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Journal of Chromatography A, 891 (2000) 149–156

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Miniaturized thin-layer radial flow cell with interdigitated ring-shaped microarray electrode used as amperometric detector for capillary electrophoresis

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Received 15 February 2000; received in revised form 30 May 2000; accepted 31 May 2000

Abstract

A chip-type thin-layer radial flow cell was developed as an amperometric detector for capillary electrophoresis. We fabricated a carbon film-based interdigitated ring-shaped array (IDRA) microelectrode with a 2 μm bandwidth and an almost 1 μm gap on a glass plate and used it as a working electrode. A fused-silica capillary was arranged above the IDRA electrode using a guide hole drilled through the acryl plate that formed the flow cell lid. A flow channel for use in connecting the outlet capillary was also fabricated in the acryl plate. We characterized the analytical performance of the IDRA electrode in the microchip flow cell in terms of linear concentration range, sensitivity and concentration detection limit. We achieved a collection efficiency and catechol redox cycle at the IDRA microelectrode of 65% and 1.71, respectively, and thus a high sensitivity and low detection limit of 392.9 pA/μ*M* and 15 n*M* for dopamine hydrochloride. We examined the reproducibility of the detector and found that the run-to-run and detector-to-detector relative standard deviations were both less than 10%.

Keywords: Instrumentation; Electrochemical detection; Detection, electrophoresis; Electrodes; Flow cell; Catecholamines

1. Introduction

Capillary electrophoresis (CE) is a highly efficient separation tool in which the migration of analytes is under the influence of an extremely high potential field across the separation capillary. Although capillaries with small inner diameters have the advantage of small volume requirements when analyzing limited volume samples, they are also needed for highly sensitive detection systems. Among the detection systems adapted for CE, electrochemical detection (ED) has been demonstrated to be an attractive choice since it is extremely sensitive and exhibits tunable selectivity. In addition, it is easy to make electrodes with the right dimensions that fit the inner diameter of the CE capillary without affecting the detection limit. Since the CE–ED technique was first developed by Wallingford and Ewing [1], it has been used for the detection of a wide variety of compounds in the pharmaceutical, biochemical and environmental fields. Several different CE–ED systems have been proposed [2–12], and this area has recently been reviewed [13–17].

Microarray electrodes have been widely used as electrochemical detectors to improve the sensitivity,

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0021-9673/00/$ – see front matter
PII: S0021-9673(00)00632-4

temporal resolution and selectivity in CE–ED measurements [18–23]. To improve temporal resolution, Gavin and Ewing [19,20] characterized the use of an array of 100 independent platinum microelectrodes for continuous electrophoretic separation in a rectangular channel, which they applied to the continuous monitoring of single cells. Microarray electrodes are also useful for improving sensitivity because of their high mass-transfer flux limit that results from radial diffusion, a low charging current, a steady-state on short time scales and a reduced ohmic IR drop in solution [24–26].

Jin and co-workers used CE with a carbon fiber microdisk array detector to detect adenine and guanine [21], and bovine serum albumin [22]. Zhong et al. developed both ring-disk wall-jet and dual adjacent carbon fiber electrode assemblies for capillary electrophoretic detection [6]. This system is beneficial in improving selectivity.

Interdigitated array (IDA) microelectrodes possess all the advantages of microelectrodes and also a relatively high Faraday current [27,28]. With IDA electrodes, a product generated at one band electrode may collect at an adjacent band electrode and revert to its initial state when the potential of the adjacent band electrode is sufficient for a reverse reaction. Then the initial state species collects again at the first electrode. As this cycle repeats, one molecule reacts many times. This redox cycling increases the currents of both the generator and collector electrodes.

The application of an IDA microelectrode as an electrochemical detector in high-performance liquid chromatography (HPLC) was first reported by Matsue and co-workers [29,30], who used a Nafion-modified IDA for the selective detection of catecholamines in the presence of L-ascorbic acid. Takahashi et al. [31,32] have used a gold film-based IDA electrode as an electrochemical detector coupled to liquid chromatography with a microbore and a conventional column for the detection of catecholamines. Carbon electrodes are widely used in electroanalytical studies because of their low residual current over a relatively wide potential window in an aqueous solution. Niwa and Morita [33] have employed a carbon film-based interdigitated ring-shaped array (IDRA) electrode in a radial flow cell for the microbore liquid chromatographic detection of catecholamines. They obtained a 3–4-fold amplification factor for epinephrine and dopamine, by multiply recycling the analyte between sequential generator/collector pairs at flow-rates of 0.01 ml/min.

We recently [34] employed a carbon film-based IDRA microelectrode with a 2 μm bandwidth and a gap of nearly 1 μm for CE–ED. Our aim was to enhance the detection sensitivity based on the redox cycling of electrochemically reversible species at the IDRA microelectrode. In the work, we used a simple device, based on a Model 9095 multimode fiber aligner, to complete the connection between the separation capillary and the one-chip IDRA microelectrode. Although the device can effectively realize end-column wall-jet amperometric detection after capillary electrophoretic separation, the wall-jet mode we used here is not superior to the thin-layer radial flow mode in terms of improving the analyte collection efficiency at the IDRA microelectrode. In the latter mode, the flow hits the center of the electrode and then radiates outward within a thin layer. This controlled, radial flow pattern maintains close contact between the working electrode and the analyte that remains in a thin diffusion layer over the entire electrode surface.

In the paper, we described a thin-layer radial flow amperometric detector that we used to connect the separation capillary and IDRA microelectrode chip with the aim of further improving the sensitivity and detection limit. We assembled the device with a sandwich structure consisting basically of an IDRA microelectrode chip, a gasket and an acrylic plate on which we fitted a separation capillary and a reference electrode. We characterized the detector in terms of separation performance, sensitivity and reproducibility by detecting certain catecholamines using amperometric detection in the thin-layer radial flow cell after capillary zone electrophoretic separation.

2. Experimental

2.1. Carbon film IDRA microelectrode

We fabricated the carbon film-based IDRA microelectrode by photolithography and dry etching from a carbon film of pyrolyzed 3,4,9,10-perylenetetracarboxylic dianhydride on quartz chips [35,36]. We provided a photograph and the dimensions of the

IDRA microelectrode in an earlier paper [34]. One of the IDRA microelectrodes (W_1) has a 10 μm diameter disk at its center. The IDRA finger widths are 2.5 μm and the gaps between the fingers are 1.3 μm, so we were able to arrange four pairs of IDRAs in an 80 μm diameter region. We calculated the areas of the W_1 and W_2 electrodes to be approximately 1250 and 1130 μm^2, respectively. The IDRA microelectrode area was exposed in solution and the other areas were covered with an insulating film. We connected the IDRA microelectrode chip to a double contact plug before fixing it to an electrochemical cell.

2.2. Thin-layer radial flow amperometric detector

Fig. 1A is a photograph of a thin-layer radial flow cell used as a detector. The actual size of the detector

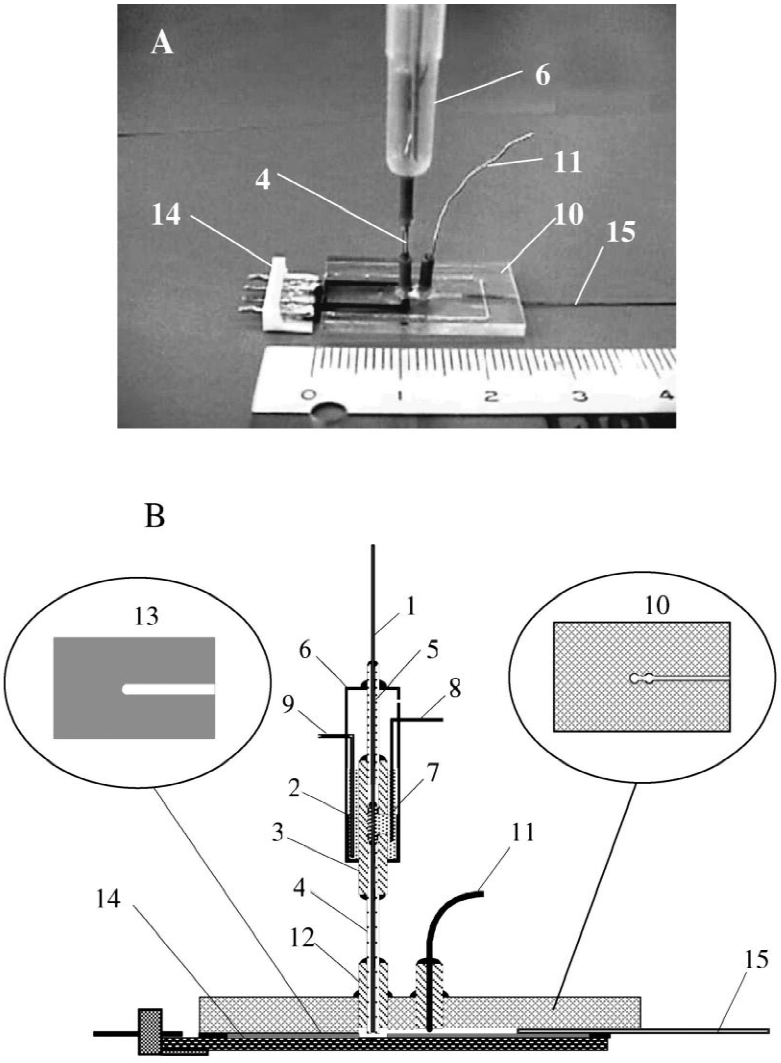

Fig. 1. A photograph of the amperometric detector (A) and a structure diagram (B). The components are numbered as follows: fused-silica capillary (1), Nafion tubing joint (2), hard plastic tubes (3 and 12), stainless steel tubes (4, 5 and 9), cylindrical plastic container (6), electrolyte solution (7), platinum wire (8), acrylic plate (10), Ag wire reference electrode (11), PTFE gasket (13), IDRA microelectrode chip (14), outlet capillary (15).

is only 25×12×4 mm^3. The main components are an acrylic plate, a gasket and an IDRA microelectrode on a chip. The detector structure is shown in Fig. 1B. We fabricated the detector as follows.

To make the detector suitable for use with large inner diameter separation capillaries, we integrated a Nafion tubing joint on a separation capillary to isolate the high separation voltage from the detection system. We fabricated the Nafion tubing joint in a way similar to that proposed by O'Shea et al. [37]. We used a capillary cutter to score the polyamide coating about 3 cm from the end of a 65 cm×25 μm I.D.×360 μm O.D. fused-silica capillary (GL Sciences, Japan). We then carefully threaded a 12 mm× 0.33 mm I.D.×0.51 mm O.D. piece of Nafion tubing (Perma Pure Products, Tom's River, NJ, USA) over the score mark. Both ends of the Nafion tubing were sealed to the separation capillary with UV curable resin. After completing the curing process, we exerted a gentle force on the Nafion tubing joint to fracture the capillary at the score point. To protect the Nafion tubing joint, we inserted it into a 2.5 cm×1.2 mm I.D.×2 mm O.D. hard plastic tube with a 1 cm×1 mm window in its wall, and it was only exposed at the center of the window. The gap between the Nafion tubing and the plastic tube was sealed along the window brim. The separation capillary was threaded into two stainless steel tubes of 2.5 cm×0.6 mm I.D.×1.0 mm O.D., which were then inserted into each end of the plastic tube. The openings at the ends of the plastic tube were sealed. The fabricated Nafion tubing joint was finally sealed in a cylindrical plastic container 6 mm in diameter and 4 cm high containing an electrolyte solution and a platinum wire used to ground the separation current. The stainless steel tube was fixed in position through a hole in the container wall to enable us to inject the electrolyte solution into the container.

Before the separation capillary with the Nafion tubing joint was installed in the thin-layer radial flow amperometric detector, it was necessary to polish the capillary outlet wrapped in the stainless steel tube so that it was flat and smooth.

The next step was to fabricate a thin-layer radial flow amperometric detector. We used a dicing saw to cut a 400×400 μm channel lengthways from the center to one side of a 25×12×2 mm^3 acrylic plate. We drilled two 2.0 mm diameter holes at an interval of 5 mm at one end of the channel on the acrylic plate (see right inset). One of the holes was used to fix an Ag wire reference electrode. The other was used when we installed the separation capillary. A 1 cm length of hard plastic tube was first inserted into the hole and sealed. We then inserted the separation capillary outlet into the plastic tube and adjusted it so that it was on the same plane as the acrylic plate surface with the channel, and finally sealed it firmly. We made a PTFE gasket with an appropriate thickness and the same size as the IDRA microelectrode chip, as shown in the left inset. The gasket was pasted on the acrylic plate with a small amount of UV curable resin with its opening positioned over the channel. An IDRA microelectrode chip was placed on the gasket and the IDRA was roughly aligned with the capillary outlet. The acrylic plate, the gasket and the IDRA microelectrode chip were sandwiched together and held in place with a clamp. We used a microscope to bring the IDRA chip into accurate alignment with the capillary outlet. We applied an appropriate amount of dilute UV curable resin along the chip edge and waited for it to diffuse into the gaps in the sandwich structure and spread across the region surrounding the IDRA and the channel. We then exposed the device to UV light for 2 min. A 5 cm×150 μm I.D.×375 μm O.D. capillary was inserted into the channel and sealed as the outlet of the detector. The complete detector fabrication process takes only 10–20 min.

2.3. CE system and apparatus

A CE system (Mebius Advanced Technology, Japan) was used to perform all electrophoretic separations. A high-voltage power supply (Otsuka Denshi, Japan) was used to apply a separation voltage from 0 to 30 kV across the ends of the separation capillary. We used an LC-4CE dual potentiostat (Bioanalytical Systems, USA), which has a clamp circuit to protect the LC-4C electronics if the high voltage ground is lost, to apply the detection potential to the IDRA microelectrode and collect the reaction currents. A DA-5 ChromGraph Interface unit was used to transfer data from the potentiostat to an Endeavor ET-6500 computer (Epson Direct, Japan) that we employed to process the data and control the high-voltage power supply.

We pretreated the separation capillary by flushing it for 1 h with 0.1 *M* NaOH. At the start of each day of use, we again flushed the capillary with 0.1 *M* NaOH for 15 min. In addition, the detector and the container for the Nafion tubing joint should be filled with running buffer before measurements are made. The stainless steel tube (4) also works as a counter electrode. The complete detector with the Nafion tubing joint was placed in a CC-4 Faraday cage (Bioanalytical Systems) during measurement. We incorporated a microampere meter, designed to measure the grounded current, between the Nafion tubing joint and the ground to monitor the work status of the joint. We controlled the electrokinetic sampling time using a computer.

2.4. Chemicals and reagents

3-(Cyclohexylamino)-1-propanesulfonic acid (CAPS) was purchased from Sigma. Dopamine hydrochloride (DA, >97%) and epinephrine (E, >98%) were purchased from Kanto (Japan). Norepinephrine (NE) was provided by Research Biochemicals International (RBI), USA, catechol (CAT, >99%) was purchased from Tokyo Kasei (Tokyo, Japan). The running buffer for separation was a 0.1 *M* CAPS solution (pH 10.0). Catecholamine stock solutions (0.01 *M*) were prepared by using 0.1 *M* perchloric acid (Wako, Osaka, Japan) and diluted to a desired concentration with running buffer. All reagents were used as received. All buffer solutions and samples were filtered through a 0.2 μm microdisk syringe filter before being used in capillary electrophoretic measurements. The solutions were prepared using water purified with Milli-Q (Millipore, Bedford, MA, USA).

3. Results and discussion

3.1. Single- and dual-mode amperometric detection of catecholamines with IDRA microelectrode in capillary electrophoresis

We first evaluated the Nafion tubing joint by measuring the currents to ground when electrophoresis was performed using a 65 cm×50 μm diameter capillary filled with 10 m*M* of phosphate buffer (pH 7.0) at high voltages varying from 0 to 30 kV in 3 kV steps. The Ohm plot exhibits a linear relationship within the voltage range and the currents are in good agreement with that obtained using a capillary of the same length but without a Nafion tubing joint. This demonstrates that the Nafion tubing joint works very effectively.

We selected catecholamines for characterizing the performance of the amperometric detector because they are the most frequently studied substances in previously reported work on CE–ED [1,2,6,20]. The typical electropherograms shown in Fig. 2 were obtained by single- and dual-mode amperometric detection with the IDRA microelectrode for DA, E, NE and CAT after electrophoretic separation with a 25 μm I.D. capillary [1]. In the single mode, only the first electrode (W_1) of the IDRA microelectrode was supplied with a potential of +0.7 V (vs. Ag elec-

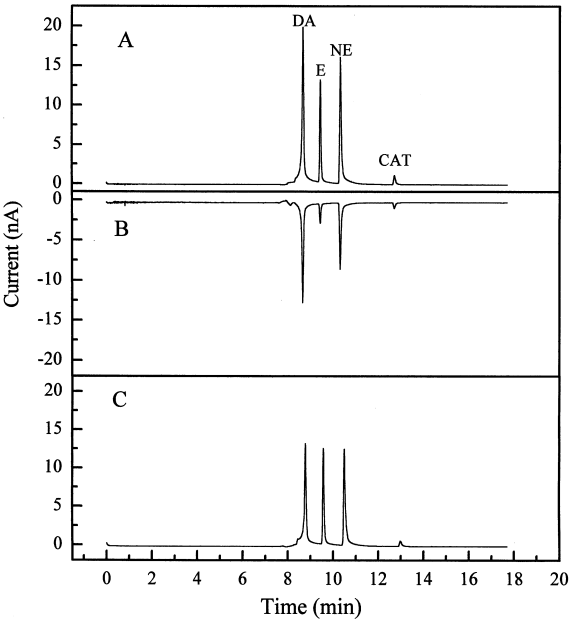

Fig. 2. Representative electropherograms of catecholamines obtained by capillary electrophoresis with an IDRA microelectrode. I_a (A) and I_c (B) are anodic and cathodic currents in the dual mode, respectively; I_s (C) is the anodic current in the single mode. Conditions: 65 cm (2 cm detection capillary)×25 μm I.D. fused-silica capillary; capillary-to-electrode distance, 10 μm; 0.1 *M* CAPS and 1 *M* NaOH added to obtain pH 10.0; separation parameters, 20 kV, 2.10 μA; electrokinetic sampling, 20 kV, 5 s; 10 μ*M* NE, CAT, DA and 20 μ*M* E; detection potentials, 700 mV on anode and −300 mV on cathode.

trode), and the second electrode (W_2) was kept open circuit. Therefore, after the analytes had been oxidized at electrode W_1, the products could not be reduced at electrode W_2 and so no redox cycling occurred. In the dual mode, the potentials of electrodes W_1 and W_2 were fixed at +0.7 and −0.4 V, respectively. In the dual mode, the redox cycling of the analytes occurred between electrodes W_1 and W_2. We expect the redox cycling to increase the Faraday currents of the IDRA microelectrode. The anodic currents for all catecholamines in the dual mode (Fig. 2A) were higher than those in the single mode (Fig. 2C). The current amplification magnitude depends on the stability of the catecholamines after electrochemical oxidation. The collection efficiencies (C_e), redox cycles (Rc) and the theoretical plate numbers (N) of separation for the catecholamines calculated from Fig. 2 are listed in Table 1. The C_e and Rc of E are the lowest because electrochemically oxidized E becomes indole by the intra-molecular cyclization reaction revealed in the cyclic voltammograms. The C_e and Rc for CAT were 65% and 1.71, respectively, which are higher than those of other catecholamines. We also achieved high anodic currents in the dual mode, C_e and Rc for DA and NE. The high Rc and C_e values at the IRDA with only four pairs of fingers seems to be due to the thin-layer radial flow-through configuration of the detector because this configuration causes the analytes eluting from the separation capillary to flow across the ring-shape IDRA microelectrode in a radial direction. Another advantage of the thin-layer radial flow amperometric detector is its relatively low dead volume of about 0.05 nl when the gasket thickness is 10 μm and the IDRA microelectrode diameter is 80 μm. This advantage allows us to retain the high resolution of capillary electrophoretic separation. The high theoretical plate numbers given in Table 1 demonstrate this advantage.

We also used capillaries with I.D.s of 15 and 50 μm to separate and detect the catecholamines. The C_e, Rc and theoretical plate numbers calculated from the electropherograms we obtained are listed in Table 1. We found that the C_e, Rc and theoretical plate numbers are basically independent of the inner diameter of the separation capillary. This also shows that the amperometric detector we designed makes it possible to use various capillaries with different inner diameters and is capable of retaining the high separation efficiency of CE. Moreover, when we used the 15 μm I.D. capillary, we did not observe any obvious noise caused by high separation voltage without the Nafion joint as compared to that with the joint. Therefore, we suggest removing the Nafion tubing joint to make the detector simpler when using capillaries with inner diameters of less than 20 μm.

3.2. Analytical performance of the amperometric detector with catecholamines

We developed the amperometric detector to improve the sensitivity of ED with the IDRA electrode in CE, since IDRA electrodes are capable of increasing the reaction current of electrochemically reversible analytes through redox cycling. To determine the degree to which detection sensitivity was improved, we investigated linear concentration ranges, sensitivities and concentration detection limits by

Table 1
Dependence of the C_e, Rc and N of catecholamines on the inner diameter of the separation capillary (conditions as in Fig. 2)

Analyte	15 μm			25 μm			50 μm		
	C_e[a] (%)	Rc[b]	N[c]	C_e (%)	Rc	N	C_e (%)	Rc	N
DA	60.3	1.64	83 410	63.0	1.51	85 080	62.8	1.58	85 320
E	19.4	1.12	124 210	18.6	1.04	129 870	18.9	1.10	128 560
NE	53.1	1.31	117 240	52.2	1.27	115 060	53.8	1.36	116 740
CAT	63.8	1.67	134 870	65.0	1.71	134 330	64.5	1.70	133 530

[a] C_e is defined as the cathodic current (I_c) at the W_2 electrode divided by the anodic current (I_a) at the W_1 electrode in dual mode.
[b] Rc is defined as the anodic current (I_a) in the dual mode divided by that (I_s) in the single mode.
[c] The N definition was calculated using the following equation derived from the separation theory of chromatography, $N=5.54(T_r/W_{1/2})^2$, where T_r is the migration time of each analyte and $W_{1/2}$ is the peak width at half the peak height. The N values were obtained with a 65 cm long separation capillary in the dual mode.

Table 2
The performance of the detector with IDRA microelectrodes for catecholamines in single and dual modes[a]

Compound	Linear range (μM)		Sensitivity (pA/μM)		Detection limit (μM)	
	Dual mode	Single mode	Dual mode	Single mode	Dual mode	Single mode
NE	0.050–500	0.070–500	157.2	123.3	0.038	0.049
E	0.050–500	0.050–500	256.7	255.2	0.023	0.024
DA	0.020–500	0.050–500	392.9	239.0	0.015	0.025
CAT	0.100–500	0.200–1000	118.4	69.6	0.051	0.100

[a] Conditions as in Fig. 2.

measuring catecholamines in the dual and single modes. We provide the results in Table 2. For all catecholamines, the sensitivities and concentration detection limits we obtained in the dual mode were improved to different extents. For NE, CAT and DA, the detection limits in the dual mode were significantly lower than those in the single mode. However, the detection limit of E deteriorated slightly because there was poorer collection efficiency at the cathode as a result of its poor electron transfer reversibility. The sensitivity of CAT in the dual mode was 1.7-times better than that in the single mode and the DA sensitivity was up to 392.9 pA/μM. The sensitivity of the amperometric detector also depends on the number of fingers in the IDRA electrode. The more pairs of fingers an IDRA electrode has, the higher the collection efficiency becomes and thus higher sensitivity can be achieved. The IDRA electrode we used in this work has only four pairs of fingers. We expect that as many pairs of IDRA microelectrodes as possible will be arranged in a small region about 300 μm in diameter to improve the sensitivity.

3.3. Reproducibility of fabrication and detection for the amperometric detector

It is important that a CE detector has good reproducibility in terms of both fabrication and measurement. We made three amperometric detectors (A, B and C) using the same fabrication procedure and materials, and carried out five parallel CE–ED measurements for each detector. Table 3 shows the results of our reproducibility study. All relative standard deviations (RSDs) are less than 10%. This demonstrates that CE–ED with the amperometric detector is highly reproducible. To obtain good reproducibility of the CE–ED system, we considered the many influences on both electrochemical detection and capillary electrophoresis and took certain measures to retain identical separation and detection conditions. The following experimental conditions are important as regards improving reproducibility. (a) We fabricated the IDRA chips, the gaskets and

Table 3
Reproducibility of anodic current (nA) in the dual mode with the amperometric detectors[a]

Detector	No. of runs	DA	E	NE	CAT
A	1	12.4	13.7	12.6	1.31
	2	12.4	12.3	11.0	1.60
	3	11.8	11.6	10.5	1.57
	4	13.1	11.2	11.5	1.61
	5	11.2	10.7	10.6	1.41
	Average	12.2	11.9	11.2	1.50
	RSD (%, n=5)	5.87	9.78	7.64	8.89
B	1	14.3	13.5	13.4	1.43
	2	13.2	13.2	12.8	1.53
	3	12.5	10.6	11.2	1.43
	4	13.9	13.8	12.8	1.65
	5	12.9	12.5	12.4	1.60
	Average	13.4	12.7	12.5	1.50
	RSD (%, n=5)	5.47	9.98	6.56	4.79
C	1	12.5	13.5	11.7	1.53
	2	12.9	12.4	11.6	1.47
	3	12.7	12.5	12.5	1.68
	4	11.3	13.7	13.5	1.62
	5	13.0	14.2	11.4	1.80
	Average	12.5	13.3	12.1	1.62
	RSD (%, n=5)	5.50	5.89	7.17	7.97
Total	Average	12.7	12.6	11.9	1.54
	RSD (%, n=15)	4.92	5.57	5.59	4.50

[a] Conditions as in Fig. 2.

the acrylic plates in batches. This can greatly reduce differences in geometry and the performance of individual detectors caused by the fabrication process. (b) The smooth section of the capillary outlet that we obtained by finely polishing it, lowered the disturbance in the thin-layer radial flow stream between the capillary outlet and the IDRA electrode. (c) To avoid fouling the IDRA electrodes when detecting high concentrations of catecholamines, we treated the IDRA electrodes between runs by using cyclic voltammetry scanning for 30 s between −0.6 and 1.2 V at 600 mV/s. (d) We rinsed the inside wall of the separation capillary by purging it with running buffer before each run and with 0.1 *M* NaOH solution once a day. (e) To obtain a reproducible sample injection in the electrokinetic mode, we used a computer to control the high sampling voltage and time.

4. Conclusion

We fabricated a thin-layer radial flow cell for connection to an IDRA electrode for CE. By using the cell, we achieved high collection efficiency and redox cycles of catecholamines at the IDRA microelectrode with values of 65% and 1.71 for CAT, respectively. A high sensitivity of 392.9 pA/μ*M* and a low detection limit of 15 n*M* for DA can be achieved. By examining the reproducibility of the detector, we found that both the run-to-run and detector-to-detector RSDs were less than 10%.

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