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Improved detection limit for catecholamines using liquid chromatography–electrochemistry with a carbon interdigitated array microelectrode

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Abstract

The detection limit of catecholamines can be lowered by using a carbon-based interdigitated array (IDA) microelectrode as a detector for liquid chromatography (LC). The IDA electrode is more sensitive than conventional glassy carbon electrodes due to the high current density caused by radial diffusion at each microband, and redox cycling between two microband arrays. Since the number of redox cycles increases at lower flow-rates, the carbon IDA is particularly useful for microbore LC. In an LC system with a 1-mm microbore column and a carbon IDA electrode, the peak height of dopamine (DA) and DOPAC did not decrease with decreasing flow-rate because of this redox cycling. A low detection limit of 5 fg (32 amol) and 9.6 fg (57 amol) was obtained for DA and DOPAC due to the high current density and low background noise level (0.1 pA) at the carbon IDA electrode. The total charge generated by oxidizing DA at the anodic array was more than the value calculated by assuming that all the DA molecules were oxidized.

1. Introduction

Liquid chromatography (LC) with electrochemical detection has many uses such as the determination of aromatic amines, thiols and amino acids [1]. Recently, a need has arisen for small sample measurement in biological analysis because various applications such as in vivo sampling [2,3] and portable analysis are becoming more widespread. Liquid chromatographic systems with 1-mm or sub-millimeter columns are frequently used for biological analysis [3–7].

In microbore chromatography, electrochemical detection is ideal for reducing detector size because electrochemical measurement is based on the surface electron transfer reaction [3,5]. Microelectrodes are particularly useful to reduce the active detection volume and therefore are used in capillary electrophoresis and capillary LC. Lower detection limits can be achieved because the radial diffusion at the microelectrode results in a high current density and high signal-to-noise ratio [8].

However, it is difficult to lower the detection limit using a single microelectrode in liquid chromatography, because the magnitude of total

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current is very small. This makes it very hard to measure the current with conventional electronics. Microarray electrodes offer a solution to this problem. They have a high current density similar to microelectrodes, but provide total current equivalent to larger electrodes. In addition, an interdigitated array (IDA) microelectrode with both oxidizing and reducing elements can generate additional current through redox cycling of the analyte. For example, a gold-film-based IDA microelectrode has been shown to be a highly sensitive detector in LC due to high mass-transfer flux resulting from redox cycling at the electrodes [9–11]. With IDA electrodes, a product generated at one microband array collects at the other array and reverts to its initial state when the potential of the adjacent electrode is sufficient for the reverse reaction. Then, having reverted to its initial state, the species collects again at the first array electrode. As this redox cycle repeats, the currents of both the anodic and cathodic array electrodes increase [12,13].

Current enhancement by redox cycling is less effective in a flow stream than in a stationary solution, because the flow stream shortens the residence time of the analyte over the array. However, the combination of a microbore column and an IDA microelectrode is expected to improve sensitivity, because the flow-rate when using the microbore column is less than one-tenth that of a conventional column [14].

Microarray electrodes fabricated by lithography, which include the IDAs, are made of metal films such as gold and platinum. Their narrow potential window in the cathodic region limits their usefulness at negative potentials. Therefore, we expect that most applications of IDAs will be at positive potentials.

Recently, the characteristics of carbon-based microarray electrodes have been reported by several groups using random and linear arrays fabricated by conventional procedures, such as the suspension of carbon particles or fibers in the insulator [15–17]. However, two of us (O.N. and H.T.) has reported the fabrication of carbon-based IDA electrodes by lithography [18,19]. These IDAs show improved performance when compared to conventional IDAs. A low detection limit of 0.5 fmol for the determination of dopamine was reported using a lithographed carbon-based IDA with a 5-μm band width and gap as a detector in LC [20].

This paper discussed the advantages of carbon-based IDA electrodes for LC systems compared with conventional glassy carbon electrodes. It also shows how the detection limit for catecholamines can be improved. We emphasize the advantages of the carbon IDA at slower flow-rate because we are planning to apply the IDA for microbore LC coupled with in vivo microdialysis or ultrafiltration sampling. The IDA should be advantageous for such application because the IDA enables current enhancement at slow flow-rates and selective determination at the cathodic array.

2. Experimental

2.1. Chemicals and reagents

Norepinephrine bitartrate (NE), dopamine-HCl (DA) and 3,4-dihydroxyphenylacetic acid (DOPAC) were obtained from Research Biochemicals (Natick, MA, USA). They were dissolved in 0.1 *M* perchloric acid (Aldrich, Milwaukee, WI, USA) to a concentration of 0.5 mg/ml. The mobile phase buffer solutions were prepared using water purified with NANOpure II (Dubuque, IA, USA), sodium octylsulfate (SOS) and 1-octanesulfonic acid (OSA) (Kodak, Rochester, NY, USA), ethylenediaminetetraacetic acid disodium salt (EDTA) (Aldrich), diethylamine-HCl (Sigma, St. Louis, MO, USA), dimethylacetamide (DMA), methanol (Burdick and Jackson, Muskegon, MI, USA), phosphoric acid, sodium hydroxide, sodium citrate dihydrate, sodium dihydrogenphosphate monohydrate and monochloroacetic acid (Mallinkrodt, Paris, KY, USA) were used to prepare the mobile phase.

2.2. IDA electrodes

The carbon-film-based IDA microelectrode was fabricated by photolithography from a carbon film of pyrolyzed 3,4,9,10-perylenetetracar-

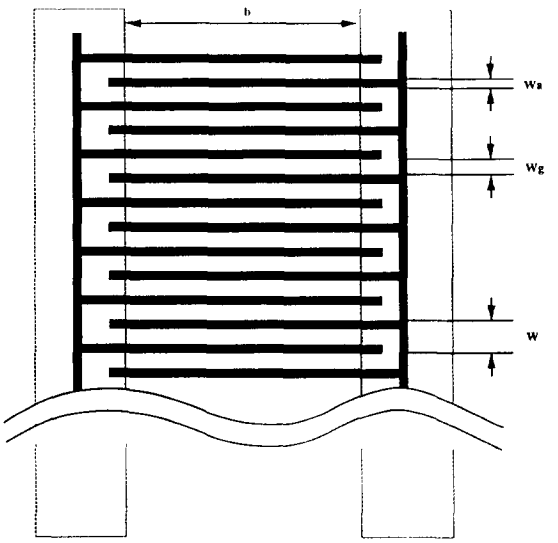

Fig. 1. Schematic representation of carbon-based IDA used in this measurement. $W = 4$ μm, $Wa = Wg$ (gap) $= 2$ μm, $b = 2$ mm. Each IDA electrode consisted of 250 pairs of microbands.

boxylic dianhydride on thermally oxidized silicon wafers [19]. Fig. 1 shows a schematic representation of the IDA used as a LC detector. The electrode consisted of 250 pairs of fingers, the finger widths (*Wa*) and gaps (*Wg*) were 2 μm and each finger was 2 mm long. A Teflon gasket with its center cut out was sandwiched between the IDA electrode and the stainless-steel counter block in order to make a thin-layer flow cell.

2.3. Apparatus and chromatographic conditions

Chromatographic measurement was performed using a BAS 200B system (Bioanalytical Systems, West Lafayette, IN, USA). A Rheodyne 8125 injection valve (Berkeley, CA, USA) with a 5-μl sample loop was used for sample injection. We used two separation columns. The first was a standard bore C_{18} reversed-phase column (100 × 3.2 mm I.D., 3 μm particle size). The second was a Sepstik microbore C_{18} reversed-phase column (150 × 1 mm I.D., 5 μm particle size) (both from Bioanalytical Systems).

For the separation of catecholamine with the standard column, the mobile phase consisted of 75 m*M* monochloroacetic acid (pH 3.0), 0.7 m*M* EDTA, 1.5 m*M* SOS and 8% DMA. The flow-rate of the mobile phase was 1.1 ml/min and the thickness of the gasket for the thin layer flow cell was 51 μm.

In contrast, a mobile phase with 50 m*M* sodium citrate dihydrate, 25 m*M* sodium dihydrogenphosphate monohydrate, 10 m*M* diethylamine hydrochloride, 27 μ*M* EDTA, 2.2 m*M* OSA, 30 ml/l methanol and 22 ml/l DMA was used for LC measurement with the microbore column. The mobile phase was adjusted to pH 3.2 with phosphoric acid. The flow-rate of the mobile phase was varied from 30 to 70 μl/min and the thickness of the gasket for the thin-layer flow cell was 16 μm. In both cases, the mobile phase was thoroughly filtered through a 0.2-μm regenerated cellulose.

All samples were diluted in deionized water from 0.5 mg/ml stock solutions prepared in 0.1 *M* perchloric acid. To obtain the amperometric signal, the potential of one finger set of the IDA electrode was held at +750 mV vs. Ag/AgCl and that of the other set was held at +50 mV. The column was kept at 30°C. The mobile phase was sparged with helium for 5 min, and then kept under a 27.6 kPa blanket of helium.

3. Results and discussion

3.1. Comparison of carbon IDA and glassy carbon disk electrodes

Fig. 2 shows a chromatogram of norepinephrine (NE) measured with a carbon IDA microelectrode in which both the anodic and cathodic arrays were potentiostated (dual mode). A 3.2-mm standard bore column was used for the measurement. The injected sample volume was 20 μl which contained 1 pg (5.9 fmol) NE. The thickness of the gasket to make the flow channel was 51 μm. The flow-rate was 1.1 ml/min. The collection efficiency, defined as the ratio of cathodic to anodic peak height, was 0.67 in spite of high flow-rate and thicker flow channel because the band width and gap of the IDA were very small (2-μm band width and gap). However, the collection efficiency was much lower than it would have been in a stationary solution,

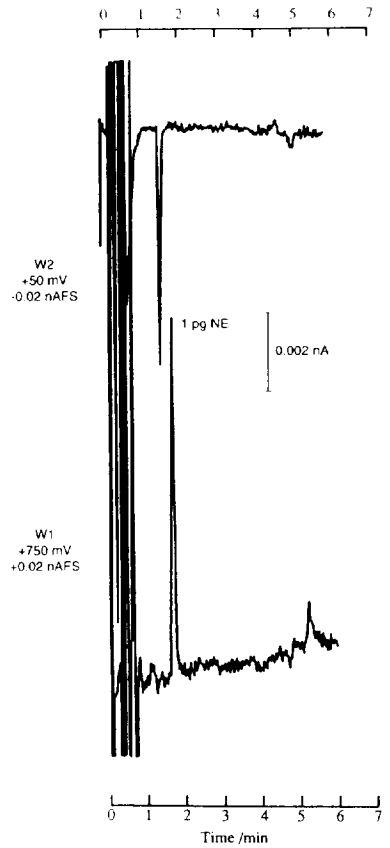

Fig. 2. Chromatograms of norepinephrine (NE) at the IDA electrode in the dual mode. The band width and gap of the IDA was 2 μm. Each array consisted of 250 microbands. The anodic and cathodic array electrodes in the IDA were potentiostated at 750 and 50 mV vs. Ag/AgCl, respectively. The standard column (100 × 3.2 mm I.D.) was used.

since the rapid flow interferes with the diffusion of NE between the arrays.

Table 1 compares NE signal strength from a 3-mm standard glassy carbon electrode and a carbon IDA microelectrode. The IDA electrode consisted of two microband arrays. Each array had a 0.01 cm^2 surface area. The surface area of the glassy carbon electrode was 0.071 cm^2. The glassy carbon electrode showed the highest peak current because the surface area of the electrode was about seven times larger than that of each IDA array. However, the table clearly indicates that the electrode with the highest current density had the lowest detection limit. When both

Table 1
Comparison of peak currents of norepinephrine at 3-mm GC and IDA electrodes

Electrode	3-mm glassy carbon	IDA (Single)[a]	IDA (Dual mode)	
			W1	W2
Potential (mV vs. Ag/AgCl)	750	750	750	50
Area (cm^2)	0.071	0.02	0.01	0.01
Peak (pA)	9.6	4.9	8.8	5.9
Sensitivity (pA/pg)	9.6	4.9	8.8	5.9
Noise (pA)	2.5	0.4	0.5	–
Current density (nA/cm^2)	0.13	0.24	0.88	0.59
Detection limit (fmol) ($S/N = 3$)	4.6	1.3	1.0	0.75

[a] Both finger sets are connected together.

arrays were potentiostated at 750 mV, the current density of the IDA electrode was about twice that of the glassy carbon electrode, because of the radial diffusion of the analyte around each microband. However, the peak area does not change when only one array is potentiostated at 750 mV (single mode). This clearly suggests the diffusion layer of NE formed at each microband overlaps in the residence time of NE over the IDA. The noise level of the IDA was much lower than that of the glassy carbon because of its smaller surface area. Therefore, the high current density and small noise level resulted in a lower detection limit. A large current density was obtained at the IDA, when the arrays of the IDA were potentiostated at 750 and 50 mV. This is because redox cycling was established between the anodic and cathodic arrays of the IDA. The peak current at the anodic array was 92% of that at the glassy carbon electrode, although the surface area of each array is seven times smaller than that of the glassy carbon. This high current density resulted in a lower detection limit than when both arrays were at 750 mV. In spite of the lower current density, the detection limit of the cathodic array was lower than that of the anodic array, because the noise level at 50 mV was much lower than at 750 mV under these conditions.

3.2. Response of catecholamines at carbon IDA in microbore LC

LC detection using a smaller column is advantageous to achieve high sensitivity, because there is less analyte dispersion than in a larger column. For example, the analyte concentration at a detector with a 1 mm diameter microbore column is theoretically 10 times higher than with a 3.2 mm diameter column [3]. The application of carbon IDAs to a small-column LC system is also advantageous, because the system is usually operated at a slow flow-rate where redox cycling of the analyte at the IDA is more efficient. The signal of 1 pg NE measured using a 1 mm diameter column was 84 pA, which was about 8.8 times larger than that obtained using a 3.2 mm diameter standard column (9.6 pA).

Fig. 3 shows flow-rate dependence of DA and DOPAC signals in dual mode (750 and 50 mV) compared with those in single mode (750 mV) in the microbore LC. The thickness of the flow cell was 16 μm in this measurement. In the thin-layer flow cell, the limiting current is proportional to the cubic root of the flow-rate. Therefore, the peak current decreases with decreasing flow-rate. The peak current of the IDA is also proportional to the cubic root of the flow-rate when it is relatively large [10,11].

In the single mode, the peak heights of DA and DOPAC decreased with decreasing flow-rate similar to a conventional electrode in a thin layer cell. In contrast, the magnitude of the signal at the anodic array did not change and the signal at the cathodic array increased with decreasing flow-rate in the dual mode. The number of redox cycles can be estimated by dividing the anodic signal in the dual mode by that in the single mode. The redox cycles of both DA and DOPAC increased from 1.1 to 1.8 and from 1.2 to 2.2, respectively, with decreasing flow-rate from 70 to 30 μl/min. This clearly indicates that the IDA electrode is advantageous for use in LC operated at a slow flow-rate. However, the number of redox cycles was still low at the lowest flow-rate. This is because the signal in the single mode is already enhanced by the redox cycling between the anodic array and the auxiliary electrode, since the distance between these electrodes is only 16 μm.

Recently, a small-volume LC system has been used to determine catecholamine neurotransmitters collected using microdialysis and ultrafiltration technique. Carbon-based IDA electrodes are advantageous as detectors for such applications because they exhibit an enhanced flow-independent signal at low flow-rates.

Fig. 4 shows the flow-rate dependence of the collection efficiency of DA and DOPAC. The collection efficiency of both analytes increases with decreasing flow-rate. However, DA showed a much higher collection efficiency than DOPAC because of the difference in the reversibility of the DA and DOPAC redox couples. The collec-

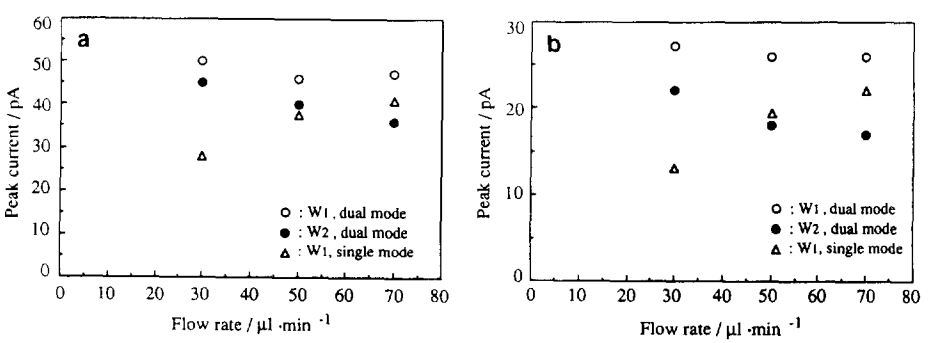

Fig. 3. Flow-rate dependence of peak heights in the dual modes compared with those in a single mode. (a) DA, (b) DOPAC. The size of the IDA was same as that used in Fig. 2. The potential of anodic and cathodic arrays were also same as those in Fig. 2. The microbore column (150 × 1 mm I.D.) was used. The injected amounts of DA and DOPAC were 1 pg.

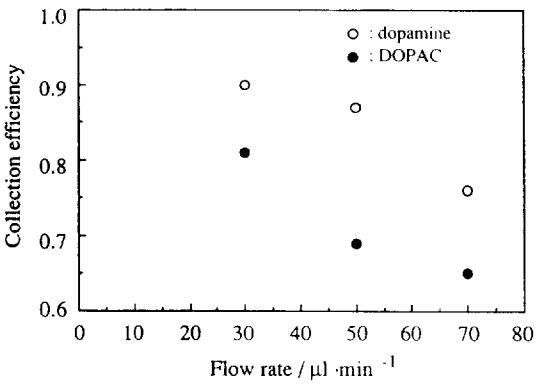

Fig. 4. Flow-rate dependence of DA and DOPAC collection efficiencies at the carbon IDA electrode. The collection efficiencies were calculated with the peak currents in Fig. 3.

tion efficiency of DA was more than 0.9 at the flow-rate of 30 μl/min, which is close to the value obtained in a stationary solution. This high collection efficiency is very important for analyzing biological fluid. Since various electroactive interfering molecules such as L-ascorbic acid and uric acid are contained in the biological samples, these interfering molecules are electrochemically oxidized at the anodic array. However, they were not reduced at the cathodic array because of their electrochemical irreversibility. Therefore, catecholamine can be determined selectively at the cathodic array while maintaining equivalent sensitivity to that at the anode due to high collection efficiency.

In the conventional flow cell in LC–electrochemical detection, only a few percent of the injected analyte reacts electrochemically at the detector. However, this percentage should increase with decreasing cell volume. It also should increase with decreasing flow-rate because the analyte molecule takes longer to pass through the detector, which increases the amount of analyte molecules that can diffuse to the electrode surface.

Figs. 5a and b show the flow-rate dependence of the total charges of DA and DOPAC oxidized at the anodic arrays of the IDA. In both cases, the total charges of DA and DOPAC in the dual mode greatly increased with decreasing flow-rate. In contrast, the total charges in the single mode changed very little as the flow-rate decreased. Since the anodic peak height did not change and the peak width only increased with decreasing flow-rate, the increases of the charge are assumed to be caused by the increase of DA and DOPAC redox cycling. The total charge of DA in the single mode increased very little, whereas there was no increase in charge for DOPAC. Since DA is more stable than DOPAC after electrochemical oxidation, the slight increase in the DA total charge was due to the redox cycling between the IDA and the axillary electrode.

3.3. Detection limit of catecholamine with carbon IDA in microbore LC

Carbon-based IDA electrodes in microbore LC are expected to achieve a low detection limit for catecholamine because of the high current

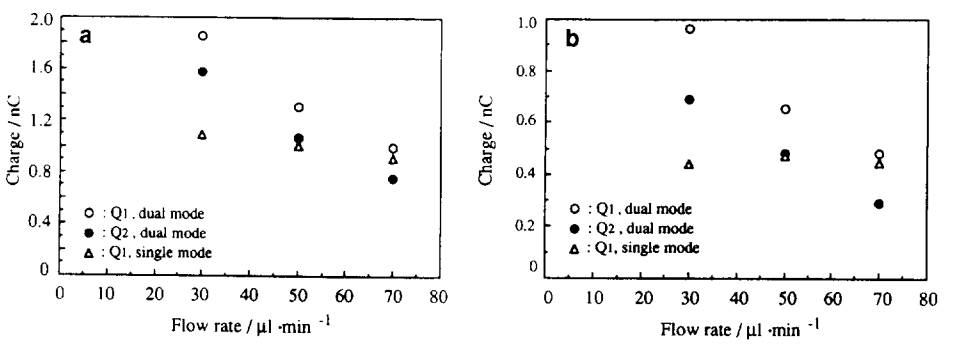

Fig. 5. Flow-rate dependence of total charges measured at the IDA anodic array in the dual and single modes. (a) DA, (b) DOPAC. The charge was calculated by integrating each peak obtained by the experiment shown in Fig. 3.

density and low noise level of the IDA and the low dispersion of the analyte in the column.

Fig. 6 is a chromatogram of DA and DOPAC measured at the carbon IDA in the dual mode. The potentials of the anodic and cathodic arrays were held at 750 and 50 mV vs. Ag/AgCl. The flow-rate was 70 μl/min and the amount of sample injected was 5 μl, containing 100 fg DA and DOPAC (0.65 fmol for DA and 0.59 fmol for DOPAC, respectively). The solvent front appeared within 2 min. The large reduction peak at 2.5 min was from oxygen reduction, because the height of this peak greatly decreased by deoxygenating a sample just before injection. In this measurement, the noise level at the anodic array was about 0.1 pA, which was lower than that at the cathodic array. The peak heights of DA and DOPAC were 6.2 and 3.2 pA, respectively. The detection limits of DA and DOPAC calculated from the peak heights and noise level were 5.0 and 9.6 fg (32 amol and 57 amol, $S/N = 3$). These detection limits are much lower than those currently reported for conventional detectors [7].

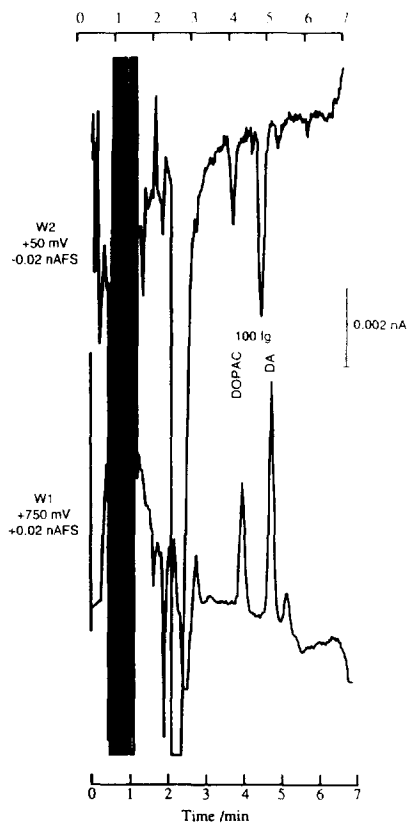

Fig. 6. Chromatograms of 100 fg DOPAC and DA at the flow-rate of 70 μl/min. The injected sample volume was 5 μl. The size of the IDA was same as that used in Fig. 2. The microbore column (150 × 1 mm I.D.) was used.

The carbon-based IDA electrodes in the small-volume thin-layer cell can also be used to obtain the equivalent current to that generated by complete conversion of the analytes. If all the DA molecules injected (1 pg) are oxidized at the anodic array, the total charge generated at the electrode is 1.26 nC. However, the observed total charge with the flow-rate of 30 μl/min is 1.86 nC, which is about 1.5 times larger than the calculated amount. Amperometric detectors with near 100% conversion efficiency have been widely applied to catecholamine detection [21]. Carbon electrodes that have a large surface area, such as porous graphite, carbon cloth or platinum gauze are used [21]. However, these electrodes generate a large background current that increases the noise. In spite of the low noise level, the charge generated at the carbon-based IDA is greater than that of conventional single electrode detector due to redox cycling. Therefore, a low detection limit is very promising using the IDA for complete conversion of analyte.

4. Conclusion

A carbon-based IDA microelectrode (2-μm band width and gap) was applied as a detector for LC. The IDA electrode had a high current density and low baseline noise compared with conventional glassy carbon electrodes, which resulted in a lower detection limit. Carbon IDA electrodes are more effective for LC coupled with microbore columns operated at lower flow-rates. The peak height of DA and DOPAC in the dual mode does not decrease with decreasing flow-rate because the redox cycling of the analyte enhances the signal effectively. As a result, low detection limits of 5 fg (32 amol) and 9.6 fg (57 amol) were obtained for DA and DOPAC due to

the high current density and low noise level (0.1 pA) at the carbon IDA electrode.

The total amount of analyte electrochemically oxidized at each injection increased with decreasing flow-rate. In the case of DA, the total charge generated at the anodic array was more than the value calculated by assuming that all the DA molecules were oxidized at the anodic array.

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