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ELECTROCHEMICAL DETECTOR FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A voltammetric detector has been developed for high-performance liquid chromatography, based on a three-electrode system with a platinum tubular working electrode. The detector has a small volume (about 1 μl) and has been used in the d.c. and differential pulse polarographic (DPP) modes. Its function was tested using various organic compounds, *e.g.*, phenol, ascorbic acid and uric acid. The detection limit is about 0.5 ng, the linear dynamic range is at least five orders of magnitude of concentration and the measurements exhibit good reproducibility. The d.c. and DPP methods of detection were about equally sensitive and the reproducibilities of the peak areas were similar, but the d.c. peak height exhibited better reproducibility than that of the DPP peaks.

The detector was used in the separation of the catecholamines adrenaline, noradrenaline, dopamine and L-dopa in a reversed-phase system. It was found that nanogram amounts can be detected, with narrow and symmetrical peaks.

INTRODUCTION

The development of high-performance liquid chromatography (HPLC) has necessitated the construction of various types of detectors, as a completely universal detector is not available. Therefore, specific detectors have great importance. In addition to common spectrophotometric, refractometric and fluorescence detectors, electrochemical detectors have received a great deal of attention, because they are sensitive, have a broad linear dynamic range, exhibit good reproducibility and are selective (except for conductimetric and high-frequency detectors), so that they do not place great demands on the purity of the samples.

Among electrochemical detectors[1], those based on the voltammetric principle have the broadest application range. Most of them use either the dropping mercury electrode, the disadvantages of which are the limited anodic potential range and difficulty of attaining a small dead volume, or planar solid electrodes in thin-layer (*e.g.*, refs. 2–4), or the "wall-jet" (*e.g.*, ref. 5) hydrodynamic systems.

Cells with tubular electrodes have rarely been used[6–8], although they are

hydrodynamically advantageous. In this work a cell with a platinum tubular working electrode and a small dead volume was constructed and tested.

EXPERIMENTAL

Reagents and apparatus

All chemicals used were of analytical-reagent grade (Lachema, Brno, Czechoslovakia) and were not further purified. The preparations of the catecholamines were obtained from the Endocrinological Institute, Prague, and the Research Institute for Pharmacy and Biochemistry, Prague, Czechoslovakia. Aqueous stock solutions were prepared at a concentration of 10^{-3} *M* and were appropriately diluted before use. The principal detector parameters were tested by direct injection of solutions into the base electrolyte stream, without a column or with a column containing an inert packing. The response to phenol was tested using 0.1 *M* sulphuric acid as the base electrolyte, and that to other substances (ascorbic acid and uric acid) using a 0.1 *M* phosphate–citrate buffer[9]. The separation and detection of catecholamines were carried out in a reversed-system with μBondapak C_{18} as the stationary phase (10 μm), packed in a 25 cm × 2.2 mm I.D. column, and a 0.1 *M* phosphate–citrate buffer[9] containing lauryl sulphate as the mobile phase (300 ml of 0.1 *M* citric acid, 160 ml of 0.1 *M* disodium hydrogen orthophosphate and 0.02 g of lauryl sulphate).

The mobile phases were freed from dissolved atmospheric oxygen by passing purified nitrogen and then degassed *in vacuo*.

For the detector construction, see Results and Discussion. The measurements were carried out with a Varian Model 8500 liquid chromatograph, LP-9 d.c. and differential pulse polarographs (DPP) (Laboratorní Přístroje, Prague, Czechoslovakia) and Varian A-25-1 and EZ-2 (Laboratorní Přístroje) line recorders. The electrochemical detection was compared with UV photometric detection using a Pye Unicam LC 3 UV detector. Small volumes of sample solutions were injected into the measuring system with microsyringes (Hamilton Micromesure, The Hague, The Netherlands). All of the measurements were performed at laboratory temperature.

RESULTS AND DISCUSSION

Detector construction

The construction of the detector was based on the following general requirements: (a) simplicity of design; (b) easy access to the working electrode to allow its mechanical polishing; (c) as small a dead volume as possible; (d) a three-electrode system that would allow work in mobile phases of low conductivity and application of measuring techniques other than d.c. voltammetry.

The basic concept of the detector is based on a cell, described earlier[8], which contained a tubular electrode system but had a large dead volume, as it was constructed for continuous monitoring in flowing systems rather than for HPLC.

The present detector is depicted in Fig. 1. It consists of two cylindrical PTFE parts. The outer part contains an inlet from the column (a steel capillary sealed in place with a piece of PTFE tubing), a depression in which the working electrode is placed and which is provided with a hole for an outlet tube. The inner part is screwed into the outer part and keeps the working electrode in place. The platinum cylindrical

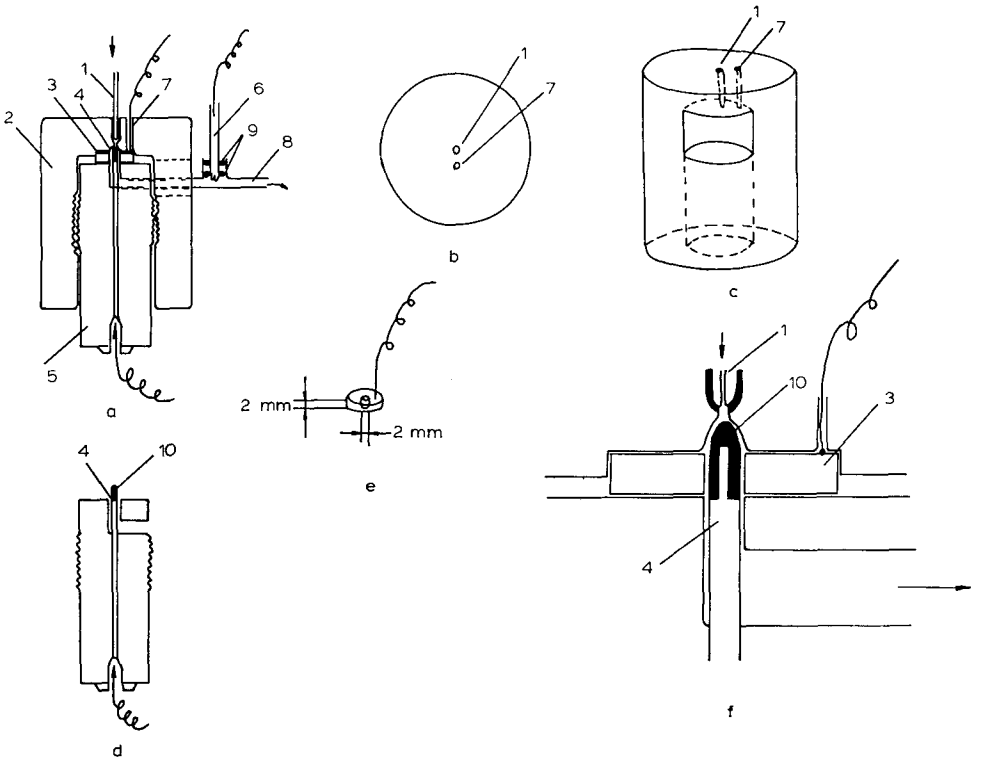

Fig. 1. The voltammetric detector. a, Cross-section through the assembled detector; b, top view; c, side view of the outer part; d, the inner part; e, working platinum tubular electrode; f, expanded view of the working–auxiliary electrode system. 1 = Inlet from the column (stainless-steel capillary, 0.23 mm I.D.); 2 = PTFE body, outer part; 3 = tubular platinum working electrode; 4 = platinum cylindrical auxiliary electrode; 5 = PTFE internal part of the detector; 6 = reference electrode (S.C.E.); 7 = channel for the lead from the working electrode; 8 = glass tube to waste; 9 = PTFE rings; 10 = PTFE insulating cap.

auxiliary electrode is placed in the channel in this inner part and reaches through the hole in the working electrode. It is equipped with a PTFE insulating cap to prevent short-circuiting of the electrodes and electrochemical interferences from the products of the reaction on the auxiliary electrode. The mobile phase thus passes through the narrow gap between the working and auxiliary electrodes (about 0.1 mm), which ensures that the dead volume of the cell is about 1 μl. The mobile phase leaves the detector through the side channel, where a glass tube is fixed with PTFE tubing and contains the reference electrode sealed by two PTFE rings. The working electrode is easily removed for mechanical polishing with metallographic papers.

Principal operating parameters of the detector

The performance of the detector was tested by injecting phenol, ascorbic acid and uric acid in a stream of base electrolyte either into a 10 cm × 1.2 mm I.D. column containing an inert packing of glass beads or directly into the detector through a short capillary. The injection of phenol into a short inert column sometimes led to peak tailing, whereas direct injection into the detector produced narrow and symmetri-

cal peaks (see Fig. 2). The limit of detection, the linear dynamic range, the reproducibility of the peak height and area and the dependence of the detector response on the mobile phase flow-rate were determined.

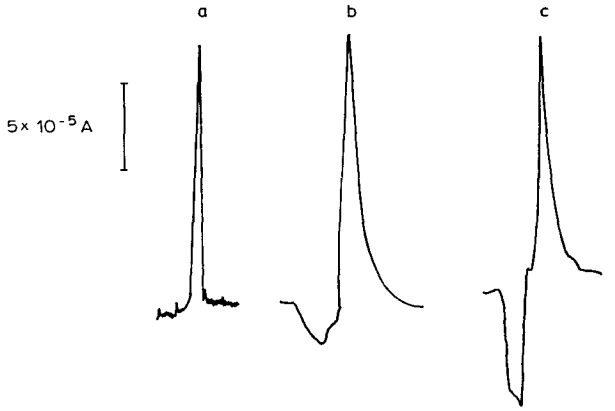

Fig. 2. d.c. and DPP detector responses to phenol. $2.7 \cdot 10^{-4}$ *M* aqueous phenol solution; 4 μl injected; 0.1 *M* H_2SO_4 mobile phase, flow-rate 130 ml/h; working electrode potential, +0.8 V (*vs.* S.C.E.); pulse height, 50 mV; pulse frequency, 1 sec. a = d.c. peak (direct injection to the detector); b = d.c. peak (injection through an inert column); c = DPP peak (injection through an inert column).

The detection limit as the peak height corresponding to twice the standard deviation of the noise, was determined by injecting 10^{-4} *M* aqueous solutions of phenol, ascorbic acid and adrenaline. The value found was approximately 0.5 ng in all instances.

The dependence of the detector response on the concentration of the test substances (the linear dynamic range) was studied using aqueous solutions of phenol. Amounts of $0.5–5 \cdot 10^4$ ng of phenol were injected and both the peak height and peak area were measured. It was found that the detector response is linear at least over the whole concentration range studied (five orders of magnitude).

The reproducibility of the detector response was found for phenol in the d.c. and DPP modes. Amounts of 4 μl of a 10^{-4} *M* solution were injected and the peak heights and areas measured. The results are given in Table I.

TABLE I

REPRODUCIBILITY OF THE DETECTOR RESPONSE

An amount of 4 μl of a 10^{-4} *M* aqueous phenol solution injected; 0.1 *M* H_2SO_4 mobile phase; flow-rate, 150 ml/h; working electrode potential, +0.8 V (*vs.* S.C.E.); pulse height, 50 mV; pulse frequency, 1 sec. Average values determined from ten measurements, 95% probability level; C.V. = coefficient of variation (%).

Detection	*Peak height (mm)*	*C.V. (%)*	*Peak area (arbitrary units)*	*C.V. (%)*	*Peak half-width (mm)*	*C.V. (%)*
d.c.	143.0	2.8	56.0	4.6	3.0	5.0
DPP	50.7	13.6	20.2	4.1	2.3	5.8

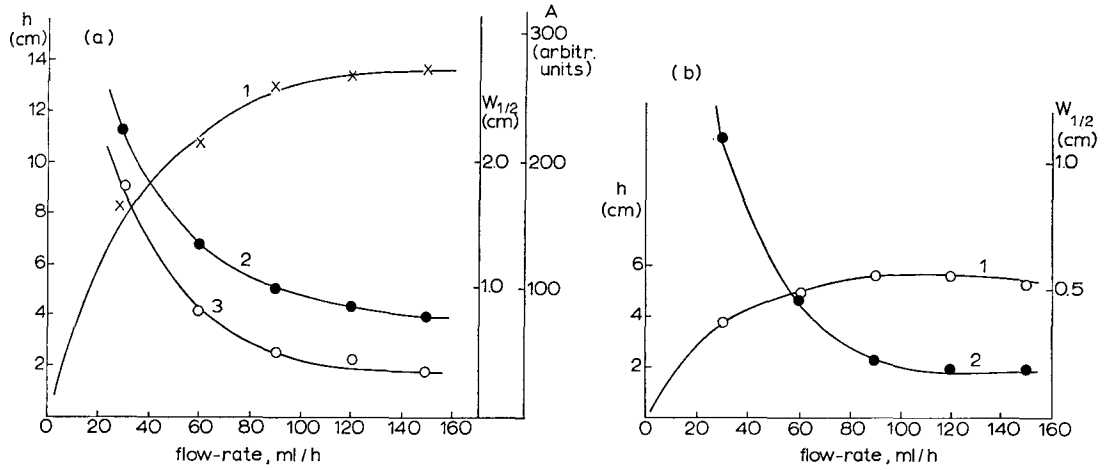

Fig. 3. Dependence of detector response on the flow-rate of mobile phase. (a) d.c. measurement: $2.7 \cdot 10^{-4}$ *M* aqueous phenol solution, 4 μl injected; 0.1 *M* H_2SO_4 mobile phase; working electrode potential, +0.8 V (*vs.* S.C.E.). Curve 1, peak height, ***h*** (cm); curve 2, peak area, *A* (arbitrary units); curve 3, peak half-width, $w_{1/2}$ (cm). (b) DPP measurements: $9.5 \cdot 10^{-4}$ *M* aqueous uric acid solution, 6 μl injected; 0.1 *M* H_2SO_4 mobile phase; working electrode potential, +0.8 V (*vs.* S.C.E.); pulse height, 50 mV; pulse frequency, 1 sec. Curve 1, peak height, *h* (cm); curve 2, peak half-width, $w_{1/2}$ (cm).

The dependence of the detector response on the mobile phase flow-rate was determined for phenol (d.c. measurement) and uric acid (DPP measurement). The results are summarized in Fig. 3.

The DPP detector response also depends on the pulse height, as shown in Fig. 4.

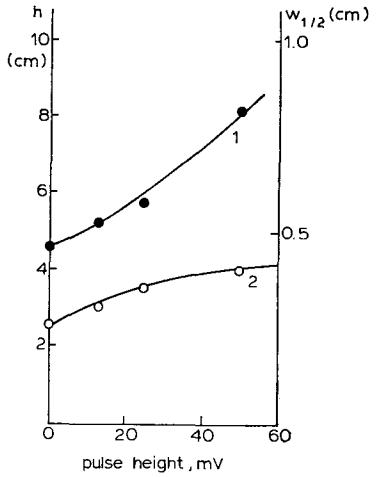

Fig. 4. Dependence of DPP detector response on pulse height. $9.5 \cdot 10^{-4}$ *M* aqueous uric acid solution, 10 μl injected; 0.1 *M* H_2SO_4 mobile phase, flow-rate 60 ml/h; working electrode potential, +0.8 V (*vs.* S.C.E.), pulse frequency, 1 sec. Curve 1, peak height, *h* (cm); curve 2, peak half-width, $w_{1/2}$ (cm).

It follows from the results obtained that the measurements in both the d.c. and DPP systems are sufficiently sensitive and reproducible and the linear dynamic range is broad. There is virtually no difference in the sensitivities of the d.c. and DPP measurements. The reproducibility of the peak areas is similar in both instances, whereas the peak height is more reproducible in the d.c. measurement.

The d.c. and DPP responses exhibit dependences on the experimental conditions that would be expected from electrochemical theory. It is sometimes claimed that the DPP measurement is less sensitive to changes in the mobile phase flow-rate, as could be expected from the fact that the diffusion layer at the electrode is thinner in non-stationary measurements. However, our measurements showed little differences in this respect between the d.c. and DPP measurements. Moreover, the DPP measurement is more sensitive to the presence of surfactants, which alter the capacity of the electric double layer, and suffers from a larger background current. For these reasons, the d.c. measurements seem more advantageous, also in view of the simpler measuring circuit.

The main problem in the use of the detector is the magnitude of the background current, and it is therefore important that the mobile phase is perfectly free of dissolved oxygen and of electroactive impurities. In our measurements, the currents corresponding to the solutes studied were relatively large (of the order of 10^{-7} A at the lowest concentrations), but were superimposed on a background current more than one order of magnitude higher. It can therefore be expected that careful purification of the mobile phase will lead to a considerable decrease in the background current and consequently to a futher decrease in the detection limit. Even under the present conditions the detection limit is the same as that of UV detection at the highest detector sensitivity, as was found by comparing the responses of the electrochemical detector and a UV detector to phenol and ascorbic acid at 240 nm.

The electrode activity did not change, even during prolonged measurements, and the background current did not exhibit the drift that is sometimes reported for electrochemical detectors[10].

Measurements with a wall-jet detector showed that the proposed detector is more sensitive. Its performance is similar to that of thin-layer detectors with planar electrodes, but from the constructional point of view it is easier to attain a small dead

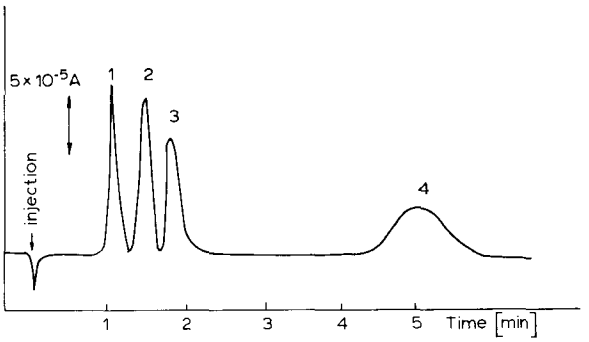

Fig. 5. Separation of catecholamines. 1 = Noradrenaline; 2 = L-dopa; 3 = adrenaline; 4 = dopamine. Flow-rate, 60 ml/h; injected amounts, 20 ng of each compound. d.c. detection, working electrode potential +0.75 V (*vs.* S.C.E.).

volume. As shown by calculations of the theoretical currents for tubular and planar systems (for the appropriate equations, see refs. 11 and 12), this system behaves similarly to detectors with planar electrodes. This could be expected as the curvature of the electrode surface can be neglected in view of the very narrow gap between the working and auxiliary electrodes.

Separation and electrochemical detection of catecholamines

Practical use of the detector described is demonstrated with the analysis of catecholamines. Fig. 5 shows a chromatogram of a mixture of adrenaline, noradrenaline, L-dopa and dopamine on a column packed with μBondapak C_{18} chemically bonded phase, using a citrate–phosphate buffer containing lauryl sulphate as the mobile phase. Good separation was attained, with narrow and symmetrical peaks for catecholamines. It can be seen that the detector can be used successfully for the detection of catecholamines in nanogram and sub-nanogram amounts.

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