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ELECTROCHEMICAL DETECTOR FOR LIQUID CHROMATOGRAPHY BASED ON A RETICULATED VITREOUS CARBON ELECTRODE IN A THIN-LAYER CELL

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SUMMARY

The use of a thin-layer cell with a reticulated vitreous carbon working electrode for detecting oxidizable compounds in high-performance liquid chromatography is described. High current peaks are obtained for low analyte concentrations owing to the relatively large electrode area. A comparison of this cell and a conventional thin-layer amperometric detector shows a three-fold improvement in the signal-to-noise ratio. The nature of the noise appears to be different from that of the amperometric cell. Mass transport properties, sensitivity, precision and linearity of response are reported. L-Dopa, acetaminophen, epinephrine, norepinephrine, dopamine and hexacyanoferrate(II) were used as test systems to give detection limits at the picogram level.

INTRODUCTION

Electrochemical cells have been proved to be both sensitive and selective detectors in liquid chromatography[1,2]. Solid electrode detectors are advantageous for this purpose owing to their applicability to both oxidizable and reducible species. Many electrode designs have been reported for use in liquid chromatographic detection. The most common solid electrode detectors employ the planar thin-layer hydrodynamic system[3,4] or the wall-jet system[5,6]. In these systems only a negligible fraction (0.1–5%) of the electroactive species passing through the detector is electrolyzed. In addition, various flow cells with large area electrodes, in which complete (100%) or partial (5–99%) electrolysis is obtained, have been reported. These include cells with electrodes such as large planar glassy carbon[7,8], carbon cloth[9] or platinum chips[10]. Most of the above detectors are generally sensitive to sub-nanogram levels (depending on the applied potential, compound characteristics and chromatographic retention).

This paper describes a flow cell for liquid chromatographic detection, composed of a reticulated vitreous carbon (RVC) working electrode in a thin-layer cell. RVC is a porous three-dimensional carbonaceous material that is well suited as a versatile electrode material[11]. It combines the electrochemical properties of glassy

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carbon with many hydrodynamic and structural advantages. Various electrochemical flow cells with an RVC working electrode have been found useful in connection with continuous flow[12–14] and flow injection[15–17] systems. However, the utility of RVC flow-through electrodes for monitoring chromatographic column effluents has not previously been reported. In contrast with most previously reported RVC flow cells, the electrodes used in this study have volumes of 10–20 μl. This is achieved by placing a thin RVC disk in the center of a commercial thin-layer channel amperometric detector. The resulting configuration is shown to be advantageous for the chromatographic detection of oxidizable species, because of its improved signal-to-noise characteristics. Its properties and advantages were explored in this study.

EXPERIMENTAL

Apparatus

The liquid chromatographic system (Bioanalytical Systems LC-303) consisted of a dual piston pump (PM-30A), a Rheodyne Model 7125 injector (20-μl loop) and a Biophase ODS-5 μm reversed-phase column (25 cm × 4.6 mm I.D.). Chromatograms were recorded with a Princeton Applied Research Model 174A polarographic analyzer and a Houston Ommiscribe strip-chart recorder. A 1-sec low-pass filter time constant was used.

A commercial glassy carbon thin-layer electrochemical transducer (Model TL-5, Bioanalytical Systems) was used. A thin reticulated vitreous carbon (RVC) disk (2 × 3-S, 0.55 cm diameter, 0.83 mm thickness, 100 pores per inch; ERG, Oakland, CA, U.S.A.) was centered over the face of the glassy carbon electrode. Electrical contact was made through the glassy carbon electrode. Six 0.005-in. PTFE gaskets (Model TG-5M, Bioanalytical Systems) were required to bolt the upper and lower transducer blocks together, exerting pressure on the RVC disk (Fig. 1). In some experiments the RVC disk thickness was halved (to reduce the dead volume of the cell) by sanding on 600-grit silicon carbide sandpaper. In this instance only three gaskets were required. The geometric dead volumes of the cell were 60 and 30 μl for the whole and halved disk electrodes, respectively. Small air bubbles were removed from the RVC disk by allowing buffer or mobile phase to flow through the cell for

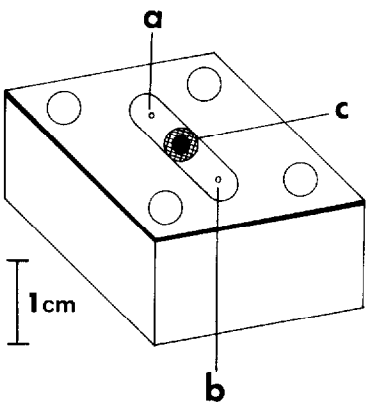

Fig. 1. Expanded view of the thin-layer cell: (a, b) solution inlet and outlet; (c) RVC disk. The upper block is not shown; the dark line represents the thickness of the gaskets.

an extended period (usually overnight). The reference and counter electrodes were located in a downstream compartment (Model RC-2A, Bioanalytical Systems). All potentials are reported *versus* a silver–silver chloride reference electrode (Model RE-1, Bioanalytical Systems). For comparison with a conventional amperometric detector, the RVC disk was removed and the glassy carbon disk (3 mm diameter) served as the working electrode using a single PTFE gasket. The glassy carbon disk was polished to a mirror finish using a 0.05 μm alumina slurry. All experiments were performed at ambient temperature.

Reagents

Epinephrine, L-dopa, acetaminophen, dopamine hydrochloride and norepinephrine were purchased from Sigma. Solutions of these compounds were made by dissolving the respective amounts in 0.1 *M* phosphoric acid (except acetaminophen, which was prepared in water) and diluting to 0.01 *M* with deionized, distilled water. The mobile phases were as follows: (1) 0.01 *M* potassium dihydrogen phosphate (KH_2PO_4) adjusted to pH 3.5 with phosphoric acid; and (2) 86% 0.035 *M* KH_2PO_4, 0.030 *M* sodium citrate, 3 m*M* sodium octyl sulfate adjusted to pH 4.5 with phosphoric acid and 14% methanol (v/v). Both mobile phases contained 2 g l^{-1} of disodium EDTA and were filtered through a 10–15 μm fritted-glass Büchner funnel and degassed prior to use. Potassium hexacyanoferrate(II) [$K_4Fe(CN)_6 \cdot 3H_2O$] and 0.10 *M* phosphate buffer (pH 7.4), prepared from a 1:4 mixture of KH_2PO_4 and dipotassium hydrogen phosphate ($K_2HPO_4 \cdot 3H_2O$) were used in the degree of conversion experiments. All reagents were used without further purification.

RESULTS AND DISCUSSION

For evaluation of the RVC thin-layer cell under convective conditions, continuous flow (steady-state) conditions were employed. Fig. 2 shows the dependence

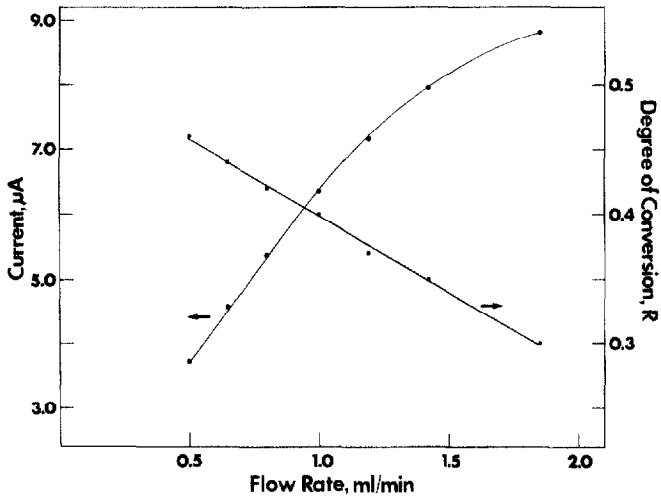

Fig. 2. Dependences of the limiting current and the degree of conversion on volume flow-rate. Solution, 10 *M* $K_4Fe(CN)_6$ in 0.1 *M* phosphate buffer (pH 7.4); applied potential, +0.9 V.

of the steady-state limiting current for hexacyanoferrate(II) on the solution flow-rate. As the flow-rate increases, the current rises rapidly at first and then more slowly. When replotted on a log–log scale, these data give a straight line with a slope of 0.68 (correlation coefficient 0.993). Different log–log dependences with slopes ranging from 0.32 to 0.73 were reported for other RVC flow-through electrode configurations[13,16]. Also shown in Fig. 2 is the dependence of the degree of conversion (electrolysis yield), R, on the flow-rate. R values were calculated from the limiting current–flow-rate data. R depends on the residence time of an element of solution in the working electrode compartment, so a decreased flow-rate provides a larger degree of conversion (up to 46% at 0.50 ml min^{-1}). At higher flow-rates the electrolytic efficiency decreases considerably (to about 30% at a flow-rate of 1.85 ml min^{-1}). Therefore, this detector is in principle not a coulometric detector but a partial electrolysis cell.

Fig. 3A and B compares chromatograms for injections of 0.39 ng of L-dopa and 0.81 ng of acetaminophen, recorded under the same conditions with the RVC thin-layer cell (curves a) and a commercial thin-layer amperometric cell (curves b). The porous electrode cell yields a 12- and 28-fold peak enhancement over the response of the amperometric cell for L-dopa and acetaminophen (note the significant current scales employed in this experiment). At the same time, a 2.5–10-fold increase in the noise level is observed in the case of the RVC cell. Overall, signal-to-noise ratios of 200 and 40.4 are observed for L-dopa and acetaminophen, respectively,

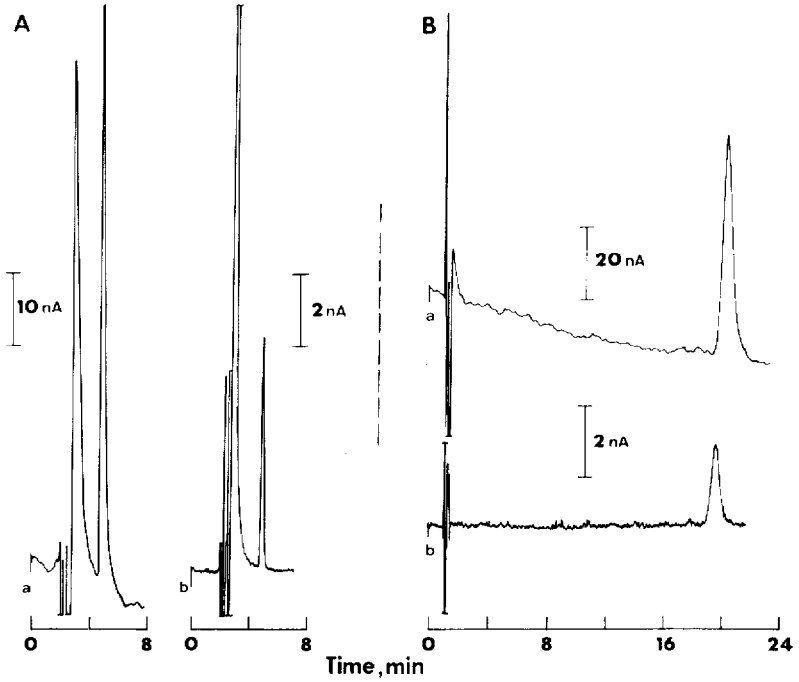

Fig. 3. Chromatograms obtained using (a) RVC and (b) amperometric detectors. (A) 0.39 ng of L-dopa; (B) 0.81 ng of acetaminophen. Flow-rate, (A) 1.0 and (B) 2.0 ml min^{-1}; applied potential, +0.6 V; mobile phase, 0.01 M KH_2PO_4 (pH 3.5).

using the RVC cell. Based on a signal-to-noise ratio of 2, these data correspond to detection limits of 3.9 pg (0.02 pmol) of L-dopa and 40 pg (0.26 pmol) of acetaminophen. In comparison, the amperometric detector yields a value 9.5 pg (0.05 pmol) of L-dopa and 116 pg (0.77 pmol) of acetaminophen. Thus, a three-fold improvement in the detectability is obtained with the RVC cell. It has been claimed[7,18,19] that an increase in the electrode area does not improve the detectability, as both the signal and the noise increase. Unlike an increase of the surface area of a planar electrode where the same hydrodynamic conditions prevail (*i.e.*, the above claim is appropriate), the area increase due to the use of a thin porous disk instead of a planar disk involves totally different considerations. This is due to the different electrode characteristics and hydrodynamic conditions that may affect the signal and noise in different patterns. The RVC flow cell behaves as a porous electrode reactor[13], where the linear flow-rate of the solution increases on entering the electrode to an interstitial flow-rate given by U/a (U being the solution flow-rate and a the cross-sectional area of the pores). As a result of this change, the transport of the analyte to the electrode surface increases. Overall, the increased signal observed with the RVC detector is the result of increasing the surface area and changing the hydrodynamic conditions. Similarly, the nature of the noise may depend on the electrode characteristics and hydrodynamic conditions. The sources of noise in electrochemical flow cells are not completely understood[20].

As shown in Fig. 3, relatively high-frequency noise is obtained in the conventional amperometric detector; the noise in this detector is usually attributed to fluctuations in the flow-rate caused by the piston pump mechanics. The noise in the RVC cell is made of lower frequency baseline fluctuations, the sources of which may be chemical or thermal[20]. Although the absolute noise of the RVC cell is much larger than that of the amperometric cell, it does not increase as much as the signal, resulting in improved detectability. Compared with other porous carbon electrodes (*e.g.*, carbon cloth or fiber bundles), RVC does not have a tremendous microscopic surface area, resulting in low background currents. As the noise generated by the RVC electrode is of a different frequency range than the signal, electronic filtering can be used to reduce the noise level.

Fig. 4 illustrates the effect of the electrode surface area and the detector volume on the height and broadening of the chromatographic peaks. A mixture of four catecholamines present at the 20 ng level was employed. The detector volumes associated with the two RVC disks [thickness (a) 0.8 and (b) 0.4 mm] and the planar glassy carbon disk (c) are 60, 30 and 10 μl, respectively. To illustrate the increased peak currents resulting from the increased surface area, the same current scale was employed. The highest signal-to-noise ratio is observed with the 0.8 mm thick RVC disk. The amperometric cell shows minimum band broadening, as expected from its smallest volume. The RVC cells result in some extra-column band spreading that increases as the cell volume changes from 30 to 60 μl. The volume of the RVC cell could be further reduced by reducing the length and width of the flow channel (utilizing gaskets of different shapes). Further reduction in the channel height is not possible as the RVC becomes fragile at thicknesses smaller than 0.4 mm. The additional detector volume associated with the increased area of the RVC electrode (as well as any porous electrode) would limit its applicability for modern low peak volume separations such as microbore liquid chromatography.

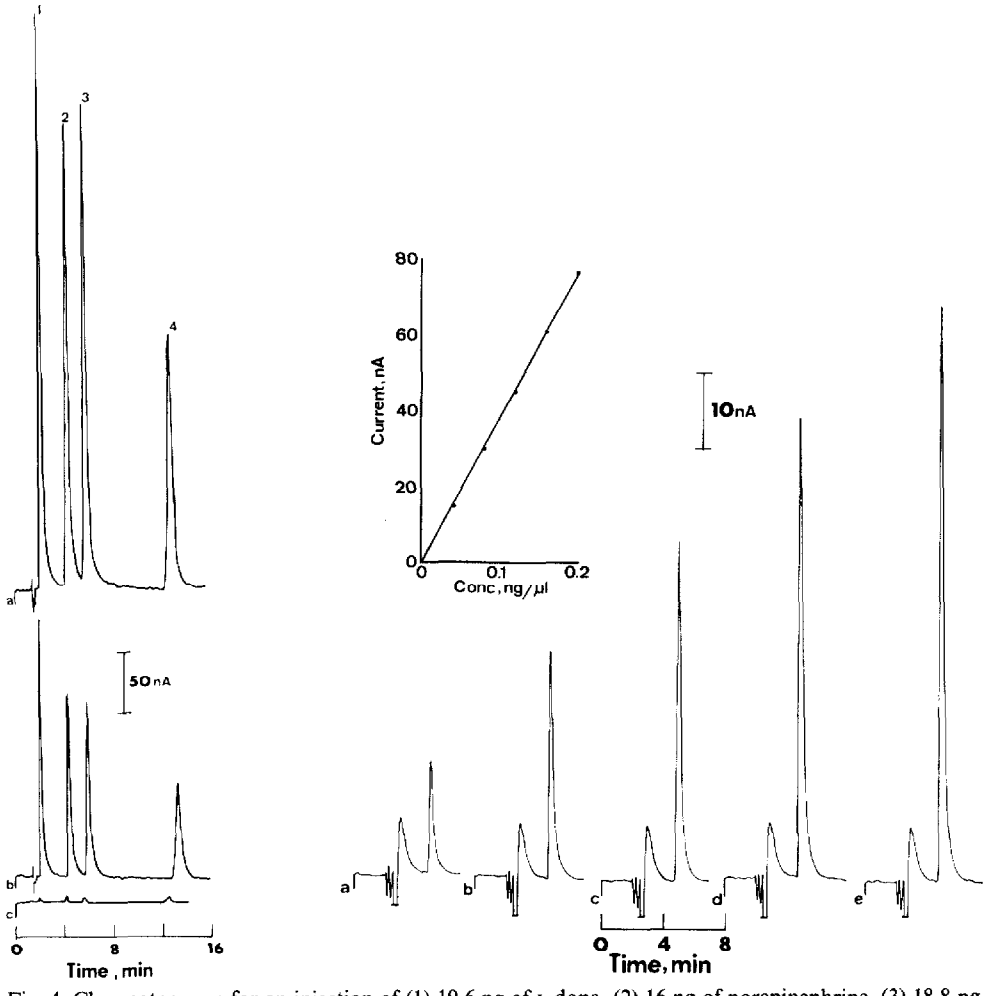

Fig. 4. Chromatograms for an injection of (1) 19.6 ng of L-dopa, (2) 16 ng of norepinephrine, (3) 18.8 ng of epinephrine and (4) 17 ng of dopamine obtained at (a) a 0.8 mm thick RVC disk, (b) a 0.4 mm thick RVC disk and (c) a planar glassy carbon disk. Flow-rate, 1.5 ml min $^{-1}$; applied potential, +0.6 V; mobile phase, 86% 0.035 *M* KH_2PO_4, 0.030 *M* sodium citrate, 0.003 *M* sodium octyl sulfate (pH 4.5) and 14% methanol (v/v).

Fig. 5. Chromatograms for five injections (a–e) of increasing increments of 0.04 ng/μl L-dopa. Conditions as in Fig. 2A.

Fig. 5 shows chromatograms for L-dopa in the 0.04–0.20 ng/μl concentration range (corresponding to 0.8–4.0 ng for the 20 μl injection). The peak response increases linearly with increasing concentration; the slope of the calibration graph corresponds to a sensitivity of 384 nA μl ng^{-1} (correlation coefficient 0.999, intercept −0.7 nA). Such a linear response is expected for partial electrolysis detectors whose response is given by[13] $i = nFCUR$ (where n, F and C have the conventional meanings). These data, coupled with the low noise level, indicate that the RVC flow cell is suitable for trace detection. The precision of the results was measured by nine

repeated injections of a 0.20 ng/μl (4.0 ng) L-dopa solution (conditions as in Fig. 2A). The mean peak response found was 76 nA with a range of 74–79 nA. The relative standard deviation over the complete series was 2.4%. Thus reproducible results are obtained at the nanogram level.

As in any electrochemical detector, hydrodynamic voltammograms must be constructed for selecting the applied potential used in the chromatographic run. As the overall current associated with porous electrodes is significantly larger than that of an amperometric cell, different ohmic drops and voltammograms are expected. The increase in current is compensated for by the reduced resistance resulting from the use of a thicker flow channel. Overall, the above data show that the RVC flow cell results in higher sensitivity than conventional amperometric detectors, and detection limits in the lower picogram range. RVC is inexpensive, and the cell construction permits easy and fast replacement of electrodes. Efforts are under way to improve the cell design by reducing its volume.

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