholamine concentration $= 2 \times 10^{-4}$: (a) dopamine; (b) adrenaline. Volume injected $= 350$ μl; temperature $= 65°C$; flow rate $= 2.0$ ml min^{-1}; reactor length $= 2.7$ m.
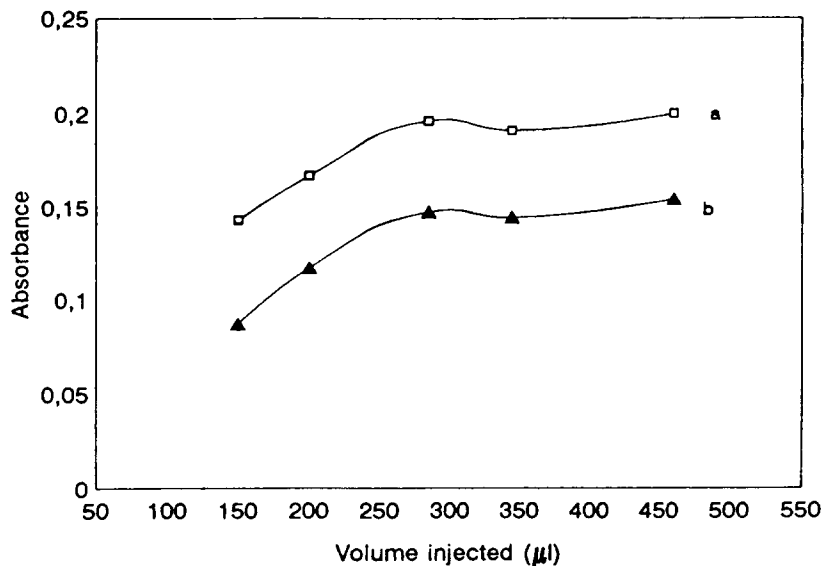

Fig. 5. Absorbance versus volume injected, run at 390 nm. Catecholamine concentration $= 2 \times 10^{-4}$ M: (a) dopamine; (b) adrenaline. Temperature $= 65°C$; flow rate $= 2.0$ ml min^{-1}; reactor length $= 2.7$ m; NaOH concentration $= 0.4$ N.

As observed in Fig. 2, the absorption spectrum of a dopamine solution in basic medium is very dependent on time. Similar behaviour was observed for adrenaline solutions. We have selected 390 nm as the wavelength for obtaining the FIA-grams.

3.1. Effect of the reaction variables

The influences of the reaction variables were studied for both catecholamines by changing each variable in turn while keeping all others unchanged. The variables studied were: volume in-

jected; length of the reactor; flow rate; hydroxide concentration; and temperature. Reactor length was varied between 100 and 400 cm; a reactor of 270 cm length was selected as appropiate (see Fig. 3).

Fig. 4 shows the influence of the sodium hydroxide concentration on the peak height for dopamine and adrenaline. A 0.4 M solution of sodium hydroxide was selected as suitable. Sodium hydroxide solution must be contained in a polyethylene recipient.

Small peaks are obtained when the reactor remains at room temperature, so the temperature of the reactor bath was varied between 20 and 85°C and 65°C was chosen as appropriate.

The volume injected was varied between 150 and 450 μl. The peak height increased when the volume injected was increased up to 300 μl (Fig. 5). At higher volumes the peak height remained virtually constant. The volume chosen was 350 μl.

Water and sodium hydroxide solution were pumped at the same flow rate. The flow rate was varied between 1.25 and 4.25 ml min^{-1}. The peak height did not increase significantly for flow rates up to 1.25 ml min^{-1}, but the peak width decreased when the flow rate increased (Fig. 6). A flow rate of 2.0 ml min^{-1} was chosen as suitable.

3.2. Analytical parameters

Regression analysis of Beer's law plotted at 390 nm with sodium hydroxide reagent gave good correlation up to a concentration of 2×10^{-4} M of the assayed solution of catecholamine.

The relative standard deviation (RSD; %) was <0.5 ($n = 10$). The sampling rate was 130 measurements h^{-1}. The results are summarized in Table 1.

3.3. Analysis of pharmaceutical preparations

The proposed method was applied satisfactorily to the determination of adrenaline and dopamine in the following pharmaceutical preparations:

Adrenalina (1 mg ml^{-1} adrenaline hydrochloride) Llorente SA (Spain);

Clorhidrato de dopamina Grifols (40 mg ml^{-1}, dopamine hydrochloride, 50 mg ml^{-1} sodium bisulfite) from Laboratorios Grifols SA (Spain):

Aprical dopamina (40 mg ml^{-1} dopamine hydrochloride) from Berenguer-Infale (Spain).

The concentrations of the catecholamines were calculated by direct measurements on the appropriate calibration graph. The results obtained are summarized in Table 2. As can be seen, for all the formulations the results were in good agreement with values from the labelled contents.

4. Conclusions

A spectrophotometric–FIA procedure is proposed for the determination of adrenaline and dopamine to be used in the control analysis of the pharmaceutical formulations.

The method, based on the aminochrome formation in alkaline medium, has several advantages. A stable blank signal of low absorbance is obtained. The proceudre is very simple, fast and sensitive and can be fully automated. Therefore, this technique is very suitable for routine analytical applications.
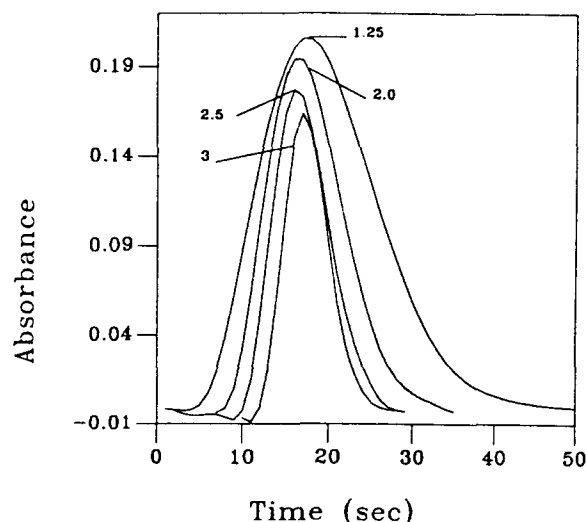

Fig. 6. Variation of peak height and width with flow rate under the proposed conditions with the flow-injection technique (numbers above peaks are flow rates (ml min^{-1}).

Table 1
Parameters of the calibration graphs used for the determination of dopamine and adrenaline when catecholamine concentrations are given in terms of moles per liter

Parameter	Catecholamine	
	Dopamine	Adrenaline
Slope	969.32	752.33
Intercept	1.504×10^{-3}	-3.537×10^{-3}
Correlation coefficient	0.9999	0.9942
Std. deviation of slope	8.1	41
Std. deviation of intercept	9.9×10^{-4}	2.5×10^{-4} M
Std. deviation of correlation	1.3×10^{-3}	3.2×10^{-3}
t^a(theoretical)	2.571	2.571
t^a(experimental)	0.552	1.616
Slope without intercept	979.86	702.72
Dynamic linear range (10^{-5} M)	2–20	2–20
Detection limit (M)	7.6×10^{-7}	9.7×10^{-7}
Quantification limit (M)	2.5×10^{-6}	3.3×10^{-6}
Precision (RDS) (%)	0.481	0.423
Sampling rate (h^{-1})	130	130

[a] Student t test.

Table 2
Determination of dopamine and adrenaline in pharmaceutical preparations

	Adrenalina Llorente	Aprical dopamina	Dopamina Grifols
Catecholamine	Adrenaline	Dopamine	Dopamine
Nominal value (mg ml^{-1})	1	40	40
n	12	9	9
Mean	1.02	40.02	38.14
SD^a	5.08×10^{-2}	1.71	1.40
SEM^b	1.47×10^{-2}	0.57	0.47
95% Confidence interval	0.985–1.05	38.71–41.34	37.06–39.21

[a] Standard deviation. [b] Standard error of the mean.

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