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CURRENT AMPLIFICATION IN DUAL ELECTROCHEMICAL DETECTION FOR MICRO HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A dual electrochemical detector comprising two working electrodes (anode and cathode) in parallel-opposed configuration has been studied. In such a detector, the product of the reaction at one electrode is detected at the opposite electrode, where starting species is regenerated. Equations relating detector current to the system variables have been derived, and theoretical predictions experimentally verified with working electrodes 0.2 cm wide and 1 cm long. The current amplification in the detector at low flow-rates of 1.4–11.2 μl/min was quantitatively demonstrated by using ferricyanide as an analyte. The detector was successfully utilized for the selective and sensitive detection of catecholamines in human serum by micro high-performance liquid chromatography.

INTRODUCTION

An innovative approach to electrochemical detection (ED) in high-performance liquid chromatography (HPLC) involves the use of dual electrochemical detectors having two working electrodes[1–13]. The orientation of the working electrodes with respect to the flow axis may be parallel-adjacent, series and parallel-opposed, as termed by Roston and Kissinger[7]. Use of parallel-adjacent and series dual electrochemical detectors can result in improved selectivity and peak identification in comparison with the ordinary electrochemical detector having one working electrode. On the other hand, a parallel-opposed dual electrochemical detector may provide high gain as well as good selectivity and peak identification.

In a previous paper[13], we reported the utilization of a dual electrochemical detector having two working electrodes (anode and cathode) in parallel-opposed configuration for the determination of catecholamines in healthy human serum, which was injected directly after ultrafiltration into the micro high-performance liquid chromatograph with a preconcentration column. It was demonstrated that the detector is a powerful tool for selective detection of objective species, whose electrode

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reactions are reversible and quasi-reversible, from other electroactive species, whose electrode reactions are irreversible, and can provide an enhancement in sensitivity by recycling oxidation and re-reduction between the anode and cathode at low mobile phase flow-rates. Current amplification in the parallel-opposed dual electrochemical detector was qualitatively demonstrated in micro high-performance liquid chromatography (MHPLC)[13], which uses very low flow-rates.

        Weber and Purdy[10,14] recently developed the detailed theory for the parallel-opposed configuration and approximated it experimentally by using working electrodes having very large surface areas (10 × 1.5 cm) at flow-rates larger than 100 μl/min. Far less is known about the noise in ED. In the absence of specific information it has become customary to assume that the noise is proportional to electrode area[15]. Thus if one can increase the sensitivity of an electrochemical detector without increasing the electrode area, it is likely that the device will yield improved limits of detection. In this paper, Weber and Purdy's equations are modified, and the theoretical predictions are experimentally verified by using working electrodes (1 × 0.2 cm) whose areas are 75 times smaller than Weber and Purdy's. The current amplification in the parallel-opposed configuration for MHPLC has also been estimated.

THEORETICAL

        The parallel-opposed dual electrochemical detector has two working electrodes, an anode and a cathode, placed parallel and close to each other. The solution containing the electroactive species flows in a thin channel between them. In such a detector the product of the electrode reaction at one electrode can diffuse to the opposite electrode where starting material may be reformed.

        Fig. 1 shows the dimensions of the channel. The fluid flows in the x direction, diffusion towards the electrodes occurs in the z direction and the width of the electrodes is measured in the y direction. The length of the electrodes is L, their width W. The electrodes are positioned a distance of at least l from the entrance to the channel of width W and height b to ensure that the flow profile in the z direction is fully
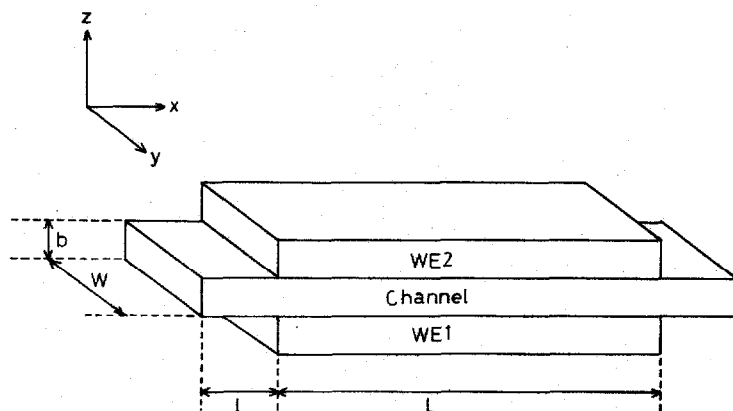

Fig. 1. Coordinates and dimensions of the channel of the parallel-opposed electrochemical detector. x = Direction of flow; z = direction of diffusion; W = electrode width; L = electrode length; l = entry length; b = channel height; WE1 = working electrode 1; WE2 = working electrode 2.

developed laminar flow. It is convenient to consider the length along the electrode, x, in dimensionless form

$$r = x \, (DW/Ub) \tag{1}$$

where D is the solute diffusion coefficient and U is the solvent volume flow-rate. Using the diffusion layer treatment[16], an approximate description of the currents from the detector has been obtained by Weber and Purdy[10,14]. According to them, the current from only one working electrode (WE1) at "infinite" cathodic potential for the oxidant as an analyte, for example, is

$$I = nFUC\psi \tag{2}$$

where n is the electron transfer number, F the Faraday constant, C the bulk concentration of analyte and ψ the electrolytic efficiency, which is given by

$$\begin{aligned}
\psi &= 1.40 \, \delta^2 - 0.80 \, \delta^3 \text{ for } 0 < r < 0.33 \\
&= 1.00 - 0.40 \exp [2.51 \, (0.33 - r_L)] \text{ for } r \geqslant 0.33
\end{aligned}$$

where $r_L = 0.94 \, \delta^3 - 0.60 \, \delta^4$ yields δ^{14}. Here r_L is r at $x = L$ and δ is the ratio of the thickness of the diffusion layer to the channel height at $r = r_L$. The current from one working electrode (WE1) with $r_L \gtrsim 2$ corresponds to coulometric conversion and is given by

$$I_s = nFCU \tag{3}$$

because ψ is virtually 1.

Consider a reversible or quasi-reversible redox couple and the case that the only oxidant is initially in the flow stream and at concentration C. The two working electrodes, WE1 and WE2, of the detector are set at potentials where the oxidant is sufficiently reduced and its reductant is sufficiently oxidized, respectively. The unitless distance δ is measured from WE1.

Current from WE1. For $0 < r \leqslant 0.33$, the cathode generates a current

$$i = 0.60 \, nFCU \tag{4}$$

from eqn. 2. For the remainder of this electrode $(0.33 < r \leqslant r_L)$

$$i = nFCU \, (r_L - 0.33) \tag{5}$$

because the diffusion layer thickness becomes b $(\delta = 1)$ and thus $\psi = 1$. The entire cathode, WE1, will generate the sum of these two currents:

$$I_c = nFCU \, (r_L + 0.27) \tag{6}$$

Current from WE2. For $0 < r \leqslant 0.33$, there is no current at the anode, because any product (reductant) at the cathode does not reach the anode. For $0.33 < r \leqslant$

0.67, the product reaches the anode, but its linear concentration gradient of C/b has not been completely established between the anode and cathode with a laminar flow. The amount of product oxidized at the anode under these conditions is simply considered to be equal to the amount of analyte (oxidant) reduced at the cathode for $0 < r \leqslant 0.33$. Thus, the anode generates a current:

$$i = -0.60 \, nFCU \tag{7}$$

Thereafter ($0.67 < r \leqslant r_L$) the linear concentration gradient of reductant is completed and equal to C/b. Thus, the anode generates a current:

$$i = -nFCU \, (r_L - 0.67) \tag{8}$$

The entire anode, WE2, will generate the sum of these two currents:

$$I_a = -nFCU \, (r_L - 0.07) \tag{9}$$

Eqns. 6 and 9 can be rewritten in terms of the cell dimensions as:

$$I_c/nFC = LDW/b + 0.27 \, U \tag{10}$$

$$-I_a/nFC = LDW/b - 0.07 \, U \tag{11}$$

The collection efficiency, Φ_c, the ratio of the anodic current to the cathodic current in this case, is important for selective detection in HPLC and is represented as:

$$\Phi_c \equiv |I_a|/I_c = 1 - \frac{0.34 \, U}{LDW/b + 0.27 \, U} \tag{12}$$

$$\approx 1 - \frac{0.34}{LDW/b} \cdot U \text{ for small } U$$

It is expected that the collection efficiency is nearly equal to 1 at very low flow-rates. The current amplification efficiency, Φ_a, is defined as the ratio of the cathodic current to the coulometric current

$$\Phi_a \equiv I_c/I_s = 0.27 + (LDW/b)(1/U) \tag{13}$$

while the effective amplification efficiency, Φ_e, may be defined as the ratio of the anodic current to the coulometric current:

$$\Phi_e \equiv |I_a|/I_s = -0.07 + (LDW/b)(1/U) \tag{14}$$

Eqn. 14 predicts the catalytic current amplification at very low flow-rates.

   Although this entire theory is couched in terms of the oxidant as an analyte, it is evident that the conclusions are easily extended to embrace reductant.

EXPERIMENTAL

The cell used for the parallel-opposed electrochemical detector was shown in Fig. 2 of the previous paper[13]. The thin-layer cavity was constructed of two fluorocarbon resin blocks separated by a PTFE sheet 30 μm or 50 μm thick and 2 mm wide. Two working electrodes were made from glassy carbon plates (1 cm long) facing one another across the solution. Thus the geometric area of each working electrode is 0.2 cm^2 (1 $\times$ 0.2 cm). The reference electrode, silver/silver chloride, was held in a cylindrical hole in one of the blocks. A platinum tube served both as the counter electrode and the exit line.

For the comparison of experiment results with theoretical predictions, a solution of 10 μM or 100 μM potassium ferricyanide in Britton–Robinson (B-R) buffer of pH 1.8 containing 1 mM sodium 1-heptanesulphonate (HSA), 0.1 mM EDTA (disodium salt) and 50 mM sodium perchlorate was passed through the damper column (PTFE tube packed with 30-μm particles, Dipax 3, 5 cm $\times$ 0.5 mm I.D.) and then the cell by using a micro feeder (Azuma Denki, Model MF-2) and a micro syringe (Terumo, Model GAN-100). The current blanks were measured by delivering the same buffer solution but without potassium ferricyanide. The flow-rate used was 1.4–11.2 μl/min and was checked by timing the passage of the solution through a tube of 0.5 mm I.D.
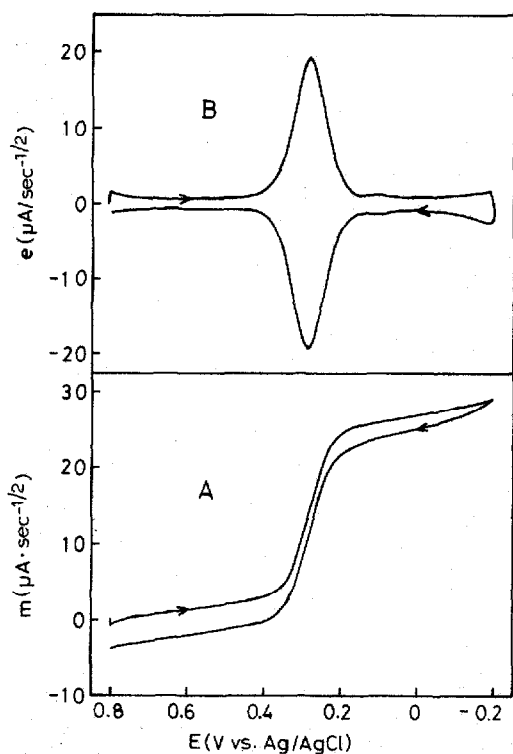

Fig. 2. Cyclic semi-integral (A) and semi-derivative (B) voltammograms of 1 mM ferricyanide in B-R buffer of pH 1.8 containing 1 mM HSA, 0.1 mM EDTA and 50 mM perchlorate at a scan rate of 100 mV/sec. m and e are, respectively, the semi-integral and the semi-derivative of the current with respect to time.
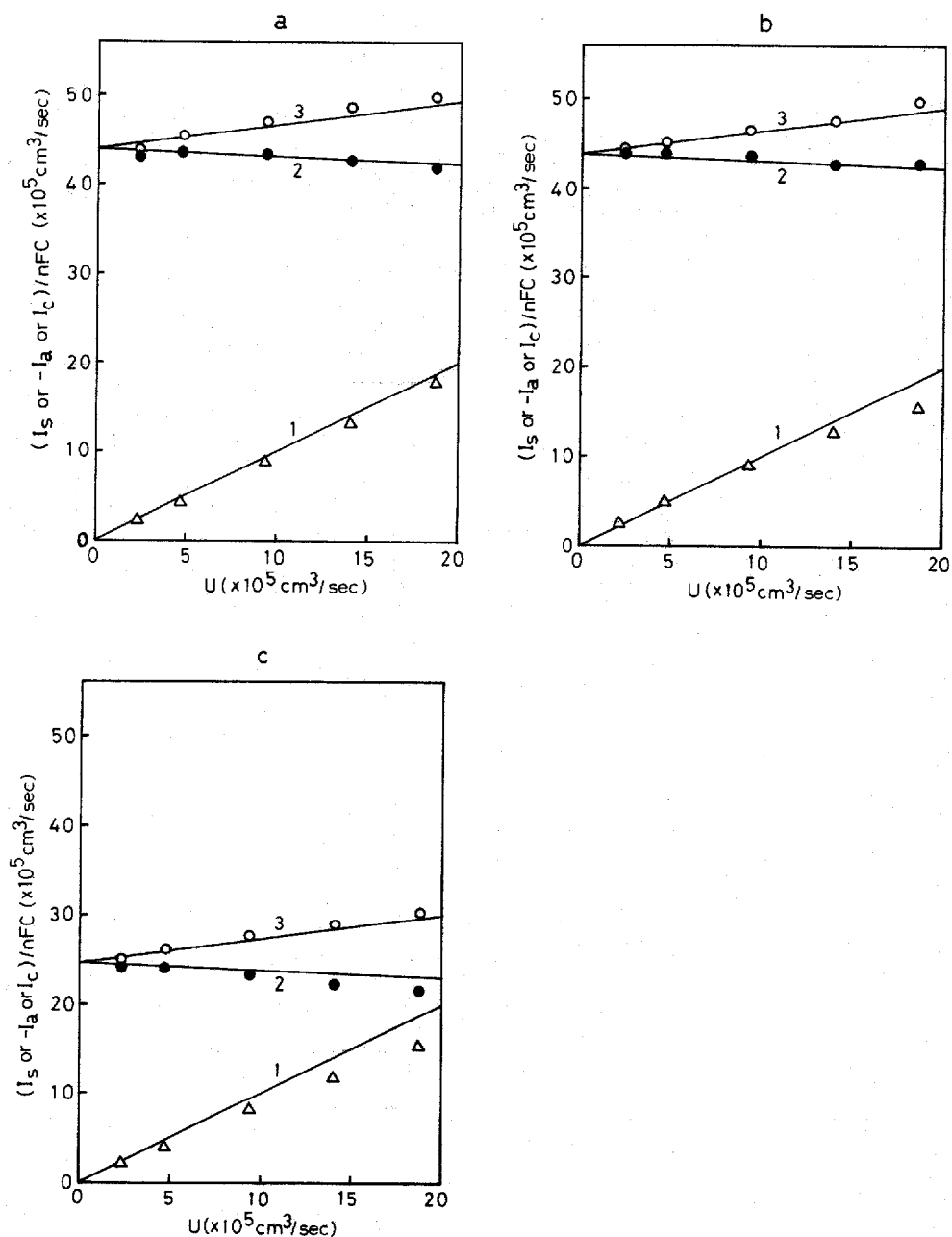
Fig. 3. Dependence of detector currents on flow-rate. The lines represent the theoretical slopes of eqns. 3, 10 and 11; the points represent experimental data. Sample: ferricyanide in the B-R buffer of pH 1.8. Potentials (V *vs.* Ag/AgCl): cathode (WE1) 0.00; anode (WE2) 0.60. Electrode dimensions: $L = 1.0$ cm, $W = 0.2$ cm. (a) $C = 100 \, \mu M, b = 30 \, \mu m$; (b) $C = 10 \, \mu M, b = 30 \, \mu m$; (c) $C = 10 \, \mu M, b = 50 \, \mu m$. Curves: 1, I_s; 2, $-I_a$; 3, I_c.

The chromatographic system and reagents used for determination of catecholamines were as in the previous paper[13]. The instruments used for cyclic semi-integral and semi-differential voltammetry were described previously[8].

RESULTS AND DISCUSSION

Electrochemical behaviour of ferricyanide

The electrochemical behaviour of ferricyanide on a glassy carbon electrode in the mobile phase for MHPLC was studied by cyclic semi-integral and semi-differential voltammetry[17–23]. Fig. 2 shows typical voltammograms of ferricyanide. The cathodic and anodic peak potentials (V vs. Ag/AgCl) in the semi-derivative voltammogram were 0.28_3 and 0.28_7 V, respectively, in this medium. The potentials of 0.00 V and 0.60 V were chosen as the "infinite" cathode and anode potentials of ferricyanide and its product (ferrocyanide) for the parallel-opposed electrochemical detector, from inspection of the semi-integral voltammogram.

Verification of theoretical predictions

The dimensions of the cell used for the detector were $L = 1.0$ cm, $W = 0.2$ cm and $b = 0.003$ or 0.005 cm, the flow-rate was $< 19 \times 10^{-5}$ cm³/sec and thus $r_L \gtrsim 1.5$. In this case, the theory predicts approximately coulometric conversion for the current from one working electrode. Eqns. 3, 10 and 11 were tested at different concentrations of ferricyanide and different channel heights, Fig. 3. Coulometric conversion was nearly achieved for the current from one working electrode in the cell of channel height 30 μm. When the channel height was 50 μm the current tended to deviate from coulometric conversion at higher flow-rates, as predicted. Both the cathode and anode currents from the detector were linearly dependent on the flow-rate in all cases. According to the theory, the intercepts of plots of $(I_c$ or $-I_a)/nFC$ versus U with the axis at $U = 0$ correspond to LWD/b. The experimental intercepts ($\times$ 10^5 cm³/sec) from the cathode and anode currents for the cell of channel height 30 μm were 43.5 and 43.6 at a concentration of 100 μM, and 43.5 and 44.2 at 10 μM. At a channel height of 50 μm, the experimental values ($\times$ 10^5 cm³/sec) of 24.4 and 24.6 were observed from the cathode and anode currents. These values are to be compared with the theoretical LWD/b values ($\times$ 10^5 cm³/sec) of 46.7 for $b = 30$ μm and 28.0 for $b = 50$ μm, respectively, by assuming that the average diffusion coefficient of ferricyanide and ferrocyanide is 7×10^{-6} cm²/sec[24]. All the experimental slopes of the lines for the cathode and anode currents coincided well with the theoretical slopes, as shown in Fig. 3.

It should be noted that the currents from this detector become nearly independent of flow-rate at very low flow-rates, while the currents from coulometric and amperometric detectors are proportional to the flow-rate and to its square root, respectively.

Current amplification efficiencies and collection efficiency

Eqns. 12–14 were tested (Fig. 4). All the experimental data concerning current amplification and collection efficiencies were in good agreement with the theoretical predictions. Collection efficiencies from 0.98 to 0.84 were obtained in the flow-rate range 1.4–11.2 μl/min. These values are much higher than those (<0.37) obtained
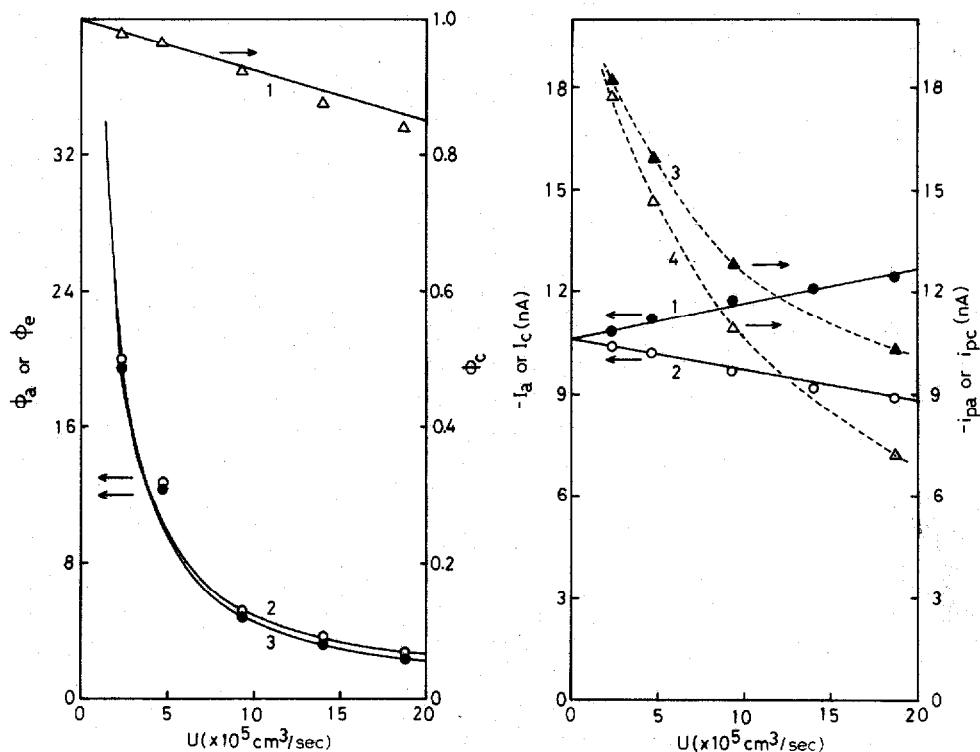
Fig. 4. Dependence of current amplification efficiencies and collection efficiency on flow-rate. The lines represent the theoretical expectations from eqns. 12–14; the points represent experimental data. Sample: 100 μM ferricyanide in the B-R buffer of pH 1.8. Channel height: 30 μm. Curves: 1, Φ_c; 2, Φ_a; 3, Φ_e. Other conditions as in Fig. 3.

Fig. 5. Dependence of steady-state detector currents, I, and chromatographic peak currents, i_p, on flow-rate. Cell dimensions: $L = 1.0$ cm; $W = 0.2$ cm; $b = 50$ μm. Potentials (V $vs.$ Ag/AgCl): anode (WE1) 0.60; cathode (WE2) 0.20. Curves: 1, $-I_a$; 2, I_c; (sample: 50 ng/ml DHBA in the mobile phase); 3, $-i_{pa}$; 4, i_{pc} (sample injected: 0.5 ng DHBA). Mobile phase: B-R buffer of pH 1.8 containing 2 mM HSA, 0.1 mM EDTA and 50 mM perchlorate.

with a series dual electrochemical detector at a flow-rate of 1 ml/min in HPLC[7].

The effective amplification efficiency varied very from 2.4 to 19.5, when the flow-rate changed from 11.2 to 1.4 μl/min, as predicted. It should be noted that these values are much larger than those (0.7–0.8) obtained for catecholamines with a series dual electrochemical detector at a flow-rate of 8.3 μl/min in MHPLC[8].

Application to MHPLC

Catecholamines and 3,4-dihydroxybenzylamine (DHBA) are nearly reversibly oxidized in the mobile phase for MHPLC, B-R buffer of pH 1.8 containing 2 mM HSA, 0.1 mM EDTA and 50 mM perchlorate, as demonstrated by cyclic semiintegral and semi-differential voltammetry. The anodic and cathodic peak potentials *(V vs.* Ag/AgCl) in semi-derivative voltammograms were 0.48 and 0.46 for noradrenaline (NA), 0.48 and 0.46 for adrenaline (AD) and 0.51 and 0.47 for DHBA. Therefore the "infinite" anode, WE1, potential of 0.60 V and the "infinite" cathode, WE2,
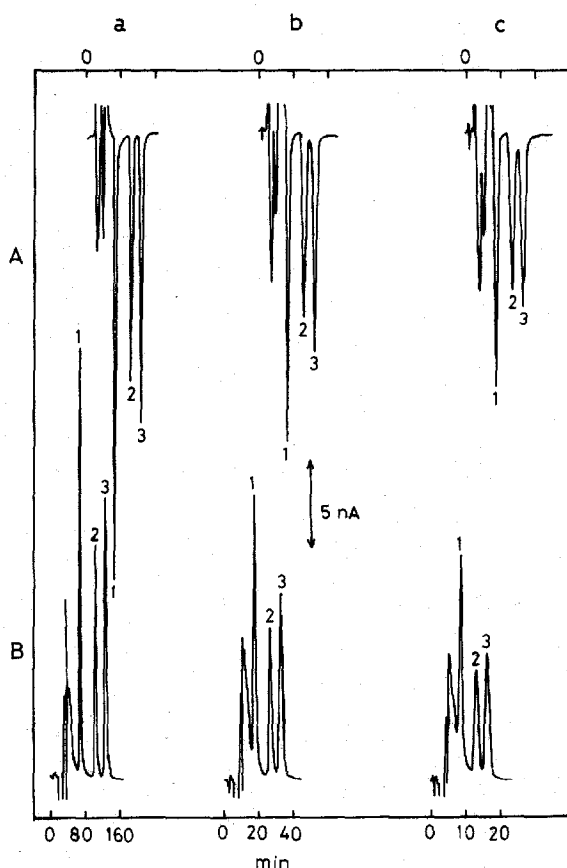
Fig. 6. Typical chromatograms of standard NA, AD and DHBA obtained by MHPLC with the parallel-opposed detector and preconcentration column. A, Anodic response; B, cathodic response. Peaks: 1 = NA; 2 = AD; 3 = DHBA. Sample: 200 μl of a standard solution of 0.5 ng/ml each of NA, AD and DHBA. Flow-rates (μl/min): 1.4 (a); 5.6 (b); 11.2 (c). Separation column: Yanapak ODS-T (15 cm × 0.5 mm I.D.). Preconcentration column: LiChrosorb Alox T (2 cm × 0.5 mm I.D.). Other conditions as in Fig. 5.

potential of 0.20 V were chosen for the detection of these three species. The dependence of the anode and cathode currents on the flow-rate are shown in Fig. 5. It can be concluded that the experimental data are in qualitative agreement with the theoretical predictions in the case of the reductant as an analyte.

A standard solution of NA, AD and DHBA was analyzed by using the same MHPLC system and detector and an alumina precolumn as in the previous paper[13]. Fig. 6 shows typical chromatograms of the three species at different flow-rates. Both the anodic and cathodic peak currents, i_{pa} and i_{pc}, of these species increased on decreasing the flow-rate. For comparison with the steady-state currents from the parallel-opposed electrochemical detector, the chromatographic peak currents of DHBA were plotted in Fig. 5. The different behaviour of the currents in the two cases is due to chromatographic band broadening and the fact that the recovery of species from an alumina precolumn may be dependent on the flow-rate. The extra-column
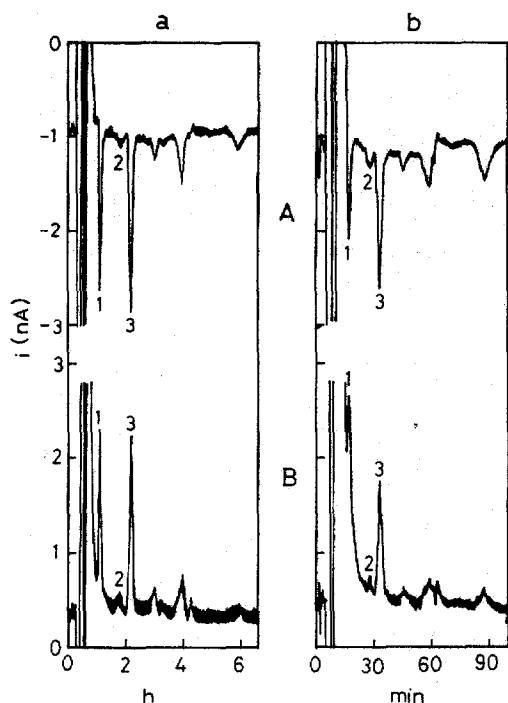
Fig. 7. Chromatograms of catecholamines in healthy human serum by internal standard addition. A, Anodic response; B, cathodic response. Peaks: 1 = NA; 2 = AD; 3 = DHBA. Sample: 200 μl of ultrafiltered human serum spiked with 100 pg DHBA. Flow-rates (μl/min): 1.4 (a); 5.6 (b). Other conditions as in Fig. 6.

band broadening was estimated from the Ouano equation[25] to be only about 1.6 μl at the largest flow-rate used (11.2 μl/min), since the detector cell volume used was only about 1.3 μl. This is about one-twentieth of the corresponding chromatographic band broadening. It should be noted that larger chromatographic peak currents, leading to high sensitivity, are obtained at lower flow-rates in this MHPLC system. The chromatographic cathodic to anodic peak ratios were 0.96, 0.79 and 0.68 for NA, 0.95, 0.81 and 0.66 for AD and 0.97, 0.86 and 0.70 for DHBA at the flow-rates of 1.4, 5.6 and 11.2 μl/min, respectively. The electrolytic efficiencies (%) obtained from the cathodic peak areas were 1132 for NA, 1000 for AD and 1324 for DHBA, by assuming $n = 2$. The deviations of the chromatographic peak currents from the theoretical predictions are due to the fact that the recovery of species from an alumina precolumn is not 100 % and the electrode potentials for this detector are not completely "infinite".

Fig. 7 shows the chromatograms of catecholamines in ultrafiltered human serum (200 μl) spiked with 100 pg DHBA and directly injected into the MHPLC system with the parallel-opposed detector and a preconcentration column. The selective and sensitive detection of catecholamines on the basis of electrochemical reversibility can be performed by using the cathodic chromatograms obtained from this detector at very low flow-rates.

CONCLUSIONS

The steady-state currents from the parallel-opposed electrochemical detector are only a weak function of the flow-rate. However, the number of coulombs corresponding to a chromatographic peak dramatically increases as the flow-rate is lowered. The current from the second working electrode is responsive only to species whose reactions are reversible or quasi-reversible. Thus the parallel-opposed detector is the most advantageous type of electrochemical detector for MHPLC and capillary liquid chromatography, which use very low mobile phase flow-rates.

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