Notes

Surface Modification of Poly(dimethylsiloxane) Microchip by Using Cetyltrimethylammonium Bromide (CTAB)

Hyunju Choi,[†] Kyung-Bok Lee, Kuk Ro Yoon, Insung S. Choi, Seong IhI Woo,[‡] Kyungseong Choi,[†] Sang Chun Lee,[†] and Yongseong Kim[†,*]

Department of Chemistry, Korea Advanced Institute of Science and Technology (KAIST), Daejeon 305-701, Korea
[†]Division of Chemistry and Chemical Engineering, Kyungnam University, Masan 631-701, Korea
[‡]Department of Chemical and Bimolecular Engineering & Center for Ultramicrochemical Process Systems, Korea Advanced Institute of Science and Technology (KAIST), Daejeon 305-701, Korea
Received October 28, 2003

Key Words : PDMS, Microchip, Cetyltrimethylammonium bromide (CTAB), Dynamic coating, Electrosmotic flow (EOF)

In the analysis of many important biomolecules, a miniaturized microchip has shown a great potential in the areas of medicinal chemistry, biology, and environmental sciences.[1-3] A microchip equipped with sample pretreatment, separation, and detection is especially called Lab-on-a-chip, and has extensively been utilized in the analysis of DNA, proteins, amino acids, and hormones.[4-8] In the beginning, conventional materials such as quartz or glass were employed to fabricate the microchip.[9] Although those chip materials revealed a good compatibility with traditional chemistry, fabrication process is labor-intensive and expensive. Furthermore, the bonding of upper and lower part of the chip is sometimes troublesome.[10] Microchip produced by a plastic material such as poly(dimethylsiloxane) (PDMS) has shown a good material of choice since PDMS has good biocompatibility, facile bonding ability, high transparency for UV and fluorescence detection, and is cost-effective for the production.[11] In addition, it is much less fragile compared to quartz or glass, and can be constructed easily by molding or embossing. However, there are several problems associated with PDMS microchips. For example, many organic molecules and biomolecules are easily adsorbed on the PDMS surface due to the nature of hydrophobicity of a PDMS chip.[12,13] Moreover, this hydrophobicity limits the use of many organic solvents in the buffer except several alcohols. The electrosmotic flow (EOF) produced under high electric field tends to become unstable as a function of time.[14] A stable and constant electrosmotic flow is very important in the analysis since the resolving power markedly depends on the pattern of electrosmotic flow. In this study, a dynamic coating on the surface of PDMS was employed by using a cationic surfactant, cetyltrimethylammonium bromide (CTAB) in order to stabilize the electrosmotic flow. This system was applied to the analysis of RITC (rhodamine B isothiocynate)-labled homocystein and methionine as model compounds.

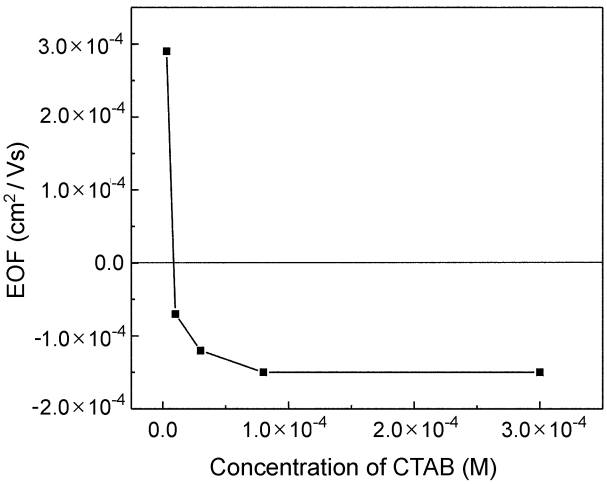

Figure 1. EOF as a function of CTAB concentration. Applied Voltage, 500 V; channel length, 4.0 cm, width, 80 μm, depth, 20 μm. EOF was measured by the current-monitoring method. Two different concentrations of TB buffer were employed for the determination of EOF.

Figure 1 shows the dependence of EOF as a function of the concentration of CTAB. EOF was measured by a current-monitoring method (see details on Experimental Section). As small amount of CTAB was gradually added in the buffer, EOF markedly dropped and then became constant at the concentration above about 0.8×10^{-4} M. The constant EOF means that the surface of PDMS chip was fully coated with CTAB under those concentrations.

The stability of EOF was investigated for the uncoated and the CTAB coated PDMS chips (Table 1). In this experiment, EOF was measured every 24 hours for 5 days using TB buffer (100 mM Tris, 20 mM boric acid, pH 9.3) with and without CTAB in the buffer. As shown in Table 1, EOF largely varied from $(2.3 \pm 0.2) \times 10^{-4}$ cm^2/Vs to $(4.1 \pm .2) \times$

Table 1. EOF measurement of uncoated and CTAB-coated PDMS chips

Days	Uncoated chip ($cm^2/V \cdot s$)	2.0×10^{-4} M CTAB-coated chip ($cm^2/V \cdot s$)
1	$(3.6 \pm 0.2) \times 10^{-4}$	$-(5.3 \pm 0.1) \times 10^{-4}$
2	$(4.1 \pm 0.2) \times 10^{-4}$	$-(5.0 \pm 0.1) \times 10^{-4}$
3	$(2.3 \pm 0.1) \times 10^{-4}$	$-(4.9 \pm 0.1) \times 10^{-4}$
4	$(2.3 \pm 0.2) \times 10^{-4}$	$-(5.0 \pm 0.2) \times 10^{-4}$
5	$(2.8 \pm 0.2) \times 10^{-4}$	$-(5.1 \pm 0.2) \times 10^{-4}$
Average EOF ($cm^2/V \cdot s$)	$(2.6 \pm 0.2) \times 10^{-4}$	$-(5.1 \pm 0.1) \times 10^{-4}$
Day-to-day Variation (range/average) $\times 100$ (%)	69%	7.8%

10^{-4} cm^2/Vs in the uncoated PDMS chip. In contrast, when the PDMS chip was dynamically coated with 2.0×10^{-4} M CTAB, EOF varied only from $-(4.9 \pm 0.2) \times 10^{-4}$ cm^2/Vs to $-(5.3 \pm 0.2) \times 10^{-4}$ cm^2/Vs.[15,16] Negative sign on EOF value means that the wall of PDMS channel became electrostatically positive due to the adsorption of positively charged cetyltrimethylammonium ion, which caused the change of the direction of EOF under the same electric field.[16] This fact provides the evidence that the wall of PDMS channel was effectively coated by CTAB. As shown above, day-to-day variation of EOF was as large as 69% in the uncoated PDMS chip. However, day-to-day variation of only 7.8% was achieved in the dynamically coated PDMS chip with CTAB.

Figure 2 shows the electropherogram of RITC-labeled homocystein and methionine in the PDMS chip. In our study, homocyetein and methionine were selected as model compounds since they are important in atherosclerotic and thromboembolic vascular disease. In methionine metabolism, homocystein is converted to methionine by methylenetetrahydrofolate reductase (MTHFR) with vitamine B_{12} (cofactor) and methyltetrahydrofolate. When this metabolism is blocked by some reasons (genetic defects of MTHFR and/or low level of cofactor), accumulation of homocystein (hyperhomocysteinemia) is caused, yielding vascular diseases. Since the traditional assay methods such as C-18 based HPLC and immunoassay for the measurement of the concentration of homocystein take long analysis time or procedure, the advent of new analytical technique is required. We believe that Lab-on-chip technique would provide a fast and cost-effective method of homocystein since the speed of separation is high under high electric field and the consumption of reagents and sample is very low. The potential use of PDMS chip for the fast analysis of homocystein and methionine is presented in Figure 2. The concentration of 2.0×10^{-4} M CTAB was arbitrarily chosen for the separation of RITC-labeled amino acids since this concentration of CTAB was in the stable region of EOF (see Figure 1). When the mixture of homocyetein and methionine were analyzed in the uncoated PDMS chip, no clear separation and band broadening were observed as shown in Figure 2(a) due to the interaction of RITC-labeled amino acids to the microchip channel surface. However, when the concentration of 2.0×10^{-4} M CTAB was employed for the dynamic coating of PDMS channel, almost baseline resolution of homocyetein and methionine was obtained, meaning that CTAB effectively provided the stable EOF, resulting in better resolving power. A Lab-on-a-chip equipped with the capability of the pretreatment process for the analysis of homocystein in blood serum is under progress in our laboratory.

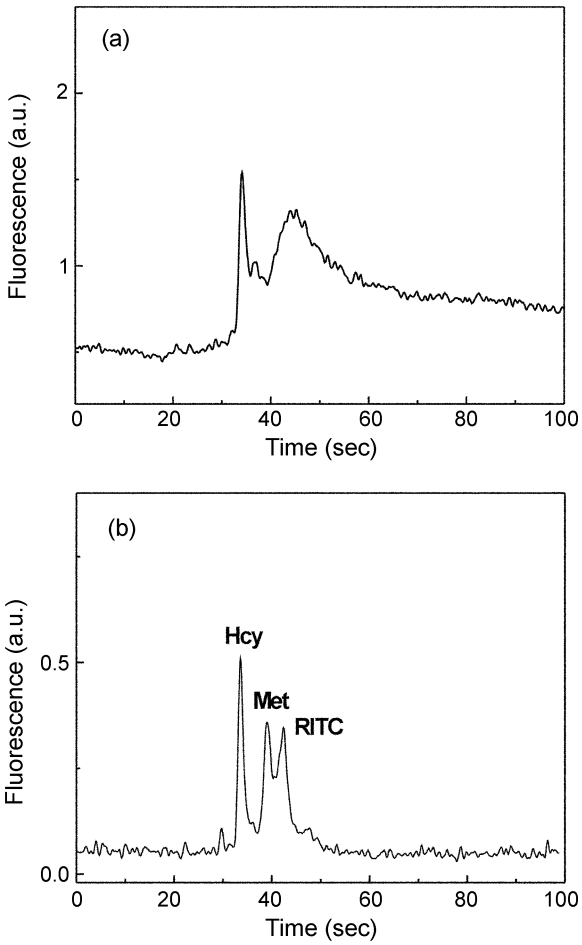

Figure 2. (a) Electropherogram of RITC-labeled amino acids in the uncoated PDMS chip. TB buffer (100 mM Tris, 20 mM boric acid, pH 9.3); electric field strength, 400 V/cm; injection time, 1 sec; a cross shape chip with separation channel length, 4.0 cm, width, 80 μm, depth, 20 μm; sample, 7.2 μM homocystein (Hcy), 6.7 μM methionine (Met); RTIC labeling, 1 : 1 (amino acid: RITC) for 24 hrs at dark at room temperature. (b) Electropherogram of RITC-labeled amino acids in CTAB coated PDMS chip. CTAB, 2.0×10^{-4} M; electric field strength, -450 V/cm; the other conditions were the same as those in Fig. 2(a).

Experimental Section

A master for PDMS microchip was constructed by using soft lithographic technique. The master had T-shaped convexed channel with 80 μm in width, 20 μm in height. Onto this master, PDMS oligomer and curing agent (10 : 1)

was poured and hardened at 75 °C for 3 hrs (for the lower part of PDMS chip). The upper and lower parts of PDMS chip were then bonded by Tesla coil (BD-10A, Electrotechnique Production, Inc., Chicago, IL, USA) with 2 min oxidation.

EOF was measured by a current-monitoring method.[17] Briefly, the reservoirs on both sides of separation channel were filled with TB buffer (100 mM Tris, 20 mM boric acid, pH 9.3). When the current became stable with 125 V/cm field strength, the reservoir on either side was replaced with lower concentration of TB buffer (50 mM Tris, 10 mM boric acid, pH 9.3). Under the same electric field, the current dropped to a certain point and became constant. EOF was calculated by the following equation,

$$\text{EOF} = L / tE$$

where L is the length of the microchannel, t is the time between the stable current values with two buffer system, and E is the electric field strength.

For the dynamic coating of PDMS surface, the channel was washed with deionized water for 5 min, 0.10 M NaOH for 5 min, deionized water for 5 min, 0.10 M HCl for 5 min, and deionized water for 5 min. After the washing, 2.0×10^{-4} M of CTAB was filled in the channel and let it sit for 30 min. The buffer containing 2.0×10^{-4} M CTAB, 100 mM Tris, 20 mM boric acid (pH 9.3) was filled in the channel for sample analysis. RITC (rhodamine B isothiocynate)-labled homocystein and methionine were injected into the separation channel for 1 sec by using gated injection mode. Fluorescence signal from RITC-labeled amino acid excited by 543 nm diode laser was collected through photomultiplier and the voltage for the flow control in the microchannel was manipulated by home-made Labview program.

Acknowledgment. This project was supported by Center for Ultramicrochemical Process Systems sponsored by KOSEF.

References

1. Waters, L. C.; Jacobson, S. C.; Kroutchinina, N.; Khandurina, J.; Foote, R. S.; Ramsey, J. M. *Anal. Chem*. **1998**, *70*, 158.
2. Ceriotti, L.; Shibata, T.; Folmer, B.; Weiller, B. H.; Robers, M. A.; Derooil, N.; Verpoorte, E. *Electrophoresis* **2002**, *23*, 3615.
3. Wakida, S.; Chiba, A.; Matsuda, T.; Fukushi, K.; Nakanishi, H.; Wu, X.; Nagai, H.; Kurosawa, S.; Takeda, S. *Electrophoresis* **2001**, *22*, 3505.
4. Sung, W. C.; Lee, G. B.; Tzeng, C. C.; Chen, S. H. *Electrophoresis* **2001**, *22*, 1188.
5. Jabssini, M.; Zhang, L.; Dang, F.; Xu, F.; Almofli, M. R.; Ewis, A. A.; Lee, J.; Nakahori, Y.; Baba, Y. *Electrophoresis* **2002**, *23*, 1537.
6. Liu, Y.; Foote, R. S.; Culbertson, C. T.; Jacobson, R. S.; Ramsey, R. S.; Ramsey, J. M. *J. Microcolumn Separations* **2000**, *12*, 407.
7. Koutny, L. B.; Schmalzing, D.; Taybr, T. A.; Fuchs, M. *Anal. Chem.* **1996**, *68*, 18.
8. Effenhasuser, C. S.; Manz, A.; Widmer, H. M. *Anal. Chem.* **2000**, *72*, 5731.
9. Seiler, K.; Harrison, D. J.; Manz, A. *Anal. Chem*. **1993**, *65*, 1481.
10. Liu, Y.; Fanguy, J. C.; Bledsoe, J. M.; Henry, C. S. *Anal. Chem*. **2000**, *72*, 5939.
11. McDonald, J. C.; Duffy, D. C.; Anderson, J. R.; Chiu, D. T.; Wu, H.; Schueller, O. J. A.; Whitesides, G. M. *Electrophoresis* **2003**, *24*, 161.
12. Lee, K.-B.; Kim, Y. S.; Choi, I. S. *Bull. Korean Chem. Soc.* **2002**, *23*, 29.
13. Duffy, D. C.; McDonald, J. C.; Schueller, O. J. A.; Whitesides, G. M. *Anal. Chem*. **1998**, *70*, 4974.
14. Ocvirk, G.; Munroe, M.; Tang, T.; Oleschuk, R.; Westra, K.; Harrison, D. J. *Electrophoresis* **2000**, *21*, 107.
15. Gao, H. W.; Zhou, D. Y. *Bull. Korean Chem. Soc.* **2002**, *23*, 29.
16. Wang, S. C.; Perso, C. E.; Morris, M. D. *Anal. Chem.* **2000**, *72*, 1704.
17. Huang, X.; Gordon, M. J.; Zare, R. N. *Anal. Chem*. **1988**, *60*, 1837.