CHROMSYMP. 638

LOW DEAD VOLUME COULOMETRIC DETECTOR FOR LIQUID CHROMATOGRAPHY

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(First received April 5th, 1985; revised manuscript received May 24th, 1985)

SUMMARY

A low dead volume coulometric detector flow cell for liquid chromatography was designed. Its cylindrical flow cell structure is simple and the cell volume is extremely small. The working and counter electrodes are made of bundled carbon fibres, and the working and counter electrode compartments are separated by a cation-exchange tube. The detector is applicable to many types of compounds. The detection limit for catecholamines is 0.05 pmol.

INTRODUCTION

Electrochemical and electrical conductivity detectors, etc., have recently been demonstrated to be useful in high-performance liquid chromatography (HPLC) in addition to conventional spectroscopic methods, absorption and emission spectrophotometry. The electrochemical detector is unique owing to its selectivity and low detection limit[1-4]. Many biochemical compounds are electrochemically active, and their concentrations in biological systems are extremely low, so that the electrochemical detector is a powerful instrument in the analysis of biochemical samples.

Of electrochemical detectors for liquid chromatography, the amperometric detector has been used more often than the coulometric detector as its simple and small cell volume structure is suitable for use as an HPLC detector[1,4]. The electrolytic efficiency in the amperometric detector, however, is low, being about 30% or less with standard columns, and is easily affected by the environmental conditions, so that sometimes the poor reproducibility of its sensitivity is a problem. The coulo-

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metric detector[4-7] has an electrochemical efficiency of about 100% and the reproducibility of the signal intensity is superior to that of the amperometric detector. However, this higher electrolytic efficiency dose not necessarily lead to lower detection limits, because the background current of the coulometric detector is much higher than that of the amperometric detector. Hence a small cell volume and a lower background current are required in order to achieve lower detection limits.

The coulometric detector cell first made by Takata and Muto[3,5] had a working electrode consisting of carbon cloth or metal gauze (platinum or silver) to enlarge its surface area. The reference electrode was made of silver–silver iodide wire netting and gave a uniform potential over the entire working electrode. An ion-exchange membrane tube diaphragm separated the working and reference electrode compartments. Two glassy carbon plates were used as electrodes in Lankelma and Poppe's cell[6], one being the working electrode and the other the counter electrode. The detection limit of this detector was 1 pmol for perphenazine. Recently, Hagihara *et al.*[7] reported a coulometric detector for HPLC that could detect 10 fmol of catecholamines. The working electrode was a glassy carbon plate, the counter electrode was a stainless-steel tube and the reference electrode was made of silver netting soaked in saturated potassium chloride solution. The coulometric detector flow cells proposed so far have a flat and stacked electrode structure.

We propose here a coulometric detector flow cell with a cylindrical cell structure. The cell structure is simple and the effective flow cell volume is much smaller than 1 μl. The detection limit of this coulometric detector is 0.05 pmol for catecholamines, and many types of compounds, such as phenols, organic acids and inorganic anions, are detectable.

EXPERIMENTAL

Design of the coulometric detector flow cell

The structure of the coulometric detector flow cell is shown in Fig. 1. The working electrode was made of a bundle of carbon fibres of diameter 7 μm (Torayca
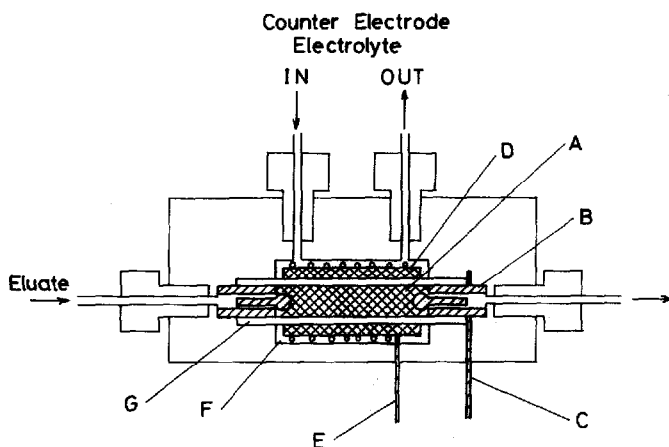

Fig. 1. Construction of the coulometric flow cell. A, Working electrode; B, platinum tube; C, working electrode lead; D, counter electrode; E, counter electrode lead; F, counter electrode compartment; G, Nafion tube.

M40; Toray, Tokyo, Japan). The electrode was constructed as follows. Pre-treatment of the carbon fibres was necessary to remove the surface coating materials. An appropriate number of carbon fibres were soaked in concentrated sulphuric acid for 10 min, washed with distilled water, neutralized with Na_2CO_3 solution until evolution of bubbles of CO_2 ceased and finally rinsed with distilled water. The pre-treated carbon fibres were soaked in distilled water. About 10 000 fibres 8 mm long were used to make the working electrode. The bundle of carbon fibres was inserted into a cation-exchange tube of 0.8 mm I.D. and 1.0 mm O.D. (Nafion tube 21T20GA-11-2048; DuPont, Wilmington, DE, U.S.A.), then two platinum tubes of 0.6 mm I.D. and 0.8 mm O.D. were inserted into the Nafion tube from each end. The platinum tubes were kept in contact with the carbon fibre working electrode electrically. The counter electrode was also made of the same carbon fibres. About 20 000 carbon fibres were cut to 8 mm in length. The working electrode Nafion tube described above was wrapped with the counter electrode carbon fibres and bound with 0.3 mm diameter platinum wire. The flow cell was installed in the counter electrode compartment. With this configuration, a uniform electrolytic potential was obtained. The maximum usable pressure of the flow cell was 25 kg/cm².

As the counter electrode electrolyte $K_3Fe(CN)_6$–$K_4Fe(CN)_6$–KNO_3–KOH solution with a concentration of 0.2 M of each component flowed through the counter electrode compartment. The potential of the counter electrode was maintained constant by chemical equilibrium, and then the electrolytic potential of the working electrode, determined as the sum of the counter electrode potential and the settled circuit potential, was constant.

Equipment

The chromatographic system consisted of a Hitachi HPLC pump (Models 635, 655 and 655-15, Hitachi, Tokyo, Japan), a pulse damper (Hitachi Model 655-1681 or a Model LOD-1, Gasukuro Kogyo, Tokyo, Japan) and a Rheodyne 7125 injection valve equipped with a 20- or 100-μl sample loop (Rheodyne, Berkeley, CA, U.S.A.).

Separation System

Catecholamines. The separation column was a 50 × 4 mm I.D. stainless-steel tube packed with Hitachi Gel 3013-C a –COOH-modified polystyrene–divinylbenzene (PS–DVB) cation exchanger (Hitachi). The mobile phase for catecholamine analysis was 2% CH_3COOH–2% CH_3CN–10^{-4} M Na_2EDTA solution, pumped at a flow-rate of (a) 1.0 ml/min and (b) 0.6 ml/min. The column was used at the room temperature. The catecholamines DOPA, noradrenaline (NA), adrenaline (A) and dopamine (DA) were purchased from Tokyo Kasei (Tokyo, Japan). The electrolytic potential for catecholamine analysis was +0.6 V *vs.* Fe $(CN)_6^{3-}$–$Fe(CN)_6^{4-}$.

Catecholamine metabolites. 3,4-Dihydroxymandelic acid (DOMA), vanillylmandelic acid (VMA), vanillyl acetic acid (VLA), homovanillic acid (HVA), 5-hydroxyindoleacetic acid (5-HIAA) and 2,5-dihydroxyphenylacetic acid were purchased from Tokyo Kasei. The separation column was Hitachi Gel 3013-O, a-OH-modified PS–DVB polymer (Hitachi)packed in a 150 × 4 mm I.D. column maintained 35°C. The mobile phase was 0.05 M tartaric acid–CH_3CN–1 M NaOH (88:12:1.5) at a flow-rate of 0.5 ml/min. The electrolytic potential for catecholamine metabolites was +0.5 V *vs.* $Fe(CN)_6^{3-}$–$Fe(CN)_6^{4-}$.

Lactic acid. A mixed sample of *d*- and *l*-lactic acids was separated with a 250 × 4 mm I.D. Chiralpak-WH column (Daicel Chemical Industries, Osaka, Japan). The mobile phase was 0.25 mM CuSO$_4$ solution at a flow-rate of 1.0 ml/min. The column was used at ambient temperature. The electrolytic potential for lactic acid was +1.1 V *vs.* Fe(CN)$_6^{3-}$–Fe(CN)$_6^{4-}$.

Inorganic anions. MCI-SCAO2 (Mitsubishi Chemical Industries, Tokyo, Japan) anion-exchange resin packed in a 150 × 4 mm I.D. column was used for the separation of inorganic anions. The mobile phase was 1 mM phthalic acid solution containing 10^{-4} M Na$_2$EDTA, the pH being adjusted to 4.0 with KOH. The pumping rate of the eluent was 1.0 ml/min. The column was used at ambient temperature. The electrolytic potential was varied from +0.8 to +1.1 V *vs.* Fe(CN)$_6^{3-}$–Fe(CN)$_6^{4-}$.

RESULTS AND DISCUSSION

Contruction of the coulometric detector cell

Number of working electrode carbon fibres. As an electrolytic efficiency of 100% is essential for a coulometric detector, we examined the effect of the number of carbon fibres used in the working electrode on the electrolytic efficiency. The number was varied from 6000 to 10 000 while the length of the working electrode was fixed at 8 mm. The results are shown in Fig. 2. When the number of carbon fibres was less than 8000 the electrolytic efficiency was in the range 20–90%. On the other hand, when the number of carbon fibres was 10 000, the efficiency became 90–100%. When the number of carbon fibres was less than 8000, the electrolytic efficiency was affected by the mode of insertion of the working electrode in the Nafion tube. A working electrode with a twisted bundle of carbon fibres displayed a higher efficiency than that with non-twisted fibres. When the number of carbon fibres was less than 8000, it was difficult to make a flow cell with a reproducibly high electrolytic efficiency.

Length of working electrode. The length of the working electrode carbon fibres
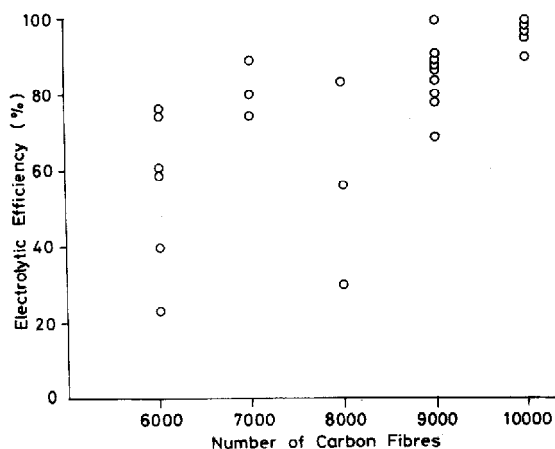

Fig. 2. Relationship between electrolytic efficiency and the number of carbon fibres. The length of the working electrode is 8 mm.
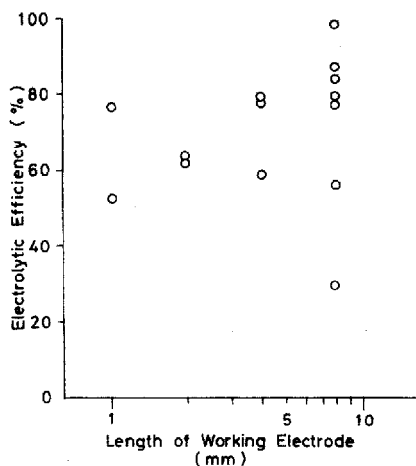
Fig. 3. Relationship between electrolytic efficiency and the length of the working electrode. The number of carbon fibres is 8000–9000.

was varied from 1 to 8 mm, keeping the number of fibres in the range 8000–9000. As shown in Fig. 3. There was a slight increase in electrolytic efficiency with increasing length of the working electrode. However, the electrolytic efficiency with a particular length of the working electrode was variable and was probably dependent on the treatment of the carbon fibres.

  Electrolytic efficiency vs. electrolytic potential. Fig. 4 shows the relationship between the electrolytic potential and electrolytic efficiency. The sample was a mixture of 100 pmol each of DOPA, noradrenaline, adrenaline and dopamine with amount. When the electrolytic potential was lower than $+0.3$ V, the catecholamines were not oxidized. With increasing potential above $+0.3$ V the electrolytic efficiency increased rapidly and reached 100% at $+0.5$ V. Each catecholamine showed similar behaviour. On the other hand, the background current increased gradually with increase in electrolytic potential. At a potential lower than $+0.3$ V, the background
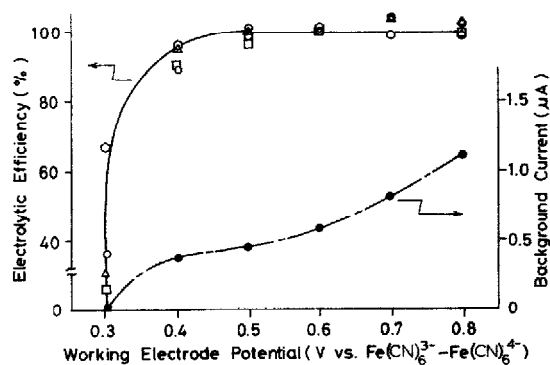

Fig. 4. Dependence of electrolytic efficiency and background current on electrolytic potential. Counter electrode electrolyte: $K_3Fe(CN)_6$–$K_4Fe(CN)_6$–KNO_3–KOH (each 0.2 M). O, DOPA; △, noradrenaline; □, adrenaline; ◇, dopamine.

current was negligibly small. For the purpose of analysis, it is preferable that the electrolytic efficiency is saturated while the background current is as low as possible. It was found that the optimum potential for catecholamine analysis was in the range +0.5 to +0.6 V.

Detection limit of the coulometric detector
    We compared the detection limit of the coulometric detector described above with that of a Hitachi 630 coulometric detector in which the working electrode consists of carbon fibre cloth. The sample was a mixture of the catecholamines DOPA, noradrenaline, adrenaline and dopamine. The flow cell volume of the Hitachi 630 coulometric detector is large (360 μl when the carbon fibre cloth working electrode is not installed) and the background current is large. The chromatographic peaks were broad in such a large flow cell, and hence the intensity and the resolution of the chromatogram were low. On the other hand, the coulometric detector described here had a small cell volume of 2 μl when the working electrode was not installed and the ultimate cell volume may be much smaller than 1 μl, and had a lower background current. The reduction in intensity and loss of resolution in the chromatogram was minimized by the extremely small cell volume, and the lower background current made it possible to achieve higher sensitivity. As can be seen from Fig. 5, the new coulometric detector showed a detection limit two orders of magnitude lower than the Hitachi 630 coulometric detector at an equal signal-to-noise ratio.
    Good linearity was obtained between the peak height and the amount of sample injected over the range 1–100 pmol.

Applications
    Catecholamines. For rapid analysis of catecholamines, we examined a packing material of smaller particle size. The column used was 50 × 4 mm I.D. packed with Hitachi Gel 3057 silica ODS (3 μm diameter particle size) (Hitachi), the mobile phase
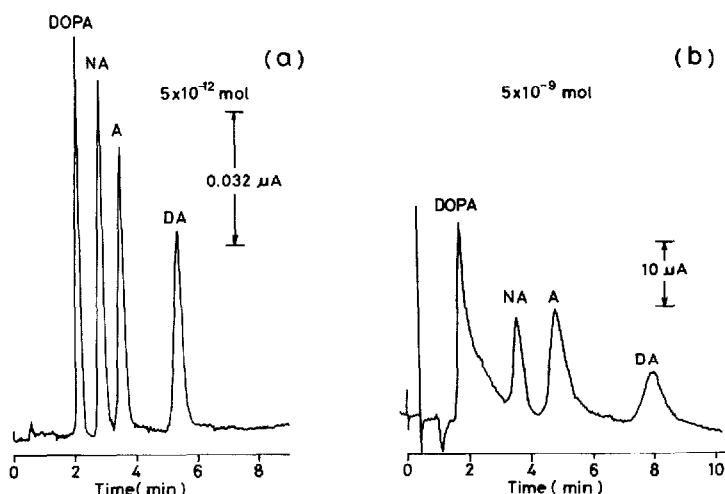

Fig. 5. Chromatogram of catecholamines. Detector: (a) coulometric detector described here; (b) Hitachi 630 coulometric detector. For conditions, see Experimental.

was an aqueous solution of 0.1 M $MgSO_4$–0.04 M KH_2PO_4–4% CH_3CN–0.1 mM Na_2EDTA at a flow-rate of 1.0 ml/min and the working electrode potential was +0.6 V. As shown in Fig. 6, all four catecholamines were eluted within 60 sec. Owing to the small cell volume, the dispersion in the flow cell was small. Hence the coulometric detector flow cell described above is particularly suitable for liquid chromatography with smaller particle columns. A 0.5 pmol amount of each catecholamine could easily be detected, and we conclude that the detection limit for catecholamines with this detector is 0.05 pmol.

Catecholamine metabolites. Fig. 7a shows the analysis of a mixture of the catecholamine metabolites DOMA, VMA, HVA and 5-HIAA and 2,5-dihydroxyphenylacetic acid.

The metabolism of catecholamines is affected by disease in neuroblasts, and the analysis of catecholamines and their metabolites is desirable in biochemistry and medicine. A 10-μl sample of human urine without pre-treatment was analyzed and the result is shown in Fig. 7b.

Lactic acid. Fig. 8 shows the separation of the *d*- and *l*-enantiomers of lactic acid on a Chiralpak-WH column with 0.25 mM $CuSO_4$ solution as the mobile phase and an electrolytic potential of +1.1 V. However, the separating conditions were not suitable for the detector system. Cu^{2+} ion in the eluent penetrates the cation-exchange tube and forms insoluble $Cu_2Fe(CN)_6$ on the surfaces of the Nafion tube and the counter electrode, with a reduction in electrolytic efficiency. A different counter electrode solution must be used that does not form a complex or insoluble compound with the mobile phase, *e.g.*, hydroquinone–benzoquinone.

Inorganic anions. Inorganic ions are usually detected with a conductivity detector (ion chromatography[8–10]). Other detectors with a selective sensitivity are needed, because the universality of the sensitivity of the conductivity detector sometimes makes it difficult to detect a specific ion of interest.

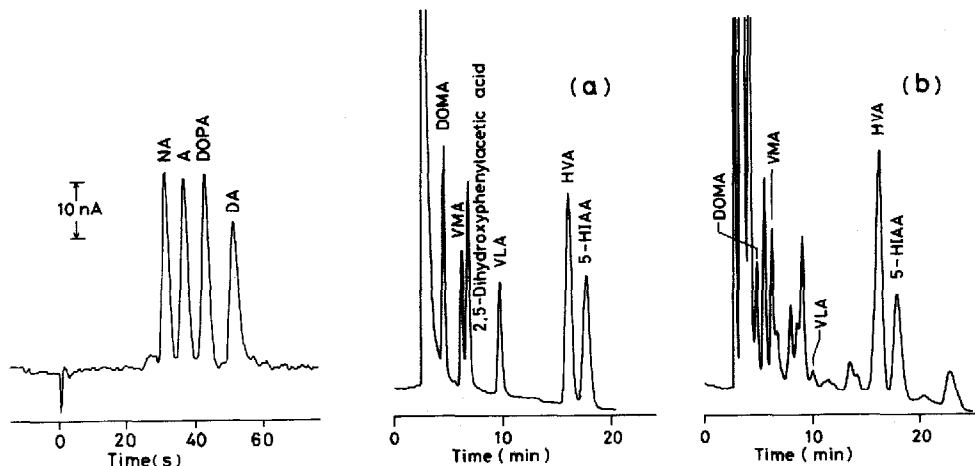Some kinds of inorganic anions and metal cations are electrochemically active,

Fig. 6. Rapid separation of catecholamines. Sample amount, 0.5 pmol each. For conditions, see text.

Fig. 7. (a) Chromatogram of catecholamine metabolites. Sample amount, 20 ng. (b) 10-μl human urine sample without pre-treatment. For conditions, see Experimental.
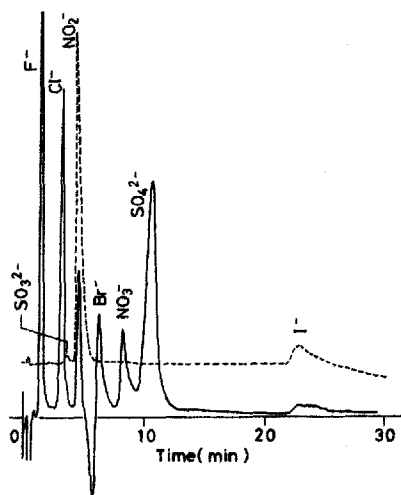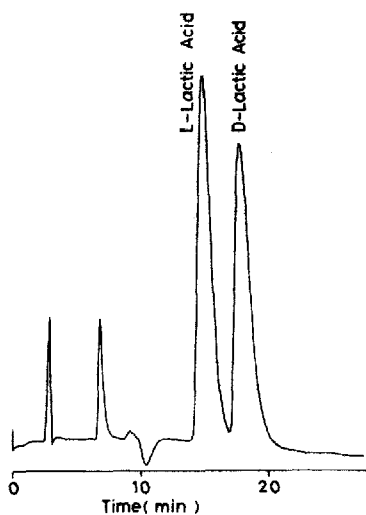
Fig. 8. Separation of lactic acid enantiomers. For conditions, see Experimental; potential, +1.1 V.

Fig. 9. Chromatogram of inorganic anions. solid line: indirect photometry at 270 nm; broken line, coulometry at +0.8 V. For conditions, see Experimental.

and an electrochemical detector is useful for such compounds owing to its unique selectivity and high sensitivity. The solid line in Fig. 9 shows inorganic anions detected by indirect photometry[11]. Each compound in the sample had a concentration of 10 ppm. The broken line shows the chromatogram of the same sample detected with the coulometric detector at a potential of +0.8 V. Of the anions F^-, Cl^-, Br^-, I^-, NO_2^-, NO_3^-, SO_3^{2-} and SO_4^{2-}, three species, SO_3^{2-}, NO_2^- and I^-, were detectable. The signal intensity of SO_3^{2-} was very low compared with the initially added sample concentration. It is expected that the SO_3^{2-} ion is readily oxidized in aqueous solution. For the SO_3^{2-} sample solution, the SO_4^{2-} signal was also observed by indirect photometry.

Two results are of interest: (1) NO_2^- was detected by our coulometric detector, whereas the detection of NO_2^- in an electrochemical detector with a silver working electrode was not reported[12]; (2) on the other hand, Br^- was not detected by the carbon fibre working electrode but it is detectable by a silver working electrode[12]. This may be explained as follows. The process $Ag + Br^- \rightarrow AgBr + e^-$ is a primary process in the detection of Br^- with a silver electrode system, whereas the corresponding process does not occur on the surface of the carbon fibre electrode.

CONCLUSION

It is easy to miniaturize a coulometric detector flow cell by use of an ion-exchange tube as a diaphragm in such a flow cell structure as described above. In this work, we constructed a flow cell with an effective cell volume of less than 1 μl. It is expected that a coulometric flow cell with a smaller volume and a lower background current could be constructed by use of a thinner ion-exchange tube, and that this flow cell will be applicable to micro-HPLC.

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