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Short communication

Electrochemical detector for microchip electrophoresis of poly(dimethylsiloxane) with a three-dimensional adjustor

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Abstract

This paper presents an electrochemical detector for poly(dimethylsiloxane) (PDMS) microchip CE with a three-dimensional adjustor which makes it possible to accurately align a separate working electrode that can be easily fabricated in laboratory to the uncertain PDMS microchannel outlet. The substantial influence of the electrode—PDMS microchannel distance was investigated. The optimal electrode-outlet distance was found to be 10 μm for the PDMS microchannel with the width of 50 μm due to its relatively slow electroosmotic flow. Adrenaline and catechol were well separated, with a linear response range from 20 μM to 1 mM, and a detection limit of 2 μM for catechol, using carbon disk electrode (diameter of 300 μm). Furthermore, arginine and histidine can be well separated and detected directly in the PDMS microchannel using a Cu disk electrode (diameter of 150 μm).
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1. Introduction

Detectors for microchip capillary electrophoresis (CE) play an important role in the miniaturization of analytical devices [1,2]. Because of its high sensitivity and easy miniaturization, electrochemical detection can be effectively used for microchip systems in three forms: amperometry, conductivity and potentiometry [3–5]. The settled location in glass or quartz microchannels ensures the satisfactory amperometric detector for microchip CE with two approaches. One is to fix the working electrode in or just outside the exit of microchannels [6–9]. Considering easy contamination of ampermetric detectors, another approach is to setup the separate detector outside the glass or quartz channels reported by Wang et al. [10,11] and Zeng et al. [12]. To avoid the disadvantages, such as the longer fabrication time and the higher cost for the glass or quartz microchannels, some kinds of polymers with simple and easy fabrication have been introduced into the construction of microchannels. Low-price poly(dimethylsiloxane) (PDMS) microchannels introduced by Whitesides and co-workers [13,14] combined with amperometric detectors could provide a very good insight for the low cost systems [15,16]. And Lunte and co-workers [17–19] reported such systems by putting the working electrode on the PDMS bottom.

However, with the soft PDMS microchip, it is much more difficult to accurately keep the same location of the microchannel in the level of micrometer each time for PDMS microchip than for the glass or quartz one. Under this circumstance, it is difficult for the PDMS microchips to be applied in previous reported systems [10–12] with separate amperometric detectors. In our previous report [16], separate amperometric detector for PDMS microchip CE was realized using a manipulator with four screws. On the basis of the improvement of that system, we here report a new electrochemical detector for PDMS microchip CE with a three-dimensional (3D) adjustor and the optimization of the influence of the microchannel outlet-electrode with that new system.

2. Experimental

All reagents are of analytical grade. Sylgard 184 was from Dow Corning (Midland, MI, USA). Arginine, histi-

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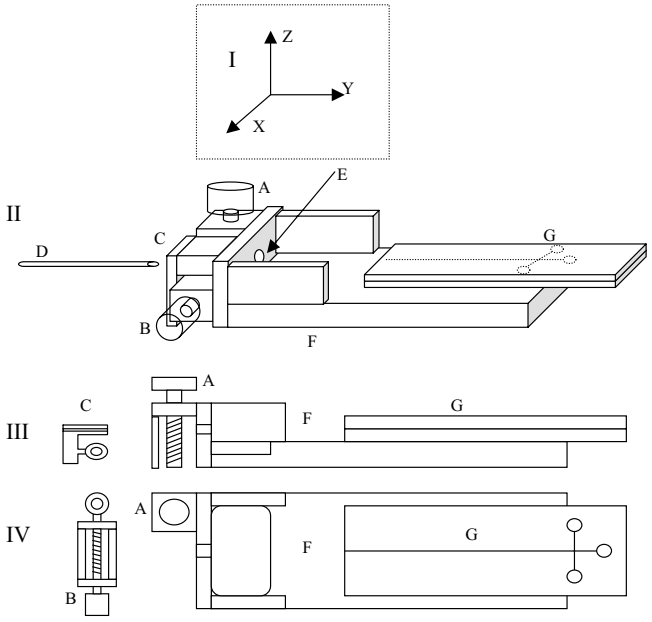

Fig. 1. Schematic representation of a PDMS microchip holder integrated with a three-dimensional adjustor for working electrode. (I) *X*, *Y* and *Z*-directions; (II) three-dimensional view of the holder; (III) side view of the holder (not including B); (IV) platform of the holder; (A) the *Z*-direction adjustor, (B) the *X*-direction adjustor, (C) working electrode holder, (D) working electrode, (E) electrode hole, (F) body of the PDMS microchip holder, (G) PDMS microchip.

dine, adrenaline and catechol were purchased from Sigma (St. Louis, MO, USA). NaOH, Na_2HPO_4, KH_2PO_4 and were purchased from Nanjing Chemical Reagents Factory (Nanjing, China). All solutions were prepared with doubly distilled water and passed through a 0.22 μm cellulose acetate filter (Xinya Purification Factory, Shanghai, China).

2.1. Apparatus

The laboratory-made Plexiglass microchip holder (Fig. 1) integrated a 3D adjustor for controlling the location of working electrode. The total system size is 12 cm long (*Y*), 5 cm wide (*X*) and 3 cm high (*Z*). The detailed state about the interface between the working electrode and the microchannel outlet can be clearly scrutinized and adjusted under a stereoscopic microscope with micro-ruler (XTB-1; Jiangnan Optical Instrument Factory, Nanjing, China). A homemade power supply provides a stable and continuously variable voltage ranging from 0 to 5000 V. A straight separation PDMS microchannel with cross sampling channels was made based on a master composed of a positive relief structure of GaAs for the channels microfabricated in No. 55 Electronic Institute (Nanjing, China) by using standard microphotolithographic technology. The sampling channel of 30 μm width and 18 μm depth and the separation channel of 50 μm width and 18 μm depth were used for all experiments. The total length of the separation channel was 4 cm.

A 0.5 mm inner diameter glass capillary was pulled to form a tip of about 300 μm diameter by a multi-function glass microelectrode puller (Shanghai Biological Institute). Then a 300 μm pencil lead (Marsmicro Polycarbon, Staedtler, Germany) was inserted into the tip of the pulled glass capillary and sealed with epoxy. A Cu wire was inserted in the other end of the glass capillary and was contacted with the pencil lead by soldering tin. Before use, the electrode was polished and ultrasonically cleaned. The 150 μm Cu disk electrodes used for the detection of amino acid were constructed according to the same procedure as in our previous report [16].

2.2. Electrophoretic and electrochemical procedure

In all cases, degassed buffer was introduced into the reservoirs and flushed through the PDMS/PDMS channel via vacuum. With a laboratory-developed program, the microchannel conditions can be judged by the separation or sampling current displayed in the computer screen and the power can be controlled switching the voltage from sampling to separation. In a routine CE procedure, separation voltage was 1600 V, sampling voltage was 800 V and sampling time was 10 s. Sampling mode was simple crossing without pinch. Electrochemical detection was carried out with a three-electrode system including a carbon or a Cu disk working electrode, Ag/AgCl reference electrode and Pt wire auxiliary electrode on an Electrochemical Workstation 832 (CH Instruments, USA) connected to a personal computer.

3. Results and discussion

When the amperometric detectors are used for microchip CE, their axial alignment plays a determining role because it would directly influence the mass transport on the electrode surface and then the detection sensitivity. With our previous equipment, the location of the working electrode was adjusted directly through the contact points of four screws. In such a point-controlling way, the adjustment of the working electrode at one direction will bring about the orientation change of the working electrode tip and the variation of amperometric response. To overcome this disadvantage, in the new equipment, the working electrode is adjusted through changing the location of the whole electrode holder (shown in Fig. 1 III). In this way, the orientation of the working electrode tip cannot be changed any longer in other two directions when it is adjusted in one direction. Then the alignment of the working electrode along with the microchannel is easy and the optimization of the outlet-electrode distance can be further performed.

In the study of amperometric detectors for glass or quartz CE, regulations of the distance between the working electrode tip and the capillary outlet and its application have been studied [20,21]. The separation efficiency can be remained when the distance is less than the diameter of capillary so

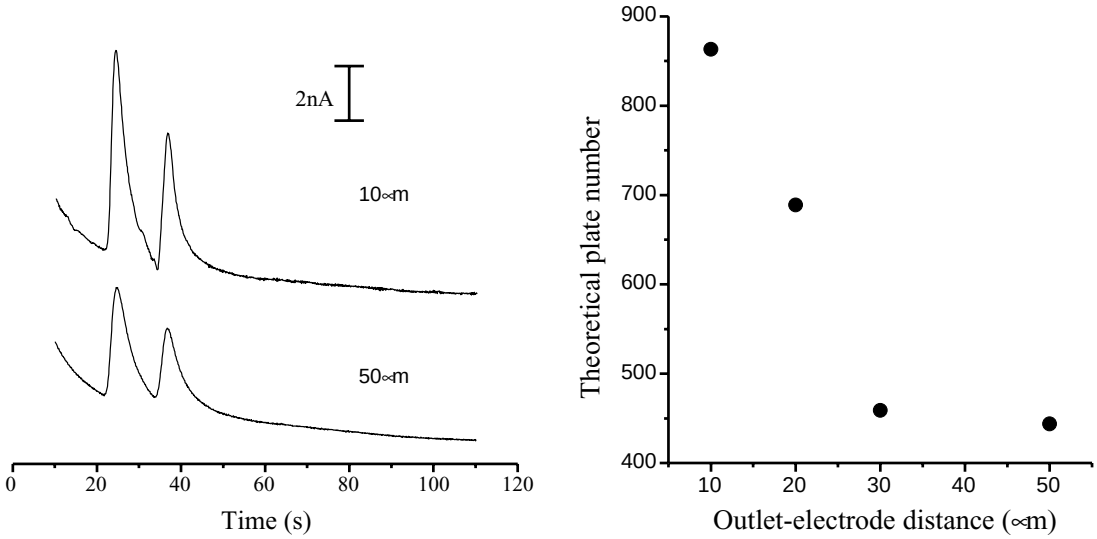

Fig. 2. Influence of the microchannel outlet-electrode distance on theoretical plate number for catechol (right) and electrophoretic separation of adrenaline (100 μM) and catechol (100 μM) (left). Experimental parameters: separation voltage: 1600 V; sampling voltage: 800 V; sampling time: 10 s; detection potential: 0.5 V (vs. Ag/AgCl); working electrode: carbon disk electrode (diameter: 300 μm); running buffer: phosphate-buffered saline (25 mM, pH 6.98).

that generally the distance is usually set at equal to the diameter of the capillary [20]. Such an important regulation has also been proved by experimental results with silica microchannels [7,10,12]. But with amperometric detectors for PDMS microchip CE, the influence of the interface between the electrode tip and the microchannel outlet on the amperometric response has not been optimized. It is well known that the migration of analytes at the microchannel outlet is mainly determined by the electroosmotic flow within certain electrode–channel distance [22]. Just outside the channel exit, analytes may begin to disperse all over the detection cell in various directions besides migrating to the working electrode surface. Considering the reported relatively slow speed of electroosmotic flow [23] in the PDMS microchannel, for the same electrode-microchannel distance, dispersion time of the analytes in the interface between the working electrode and the outlet is longer for a PDMS microchannel than for silica one, resulting in the more difficulty of effective mass transport for the analytes to the electrode and then the more broaden current peak. Therefore, in order to ensure well-defined mass transport, shorter electrode-channel distance is required for the PDMS-based system than for the silica-based one.

Our experimental results were coincident very well with above deduction. As shown in Fig. 2 (left), when the electrode-outlet distance was equal to the width of the PDMS separation channel (50 μm), the number of theoretical plates (444) for the detection of catechol was small. With the decrease of the distance from 50 to 30 μm, no obvious change of the number of theoretical plates was observed. However, with further decrease of the distance from 30 to 10 μm, the number of theoretical plate increased significantly (from 459 to 863). With a distance <10 μm, the revere interference would be observed because of the influence of separation high voltage. In addition, the detection currents at 10 μm distance for both adrenaline and catechol were more than 1.5 times as high as those at 50 μm, and the half-peak widths were considerably narrowed. These results demonstrated that a 10 μm electrode-channel distance is to be used as the optimal condition for effective separation.

Other parameters influencing the separation efficiency were also optimized. Our results displayed the influence of the separation voltage on the amperometric response and separation efficiency of adrenaline and catechol. Sixteen hundred volt was chosen as an optimal separation voltage according to the ohm curve. The detection potential of 0.5 V (versus Ag/AgCl) is chosen for experiments. In order to obtain an optimal separation efficiency and high sensitivity, sampling voltage of 800 V and sampling time of 10 s were chosen. In the case of the optimal experimental conditions, the calibration curves were linear with sensitivity of 73.8 and 44.5 nA/mM for adrenaline and catechol, respectively, and the correlation coefficients are 0.994 and 0.992 in the range of 20 μM to 1 mM. The detection limits for catechol was 2 μM ($S/N = 2$).

To further demonstrate the application of our system in the field of bioanalysis, arginine and histidine were separated and detected directly at the optimal electrode-microchannel distance of 10 μm. As shown in Fig. 3, arginine and histidine can be well separated in PDMS microchannel and directly detected with a Cu disk electrode of diameter 150 μm. Their theoretical plate number is around 800. The results confirm the validity of the 3D adjustor for the separate working elec-

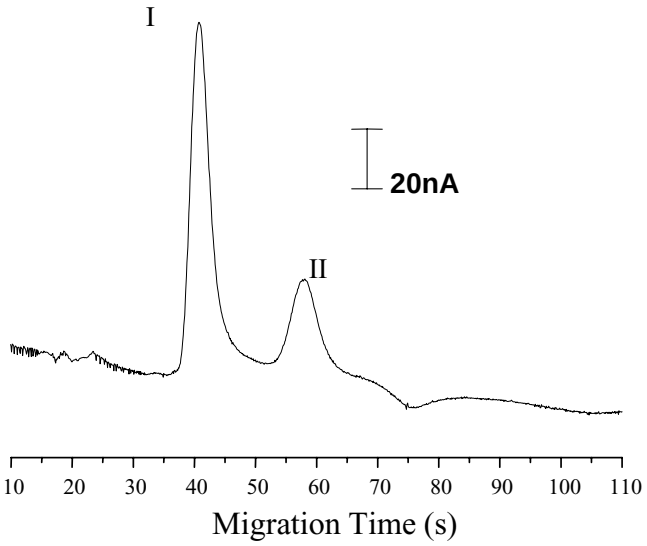

Fig. 3. Electropherogram of arginine (1.6 mM) (I) and histidine (1.2 mM) (II) in native PDMS microchannel. Experimental parameters: separation voltage: 1000 V; sampling voltage: 800 V; sampling time: 4 s; working electrode: Cu disk electrode (diameter: 150 μm); detection potential: 0.7 V; running buffer: NaOH (40 mM).

trode and the optimal PDMS microchannel–electrode distance.

4. Conclusion

An integrated three-dimensional adjustor for the separate amperometric detector is demonstrated to be an efficient approach for the separation and detection of some biomolecules in the soft PDMS microchip with the uncertain outlet location. The optimal microchannel outlet-electrode distance is to be much smaller than the width of the PDMS channel, which is obviously different from that of glass or silica channels. It would be worthy for the study of the separation and detection of more amino acids and other biomolecules in PDMS microchannel with this system.

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