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Short communication

Flow injection amperometric determination of L-dopa, epinephrine or dopamine in pharmaceutical preparations

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1. Introduction

Catecholamines are organic compounds with an alkylamine chain bonded to a benzenic ring with two hydroxyl groups, among which dopamine and epinephrine are important neurotransmitters and L-dopa is a dopamine physiologic precursor. The determination of catecholamines in biologic matrices usually requires analytical procedures which are capable of low detection limits, since they occur in low concentrations. Therefore, the use of chromatography [1–4] and some electrochemical methods [5–13], usually using modified electrodes due to the increased selectivity and sensitivity required, is very common.

The above mentioned restrictions do not apply when measuring the dosage of catecholamines in pharmaceutical preparations as they occur in much higher concentrations, and thus are amenable to the use of simple, quick and inexpensive analytical methods, which whenever possible should also be automated. In recent years several procedures have been referred to in the literature as capable of determining catecholamines in pharmaceutical preparations such as the use of titrations [14–18], spectrophotometric UV/Vis [15–21], flurimetric [21], kinetic [22,23], and chromatographic [24] methods, which however can hardly be considered simple, quick or inexpensive. Spectrophotometric detection has been used as a method in stopped-flow injection analysis (FIA) [25–31] to significantly increase the sampling rate and reduce the use of reagents. However, in many proposals in the literature the use of reagents can be significant, since one or more reagents are needed in order to generate products that can be measured by the detection systems. In some methods it is necessary to control the temperature and the resulting manifolds are complex and generally compromising the sampling rates.

In this paper a simple system, with an amperometric detector which is easily handled and that gives high sampling rates and in which the sample preparation is reduced to a simple dilution in

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water or support electrolyte, is described. The use in this system of dilute solutions and the brief contact of the material at the electrode surface drastically reduces absorption, thus requiring mechanical cleaning of the glassy carbon electrode only at the beginning of each working day. The system, without any changes, has been used to determine the dosage of L-dopa, dopamine and epinephrine in pharmaceutical preparations available on the Portuguese market.

2. Experimental

2.1. FIA manifold

This FIA manifold (Fig. 1) uses an Ismatec Mini S-840 peristaltic pump to propel the solutions, a Rheodyne 5020 valve to inject the solutions and, as the detector unit, an electrochemical system consisting of a VA 641 Metrohm detector and a 656 Metrohm electrochemical wall jet cell. The cell is composed by three electrodes, namely two EA 286/1 Metrohm glassy carbon electrodes for the working electrode and the auxiliary electrode respectively and a Ag–AgCl EA 442 Metrohm as the reference electrode. At the end of each day, the working electrode was manually cleaned and polished with an abrasive surface (Kemet, PSU8 type) which had been previously sprayed with Kemet spray (1 μ). The electrode surface was then washed with water and kept dry until the next day. Omnifit Teflon tubing (0.5 mm i.d.) and Gilson end fittings as well as connectors and home-made confluences and dampers drilled from perspex [32] were used to connect the different manifold components. A BD 112 Kipp and Zonen was used to record the analytical signals.

2.2. Reagents and solutions

Analytical grade chemicals were used without any additional purification. Deionized water with conductivity < 0.1 μS cm^{-1} was used to prepare the samples. The support electrolyte (KH_2PO_4–K_2HPO_4, pH 6.4 and I = 0.1 M) prepared by 10-fold dilution of a mixture of equal volumes 0.8 M KH_2PO_4 and 0.2 M K_2HPO_4, was used as a carrier. This solution was prepared by diluting 10.8 g KH_2PO_4 in 100 ml water. Similarly for 3.5 g K_2HPO_4. Thus the solutions had concentrations close to 0.8 M and 0.2 M respectively. The support electrolyte was obtained by mixing 50 ml KH_2PO_4 aqueous solution with 50 ml K_2HPO_4 aqueous solution and then diluting to 500 ml.

2.3. Standards and samples preparation

The L-dopa (Sigma) and epinephrine (Sigma) stock solutions (10^{-3} M) were prepared in a support electrolyte, since these compounds are not easily soluble in water. The solubility was increased by using an ultrasonic bath. The concentrations of these solutions were of 10^{-3} M. More diluted solutions, between 7×10^{-6} and 5×10^{-5} M prepared by careful dilution of the stock solutions with support electrolyte, were used for the calibrations.

The same procedures have been carried out for the stock solution of dopamine hydrochloride (Sigma) except that there is no use of a support electrolyte, since this compound is easily soluble in water. All these solutions were prepared daily and protected from light.

The determination of L-dopa was made from commercial tablets available in Portugal. Ten tablets were powdered and a quantity corresponding to 2.3×10^{-3} g of L-dopa was carefully weighed. This sample was then diluted in 50.00 ml of KH_2PO_4–K_2HPO_4 (pH 6.4) support electrolyte. The sample was afterwards diluted carefully with the same support electrolyte in order to obtain a concentration within the calibration curve range.

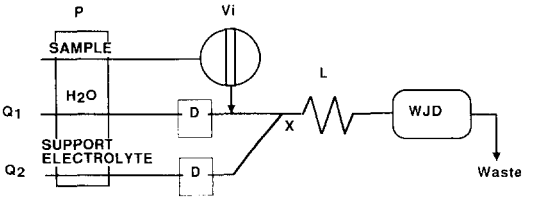

Fig. 1. Flow injection system. P, peristaltic pump; injection valve (92 μl); *Q*, flow rate (Q1 = Q2 = 1.2 ml min^{-1}; D, pulse damper; L, mixing coil (225 cm); WJD, wall jet detector.

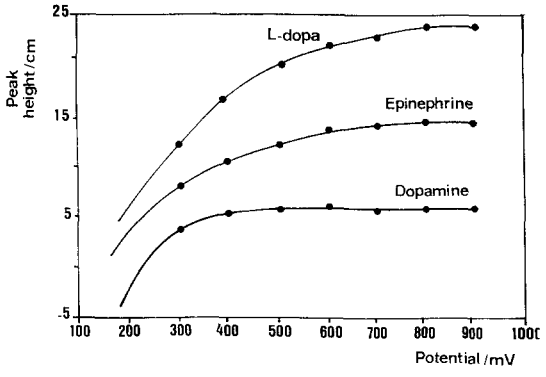

Fig. 2. Peak heights obtained for 10^{-4} M L-dopa, dopamine and epinephrine solutions as functions of the working electrode potential at a 2.4 ml min^{-1} flow rate.

For the injectable solutions an accurate volume was weighed and diluted according to their predicted composition, so that a concentration of approximately 1×10^{-4} M would be produced. The samples were then further diluted until their concentration was within a 7×10^{-6}–5×10^{-5} range. For epinephrine the dilutions were carried out with support electrolyte whereas for dopamine the dilutions were carried out with water.

The quality of the results of the analytical procedures was assessed by determining the respective recovery rates.

3. Results and discussion

3.1. FIA manifolds

The FIA system used in the determination of catecholamines was optimized by the univariant method with the purpose of maximizing the sample rate and reproducibility. The parameters studied were the value of the working electrode potential, the injection volume, the reactor length and the flow rate.

To select the value of the working electrode potential, a study of the variation of the peak height with the potential applied to each of the catecholamines, having concentrations of 10^{-4} M (Fig. 2 shows the behaviour of each catecholamine), was carried out. As may be seen, there is an increase in the peak height in all of them and then it stabilizes, but still varies according to each catecholamine. Therefore, a potential of 800 mV has been chosen as the one that presents the highest peak height for all catecholamines, without compromising the reproducibility.

Selection of the flow rate was dependent on the characteristics of the electrochemical detector whose small dead volume (about 1 μl [33]) does not allow the use of very high flow rates. Our experience showed that a flow rate higher than 2.4 ml min^{-1} at the entry to the detector was inadequate due to high pressures within the system and also because of the use of peristaltic pumps which produce a significant decrease in the analytical signals reproducibility. On the other hand, lower flow rates compromised the sampling rate. Consequently, 2.4 ml min^{-1} has been selected and is achieved by a 1.2 ml min^{-1} flow rate both in the sample transporting channel (Q1) and in the electrolyte transporting channel (Q2).

Once the flow rate was selected, the most adequate injection volume was determined and loops were constructed with the same Teflon tubing that was used for other parts of the system (0.5 mm i.d.), with lengths between 10 and 30 cm. A 25 cm loop was selected, since higher injection volumes compromised the sampling rate and lower injection volumes produced less reproducible analytical signals.

The real volume which could actually be introduced into the system (this includes the internal dead volume of the injection valve) was accurately evaluated for a loop of 25 cm length. The correspondent volume, determined by titration with samples of known concentration, of a solution obtained by 25 replicate injections [34], was 92 μl.

The optimization of this system concluded with the selection of the reactor length (L), between the confluence point (X) and the detector. With a reactor length less than 225 cm mixing between the electrolytic solution and the sample was not complete and lead to non reproducible results. Greater lengths to the detector reduced the sampling rate.

With these parameters selected for the system, a sampling rate of 100 samples h^{-1} were achieved.

3.2. Determination of catecholamines in pharmaceutical preparations

In the double-channel flow-injection manifold one channel is used for water to carry the sample plug of the pharmaceutical preparation solution to the confluence point, where the supporting electrolyte is added. The solution of the pharmaceutical products common to the portuguese market are prepared as described in Section 2.3. In these pharmaceutical preparations only one active principle is present and the other components do not give a significant signal under the experimental conditions used in the determinations.

The FIA system was calibrated by three replicate injections of different catecholamine standards with a concentration in the range 7×10^{-6}–5×10^{-5} M. Under these conditions the calibration plot showed a straight line for L-dopa ($r = 0.998$; $n = 5$), epinephrine ($r = 0.998$; $n = 5$) and dopamine ($r = 0.997$; $n = 5$). Fig. 3 shows the diagram for epinephrine standard solutions for the sample and recovery experiments. Table 1 list the mean results and the corresponding S.D. obtained with 5–8 replicate determinations of each batch. Recoveries were determined to assess the accuracy of the results. The values obtained varied between 98 and 102%.

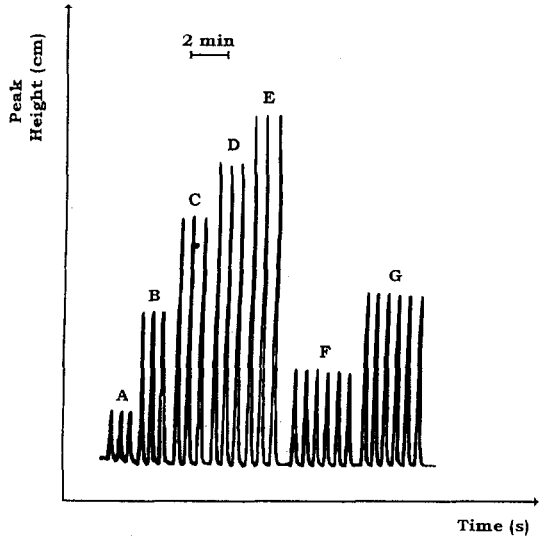

Fig. 3. Typical recorder output for a series of epinephrine standards (A = 6.77×10^{-6}, B = 2.03×10^{-5}, C = 3.38×10^{-5}, D = 4.06×10^{-5}, E = 4.73×10^{-5} M) real sample (F) and its recovery determinations (G) under the conditions proposed for the FIA technique.

Table 1
Results obtained for commercial pharmaceutical preparations by FIA with amperometric detection

Preparation[a]	Obtained (%w/w)[b]	Recovery (%)
L-dopa		
Sinemet®	74.80 ± 4.70	98 ± 8
Dopamine		
Medopa®	3.70 ± 0.06	101 ± 5
Epinephrine		
Dyspneinhal®	3.61 ± 0.31	102 ± 5
Adrenalina Palex®	0.097 ± 0.006	94 ± 4

[a] Commercially available dosage forms with commercial names written in Portuguese.
[b] Mean and S.D. of 5–8 determinations for different samples.

Reproducibility of the method was evaluated by performing ten consecutive injections from each of the solutions to be analysed. The errors obtained for L-dopa, dopamine and epinephrine were 1.9, 0.7 and 1.4%, respectively.

4. Conclusion

This FIA system enables the determination of L-dopa, dopamine and epinephrine in pharmaceutical preparations. It also enables a sampling rate of 100 samples h^{-1} and a recovery rate near 100%. Complex pre-treatment of the samples is not necessary because the preparation of the pharmaceutical formulations is done simply by dissolving in water or electrolyte. Furthermore the use of very diluted solutions and the short time of contact between the sample and the sensor drastically reduces absorption at the electrode surface, thus requiring manual cleaning of the glassy carbon only at end of the working day.

Additionally it must be stressed that this system enables the determination of the three active principles, without needing to change the system or even the working electrode potential. Therefore the this system is particularly useful for the implementation of routine analysis.

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