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Microchip Capillary Electrophoresis with Electrochemical Detection

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A novel microchip capillary electrophoresis system with electrochemical detection, using the replaceable microelectrode, is first reported. This kind of electrode can be fabricated in general laboratories and can be replaced quickly with electrodes of different materials according to the requirements of experiments. The end-column electrochemical detection on microchip CE was achieved by fixing the working electrode (such as carbon fiber, Pt, or Au, etc.) through a guide tube on the end of the separation channel. The experiment results indicate that the alignment of the electrode with the channel outlet can be carried out accurately and reproducibly, and therefore, the detection device has low noise and good reproducibility. The detection limit of dopamine is 2.4×10^{-7} M, which is the lowest result reported so far. The separation and detection of dopamine, 5-hydroxytryptamine and epinephrine using carbon fiber and Pt microdisk electrodes within 50 s was successfully performed.

Though the development of analytical techniques, for example capillary electrophoresis (CE), had displayed an attractive trend of integration and miniaturization, until the late 1980s, the exploitation of microfabrication technology reinforced this trend intensively. A flood of interest has been spurred in microchip-based analytical devices since the introduction of the concept of miniaturized total analysis system (μ-TAS).[1,2] In this field, μ-CE has become undoubtedly the most rapidly advancing and the most fruitful branch so far. The reason for this dominance of electrophoresis separation over other chromatographic techniques lies partly in the inherent simplicity of fabrication and operation combined with unique features with respect to separation speed, sample injection, and reagent consumption. Numerous articles have been published in recent years, which shows a dramatic advance in fabrication methods, operating techniques, and applications in various research fields.[3−7]

From simple single-channel structures fabricated on relatively large-scale chips to increasingly complicated and small-dimension ones, the aspect of functional integration and structural miniaturization has experienced significant development. Optional functions, including integrated sample mixing,[8,9] pre- and postcolumn derivatization or modification,[10−13] and various operating separation modes,[14−16] have been incorporated in CE chips. In addition, the devices for performing more and more large-scale array analysis have been implemented on microchips.[17,18] In sharp contrast to strong interest in the above aspect, much less attention has been given to the integration and miniaturization of detectors. Benefiting from its high sensitivity up to single molecular detection,[19] laser induced fluorescence (LIF) has so far been employed predominantly in connection with separation based on chips. Its bulky and complex optical system, however, impedes the integration of LIF detection with separation chips. The possibility of miniaturizing mass spectrometric devices has been investigated to achieve the monolithic analytical system coupled with MS detection.[20] Electrochemical detection has exhibited its impressive advantages in the use of conventional CE, such as no need of derivatization for many compounds; remarkable sensitivity, as compared with that of LIF; and ideal compatibility with integration and miniaturization of CE. All of these characteristics endow electrochemical detection with attractability for realizing on-chip detection. Originally, Ewing et al. reported an electrochemical detection scheme for an open-channel electrophoresis chip based on the photolithographic placement of the microelectrode just outside the exit of the electrophoresis channel.[21,22] Mathies et al. developed a

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microchip-based CE-EC system with a plasma-sputtered Pt thin-film microelectrode for indirect electrochemical detection of DNA.[23] Wang et al. brought out two kinds of electrochemical detection design. One consists of a fixed gold electrode sputtered around the outlet of the separation channel,[24] and the other employs a replaceable screen-printed carbon thick-film electrode mounted perpendicularly to the flow direction.[25] Lunte et al. reported carbon paste electrochemical detectors for microchip CE.[26] Additionally, electrochemical detection on microchips made of plastic,[27] ceramics,[28] and poly(dimethylsiloxane) (PDMS)[29] has been exploited. A Pt film decoupler for amperometric detection on CE chips was demonstrated recently by Chen and co-workers.[30] The good decoupling effect enables sensitive end-column detection on chips with large-size channels.

In most cases above, the on-chip electrochemical detectors are composed of film-like electrodes permanently deposited either on the inner wall of the separation channel[30] or on the external wall close to the channel outlet.[23,24,29] Such configurations are significantly limited with respect to their applications requiring frequent replacement of electrodes, especially in bioanalysis, in which electrodes will suffer fouling easily. In addition, those designs cannot provide a central position relative to separation channels for electrodes to obtain the highest coulometric efficiency and the best sensitivity.

A microsystem using the end-column electrochemical detection based on the microdisk electrode is first reported herein. The ability to replace working electrodes enhances the flexibility for analyzing a large range of analytes. Meanwhile, the higher sensitivity and coulometric efficiency result from the central alignment of the working electrode with the channel outlet.

EXPERIMENTAL SECTION

Apparatus. The microsystem with a CE chip (AMC-μChipT180, Alberta Microelectronic Corporation, Canada) was constructed as shown in Figure 1A. The waste reservoir on the chip was cut off to expose the channel outlet (Figure 1B). The chip has a 75-mm-long separation channel and a 250-μm offset double-T injection channel. The cross section of the channels was approximately semicircular with a width of 50 μm at the top and a maximum depth of 20 μm. The crucial part of the detection cell is the guiding tube used to align the working electrode with the channel outlet accurately and reproducibly. It was fabricated by pulling a ~2-mm-i.d. glass tube to form a ~30-μm-i.d. capillary tip. Subsequently, an optical microscope and a five-dimension micropositioner were employed to align axially the tip with the channel outlet with ~70 μm distance between them. Lastly, the guiding tube was fixed on the glass plate by epoxy.

During the experiments, the CE chip with the integrated detection cell was fastened to a plexiglass holder so that a cylindrical electrode could be inserted into the guiding tube with the copper conducting wire fastened to the movable electrode clamp. Driven by the precision screw and piloted by the guiding tube, the electrode tip can approach the channel outlet gradually. The elasticity of the thin copper wire will protect the electrode tip against rough movement or shock.

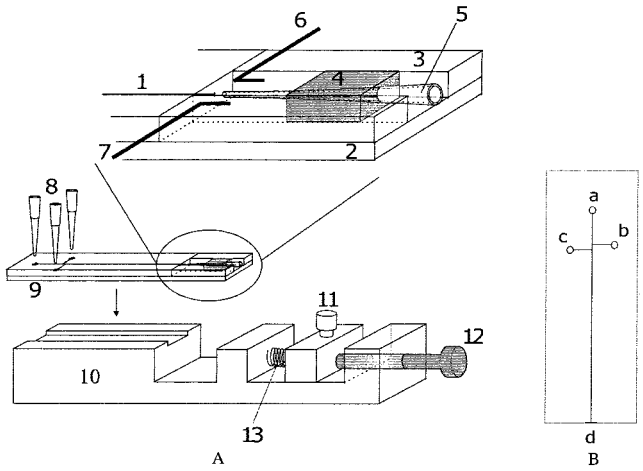

Figure 1. Capillary electrophoresis chip with electrochemical detection. A. Configuration of electrophoresis system with integrated electrochemical detection cell: 1, separation channel; 2, glass plate; 3, plexiglass block; 4, epoxy; 5, guide tube; 6, Pt wire; 7, Ag/AgCl wire reference electrode; 8, pipet tips; 9, capillary electrophoresis chip; 10, plexiglass body; 11, electrode holder; 12, precision screw; 13, spring. B. Layout of the chip: (a) buffer reservoir, (b) sample reservoir, (c) sample waste reservoir, (d) detection reservoir.

A high voltage supply (0−30 kV, 0−30 mA, Institute of Nuclear Science, Chinese Academy of Sciences) and a home-built relay box were used to provide potentials for injection and separation. Amperometric determinations and other voltammetry experiments were performed with a CHI 660A electrochemical workstation (CH Instruments, Shanghai, China) in conjunction with a PIII 550 computer. To minimize the interference of external electronic noise, the whole microsystem and the electrochemical workstation were housed in a well-grounded Farady cage.

Chemicals. Dopamine (DA), epinephrine (E) and 5-hydroxytryptamine (5-HT) were all received from Sigma (St. Louis, MO). All other reagents were of analytical grade. Ultrapure water (Water Pro Plus, Labconco) 18 MΩ was used to prepare all solutions. Stock solutions of the neurotransmitters (1 mM) were obtained weekly by dissolving them in 0.1 M $HClO_4$ and were stored in a refrigerator. Unless specified, stock solutions were diluted to the desired concentrations with 50 mM Tris−HCl buffer (pH 7.5) prior to use. All solutions for the electrophoresis experiments were centrifugalized at a rate of 12 000 rpm for 10 min to deposit microparticles in the solutions.

Microdisk Electrodes. Carbon fiber microdisk electrodes were made by using a method reported previously by our group.[31] A 0.9-mm-i.d. glass capillary was pulled on the flame of the gas lamp to form a segment with ~0.1-mm i.d., and then this segment was straight-tapered again near the outer flame to get a small tip of ~20−25-μm o.d. and >7-μm i.d. A cleaned carbon fiber (7 mm in diameter, Goodfellow Co., Oxford, U.K.) was connected to a

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copper wire with silver print conductive paint (GC Thorsen, 1801 Morgan St. Rockford, IL). The carbon fiber−copper wire was inserted from the other end of the capillary into the capillary tip with a small segment protruding from the tip, and then the tip was rapidly placed close to the outer flame of the gas lamp. The capillary tip was fused slightly to form a solid glass cylinder, which sealed the carbon fiber tightly. A portion of this cylinder with a fresh scalpel was cut under the inverted microscope to expose a disk surface of the carbon fiber. The tip was inserted into the outer flame again to fuse the insulating glass for eliminating the cracks. Finally, the copper wire and capillary interface was sealed by epoxy and the carbon fiber microdisk electrode was prepared. Such construction offers the electrode tip a gentle slope to meet the frame of the guiding tube. Pt microdisk electrodes (30 μm) were also made by the same process. The electrodes were cleaned by sonication for 10 min in acetone and ultrapure water prior to use.

Electrophoresis Procedure. Electrophoresis separation was carried out in uncoated channels that had been flushed with 0.1 M NaOH for 30 min; with ultrapure water for 10 min; and finally, with Tris−HCl buffer for 40 min. Injection was performed by applying electrokinetic injection. Voltages were applied to both the sample and buffer reservoirs, with the sample waste reservoir grounded and the detection end floated. In this injection mode, the intersection volume rather than the injection time approximately determines the final injection volume. To fill the intersection of the channel with the sample solution, sufficient time (>30 s generally) is required. Once injection was completed, a separation potential was applied to the buffer reservoir while the detection end remained ground. At the same time, two 60% separation potentials were applied to both the sample and sample waste reservoirs, respectively, to avoid the leakage of analytes from the sample channel to the separation channel.

Electrochemical Detection. The Ag/AgCl reference electrode was prepared by anodic oxidization of a clean Ag wire in 1% hydrochloric acid. In electrode characterization experiments, a standard three-electrode setup was used, and all amperometric determinations were performed using a two-electrode setup at the constant detection potential of 0.8 V (vs Ag/AgCl).[32] Before injection, the working electrode was polarized at the detection potential to obtain a stable electrochemical surface. The electropherograms with a time resolution of 0.2 s were recorded, processed, and stored directly by the computer.

RESULTS AND DISCUSSION

The cyclic voltammetric measurements in 1 mM potassium ferricyanide solution in 0.5 M KCl (pH 3) were performed routinely to determine the electrochemical behavior of the homemade microelectrodes. The electrodes that could show steady and well-defined sigmoidal voltammograms were considered qualified for electrochemical detection.[31]

For electrochemical detection, the influence of separation voltage on detection is a significant concern with respect to improving detection sensitivity. Figure 2 exhibits the influence of separation voltage on the detection of 0.1 mM dopamine (DA) and epinephrine (E). The very low noise level (<1.6 pA, peak-to-peak) and the slight increase of the noise (from 0.6 to 1.6 pA)

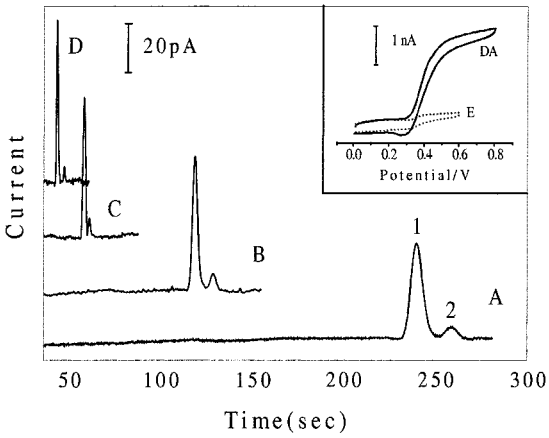

Figure 2. Influence of the separation voltage on the detection of 0.1 mM dopamine (1) and epinephrine (2). Separation voltages: (A) 500, (B) 1000, (C) 2500, and (D) 3000 V. Conditions: injections were performed at 60% separation voltage for ~50 s; separation and detection were performed as detailed in the Experimental Section using a channel-electrode spacing of 30 ± 5 μm. The attachment is the cyclic voltammograms of 0.1 mM DA and E in 50 mM Tris−HCl buffer solution (pH 7.5) at 100 mV/s scan rate using the same carbon fiber microdisk ekectrode.

indicate an effective isolation of the high voltage. As expected, by enhancing the separation electric field, the migration time and the half-peak width dramatically decrease, from 241.0 to 42.2 s and from 9.8 to 1.4 s, respectively. Meanwhile, a relatively gentle ascent of the amperometric signal with the increase of voltage was observed, because as the migration speed of analyte zone in the channel is enhanced, the wall-jet effect on amperometric detection is also strengthened and, thus, results in the larger peak current.[35] However, the increasing interference of the high voltage leads to a more complex variation of separation efficiency and detection sensitivity, represented by the theoretical plate number and the limit of detection, respectively. For DA, the highest theoretical plate number (4800) and the lowest detection limit (8.2 $\times$ 10^{-7} M) emerged at 2500 and 1000 V, respectively. The limit of detection was estimated according to the triple of the peak-to-peak noise. Thus, the general separation potential was chosen from 1000 to 2500 V.

Another important concern is achieving and maintaining the proper alignment of the working electrode. Poor axial placement of the electrode relative to the capillary bore will cause a significant decrease in detection coulometric efficiency and, hence, lower sensitivity.[32] The end-column electrochemical detection is limited in conjunction with a narrow capillary (≤25 μm). The difficulties in precise and reproducible alignment of the electrode with such a narrow capillary impede the routine use of this mode. Recent research on decoupler-free electrochemical detection using 50-[36,37] and 75[38]-μm-i.d. capillaries overcomes these difficulties. Additionally, great efforts have been put on developing various end-column detectors.[32,35,39,40]

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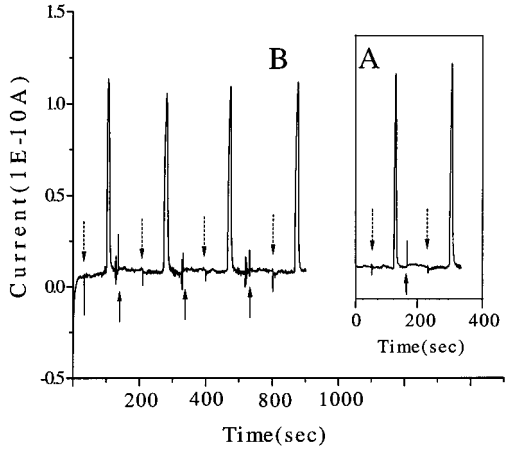

Figure 3. Electropherogram for consecutive injections of 0.1 mM dopamine. Separation voltage, 2 kV; other conditions as in Figure 2; (A and B share the same ordinate) → sampling ····> separating.

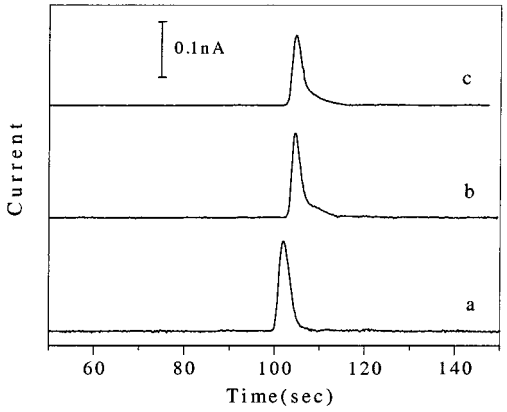

Figure 4. Electropherograms for 0.1 mM 5-HT at the channel-to-electrode of (a) 0 ± 5, (b) 30 ± 5, and (c) 50 ± 5 μm. Other conditions as in Figure 3.

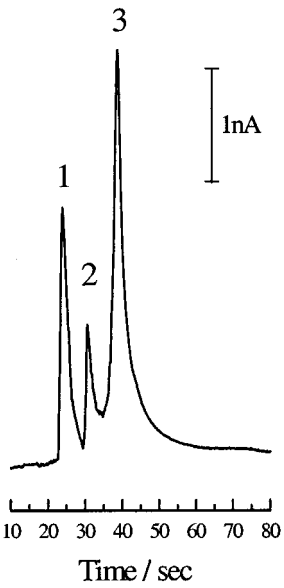

Figure 5. Electropherogram for 0.1 mM (1) dopamine, (2) 5-HT, (3) epinephrine. Conditions: 25 mmol/L Tris−HCl buffer, pH 7.4; injection voltage, 2 kV; separation voltage, 4 kV; detection at 0.8 V (vs Ag/AgCl).

In our design, a tapered glass tube (termed guiding tube) with a tip of ~30-μm i.d. was fixed with accurate alignment with the channel outlet. Such a configuration ensures that the radial deviation between the channel outlet and the electrode (~20−25-μm o.d.) can be controlled to less than ±5 μm. Matysik discussed the effect of capillary-to-electrode positioning on hydrodynamic mass transport contribution and dispersion and demonstrated that a remarkable detection reproducibility can be obtained if the electrode is located in a small range away from the axis of capillary.[38] On the basis of this result, the configuration of our design is expected to have a satisfactory reproducibility. Figure 3 shows the result of investigating the reproducibility of the system using the same electrode. In Figure 3A, two consecutive injections and separations were performed normally, and then we gently vibrated the chip holder to change the radial position of the working electrode before every injection, but almost the same results as Figure 3A were obtained, as shown in Figure 3B. The relative standard deviation for the peak areas is 4.2% and for peak height is 3.2% (n = 6), displaying very good reproducibility of the electrochemical detector.

Figure 4 examines the effect of the channel-to-electrode distance upon the detection. In general, the closer the electrode is positioned away from the capillary outlet, the higher the sensitivity is because of the reduced dispersion of the analyte zone when ejected from the end of the capillary to the electrode surface. However, a very small capillary-to-electrode distance will result in relatively intense interference of separation voltage on detection, decreasing the sensitivity. Using a microdisk electrode with a diameter smaller than that of the capillary and selecting an appropriate capillary-to-electrode distance can ensure effective hydrodynamic transport of the analyte zone toward the electrode surface and minimize the influence of the high electric field.[38] The electropherograms of 0.1 mM 5-HT solutions at different electrode positions of 0 ± 5, 30 ± 5, and 50 ± 5 μm display no dramatic variations in signal strength (from 127.9 to 164.4 pA), noise level (from 0.6 to 1.0 pA), and retention time (from 101.7 to 106.8 s). These results indicate that the good efficiency of the hydrodynamic mass transport can be maintained when the capillary-to-electrode distance is limited within a small range (<70 μm in the case of our design). The highest plate number (14 200) and the lowest detection limit (1.4 μM, S/N = 3) are both obtained at 30 ± 5 μm (n = 3) as a result of the combination of low noise level and good efficiency of mass transport.

Our design facilitates rapid replacement of electrodes. Additionally, this microsystem allows using electrodes of various materials. Figure 5 demonstrates the utility of the 30-μm Pt microdisk electrode for analyzing a mixture of DA, E, and 5-HT. A larger guiding tube (~46-μm i.d.) was used here to match the size of the Pt electrode. Also revealed by this advantage is the significant convenience of using a variety of chemically modified electrodes in chip-based CE.

One criterion of the detector is the detection performance at low analyte concentrations. As depicted in Figure 6, a well-defined peak of dopamine was recorded at the 5×10^{-7} M concentration, which is close to the detection limit. The calculated concentration limit of detection is 2.4×10^{-7} M (S/N = 3) (Table 1). This value is one order of magnitude lower than those reported for the use

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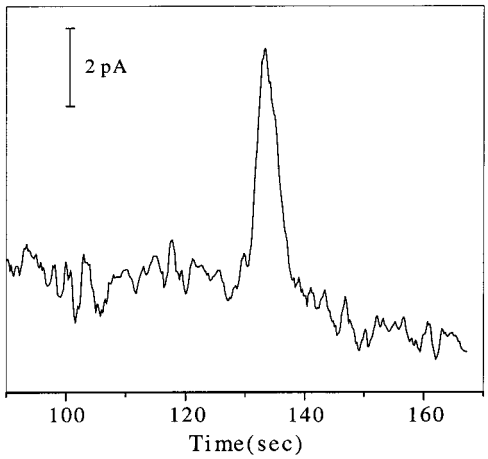

Figure 6. Electrophoretic separation of 5×10^{-7} M dopamine at 1 kV; other conditions as in Figure 2.

Table 1. Comparison of Detection Limits for the Neurotransmitters in Electrochemical Detection with Microchip Capillary Electrophoresis

catecholamines	electrodes	detection limits (M)	ref
dopamine	Pt film[a]	3.7×10^{-6} (S/N = 2)	23
dopamine	Au film[a]	1.0×10^{-6} (S/N = 2)	24
dopamine	carbon film[b]	3.8×10^{-7} (S/N = 3)	25
dopamine	carbon fiber[c]	2.4×10^{-7} (S/N = 3)	this work
catechol	Au band[d]	4.0×10^{-6} (S/N = 3)	29
catechol	carbon paste[e]	1.0×10^{-6} (S/N = 3)	26

[a] Plasma-sputtered metal thin film on the outlet of the separation channel. [b] Screen-printed carbon thick film on the ceramic plates. [c] The end-column electrochemical detection based on the microdisk electrodes. [d] Deposited gold band on glass plate. [e] Filled the carbon paste in the electrode channel.

of permanently fixed electrodes[23,24,29] and slightly excels the one obtained with the carbon thick-film electrode(3.8×10^{-7} M).[25] The detection limit can be decreased further by further optimizations of parameters in separation and detection.

The high sensitivity should be attributed to the introduction of the end-column detection based on the microdisk electrode. The signal of amperometric detection is dependent on the efficiency of the oxidation of the analyte. In end-column amperometric detection, such efficiency is proved to be critically dependent on the precise alignment of the working electrode with the outlet of the separation channel.[38] With the same separation and detection conditions, the central alignment of the working electrode with the capillary outlet leads to the highest coulometric efficiency and the best detection sensitivity.[32] As compared to the previous configurations with deposited thin-film electrodes,[23,24,28,29] our design offers a central placement of electrodes, which permits the electrode surface to touch a larger portion of the analyte plug when it is pushed out of the separation channel by the applied potential; thus, a higher sensitivity can be expected.

CONCLUSIONS

A microchip capillary electrophoresis system with end-column electrochemical detection using homemade microdisk electrodes is reported. Several attractive advantages are associated with the novel design. It eliminates the limitation of the existing configurations that results from depositing immobile electrodes either on the inner walls of the separation channels[31] or on the off-channel walls.[23,24,29] The flexibility of exchanging working electrodes benefits its practical applications greatly, particularly those requiring convenient electrode replacement. For example, compared to the fixed electrode, such a configuration allows easier surface modification of the electrode without adding adverse effects to the separation. In addition, a more extensive scope of substances can be analyzed in one device because of the ability of using different material electrodes or modified electrodes. The microsystem is provided with relative simplicity, remarkable reliability, high sensitivity, and attractive applicability, as indicated in the above sections. Work on optimization of the design and practical applications is in progress in our laboratory.

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