International Journal of Pharmaceutics, 3 (1979) 239–246

DETERMINATION OF CATECHOLAMINES IN PHARMACEUTICAL PREPARATIONS BY DIFFERENTIAL PULSE POLAROGRAPHY AT THE GLASSY CARBON ELECTRODE

JILL BALLANTINE and A.D. WOOLFSON *

Department of Pharmacy, The Queen's University of Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast BT9 7BL (N. Ireland)

(Received January 11th, 1979)
(Revised version received February 21st, 1979)
(Accepted March 1st, 1979)

SUMMARY

A polarographic method has been developed for the assay of catecholamines in pharmaceutical preparations. The compounds were determined by direct anodic oxidation at the glassy carbon electrode using differential pulse polarography. Peak current was found to be linearly related to catecholamine concentration and the glassy carbon surface was not fouled by the products of the electrochemical oxidation.

The effects of degradation products and of antioxidant on the polarographic method were investigated. The method was used to determine adrenaline and noradrenaline in solution formulations, and isoprenaline and methyldopa in tablets, and was found to give accurate and reproducible results. The polarographic method had the advantages of rapidity and simplicity, making it suitable for application in routine quality control of these preparations.

INTRODUCTION

Numerous methods have been reported for the determination of catecholamines in pharmaceutical preparations. The British Pharmacopoeia (1973) utilizes both optical rotation and colorimetric methods for the determination of catecholamines such as adrenaline. However, spectrofluorimetry, based on the trihydroxyindole reaction of Loew (1918), is generally accepted as the preferred method. These techniques have in common the fact that a derivative compound, rather than the catecholamine itself, is determined. Further, each has certain disadvantages with respect to assay time, specificity or a marked dependence on reaction conditions.

* To whom reprint requests should be addressed.

Electrochemical determinations of catecholamines may be achieved either by reduction of derivative compounds or by direct anodic oxidation of the catecholamine nucleus. One of the first polarographic investigations on catecholamines established the electrochemical activity of adrenochrome, an adrenaline oxidation product, which can subsequently be reduced at the dropping mercury electrode (Brezina and Zuman, 1958). Other indirect methods have been described in which the catecholamine is first converted to an electroreducible form by oxidation (Morvay and Kiss, 1969; Henderson and Friedberg, 1955) or by formation of the nitroso derivative (Vilvala and Halmekoski, 1976).

Sartori and Cattaneo (1945) reported the direct anodic oxidation of adrenaline at the dropping mercury electrode. However, it has now been established that direct anodic oxidation of catecholamines at the dropping mercury electrode is impractical due to the electrolytic dissolution of mercury at positive potentials. The use of a solid electrode allows a greater anodic range to be achieved and hence the direct anodic oxidation of the catechol nucleus becomes possible. Cantin et al. (1975) successfully determined catecholamines in various formulations by use of the rotating platinum electrode, whereas Sternson et al. (1976) oxidized noradrenaline at the stationary carbon paste electrode. These two electrodes are generally considered unsatisfactory for routine analytical work due to problems in obtaining a reproducible electrode surface.

Initial studies on the use of the glassy carbon electrode and differential pulse polarography for the determination of adrenaline (Ballantine and Woolfson, 1978) suggested that this method may meet many of the requirements for the rapid and routine determination of catecholamines in pharmaceutical preparations. The present study assesses the proposed method in terms of these requirements and report its application to the determination of catecholamines in various formulations.

MATERIALS AND METHODS

Reagents

Adrenaline, noradrenaline, isoprenaline and methyldopa were of B.P. quality. All other reagents employed were of Analar grade.

Instrumentation

Polarographic determinations were made with a PAR 174A polarograph (P.A.R. Corporation) and a three-electrode system consisting of a glassy carbon working electrode (Metrohm Ltd.) and platinum auxiliary and saturated calomel reference electrode. The polarographic cell was of amber glass, capacity 10 cm^3, and contained 1 M sulphuric acid as the supporting electrolyte. Polarograms were recorded on a JJ PL 120 XY Plotter (Educational Measurements Ltd.)

Fluorimetric measurements were made using a Perkin–Elmer Model 1000 spectrofluorimeter, and spectrophotometric determinations with a Pye–Unicam SP6-500 spectrophotometer.

Polarographic conditions

Differential pulse polarograms were recorded in 1 M H_2SO_4, with an inital potential of +0.2 V v SCE and a scan rate of 5 mV/s in the anodic direction. The pulse modulation

amplitude was 50 mV and the current range (sensitivity) varied between 0.2 and 0.05 mA as required.

Effect of antioxidant on peak current

Adrenaline standard solutions (10^{-4} M) in 1 M H_2SO_4 were prepared containing, respectively, 0.01, 0.05, 0.1 and 0.2% w/v of sodium metabisulphite. Differential pulse polarograms of the solutions were recorded and the resulting peak currents noted.

Effect of degradation products on the polarographic assay of adrenaline

Aqueous adrenaline solutions (0.1% w/v) containing, respectively, 0, 0.1 and 0.5% w/v sodium metabisulphite were stored at 90°C over a period of 5 days. The concentration of undegraded adrenaline was determined by differential pulse polarography and the results compared with the fluorimetric method described by Prasad et al. (1973) in which iodine was used as an oxidizing agent to obtain the fluorescent trihydroxyindole derivative of adrenaline.

Analytical procedure

The proposed polarographic method was applied to the determination of adrenaline and related compounds in both official and commercial solution and solid dosage formulations. The general procedure employed was as follows.

(A) Solutions

An aliquot (0.05 cm^3) of the unknown solution was added to 10 cm^3 of 1 M H_2SO_4 in the polarographic cell. The peak current was obtained from the resulting differential pulse polarogram. A second differential pulse polarogram was recorded following addition of 0.05 cm^3 of a catecholamine standard solution of similar concentration to the unknown and containing an equivalent concentration of antioxidant. The concentration of the unknown was readily calculated from Eqn. 1.

$$\frac{\text{Molarity of unknown}}{\text{Molarity of standard}} = \frac{ix}{i_t + (i_t - i_x)\,V/v} \qquad (1)$$

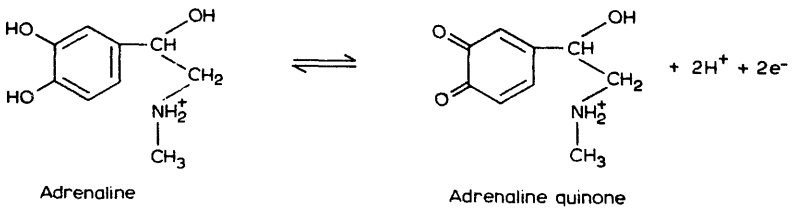
where i_x = peak current of unknown: i_t = peak current of (unknown + standard); V = volume of unknown solution in cell; and v = volume of standard addition.

(B) Tablets

A weight of powdered sample sufficient to give a 10^{-3} M catecholamine concentration was diluted to 100 cm^3 with listilled water (specifically in the case of methyldopa 0.1 M H_2SO_4). A 0.05 cm^3 aliquot of this solution was polarographed in 10 cm^3 of 0.1 M H_2SO_4 and the peak current compared with that resulting from a standard addition (0.05 cm^3) of a catecholamine solution of equivalent concentration. The molarity of the unknown was calculated from Eqn. 1 and thus the catecholamine content of the tablets determined.

RESULTS AND DISCUSSION

The direct anodic oxidation of catecholamines at solid electrodes is based on conversion of the catecholamine to its corresponding quinone at low pH. The oxidation of adrenaline at a planar carbon paste electrode has been studied by Hawley et al. (1967) using cyclic voltammetry. In 1 M H_2SO_4 oxidation resulted in a single anodic peak corresponding to the reaction in Fig. 1, in which a reversible two electron transfer is involved.



Adrenaline — Adrenaline quinone

Fig. 1.

At higher pH values more than one oxidation step is involved. Thus, for analytical purposes a medium or low pH, i.e. 1 M H_2SO_4, is used, to preclude further oxidation, resulting in a polarogram showing a single oxidation step.

Glassy carbon in a proprietary type of highly impermeable carbon, the properties of which have been extensively reviewed by Yamada (1968). Commercially available glassy carbon electrodes (G.C.E.) generally consist of an isotropic glassy carbon disc sealed into an inert plastic body. Only the glassy carbon tip is electrochemically active. The application of the G.C.E. to voltammetry has been studied by Zittel and Miller (1965), who quoted a potential range from about +1.2 to –0.8 V v SCE in acid medium. However, the electrode has been little used for routine analysis. In common with other solid electrodes, fouling of the electroactive surface by the products of the electrochemical reaction may occur. In the case of the G.C.E., though, the surface is readily cleaned by polishing with alumina (particle size 0.3 μm). The extent to which fouling may occur depends on the type of compound being oxidized, but presents no particular problem with catecholamines.

Although the normal range of voltammetric techniques is applicable to the G.C.E., the present study exclusively involved the use of the differential pulse mode, chosen both for its greater sensitivity and the fact that the polarogram is in the form of a well-defined peak.

The catecholamines studied were adrenaline, noradrenaline, isoprenaline and methyldopa, the peak potentials of which in 1 M H_2SO_4 were, respectively, +0.550, +0.535, +0.470 and +0.520 V v SCE. Initial studies (Ballantine and Woolfson, 1978) established the linearity of peak current with adrenaline concentration over the range 10^{-1} to 10^{-5} M. A similar situation was found to exist for the other catecholamines. Having established linearity of peak current with concentration it thus became possible to use a standard addition technique, thereby considerably reducing the analysis time.

The ease of oxidation of the catechol nucleus requires the addition of an antioxidant, generally sodium metabisulphite, to aqueous formulations. Cantin et al. (1975) have determined adrenaline, noradrenaline, and isoprenaline at the rotating platinum elec-

Adrenaline Concentration	Sodium Metabisulphite Concentration	Peak Current	Polarographic Peak
1.0×10^{-4}M	0	13μA	a
1.0×10^{-4}M	0.01%	33μA	b
1.0×10^{-4}M	0.05%	74μA	c
1.0×10^{-4}M	0.1%	75μA	d
1.0×10^{-4}M	0.2%	74μA	e

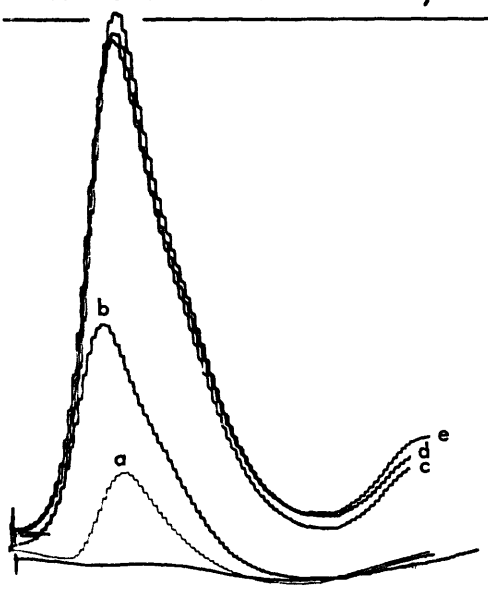


Fig. 2. Effect of increasing sodium metabisulphite concentration on peak current.

trode using DC polarography. They reported an enhanced polarographic wave for these drugs in the presence of sodium sulphite. This enhancement was ascribed to a sulphite wave overlapping with that for the catecholamine. However, in the present study, it was found that no polarographic wave exists for sulphite in the region where oxidation of the catecholamines occurs. The effect of increasing sodium metabisulphite concentration on the peak current resulting from a differential pulse polarogram of a 10^{-4} M adrenaline solution was investigated. The results obtained (Fig. 2) indicated that peak current enhancement is dependent on antioxidant concentration. Peak current enhancement was found to be constant for sodium metabisulphite concentrations of 0.05% and greater. Consequently, standard solutions of the drugs used were prepared containing an equivalent amount of antioxidant to that found in the formulation being analyzed. It would appear that enhancement of the peak current is due to a regeneration catalytic current (Heyrovsky and Zuman, 1968) resulting from reduction of the product of the electrode reaction, thus effectively increasing the concentration of the electroactive species at the electrode surface.

TABLE 1

COMPARISON OF POLAROGRAPHIC (P) AND FLUORIMETRIC (F) DETERMINATIONS OF THE PERCENTAGE OF UNCHANGED ADRENALINE DURING STORAGE AT 90°C

	Storage time (h)									
	24		48		72		96		120	
	P	F	P	F	P	F	P	F	P	F
0% sodium metabisulphite	70.3	66.1	56.4	51.2	46.3	44.5	33.3	36.3	29.4	31.2
0.1% sodium metabisulphite	92.0	93.5	80.9	79.1	72.3	74.2	60.9	63.0	48.9	51.9
0.5% sodium metabisulphite	96.6	96.0	84.4	89.0	78.6	79.5	64.5	66.5	53.3	54.1

One particular requirement of assay methods for adrenaline is that they should detect only unchanged adrenaline and not the decomposition products caused by the oxidation of adrenaline. A comparison was made between results obtained by differential pulse polarography at the G.C.E. and those obtained by the fluorescence procedure of Prasad et al. (1973) which has been shown to measure only unchanged adrenaline. Results (Table 1) were similar for both methods.

The polarographic method was applied to the determination of catecholamines in various commercial formulations. In all cases results were compared with a standard or official method. Adrenaline was determined in Adrenaline Injection B.P. and in Bupivacaine and Adrenaline Injection. The results obtained (Table 2) were comparable with the B.P. assay for adrenaline in Lignocaine and Adrenaline Injection B.P. Statistical analysis indicated a satisfactory level of accuracy and precision. Similarly, noradrenaline in Noradrenaline Solution, strong, sterile B.P.C. was successfully determined by the polarographic method (Table 2).

Isoprenaline and methyldopa were determined in Isoprenaline Tablets B.P. and Methyldopa Tablets B.P., results being comparable with the official method (Table 2). Again the polarographic assay showed a satisfactory level of both accuracy and precision.

The major advantages of the proposed polarographic method using the G.C.E. are its rapidity and simplicity. A typical analysis time using a standard addition method in which only a sample and standard are required to be polarographed was in the region of 5 min. Reproducibility of the glassy carbon surface has been shown to present no difficulty, and the electrode surface is easily cleaned. In particular, the determination of adrenaline in the local anaesthetic formulation had the advantage of not requiring separation of the local anaesthetic and adrenaline components, as in the official method for adrenaline in Lignocaine and Adrenaline Injection B.P. Tablet assays may be carried out either in batch form as described in the B.P. or as a single tablet assay. Thus the polarographic method using the G.C.E. would appear to have useful potential in the routine quality control of preparations of various catecholamines.

TABLE 2

RESULTS FOR THE DETERMINATION OF CATECHOLAMINES IN VARIOUS FORMULATIONS

Preparation	Catecholamine	Method	Recovery, %	S.D., %
Adrenaline Injection B.P.	Adrenaline	Polarographic	95.9, 97.6, 98.7, 93.8, 95.3, 97.0	1.75
	Adrenaline	B.P. [a]	95.4, 98.5, 98.5, 96.3, 99.1, 99.1	1.57
Bupivacaine and Adrenaline Injection	Adrenaline	Polarographic	96.8, 97.8, 97.6, 96.9, 97.6, 97.6	0.43
	Adrenaline	B.P. [a]	97.9, 96.2, 97.9, 97.9, 98.9, 98.9	1.00
Noradrenaline, strong, sterile B.P.C.	Noradrenaline	Polarographic	95.1, 99.9, 99.5, 97.9, 97.1, 98.0	1.73
	Noradrenaline	B.P.C.	96.5, 97.6, 97.1, 96.8, 96.5, 97.1	0.62
Isoprenaline Tablets B.P.	Isoprenaline	Polarographic	97.6, 98.8, 97.4, 98.4, 101.4, 98.8	1.43
	Isoprenaline	B.P.	99.7, 98.6, 99.7, 99.2, 99.7, 98.6	0.54
Methyldopa Tablets B.P.	Methyldopa	Polarographic	95.5, 95.3, 96.7, 96.7, 96.1, 96.9	0.68
	Methyldopa	B.P.	98.8, 96.6, 96.6, 97.7, 97.7, 97.7	0.83

[a] B.P. – as in Lignocaine and Adrenaline Injection B.P.

ACKNOWLEDGEMENT

One of us (Jill Ballantine) gratefully acknowledges receipt of a postgraduate studentship from the Department of Education for Northern Ireland.

REFERENCES

Ballantine, J. and Woolfson, A.D., Differential pulse polarographic determination of adrenaline at the glassy carbon electrode. J. Pharm. Pharmacol., 30 (1978) 46P.

Brezina, M. and Zuman, P., Polarography in Medicine, Biochemistry and Pharmacy, Wiley-Interscience, New York, 1958.

British Pharmacopoeia, H.M.S.O., London, 1973.
Cantin, D., Alary, J. and Coeur, A., Polarographic determination of polyphenolic amines. Analusis, 3 (1975) 5–10.
Hawley, M.D., Tatawawadi, S.V., Piekarski, S. and Adams, R.N., Electrochemical studies of the oxidation pathways of catecholamines. J. Am. Chem Soc., 89 (1967) 447–450.
Henderson, J. and Freedberg, A.S., Polarographic measurement of l-noradrenaline and l-adrenaline. Anal. Chem., 27 (1955) 1064–1066.
Heyrovsky, J. and Zuman, P., Practical Polarography, Academic Press, London, 1968.
Loew, O., The character of the toxic action of suprarenine. Biochem. Z., 85 (1918) 295–306.
Morvay, J. and Kiss, L., Polarographic determination of adrenaline with potassium tetrathiocyanate. Acta. Pharm. Hung., 39 (1969) 198–201.
Prasad, V.K., Ricci, R.A., Nunning, B.C. and Granatek, A.P., Improved, rapid spectrophotofluorometric method for assay of epinephrine: a comparative study. J. Pharm. Sic., 62 (1973) 1130–1135.
Sartori, G. and Cattaneo, C., Polarographic study of adrenaline. Gaz. Chim. Ital., 72 (1945) 525–528.
Sternson, L.A., Sternson, A.W. and Bannister, S.J., A differential pulse polarographic assay for O-methylation of catechols by catechol-O-methyltransferase. Anal. Biochem., 75 (1976) 142–152.
Vilvala, R. and Halmekoski, J., Investigation of the nitroso derivatives of some sympathomimetic amines (III). Farm. Aikak, 85 (1976) 9–13.
Yamada, S. A Review of Glasslike Carbons. Tokai Carbon Co., Ltd., Tokyo, Japan, 1968.
Zittel, H.E. and Miller, F.J. A glassy-carbon electrode for voltammetry. Anal. Chem., 37 (1965) 200–203.