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DUAL ELECTROCHEMICAL DETECTOR FOR MICRO HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND ITS APPLICATION TO THE SELECTIVE DETECTION OF CATECHOLAMINES

MASASHI GOTO*, EIZO SAKURAI and DAIDO ISHII
Department of Applied Chemistry, Faculty of Engineering, Nagoya University, Chikusa-ku, Nagoya-shi 464 (Japan)
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SUMMARY

A dual electrochemical detector having two working electrodes (anode and cathode) suitable for micro high-performance liquid chromatography has been characterized for the selective detection of catecholamines on the basis of their electrochemical reversibility. The rapid determination of applied potentials for such electrochemical detectors in liquid chromatography by means of cyclic semi-differential voltammetry is described. The dual electrochemical detector has been successfully utilized for the selective determination of catecholamines in human urine injected directly into a micro high-performance liquid chromatograph with an alumina preconcentration micro-column.

INTRODUCTION

Micro high-performance liquid chromatography (MHPLC)[1] using packed columns of I.D. less than 1 mm and packed or open-tubular capillary liquid chromatography (capillary LC)[2,3] are attractive in that the consumption of the mobile phase and sample is much less than that in conventional HPLC. Flow-rates of the mobile phase employed in MHPLC and capillary LC are typically 0.1–30 μl/min. These extremely slow flow-rates require that small volume detectors be designed and this has been achieved for spectrophotometric detection by UV[1] and fluorescence methods[4]. The usefulness of electrochemical detectors in HPLC has already been recognized[5–7].

Goto *et al.*[8] and Hirata *et al.*[9] independently designed sub-microlitre electrochemical detectors having one working electrode for use in MHPLC and capillary LC, and they were successfully utilized for the determination of aminophenol isomers separated on a micro-column and of acidic and neutral metabolites of tyrosine and tryptophan in human urine separated on a packed capillary column, respectively.

In a preceding paper[10], an electrochemical detector having two working electrodes (dual electrochemical detector) was designed for use in MHPLC and was successfully utilized for the selective detection of catecholamines based on their elec-

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trochemical reversibility. The principle of selective detection by the dual electrochemical detector is as follows. The anode and cathode in the twin electrode thin-layer electrolytic cell are set at potentials where the reductant (sample) is oxidized and its oxidant is reduced, respectively. The reductant of reversible or quasi-reversible species is oxidized at the anode and the product of this electrode reaction is re-reduced at the cathode, whereas the reductant of irreversible species is not re-reduced at the cathode. Therefore, the reversible and/or quasi-reversible species present in irreversible species can be selectively detected by measuring the re-reducing or re-oxidizing current.

Care is required in selecting the applied potentials of such electrochemical detectors in LC. To maximize the analytical response and selectivity and minimize background interference, the applied potentials should be held at the minimum potential for oxidation or the maximum potential for reduction, at which currents reach the limiting values for the sample under investigation. The optimum applied potentials can be determined directly by hydrodynamic voltammetry (HDV), in which peak current (i_p) is measured against applied potential (E), point by point, for the sample injected into the stream flowing through the electrochemical detector under the experimental conditions of LC. However, HDV measurements require several hours for completion. Alternatively, the approximate applied potentials for electrochemical detectors can be established, indirectly, much more rapidly by cyclic voltammetry (CV), which may be carried out on a quiet solution (without stirring) in a separate electrochemical cell[11]. Cyclic semi-differential voltammetry (CSV)[12–15] provides higher sensitivity and better resolution than ordinary CV. The technique measures the semi-derivative of current with respect to time (e) *versus* applied potential under the same experimental conditions as in CV. We report here the cyclic semi-differential voltammetric determination of applied potentials for the dual electrochemical detector in MHPLC, using catecholamines in human urine as the test samples.

EXPERIMENTAL

Apparatus

The MHPLC system with a pre-concentration micro-column and a dual electrochemical detector as described previously[10] was used for HDV measurements and the selective determination of catecholamines in human urine with direct injection. The design of the twin-electrode thin-layer electrolytic cell for the dual electrochemical detector is shown in Fig. 2 in a preceding paper[10]. The thin-layer cavity was constructed of two fluorocarbon resin blocks separated by a PTFE sheet 50 μm thick and 2 mm wide. Two working electrodes were made with glassy carbon disks of 3 mm diameter contained in one of the blocks. The silver/silver chloride reference electrode was held in a cylindrical hole in the other block. A platinum tube served as the counter electrode and the exit line. A micro alumina precolumn (2 cm × 0.5 mm I.D.) and a micro ODS column (15 cm × 0.5 mm I.D.) were used for enriching catecholamines and separating them.

A cyclic voltammetric instrument (Bioanalytical Systems, Model CV-1B) and a home-made analogue semi-differentiating circuit as shown in Fig. 1 in a previous paper[16] were used for CSV measurements. The output of the cyclic voltammetric instrument was fed to the analogue circuit for semi-differentiation through a voltage

follower and a resistor in order to minimize electrical noise. An *x–y* recorder (Yokogawa Co., Model 3086) was used to record the cyclic *e versus E* curves (cyclic semi-derivative voltammograms). A glassy carbon disk of 3 mm diameter was used as the working electrode. The surface of the disk was polished to a mirror finish with alumina powder (0.05 μm) on an acrylic resin plate before use. A silver/silver chloride electrode and a platinum wire were used for the reference and the counter electrode, respectively.

Reagents

Analytical-reagent grade chemicals were used without further purification. All solutions were prepared from distilled, deionized water. For standard samples, noradrenaline (NA), adrenaline (AD), dopamine (DA) and *l*-dopa (LD) were dissolved in Britton–Robinson buffer (B–R buffer) of pH 1.8. The mobile phase used in MHPLC and the solvent used in CSV were B–R buffer of pH 1.8 containing 0–0.5 m*M* sodium 1-heptanesulphonate (HSA) as the ion-pair reagent and 0–1 m*M* EDTA (disodium salt) as the masking reagent for iron(II) ion. The buffer solution for pre-treatment of the micro alumina pre-concentration column and pH adjustment of the sample in the MHPLC system was 1 *M* Tris buffer of pH 8.7 containing 0.25% EDTA (disodium salt) and 0.05% sodium hydrogen sulphite as the stabilizing reagents for catecholamines.

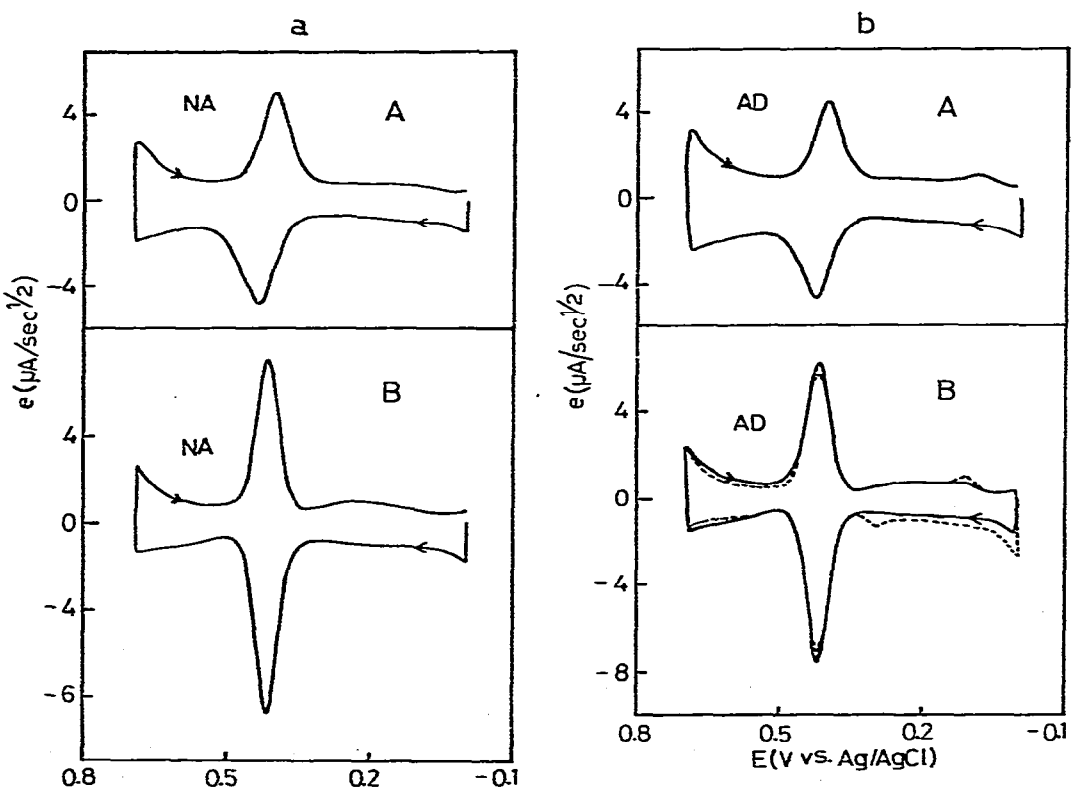

Fig. 1.

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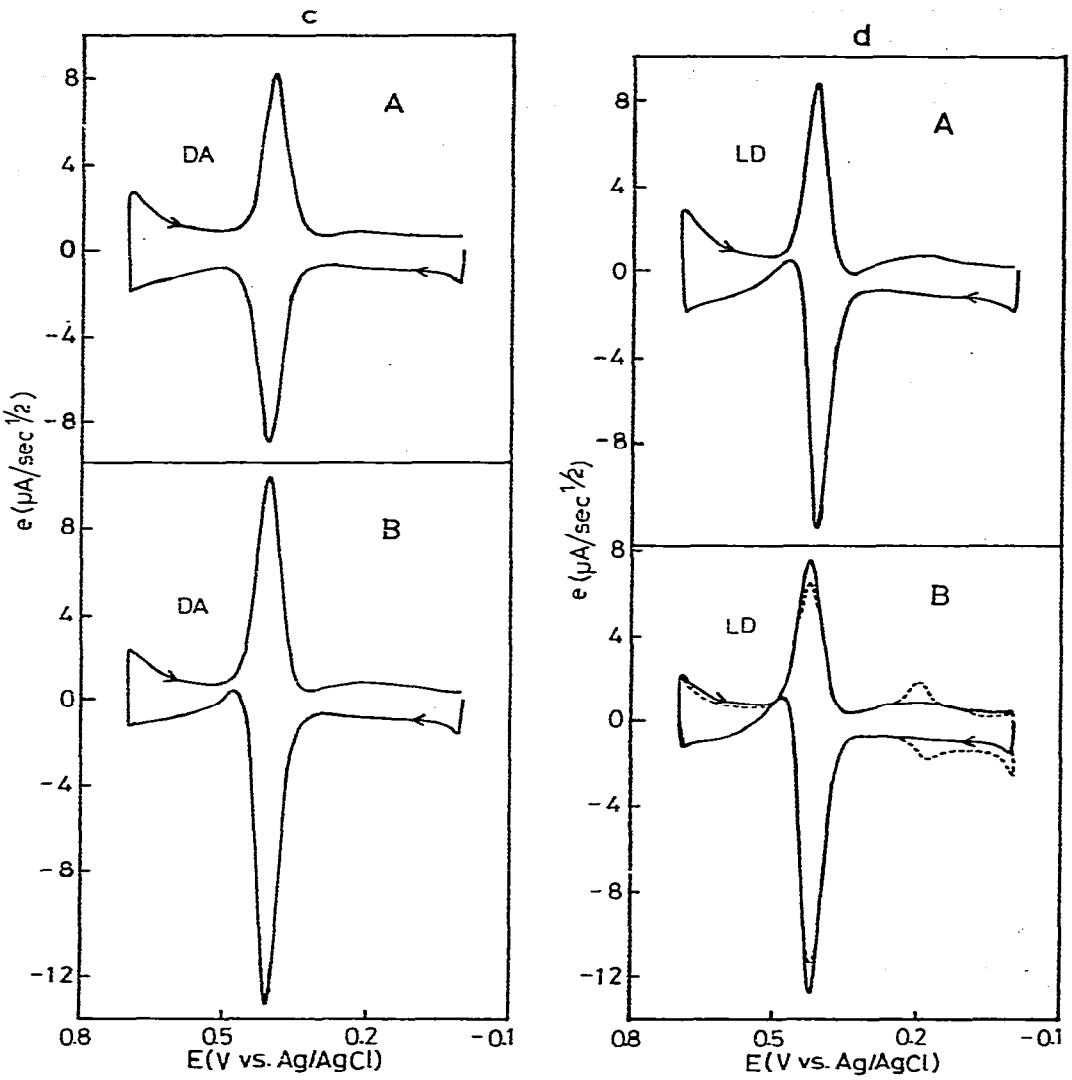


Fig. 1. Cyclic semi-derivative voltammograms of 0.1 m*M* catecholamines in B–R buffer of pH 1.8 alone (A) and containing 0.2 m*M* HSA (B). Scan rate: 100 mV/sec. (a) Noradrenaline; (b) adrenaline; (c) dopamine; (d) *l*-dopa.

RESULTS AND DISCUSSION

Cyclic semi-differential voltammetry of catecholamines

The cyclic semi-differential voltammetric behaviours of NA, AD, DA and LD were studied in B–R buffer alone and containing HSA and/or EDTA. Fig. 1 shows typical cyclic semi-derivative voltammograms of the four catecholamines in B–R buffer of pH 1.8 alone and containing 0.2 m*M* HSA. Theoretically, the anodic peak potential (E_{pa}) in the cyclic *e versus E* curves coincides with the cathodic peak potential (E_{pc}) for the reversible system[13]. The peak potential differences of NA, AD, DA

TABLE I

PARAMETERS FOR CSV OF CATECHOLAMINES IN DIFFERENT MEDIA

Scan rate, 100 mV/sec; sample concentration, 0.1 m*M* each. Medium: (A) B–R buffer of pH 1.8 alone; (B) A plus 0.2 m*M* HSA; (C) A plus 0.5 m*M* HSA; (D) C plus 0.1 m*M* EDTA.

Catecholamine	*Medium*	*Peak potential (V vs. Ag/AgCl)*		*Peak potential differences ($E_{pa} - E_{pc}$) (mV)*
		E_{pa}	E_{pc}	
NA	A	0.43_0	0.41_0	20
	B	0.42_5	0.42_5	0
	C	0.42_0	0.41_5	5
	D	0.41_3	0.41_2	1
AD	A	0.43_0	0.40_2	28
	B	0.42_5	0.42_0	5
	C	0.42_0	0.41_7	3
	D	0.41_7	0.41_3	4
DA	A	0.40_0	0.39_0	10
	B	0.41_0	0.40_5	5
	C	0.40_0	0.39_8	2
	D	0.40_0	0.39_8	2
LD	A	0.41_3	0.41_5	− 2
	B	0.42_3	0.42_7	− 4
	C	0.42_0	0.42_2	− 2
	D	0.42_1	0.42_5	− 3

and LD in B–R buffer of pH 1.8 alone were 20, 28, 10 and 2 mV, respectively (see Table I). This indicates that the electrode reactions of NA, AD and DA are quasi-reversible, while that of LD is reversible in this medium. It is interesting that the electrode reactions of all four catecholamines become reversible on adding ion-pair reagent to the medium, as can be seen in parts B in Fig. 1 and Table I. The asymmetric anodic peak shape for DA and LD in Fig. 1 indicates that they tend to be adsorbed on a glassy carbon electrode. On the first anodic scan for AD, an anodic peak occured at 0.43 V (*vs.* Ag/AgCl), which corresponds to the oxidation of AD to the open-chain quinone. On potential reversal the re-reduction of this quinone was observed at 0.42 V. On subsequent cycles, these peaks were diminished in intensity and another cathodic peak at 0.11 V as well as a new anodic peak at 0.30 V appeared, as shown by the dotted line (the fourth cycle) in Fig. 1b. The former corresponds to the reduction of the cyclized product (adrenochrome) to leucoadrenochrome, while the latter is that of the re-oxidation of leucoadrenochrome to adrenochrome[17]. On subsequent cycles, the similar new cathodic peak at 0.20 V and anodic peak at 0.18 V appeared, as shown by the dotted line (the fifth cycle) in Fig. 1d for LD, while only one anodic and one cathodic peak were observed for NA and DA. The reversibility of catecholamines was virtually independent of the concentration of ion-pair reagent and EDTA present in the medium (see Table I).

Cyclic voltammetric determination of approximate applied potentials for electrochemical detectors in LC

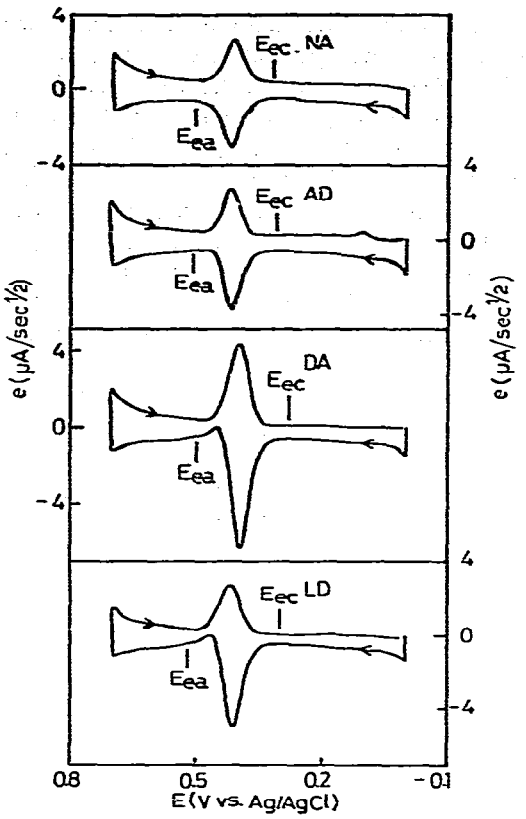
Fig. 2 shows the cyclic semi-derivative voltammograms of catecholamines at a scan rate of 40 mV/sec in the same medium used as the mobile phase in MHPLC. For selective detection of catecholamines by the dual electrochemical detector, the anode and cathode of the twin electrode cell should be set at the end potentials (E_{ca} and E_{cc}), at which the oxidation and re-reduction wave in the cyclic *e versus E* curve are complete, respectively. As shown in Table II, the end potentials of catecholamines tended to shift to larger overpotentials as the scan rate of the potential increased, whereas their peak potentials were virtually constant, independent of the scan rate. It is expected, moreover, that the end potentials in CSV and the minimum or maximum potentials for the limiting currents in HDV for slow electron transfer reactions will shift to larger overpotentials as the scan rate of the potential or the flow-rate of the

TABLE II

PARAMETERS FOR CSV OF CATECHOLAMINES IN B–R BUFFER OF pH 1.8 CONTAINING 0.5 m*M* HSA PLUS 0.1 m*M* EDTA AT DIFFERENT SCAN RATES

Sample concentration: 0.1 m*M* each.

Catecholamine	*Scan rate (mV/sec)*	*Peak potential (V vs. Ag/AgCl)*		*End potentials (V vs. Ag/AgCl)*	
		E_{pa}	E_{pc}	E_{ca}	E_{cc}
NA	10	0.42	0.42	0.48	0.34
	20	0.42	0.42	0.49	0.33
	40	0.42	0.41	0.50	0.32
	60	0.42	0.42	0.52	0.28
	80	0.42	0.42	0.53	0.26
	100	0.41	0.41	0.55	0.22
AD	10	0.42	0.42	0.49	0.35
	20	0.42	0.42	0.50	0.33
	40	0.42	0.42	0.51	0.31
	60	0.42	0.42	0.52	0.29
	80	0.42	0.41	0.53	0.27
	100	0.42	0.41	0.55	0.22
DA	10	0.40	0.40	0.47	0.32
	20	0.40	0.40	0.48	0.30
	40	0.40	0.40	0.50	0.28
	60	0.40	0.40	0.52	0.26
	80	0.40	0.40	0.54	0.25
	100	0.40	0.40	0.56	0.21
LD	10	0.42	0.42	0.48	0.34
	20	0.42	0.42	0.50	0.32
	40	0.41	0.42	0.52	0.30
	60	0.41	0.42	0.54	0.29
	80	0.41	0.41	0.56	0.28
	100	0.42	0.42	0.59	0.21



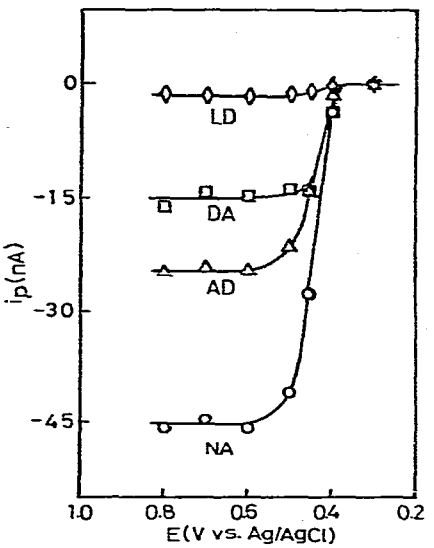


Fig. 2. Cyclic semi-derivative voltammograms of 0.1 m*M* catecholamines in B–R buffer of pH 1.8 containing 0.5 m*M* HSA plus 0.1 m*M* EDTA at a scan rate of 40 mV/sec.

Fig. 3. Hydrodynamic voltammograms of catecholamines using one working electrode. Mobile phase, B–R buffer of pH 1.8 containing 0.5 m*M* HSA plus 0.1 m*M* EDTA; flow-rate, 8.3 μl/min; sample injected, 5 ng each.

mobile phase increases, respectively[11]. Therefore, we recommend that one should determine the approximate applied potentials for electrochemical detectors in LC from the end potentials by CSV at a scan rate of 100 mV/sec and then determine the optimum applied potentials by HDV at a few potentials around them.

Hydrodynamic voltammetric determination of optimum applied potentials for electrochemical detectors in LC

Micro high-performance liquid chromatograms after pre-column enrichment of catecholamines were measured by using a twin electrode thin-layer electrolytic cell at different applied potentials. The mobile phase used was B–R buffer of pH 1.8 containing 0.5 m*M* HSA plus 0.1 m*M* EDTA and its flow-rate was 8.3 μl/min. Fig. 3 shows the i_p *versus* E curves (hydrodynamic voltammograms) of catecholamines using only one working electrode in the twin electrode cell. The minimum potentials, at which their oxidation peak currents reach the limiting values, were about 0.60 V for all four catecholamines.

Fig. 4 shows the hydrodynamic voltammograms using two working electrodes in the twin electrode cell under a constant potential of the upstream electrode (anode) of 0.80 V at different applied potentials of the downstream electrode (cathode or anode). It was found from the responses of the downstream electrode that the maximum potentials, at which their re-reduction peak currents reach the limiting values, were about 0.20 V, independent of the species of catecholamines. Therefore, the optimum applied potentials of the anode and cathode for selective detection of catecholamines in other electroactive species should be 0.60 and 0.20 V, respectively.

The limiting peak current ratios of re-reduction to oxidation of catecholamines were 0.68, 0.68, 0.78 and 0.71 for NA, AD, DA and LD, respectively, under the conditions shown in Fig. 4, in which no electric filter was used to record chromatograms. It should be noted that the limiting peak currents for oxidation of catecholamines in measurements using one working electrode increased by 13–22% when the potential of the downstream electrode was held at 0.20 V or more negative potentials compared with that using two working electrodes. This indicates that the oxidation and re-reduction of catecholamines are repeated between two working electrodes in the thin-layer cell because of the slow flow-rate of the mobile phase, *e.g.*, 8.3 μl/min.

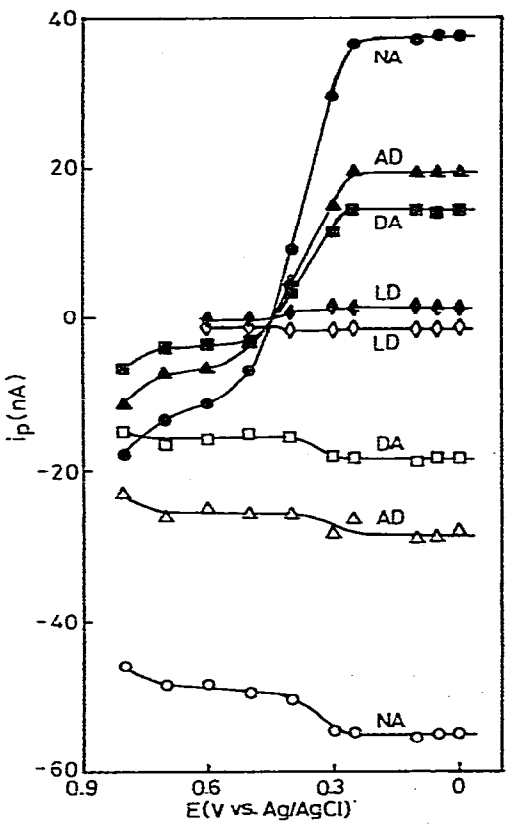


Fig. 4. Hydrodynamic voltammograms of catecholamines using two working electrodes. Upstream electrode potential: 0.80 V *vs.* Ag/AgCl. ○, △, □ and ◇, upstream electrode responses; ●, ▲, ■ and ◆, downstream electrode responses. Other conditions as in Fig. 3.

Kissinger *et al.*[18] predicted amplification of the electrochemical detector response obtained by recycling the electroactive functional group between its reduced and oxidized forms at extremely low flow-rates. One attempt to implement this concept failed because pellicular columns were used at too great a flow-rate[19]. This work is the first experiment in which an amplification effect based on such a concept was achieved.

Selective detection of catecholamines in human urine

Chromatograms of catecholamines in 100 μl of human urine injected directly without any pre-treatment into the MHPLC system with a micro pre-column were measured using the dual electrochemical detector by the procedures described previously[10]. Fig. 5 shows examples of the selective detection of catecholamines in urine from healthy humans under conditions in which the applied potentials of the anode and cathode were held at 0.60 and 0.20 V, respectively. Of particular interest in parts A is the peaks appearing as the background of NA and AD in Fig. 5a and as that of LD in Fig. 5b. By recording the re-reduction current, the interferences from the

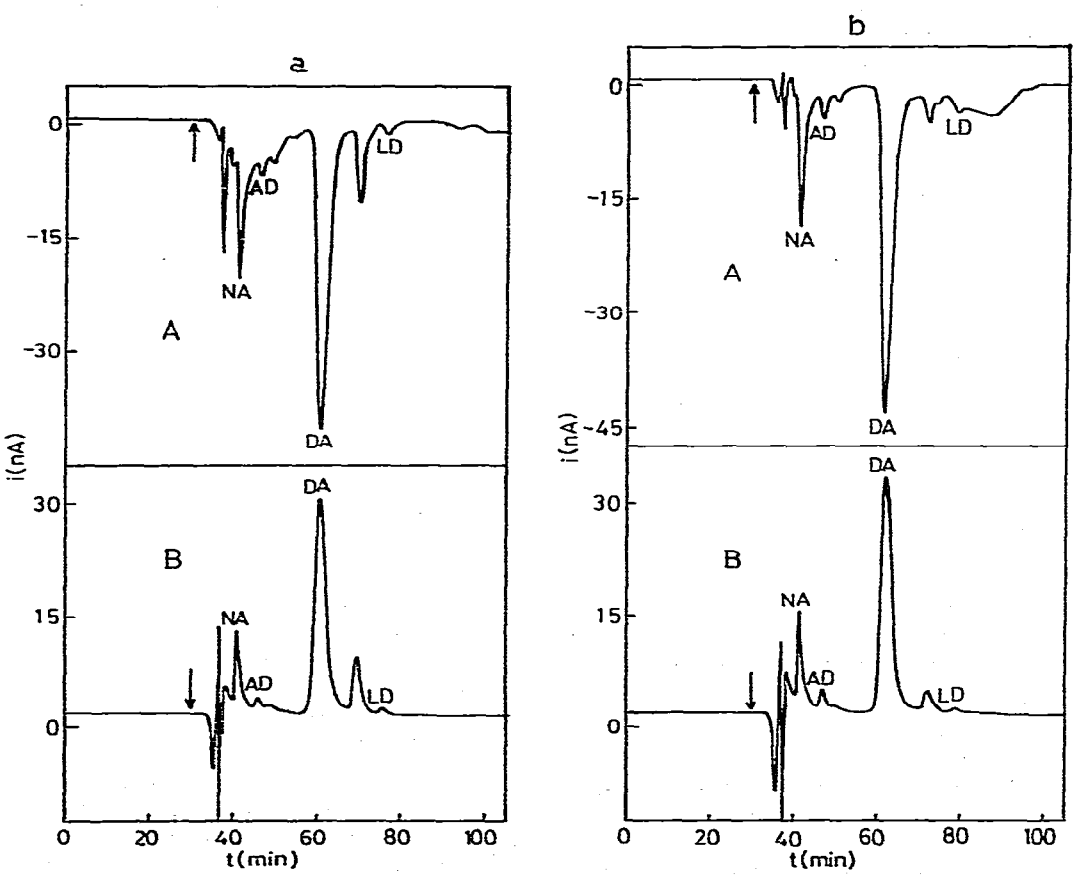


Fig. 5. Selective detection of catecholamines in human urine using a dual electrochemical detector in MHPLC. (A) Anodic response; (B) cathodic response. Sample: 100 μl of healthy human urine. Applied potentials:anode 0.60; cathode 0.20 V *vs.* Ag/AgCl. Other conditions as in Fig. 3.

compounds responsible for these peaks could be removed, as shown in parts B in Fig. 5 on the basis of their electrochemical irreversibility.

CONCLUSIONS

HDV measurements can determine precisely the optimum applied potentials for electrochemical detectors in LC, but require several hours or more for completion, owing to the time required for the background current to stabilize after each change of electrode potential. On the other hand, CV or CSV measurements can be made much more rapidly, requiring less than 1 min for one cycle, and provide both the potentials for oxidation (or reduction) and re-reduction (or re-oxidation) of the samples at the same time. CSV is a more suitable technique than CV for determining the approximate applied potentials for electrochemical detectors, because the peaks observed in CSV are sharp and symmetrical whereas those in CV are broad and asymmetric.

The dual electrochemical detector with an anode and cathode is a powerful instrument for the selective detection of reversible and/or quasi-reversible species present in many irreversible species and may provide an enhancement in sensitivity by recycling oxidation (or reduction) and re-reduction (or re-oxidation) between the anode and cathode for reversible and/or quasi-reversible species at the extremely slow flow-rates.

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REFERENCES

1 D. Ishii, K. Asai, K. Hibi, T. Jonokuchi and M. Nagaya, *J. Chromatogr.*, 144 (1977) 157.
2 D. Ishii and T. Takeuchi, *J. Chromatogr. Sci.*, 18 (1980) 462.
3 M. Novotny, *J. Chromatogr. Sci.*, 18 (1980) 473.
4 Y. Hirata and M. Novotný, *J. Chromatogr.*, 186 (1979) 521.
5 P. T. Kissinger, *Anal. Chem.*, 49 (1977) 448.
6 K. Brunt, *Pharm. Weekbl.*, 113 (1978) 689.
7 R. J. Ruch, *Talanta*, 27 (1980) 147.
8 M. Goto, Y. Koyanagi and D. Ishii, *J. Chromatogr.*, 208 (1981) 261.
9 Y. Hirata, P. T. Lin, M. Novotný and R. M. Wightman, *J. Chromatogr.*, 181 (1980) 287.
10 M. Goto, T. Nakamura and D. Ishii, *J. Chromatogr.*, 226 (1981) 33.
11 J. L. Anderson, D. E. Weisshaar and D. E. Tallman, *Anal. Chem.*, 53 (1981) 906.
12 M. Goto and D. Ishii, *J. Electroanal. Chem.*, 61 (1975) 361.
13 P. Dalrymple-Alford, M. Goto and K. B. Oldham, *J. Electroanal. Chem.*, 85 (1977) 1.
14 P. Dalrymple-Alford, M. Goto and K. B. Oldham, *Anal. Chem.*, 49 (1977) 1390.
15 M. Goto, M. Kato and D. Ishii, *Anal. Chim. Acta*, 126 (1981) 95.
16 M. Goto, K. Ikenoya, M. Kajihara and D. Ishii, *Anal. Chim. Acta*, 101 (1978) 131.
17 M. D. Hawley, S. V. Tatawawadi, S. Piekarski and R. N. Adams, *J. Amer. Chem. Soc.*, 89 (1973) 447.
18 P. T. Kissinger, K. Bratin, G. C. Davis and L. A. Pachla, *J. Chromatogr. Sci.*, 17 (1979) 137.
19 R. J. Fenn, S. Siggia and D. J. Curran, *Anal. Chem.*, 50 (1978) 1067.