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Direct determination of thiolated amino acids using poly(dimethylsiloxane) microchip capillary electrophoresis coupled with electrochemical detection

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Abstract : The determination of the biomarkers homocysteine, glutathione and N-acetyl-L-cysteine has been investigated using microchip capillary electrophoresis coupled with pulsed amperometric detection (PAD). The microchip configuration consists of a first layer of polydimethylsiloxane (PDMS) composed of injection and separation channels, reservoirs and a gold microwire sealed with a second layer of PDMS. A gold microwire was used as the working electrode and platinum microwire located in the waste reservoir was used as a counter electrode. The experiments were carried out with 20 mM boric acid buffer (pH 8.5) and compared to 20 mM MES and 1 mM SDS buffer (pH 6.0) at the detection potential of 0.7 V. The effect of including injection and separation potential, pH and injection time, and PAD parameters were studied to optimize the separation and detection. The results showed that homocysteine, glutathione and N-acetyl-L-cysteine could be separated and detected in less than 100 s with 20 mM MES and 1 mM SDS buffer (pH 6.0), with more reproducibility and sensitivity than a standard boric acid buffer. This method was applied to determine homocysteine, glutathione and N-acetyl-L-cysteine in human plasma, with the intent indicating cardiovascular disease.

Keywords: PDMS Microchip capillary electrophoresis, Pulsed amperometric detection, homocysteine, glutathione and N-acetyl-L-cysteine

Introduction

Microchip capillary electrophoresis (μCE) has been of strong interest in biochemical analysis. Due to it is high sensitivity, portability, rapid, low cost, and low consumption of samples and reagents [1]. Initially, most microfluidic systems were fabricated from glass, quartz or silicon. These materials are not accessible to every laboratory and expensive. Therefore, polymer materials such as poly(methyl methacrylate) (PMMA), polycarbonate (PC), and poly(dimethylsiloxane) (PDMS) have been used for construction of microchips instead of glass and quartz. In particular, poly(dimethylsiloxane) (PDMS) becomes a popular material due to its easy fabrication, low curing temperature, robust and good optical transparency [2].

Detection modes for microchip-CE include laser induced fluorescence (LIF) and electrochemical detection (ECD). LIF is expensive and requires analyte derivatization. Thus, electrochemical detection is an attractive detection mode because it is suitable for small molecules, high selectivity for easily oxidized analytes, compatible with microelectronic technology, and does not require analyte derivatization [3]. Unfortunately, dc amperometry is subjected to electrode fouling. This effect occurs when measuring thiols, phenols, and carbohydrates. For avoid this effect, pulsed amperometric detection (PAD) is used for detection. PAD is a three-potential waveform technique. First, the cleaning electrode surface step is applied at a high positive potential, followed by reactivating the electrode surface at a negative potential. Finally, measurement of the analytes is accomplished at a moderate positive potential [4].

Sulfur-containing amino acids such as homocysteine (Hcy), glutathione (GSH) and *N*-acetyl-L-cysteine (NAC) are important biomarkers for biological systems and metabolism. Increased levels of Hcy are related to cardiovascular disease [5]. Decreased levels of GSH are a risk to immune deficiency, heart diseases, and diabetes [6]. NAC is used for treatment of congestive and paracetamol intoxication [7]. Conventional methods such as liquid chromatography [8] and capillary electrophoresis have been reported for the determination of thiols compounds.

In this report, the use of a PDMS microchip coupled with PAD is used to detect Hcy, GSH and NAC. Dopamine and catechol were selected to evaluate the PDMS microchip. The effects of separation and detection for the analytes were studied.

Experimental

Fabrication of PDMS microchip

The fabrication of PDMS microchip was slightly modified from the previous report [9]. Briefly, Sylgard 184 silicone elastomer and curing agent were mixed in the ratio of 10:1 (w/w) and degassed by vacuum. The mixture was poured onto silicon wafer both a molding master and a blank wafer and then was cured in an oven at least 2 h at 65^{O}C. The cured PDMS was peeled off from the mold and reservoirs (6 mm diameter) were made at the end of each channel. Next, The 25 µm gold wire was placed in predesignated electrode channel. Then, the PDMS layers were treated and oxidized for 45 s in air plasma and immediately attached together to form an irreversible bond. The extremities of the electrode channel were sealed with super-glue. Finally, an electrical connection of the working electrode was produced by silver paint and a copper wire. The configuration of PDMS microchip is shown in Fig.1.

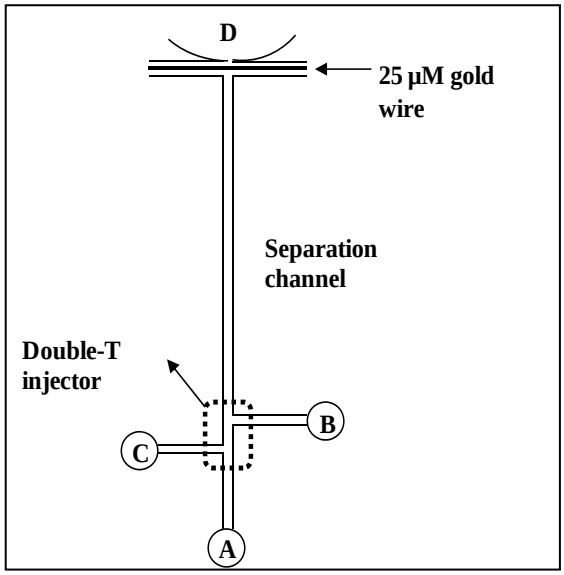

Figure 1 : *Pattern of PDMS microchip (A) buffer reservoir, (B) sample reservoir, (C) sample waste reservoir and, (D) waste reservoir. Channels: 50 µm width, 50 µm depth. Separation channel: 52 mm length. Double-T injection channel: 100 µm length.*

Electrochemical detection

The detection of dopamine and catechol was studied to demonstrate the performance of our devices using amperometry. The detection of thiol compounds was studied using PAD [10]. Both amperometry and PAD experiments were run with a two-electrode configuration. The gold wire was used as the working electrode. The second electrode, a Pt wire placed in the waste reservoir (D in Figure.1) was used as the counter electrode. Prior to use, the channels were treated with 0.1M NaOH to generated negative charge at the PDMS surface.

Results and discussion

Microchip capillary electrophoresis characterization

The PDMS microchip was first characterized to demonstrate performance of PDMS microchip using dopamine and catechol as model compounds. An electropherogram of 250 µM dopamine and catechol using 20 mM MES (pH 7.0) as the running buffer and 0.8 V the detection potential is shown in Fig. 2. The migration time of dopamine and catechol were found to be 45 and 73 s, respectively. These results indicated that this PDMS microchip can be used for separation of the thiol analytes.

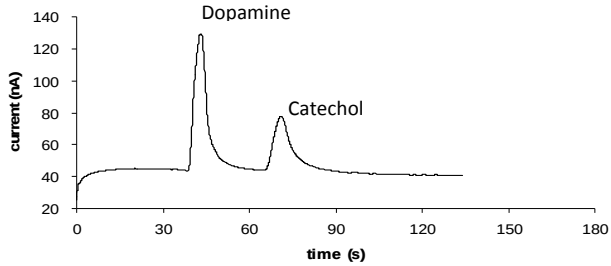

Figure 2 : Electropherogram of 250 µM dopamine and catechol. Experimental conditions: detection potential, 0.8 V; separation voltage, 1300 V; pinched injection time, 20 s; running buffer, 20 mM MES (pH 7.0).

Analysis of thiol compounds

The electropherogram of the separation of Hcy, GSH and NAC are shown in Fig. 3. The separation of these compounds was performed using 20 mM boric acid (pH 8.5) as the running buffer. The migration times of Hcy, GSH and NAC were 37 s, 54 s and 64 s, respectively.

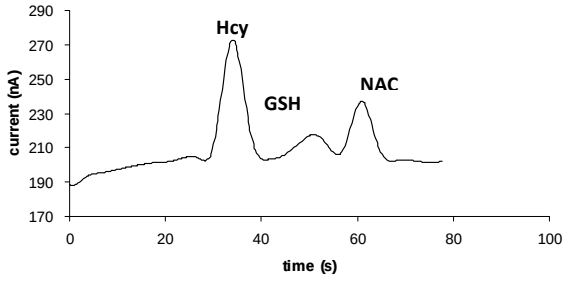

Figure 3 : *Electropherogram of 250 µM Homocysteine, glutathione and N-acetyl-L-cysteine. Experimental conditions: detection potential, 0.7 V; separation voltage, 1300 V; pinched injection time, 20 s; running buffer, 20 mM boric acid (pH 8.5).*

The running buffer was changed from 20 mM boric acid (pH 8.5) to 20 mM MES with 1 mM SDS (pH 6.0). SDS was added to stabilize the electroosmotic flow (EOF). The peak shape, sensitivity and reproducibility of Hcy, GSH and NAC were greatly improved when used 20 mM MES and 1 mM SDS as the running buffer. Fig. 4 shows electropherogram of Hcy, GSH and NAC with using 20 mM MES and 1 mM SDS (pH 6.0). The migration times of Hcy, GSH and NAC were 40 s, 55 s and 63 s, respectively.

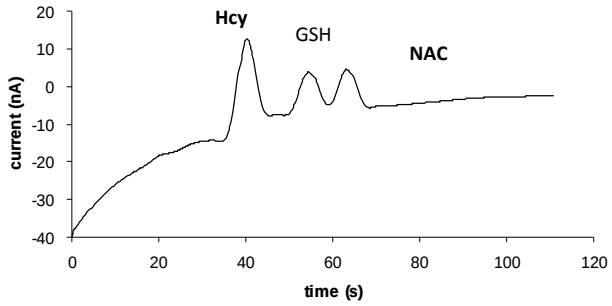

Figure 4*: Electropherogram of 150 µM Homocysteine, 200 µM glutathione and 200 µM N-acetyl-L-cysteine. Experimental conditions: detection potential, 1.0 V; separation voltage, 1300 V; pinched injection time, 20 s; running buffer, 20 mM MES and 1 mM SDS (pH 6.0).*

Conclusion

The PDMS microchip capillary electrophoresis was constructed and used for separation of thiol compounds using pulsed amperometric detection. From the results, the use of SDS with MES buffer provided a better reproducibility and sensitivity. Therefore, MES buffer system including of SDS may be selected as the suitable buffer system for further experiments.

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