Track-etched membrane-based dual-electrode coulometric detector for microbore/capillary high-performance liquid chromatography

Hitoshi Mizuguchi[a,*], Daichi Nishimori[a], Tomohiko Kuwabara[a], Masaki Takeuchi[b], Masamitsu Iiyama[c], and Toshio Takayanagi[a]

[a]Graduate School of Science and Technology, Tokushima University, Tokushima 770-8506, Japan

[b]Institute of Biom

edical Sciences, Tokushima University, Tokushima 770-8505, Japan

[c]Nomura Micro Science Co., Ltd., 2-4-37, Okada, Atsugi, Kanagawa 243-0021, Japan

* Corresponding author. *E-mail address: mizu@tokushima-u.ac.jp*

Abstract

The electrochemical flow cell containing track-etched microporous membrane electrodes was applied to a dual-electrode coulometric detector for microbore/capillary HPLC with a small injection volume and low eluent flow rate. The proposed flow cell with a 0.1-mm diameter inlet channel gave a detection volume of 0.08 nL per electrode, which was determined by the eluent flow through the electrode. For the dual-electrode detector, the calculated volume was 0.24 nL. The efficiency of electrooxidation of L-ascorbic acid increased as the flow rate decreased and was close to 100% when the flow rate was below 50 μL min^{-1}, which is a common flow rate in microbore or capillary liquid chromatography. Catecholamines, such as noradrenaline, adrenaline, and dopamine, were detected by total conversion with two-electron oxidation in the potential range from 0.8 to 1.0 V vs. Ag/AgCl after separation with a microbore column. These peaks were accompanied by corresponding cathodic peaks derived from quasi-stable electrooxidation products of the catecholamines. The detection limits of noradrenaline, adrenaline, and dopamine were 0.1, 0.1, and 0.2 μM, respectively. The RSD values for five replicate measurements of 5.0 μM of these compounds were 0.9%, 0.7%, and 1.5%, respectively. Coulometric detection was also demonstrated by determination of catecholamines in pharmaceuticals.

Keywords: Track-etched membrane filter; Coulometric detection; Microbore/capillary liquid

chromatography; Electrochemical detector; Dual-electrode system

1. Introduction

High-performance liquid chromatography (HPLC) coupled with an amperometric or coulometric detector is widely used for the determination of trace substances, especially biologically active species, owing to the advantages of a generally higher sensitivity than UV or fluorimetric detection and a selectivity that is based on oxidation or reduction of certain substances [1–5]. Since an amperometric detector for modern HPLC was proposed by Kissinger and co-workers in the early 1970s [6], the continuous improvement of electrochemical detectors has offered superior analytical performances and wider applications. In the amperometric detection mode, a thin-layer flow cell [6–8] or wall-jet type flow cell [9–11] is commonly used. In these cases, the detector volume, which includes any dead volume in the eluent stream, should be as low as possible to avoid undue broadening of the chromatographic peaks. The conversion efficiency during passage of the analyte through the detector increases as the thickness of the solution layer decreases; however, a small percentage (generally 5%–10%) of the analyte is electrolyzed at the working electrode. Coulometric flow cells containing electrodes with surface areas much greater than those of the amperometric detectors have been used for total conversion (nearly 100%) of the analyte [12–14]. The current responses increase as the efficiency of electrolysis improves, but the concomitant background current and noise tend to increase with a larger surface area. The detector volumes of coulometric flow cells are generally much larger than

those of amperometric flow cells.

Electrochemical detection has also been used for microbore or capillary HPLC because of its applicability to small amounts of samples [15–17], and capillary HPLC integrated with a microdialysis sampling system has also recently been reported [18–20]. In the case of microbore or capillary HPLC, a detector with a lower volume than that for analytical-scale HPLC is needed because of the small injection volume and low eluent flow rate, and miniaturized amperometric detectors have been used [15–17, 21]. The detector volume of the wall-jet type flow cell can be adjusted using a thin spacer that defines the flow channel thickness; however, the minimum volume is limited by the thickness that can be produced by the spacer material [22]. There have been very few reports on coulometric flow cells for miniaturized HPLC systems, and glassy carbon powder packed in a stainless steel tube is limited to use as a post column reactor [23].

Dual-electrode detectors have also been used with HPLC for a long time [1–5]. A dual-electrode detector consists of two working electrodes with separately controlled potentials in an eluent stream. Many papers have described different types of dual-electrode systems, such as two planar electrodes embedded in series [24–27], parallel [27,28], and opposite configurations [27,29,30]. A wall-jet type ring-disk electrode [31,32], an interdigitated microarray dual-electrode [33–35], and a split-disk dual-electrode [36] have also been reported as HPLC detectors. These dual-electrode detectors provide different analytical performances, such as selectivity

improvement by multiple potential detection [24–26,28,29], elimination of interfering species prior to the detection of analytes [31–33], peak identification in complex samples [27], and current amplification by redox cycling [29,30,34–36]. In the coulometric detection mode, flow-cell units containing porous carbon electrodes are most commonly used in multiple detection systems [1–5,14,37]. Placing the coulometric flow-cell units in series provides effective detection modes, such as screen, difference, and redox modes, to separate the peak of the analyte from complex matrices or to stably detect the analytes. However, conventional dual-electrode systems containing porous carbon are not suitable for microbore or capillary HPLC because the detector volume is much larger than the injected sample volume. As far as we know, there have been no reports on the effective combination of microbore or capillary liquid chromatography and a dual-electrode system containing coulometric detection units placed in series. Coulometric detectors are generally prone to encounter difficulties in handling and maintenance owing to poisoning of the working electrodes. Particularly, mechanical activation, such as polishing, is not applicable to porous electrodes that often contain expensive materials. A larger surface area for lifetime improvement is also not a viable solution because of the increase in background current. In this context, the development of inexpensive as well as easily replaceable electrodes is a practical solution for this problem [38,39].

The authors have previously proposed an electrochemical flow cell on which track-etched

microporous membrane electrodes are mounted [40–44]. The electrode was prepared by making a coating of platinum or gold on a track-etched microporous membrane filter, which possessed a smooth flat surface and cylindrical pores with uniform diameters produced by first bombarding membrane sheets with high energy particles to create tracks and then chemical etching [45]. Electrolysis with high conversion efficiency (close to 100%) is possible while passing the sample solution through the electrodes because of restriction of diffusion layer growth at the entrances of the pores. The high conversion efficiency resulted in the detection of ultra-trace mercury by anodic stripping voltammetry in a shorter deposition time with a smaller amount of sample solution under the flow condition [41]. Furthermore, various multiple electrode systems can also be constructed simply by stacking electrodes and template membrane filters that had a thickness of 10 μm. The configuration of a series of dual-electrode detectors produced a simultaneously high conversion efficiency (close to 100%) as well as a high collection efficiency (close to 100%) [40]. The collection efficiency was defined as the ratio of the peak currents observed at the first and the second electrodes, that is, the magnitude of the fraction of the first electrolysis product that was detected at the second electrode. Enzyme-based flow biosensors that can detect analytes while removing interferents have also been demonstrated using these electrode systems [42,43]. The design of these electrode systems and their applications have been outlined in a short review [44]. More recently, an improved design and low-cost method of fabrication of the electrode

system using track-etched membranes were proposed by Hauke et al [46]. By considering this trend, the detector system using track-etched microporous membrane electrodes is expected to become a reasonable choice among the various electrochemical flow cells because of its inexpensive fabrication and easily replaceable electrodes.

The aim of the current study was to apply an electrochemical flow cell containing track-etched microporous membrane electrodes to a dual-electrode coulometric detector for microbore or capillary HPLC using a small injection volume and low eluent flow rate. In our previous study, a flow cell with a 2.0-mm diameter inlet channel was used. The diameter of the inlet channel was reduced to 0.1 mm in the present study to meet the requirements for use as an electrochemical detector for microbore or capillary HPLC. The proposed design has a much lower detector volume than conventional designs because the radial cross section of the eluent flowing through the electrode system is dependent on the shape of the inlet channel of the flow cell at the front surface of the first electrode. A new detector design for electrochemical detection coupled with microbore HPLC and its analytical performance are described.

2. Experimental section

2.1. Reagents

Standard solutions of adrenaline, noradrenaline, dopamine, and L-ascorbic acid were prepared

daily by dissolving L-adrenaline bitartrate (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan), L-noradrenaline bitartrate (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan), dopamine hydrochloride (Nacalai Tesque, Inc., Kyoto, Japan), and L-ascorbic acid (Kanto Chemical Co., Inc., Tokyo, Japan), respectively, in water. Sodium 1-octanesulfonate was purchased from Tokyo Chemical Industry Co., Ltd., Tokyo, Japan. All other reagents were guaranteed reagent grade and were used without further purification. All water used was 18 MΩ cm deionized water, which was obtained with a water purification system (Milli-Q® Gradient A10, Millipore).

2.2 Electrochemical detector

The track-etched microporous membrane electrode was prepared by sputter deposition of platinum onto both sides of a Nuclepore™ track-etched membrane filter (Whatman, pore size 0.40 μm, porosity 13%, thickness 10 μm), which was used as a template for the electrode. For platinum coating, an ion sputter coater (Hitachi model E-1030) was used and the distance between the target plate and the membrane filter was 35 mm. The coating time and current were 200 s and 30 mA, respectively. The electrode was cut into a rectangular shape with a width of 2 mm for the working electrode or a width of 6 mm for the counter electrode. The electrodes were sequentially arranged as working and counter electrodes along the direction of eluent flow by alternately stacking the electrodes and unmodified track-etched membrane filters (pore size 5.0 μm, thickness

10 μm) (Fig. 1). These electrodes were sandwiched between the top and main body of the flow cell, which contained a polytetrafluoroethylene (PTFE) line-filter (LRF-1; GL Sciences Inc., Tokyo, Japan) to prevent distortion of the electrodes. Although the structure of the flow cell was based on that of our previous report [40], the current flow-cell design featured an inlet channel in the top body with an inner diameter of 0.1 mm and length of 1.0 mm. A silver–silver chloride reference electrode (Model RE-3V; ALS Co., Ltd., Tokyo, Japan) was placed 10 mm downstream of the counter electrode. All the electrodes were connected to a multi-channel potentiostat (Model HA1010mM4A; Hokuto Denko Co., Tokyo, Japan) equipped with a function generator (Model HB-111A; Hokuto Denko Co.) and Chromato-PRO data processor (RunTime Co., Hachioji, Japan).

2.3. Chromatography systems

In this study, analytical-scale HPLC and microbore HPLC were employed for evaluation of the electrochemical detector. Analytical-scale HPLC was performed using a reversed-phase column Merck LiChrospher® 100 RP-18 LiChroCART® (4 mm I.D. × 125 mm, particle size 5 μm) with 0.2% (w/v) phosphoric acid solution as the mobile phase. For microbore HPLC, an internal sample injector (0.2 μL injection volume) (Model C4-1004-.02, Valco Instruments Co., Inc.) was connected between a HPLC pump (Model LC-20AD; Shimadzu Corp., Kyoto, Japan)

and a MonoCap C18 WideBore column (0.5 mm I.D. × 50 mm) (GL Sciences Inc., Tokyo, Japan). The mobile phase was 0.2% (w/v) phosphoric acid solution. For analysis of catecholamines, a column of Capillary EX InertSustain C18 (0.7 mm I.D. × 50 mm, particle size 3 μm) (GL Sciences Inc., Tokyo, Japan) was used with a mobile phase of 3% acetonitrile (HPLC grade, Kanto Chemical Co., Inc., Tokyo, Japan) and 97% aqueous solution containing 0.45 M acetic acid-acetate buffer (pH 4.1), 0.15 mM disodium salt of EDTA, and 1.75 mM sodium 1-octansulfonate. Dissolved oxygen in the eluent was removed by bubbling with argon gas for 30 min followed by ultrasonic degassing. All analyses were performed after activation of the working electrodes by repetitive potential cycling from 0 to +1.0 V *vs*. Ag/AgCl in the carrier electrolyte.

3. Results and discussions

3.1. Evaluation of the detector performance

In this study, the electrochemical performance of the proposed detector was evaluated using L-ascorbic acid. The chromatographic responses against the injection of L-ascorbic acid solutions are depicted in Fig. 2. The linear relationships against the concentration of L-ascorbic acid were also obtained in both the peak height and the charge, which was converted from the peak area (Fig. S1). Peak height tended to be higher for a higher flow rate, whereas the charge became larger for a lower flow rate. Figure 3 shows the relationship between the volumetric flow rate and

conversion efficiency. The open symbols are for data obtained from analytical-scale HPLC, and the closed symbols are from microbore HPLC. The efficiency increased with decreases in the flow rate, and was close to 100% at a flow rate lower than 0.05 mL min^{-1} (Fig. 3 (A)). According to the theoretical consideration under a mass-transfer-controlled, limiting-current condition with a flow-through porous electrode [47], the relationship between volumetric flow rate (v) and conversion efficiency (R) is expressed by

$$R = 1 - \exp(-m_o sAL/v)$$

where m_o, s, A, and L are the mass-transfer coefficient, specific area, cross-sectional area, and length of the porous electrode, respectively. The specific area, s, is defined by $s = a/LA$, where a is total internal area of the electrode. These parameters were constant, except for v, under the experimental conditions of this study. The solid lines in Fig. 3 were calculated using the data obtained from analytical-scale HPLC. The result showed that an efficiency approaching 100% at a flow rate lower than 0.05 mL min^{-1} is theoretically acceptable.

In this study, template filters with different pore sizes and porosities were also tested for comparison because the conversion efficiency was expected to be affected by the pore size and porosity of the electrode. In our previous study [40], the conversion efficiency increased with decreases in the pore size of the track-etched membrane filter used as the template. This phenomenon can be explained by the restriction of diffusion layer growth as smaller pore sizes

were employed. In the case of a pore size of 0.10 μm (Fig. 3 (B)), however, the efficiency was substantially lower than that of the electrode with a pore size of 0.40 μm (Fig. 3 (A)). The porosity of the template filter with a pore size of 0.10 μm was 2.4%, and was much lower than that of the filter with a pore size of 0.40 μm (porosity 13%). This lower efficiency was seemingly caused by a shorter residence time in the electrode pores because of the lower porosity. It is expected that higher porosity would improve the efficiency. However, this requires caution because a higher porosity tends to result in many merged pores, which will behave as pores with larger sizes. The efficiencies with the higher porosity electrodes were not so high even with the gradual increase in the low flow rate (Fig. 3 (C) and (D)). In subsequent experiments, electrodes prepared using the template membrane filter with a pore size of 0.40 μm were used.

3.2. Chromatographic separation and coulometric detection of catecholamine

The chromatogram obtained in this study is shown in Fig. 4. Catecholamines can be separated by reversed-phase HPLC. According to the literature [48–50], the retention and resolution of catecholamines are affected and improved by the surfactant chain length of the counter anion (see also Table S1) and the content of acetonitrile as an organic modifier in the mobile phase. The chromatography conditions used in this work provided sufficient separation. Noradrenaline, adrenaline, and dopamine were detected by both anodic and corresponding cathodic peaks, which

reflected electrooxidation of these catecholamines and reduction of the products generated on the first electrode. Electrooxidation of these catecholamines is accompanied by side reactions such as cyclization followed by additional redox reactions of byproducts [51–54]. The rate of cyclization is dependent on pH, and the cyclization of adrenaline is somewhat faster than that of noradrenaline or dopamine [51]. In fact, a cyclic voltammogram of adrenaline in the same solution as the eluent contained some redox peaks derived from side reactions [51]. A quasi-reversible system was seen for noradrenaline and dopamine. Despite these difference in the voltammetric behaviors, similar responses from these catecholamines were observed in the proposed detector system. In the case of L-ascorbic acid, only an anodic peak at the first working electrode (WE1) was obtained under this condition. This result suggests that the electrooxidation product has vanished before reaching the second working electrode (WE2) because L-ascorbic acid produces electrochemically inactive species by a fast hydrolysis reaction [55,56]. Therefore, an analyte that produces a relatively stable species can be detected selectively at WE2 when removing interferents such as L-ascorbic acid.

It should be noted that additional peak broadening was not observed in the chromatogram obtained at WE2 compared with that obtained at WE1. This indicated that the radial dispersion of catecholamines while flowing through the proposed electrode system is small enough to ignore. The radial cross section of the eluent flowing through the electrode system is dependent on the

shape of the inlet channel of the flow cell at the front surface of WE1. Actually, the background current at WE1 was much lower than when using the top body of the flow cell with an inlet channel diameter of 1.0 mm (Fig. S2). Assuming that radial dispersion of the eluent never occurred in the proposed electrode system, the detection volume, which is defined by the eluent flow through the electrode, was geometrically calculated to be 0.08 nL per electrode (Fig. 1g or 1h). Although there is a relatively insulating space because of the absence of a platinum layer in the middle of each pore [40], the volume in this calculation includes such a space. For the dual-electrode system, the total detection volume was 0.24 nL (g, k1, and h in Fig. 1). This value was smaller than the inlet channel volume (Fig. 1f), which was estimated to be 7.8 nL. The estimated volumes were much smaller than that of other electrochemical detectors in the literature [15–17,20,21], especially coulometric flow cells. For conventional coulometric flow cells with large inner volumes [14,37], peak broadening in the chromatogram obtained with the second electrode would be inevitable with a small injection volume and low eluent flow rate. Therefore the proposed flow cell is suitable for coulometric detection by microbore or capillary HPLC, which is carried out with a small injection volume and low flow rate.

The effects of applied potential of WE1 on the conversion and collection efficiencies are shown in Fig. 5. The conversion efficiency was calculated using the peak area obtained at WE1 based on two-electron oxidation of the catecholamines. The collection efficiency was obtained as

the ratio of the peak areas observed at WE1 and WE2. The conversion efficiencies of the catecholamines were close to 100% in the potential range from 0.8 to 1.0 V *vs*. Ag/AgCl, whereas the charge derived from the anodic oxidation increased significantly up to 200% at a potential above 1.2 V *vs*. Ag/AgCl. Conversely, the maximum collection efficiency was observed at around 1.0 V *vs*. Ag/AgCl and gradually decreased at a higher potential. The reason for the excessive increase in the charge on WE1 at higher potential still needs to be investigated. The large change in collection efficiency indicates different reactions occur than under the lower potential condition. In the subsequent experiments, the potential of WE1 was adjusted to 0.8 V *vs*. Ag/AgCl to demonstrate the coulometric detection of catecholamines.

3.3. Calibration

The relationship between the peak area and concentration of catecholamine is shown in Fig. 6. All three calibration curves of adrenaline, noradrenaline, and dopamine were straight lines through the origin, and exhibited the same slope. Furthermore, these slopes coincided with the theoretical value, which was calculated from the total charge based on two-electron oxidation of these compounds. This result showed that calibration-free coulometric detection was achieved under this condition. The detection limits of noradrenaline, adrenaline, and dopamine were 0.1, 0.1, and 0.2 μM, respectively. The RSD values for five replicate measurements of 5.0 μM of these

compounds were 0.9%, 0.7%, and 1.5%, respectively. In this study, amperometric detection was also demonstrated using the linear calibration plots obtained from the peak heights. The slopes of the calibration formulae that were prepared for noradrenaline, adrenaline, and dopamine were 9.0, 7.1, and 4.1 nA μM^{-1}, respectively. Simple amperometric detection with higher sensitivity could be also carried out at a higher flow rate within the limit of internal pressure.

3.4. Application to pharmaceuticals

To study the accuracy of the proposed electrochemical detector, determination and recovery tests for the catecholamines were performed using parenteral injection solutions of noradrenaline, adrenaline, and dopamine. These solutions were diluted 1000 or 20,000 times with water prior to the analyses. The analytical results are summarized in Tables 1–3. The obtained results were in agreement with manufacturer's stated contents and adequate recoveries were obtained in both coulometric and amperometric detection. The results indicated the suitability of the track-etched microporous membrane electrodes for the detection of catecholamines.

4. Conclusion

In this study, a dual-electrode coulometric detector for microbore or capillary HPLC was constructed using track-etched microporous membrane electrodes, which enabled efficient

electrolysis. The current flow cell design featured a small inlet channel (0.1 mm in diameter), which was modified from the previously reported one [40]. This structure provided an estimated detector volume of 7.8 nL for the inlet channel volume and 0.08 nL per electrode for the detection volume, which was defined by the eluent flow through the electrode. For the dual-electrode, the calculated volume was 0.24 nL. These volumes are much smaller than those of conventional electrochemical detectors, especially coulometric flow cells. The conversion efficiency of L-ascorbic acid was close to 100% at a flow rate lower than 0.05 mL min^{-1}. Chromatograms including anodic peaks derived from total conversion of catecholamines and the corresponding cathodic peaks were detected simultaneously by the proposed dual-electrode system after separation with a microbore column. Calibration-free coulometric detection of catecholamines was also demonstrated. We believe this proposed flow cell, which can be constructed easily using inexpensive materials, will become a powerful tool for electrochemical detection with a small injection volume and low eluent flow rate.

ORCID

Hitoshi Mizuguchi: 0000-0003-2396-6812

Masaki Takeuchi: 0000-0001-6193-0074

Toshio Takayanagi: 0000-0002-5767-1126

Conflict of interest

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Supplementary data

Supplementary data to this article can be found online at https:// .

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Figures

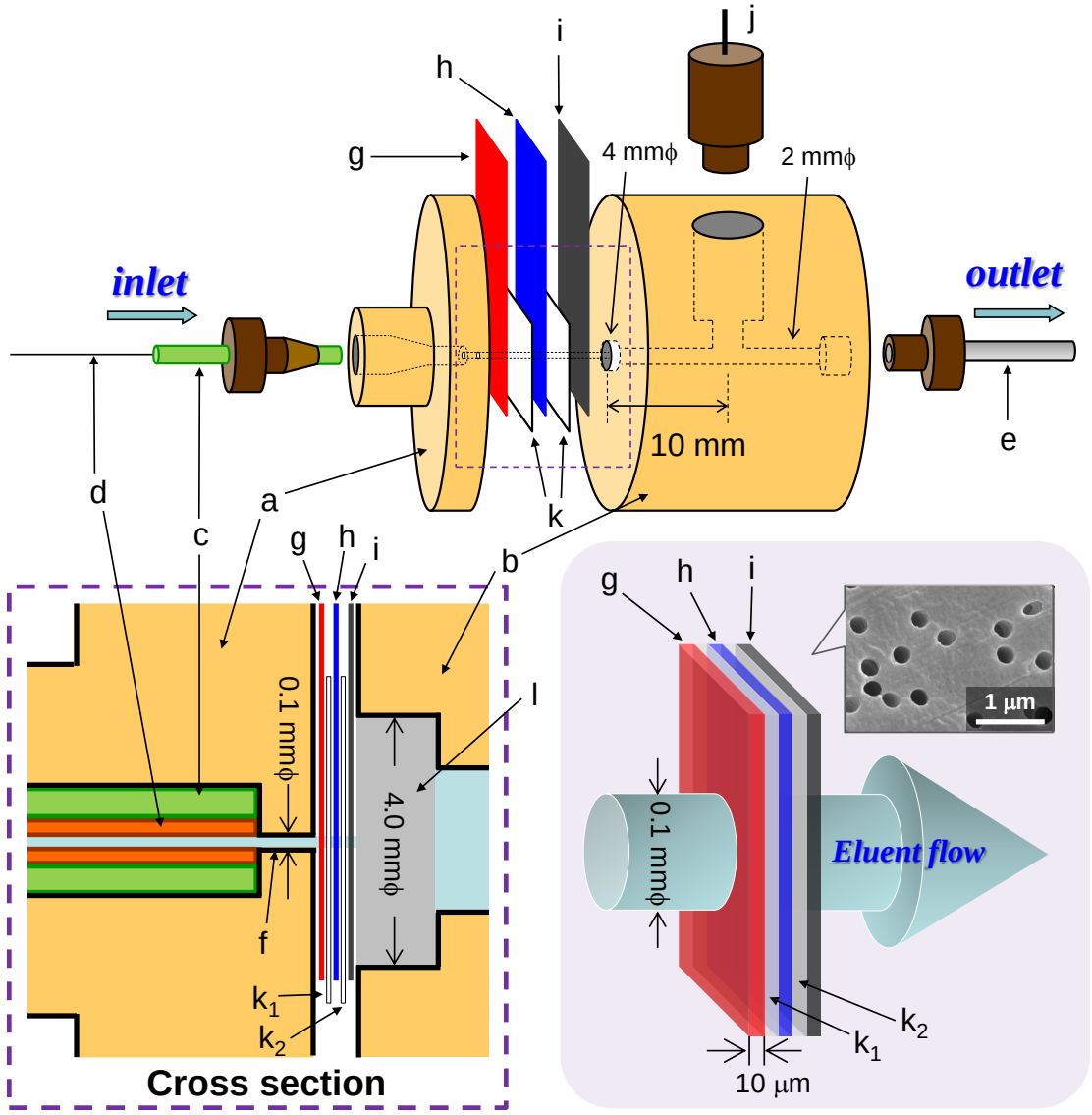

Fig. 1. Schematic representation of the flow cell constructed in this study. Top body of the flow cell (a), main body (b), tubing sleeve (c), capillary HPLC tube (d), PFA tube (e), inlet channel (f), the first working electrode (WE1) (g), the second working electrode (WE2) (h), counter electrode (i), reference electrode (j), spacer (k_1, k_2), PTFE line-filter (l).

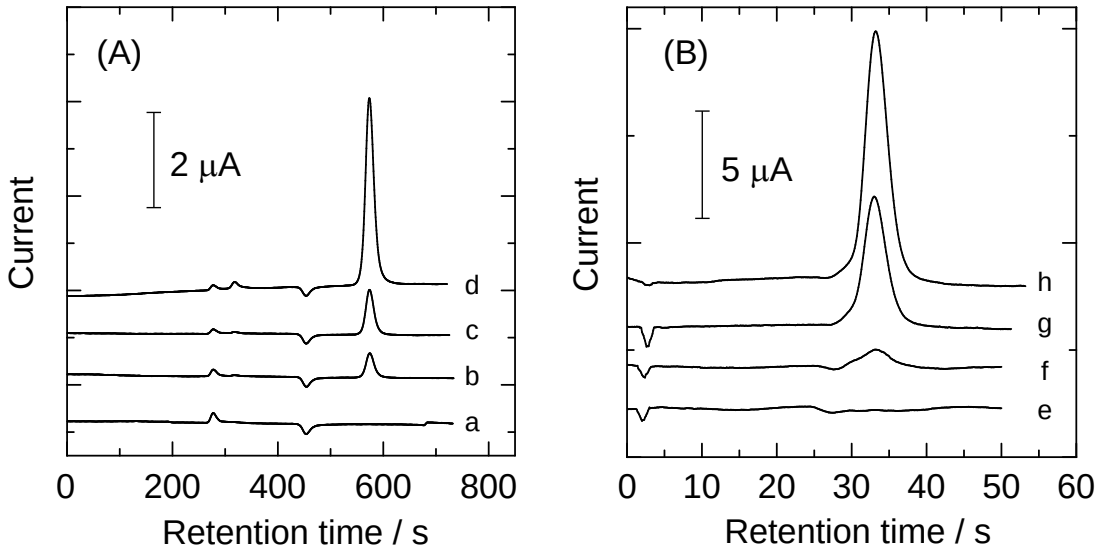

Fig. 2. Chromatographic responses of L-ascorbic acid obtained by analytical-scale HPLC at the flow rate of 0.2 mL min^{-1} (A) and microbore HPLC at 0.03 mL min^{-1} (B). The detection potential was 0.6 V *vs*. Ag/AgCl. The concentration of L-ascorbic acid were 0 (a, e), 0.0050 (b), 0.010 (c), 0.050 (d), 0.10 (f), 0.50 (g), and 1.0 mM (h).

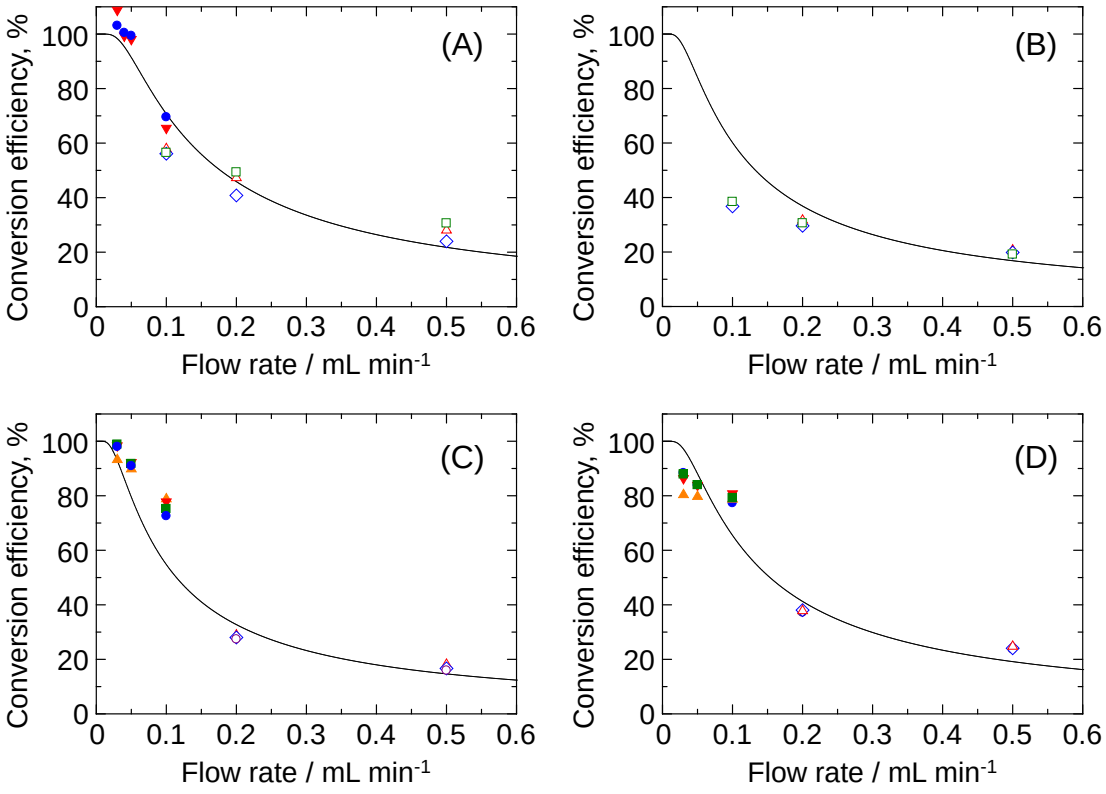

Fig. 3. Relationship between flow rate and conversion efficiency. The pore sizes and porosities of the template filters used were 0.40 μm and 13% (A), 0.10 μm and 2.4% (B), 0.20 μm and 16% (C), and 0.40 μm and 19% (D). Concentration of L-ascorbic acid were 0.0050 (open triangle), 0.010 (open rhombus), 0.015 (open circle), 0.050 (open square), 0.25 (closed triangle), 0.50 (inverted closed triangle), 0.75 (closed square), and 1.0 mM (closed circle). The solid line shows a theoretical curve calculated using the data of analytical-scale HPLC. The plots of opened symbols were data obtained from analytical-scale HPLC, and closed ones were microbore HPLC. The detection potential was +0.6 V *vs*. Ag/AgCl. The filters used for (C) and (D) were obtained from it4ip SA (Belgium).

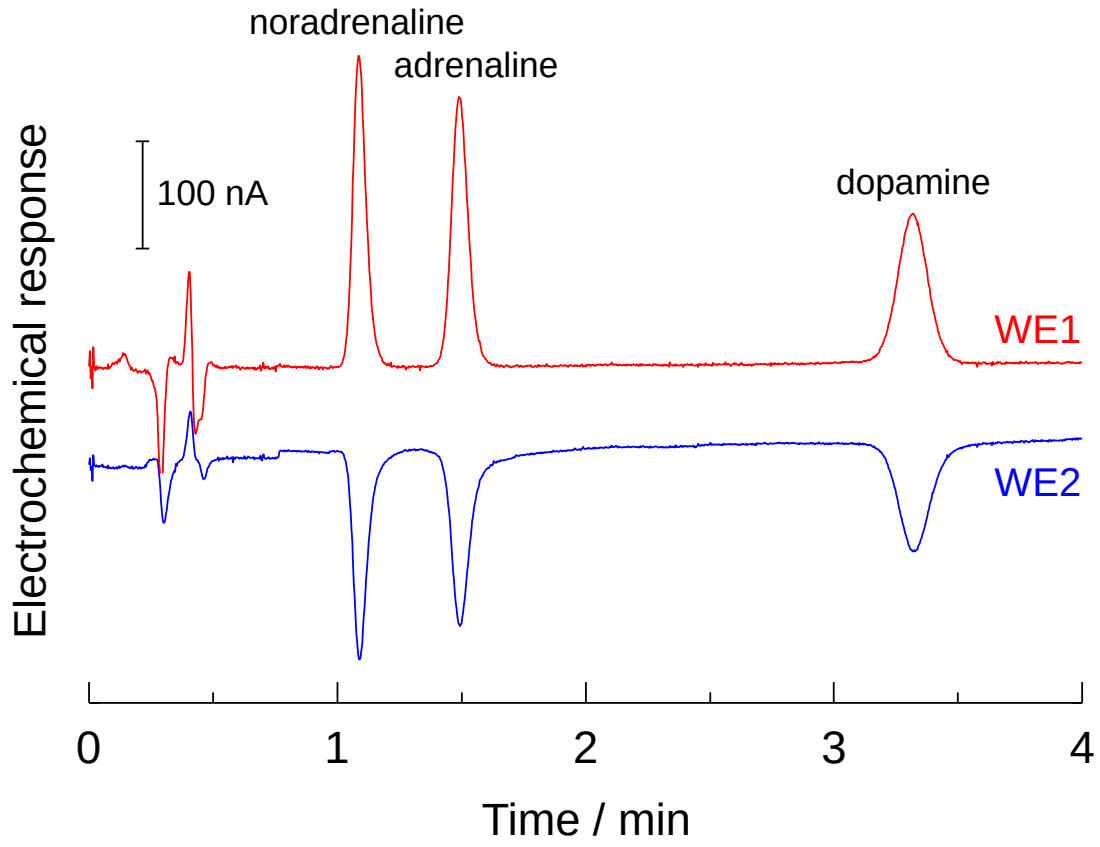

Fig. 4. Chromatograms obtained using the proposed dual-electrode coulometric detector. The electrodes, WE1 and WE2, were polarized at +0.8 and +0.2 V *vs*. Ag/AgCl, respectively. The concentration of catecholamine was 50 μM.

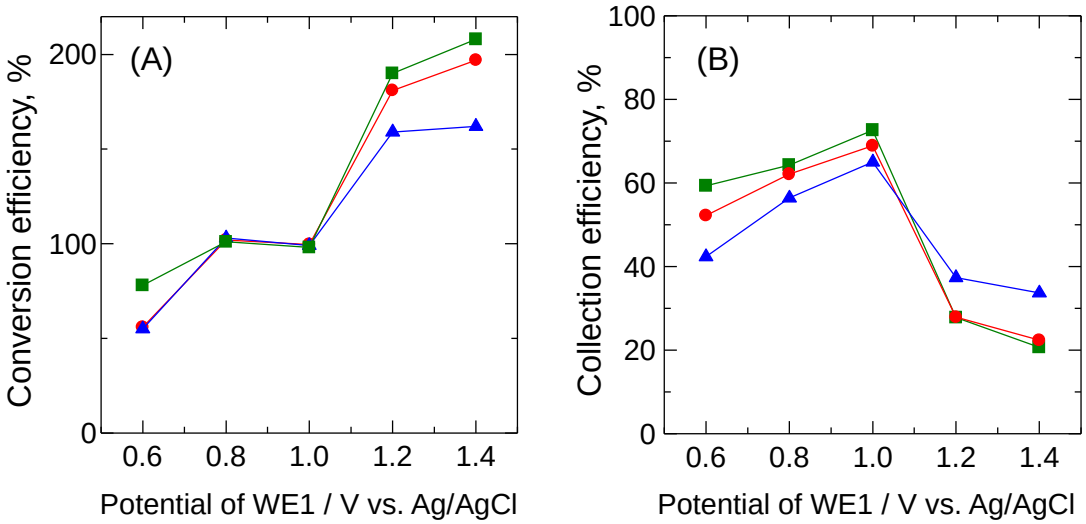

Fig. 5. Influence of the potential of WE1 on the conversion efficiency (A) and collection efficiency (B) of noradrenaline (closed circle), adrenaline (closed triangle), and dopamine (closed square). The potential of WE2 was held at +0.2 V *vs.* Ag/AgCl. The concentration of catecholamine was 50 μM.

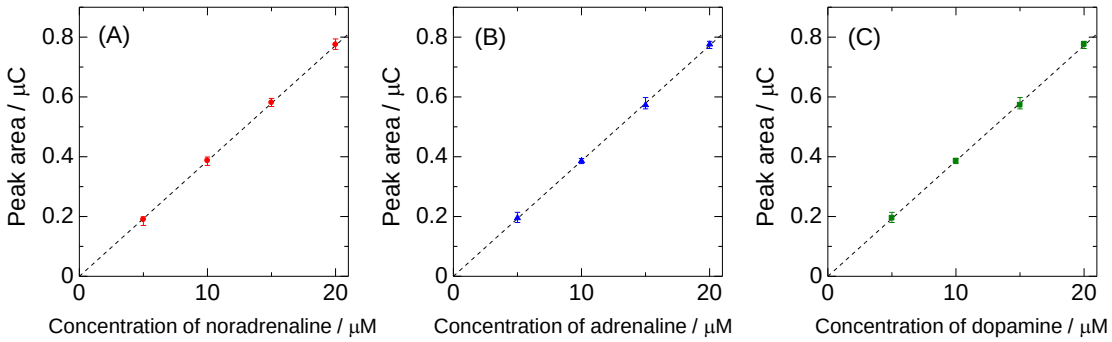

Fig. 6. Calibration plots of noradrenaline (A), adrenaline (B), and dopamine (C) in the coulometric detection. The dashed line was the theoretical value calculated using the total charge based on two-electron oxidation.

Table 1 Recovery tests of noradrenaline in parenteral injection solution

Sample	Added	Coulometric detection		Amperometric detection	
		Found[a] /mg mL^{-1}	Recovery, %	Found[a] /mg mL^{-1}	Recovery, %
Injection 1	0	0.98±0.09	–	1.02±0.01	–
	0.50	1.45±0.14	95	1.52±0.07	99.8
	1.00	1.98±0.12	100	2.06±0.05	105

The original sample solution contains 1 mg/mL noradrenaline, 0.3 mg/mL sodium hydrogen sulfite, 5 mg/mL chlorobuthanol, tonicity adjusting agents, and pH buffer agent. The sample solution was diluted with water 1000 times prior to the analysis.

[a] Average value of five replicate analysis.

Table 2 Recovery tests of adrenaline in parenteral injection solution

Sample	Added	Coulometric detection		Amperometric detection	
		Found[a] /mg mL^{-1}	Recovery, %	Found[a] /mg mL^{-1}	Recovery, %
Injection 2	0	1.03±0.10	–	1.14±0.05	–
	0.50	1.51±0.08	97	1.64±0.04	99.6
	1.00	1.92±0.10	89	2.11±0.07	96.9

The original sample solution contains 1 mg/mL adrenaline, 0.5 mg/mL sodium hydrogen sulfite, 3 mg/mL chlorobuthanol, sodium chloride, and hydrochloric acid. The sample solution was diluted with water 1000 times prior to the analysis.

[a] Average value of five replicate analysis.

Table 3 Recovery tests of dopamine in parenteral injection solutions

Sample	Added	Coulometric detection		Amperometric detection	
		Found[a] /mg mL^{-1}	Recovery, %	Found[a] /mg mL^{-1}	Recovery, %
Injection 3[b]	0	0.97±0.14	–	1.01±0.12	–
	0.50	1.46±0.10	99	1.49±0.05	97.2
	1.00	1.95±0.07	99	1.95±0.06	94.6
Injection 4[c]	0	19.5±1.2	–	18.0±1.9	–
	10.0	29.2±1.2	96	28.0±1.9	99.7
	20.0	39.4±1.5	100	39.8±2.1	109

[a] Average value of five replicate analysis.

[b] The original solution contains 1 mg/mL dopamine hydrochloride, 0.3 mg/mL sodium hydrogen sulfite, 50 mg/mL glucose, and pH buffer agent. The sample solution was diluted with water 1000 times prior to the analysis.

[c] The original solution contains 20 mg/mL dopamine hydrochloride and 0.5 mg/mL sodium hydrogen sulfite. The sample solution was diluted with water 20,000 times prior to the analysis.