

## 마이크로 전기영동 소자의 제작과 유로 면 특성에 따른 전기삼투 및 전기영동 효과

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### Fabrication of electrophoresis microchips and effects of channel surface properties on electrophoresis separation efficiency

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**Abstract** - We investigated the influence of the properties of substrate material on the separation efficiency in microchip electrophoresis. We fabricated the various microchips and studied separation efficiency in microchannels composed of a single material such as quartz, glass, polydimethylsiloxane (PDMS), and polymethylmethacrylate (PMMA), as well as hybrid micro channels composed of different materials. New fabrication process for glass chip was suggested and some treatment is added to improve fabrication process in other chip. Separation efficiency was compared by measuring migration times and bandwidths of EOF and analytes in each microchip. The efficiency is the function of migration time, which is affected by the electroosmotic flow (EOF), and bandwidth of an analyte. EOF is highly dependent upon the characteristics of a microchannel wall surface. Migration time was more reproducible in silica chips than that of PDMS chip and more band broadening was observed in the microchip composed of hybrid material due to non-uniformity of surface charge density at the walls of the channel.

#### 1. Introduction

Microchip electrophoresis is a promising analytical technique that has generated a great deal of interest. The advantages of microchip capillary electrophoresis (CE) include fast analysis time, consumption of minute amounts of sample volume, the possibility of electrokinetic control of fluids, the use of high separation field strength and easily coupling of various channels for sample concentration, mixing, dilution, reaction, etc [1,2,3].

Various separation modes and sample manipulation methods have been developed in microchip electrophoresis, enlarging the usefulness of microchip electrophoresis [4]. Moreover, microchip electrophoresis devices can be fabricated using a variety of materials such as glass, quartz, and plastics. Different surface characteristics can be obtained depending on chip material.

In microchip electrophoresis, in addition to the solutes, the buffer solution usually also moves through the capillary under the influence of an electric field. This phenomenon is termed electroosmotic flow. Typically, the width of the electric double layer is very small, so that the electroosmotic flow can be considered to be a wall-driven phenomenon. Consequently the separation efficiency is highly dependent upon the substrate materials and channel surfaces in microchip electrophoresis [5,6]. And the sample dispersion will be greater if the surface charge on the walls of the microchannel is nonuniform. Increased dispersion degrades the performance of electrokinetic separations by impacting the resolution of closely eluted peaks. It also affects the ability to control the movement of discrete samples within a micro fluidic device. One of the reasons that

electrokinetic pumping is so often used in microfluidics is that much lower sample dispersions can be achieved compared with pressure-driven pumping. Consequently, to successfully develop microfluidic devices it is essential to understand the effects of chip material and fabrication methods on the density and distribution of surface charges that ultimately determine the rate of dispersion under electrokinetic flow.

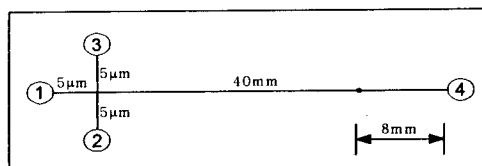
Generally microfluidic devices for capillary electrophoresis are fabricated in two step. First, closed network of channels is formed by etching, molding or machining into the substrate according to substrate materials. Second, other substrate is placed over the substrate which contains channel networks, and bonding process follows for sealing two substrate [7,8]. For an ideal plug flow, it is preferable to use same substrate material. But it may be difficult to do this using same material, because the process of bonding same material is restricted and heat used for bonding can degrade the structure of the microchannels in polymer substrate.

In this paper, we investigated the effects of chip material on electrophoretic separation efficiency in microchip electrophoresis. We fabricated microfluidic devices for capillary electrophoresis with silica substrate - quartz and glass and polymer substrate - PDMS and PMMA. We compared the separation efficiency by migration times and bandwidth of EOF and analytes in each chip, and we studied band broadening effects in capillary electrophoresis by comparing the results of the hybrid channels composed of PDMS/glass and glass/silicon dioxide membrane.

#### 2. Experimental

##### 2.1 Chemicals

2,7-Dichlorofluorescein (DCF) was from Merck (Darmstadt, Germany) and sodium fluorescein was from Junsei Chemicals (Tokyo, Japan). All reagents were of analytical grade and were used without further purification.



**Fig.1** Schematic layout and dimensions of the microchip. Dimensions are given in millimeters. Reservoirs are labeled (1) sample, (2) run buffer, (3) sample waste, and (4) buffer waste. The channel depth was 31  $\mu\text{m}$  and width at the bottom and top were 68 and 94  $\mu\text{m}$ , respectively. The dots represent the detection points, which are located at 32 mm from the injection cross.

1-mM stock solutions of fluorescein and DCF were prepared in a 10% (v/v) aqueous ethanol solution and a few drops of 0.1 M NaOH were also added before the solution was diluted to the desired sample concentration. Deionized water was obtained from a NANOpure purification system (Barnstead, Dubuque, IA, USA).

## 2.2 Fabrication of microchip

### 2.2.1 Microchip using quartz substrate

The quartz microchip was fabricated using standard photolithographic, wet etching, and bonding techniques [9]. Wet-etching mask of a Cr/Au (200Å/2000Å) layer was deposited on both side of the 500 μm thick quartz wafer by thermal evaporation. A positive photoresist (AZ1512; Clariant Corp., Somerville, NJ, USA) was then spin-coated on both side of the wafer, and the column design (Fig. 1) was transferred to the substrate using a film photomask (Ppm technology Corp., Seoul, Korea). After patterning, the patterned photoresist layer was hardened (110°C; 1 hr) and the unprotected Cr/Au layer was removed.

The microfluidic channels on quartz chip were then isotropically etched in 49% HF for 20 min (etching rate: ~1.5 μm/min) at 20°C. The channels were trapezoidal due to the isotropic etch with a depth of 31 μm and width of 68 and 94 μm at the bottom and top, respectively. The reservoir was fabricated by sandblaster using 20 μm sand with a 100 μm thick dry film resist (DFR; BF410, Tokyo Ohka Kogyo Co., Kanagawa, Japan) etch mask. The two wafers were directly bonded at room temperature and then annealed in a furnace (1000°C; 3 hrs) for concrete bonding.

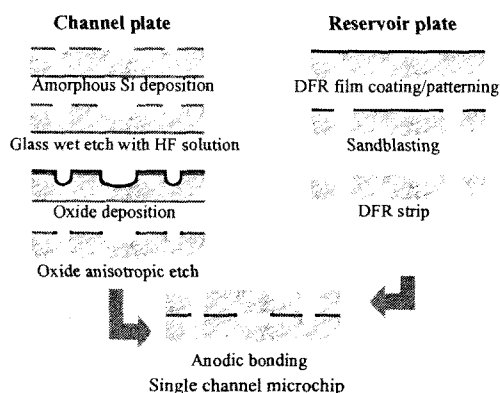
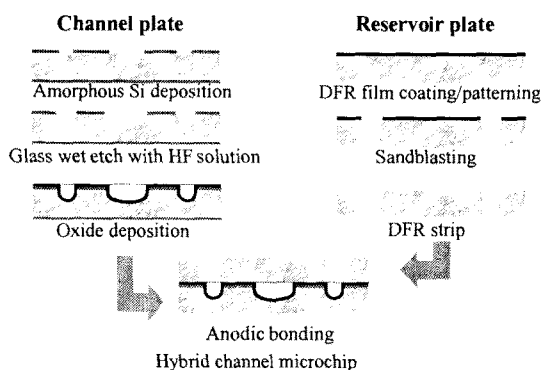


Fig.2 Proposed fabrication process for glass chip

### 2.2.2 Microchip using glass substrate

Similar to fabrication of quartz chip, microchannel in glass (borosilicate glass; pyrex 7740, Corning, New York, USA) chip was fabricated using standard photolithographic, wet etching process (Fig.2). But we used amorphous silicon layer for etch mask and anodic bonding layer. It is more reasonable to seal the microchannel by anodic bonding method, because the high temperature used for direct bonding method can degrade the structure of the microchannel besides poor bonded regions will often be made, so the process has to be done repeatedly [10]. After etching process, we remained the amorphous silicon layer and passivated the layer with silicon dioxide layer for insulation. And two substrates were bonded successfully by anodic bonding. This method is far easier and more exact than other glass bonding methods

### 2.2.3 Microchip using PMMA substrate

In PMMA electrophoresis chip, channel was fabricated based on standard lithographic, hot embossing process. The fabrication process for the PMMA electrophoresis chip is depicted in Fig. 3. Silicon deep reactive ion etching (DRIE) process was employed to create a master mold. A negative photoresist (AZ5214, Clariant Corp., Somerville, NJ, USA) was spin-coated on the silicon wafer.

After standard photolithographic process to pattern the channel, the silicon wafer was etched for 30 μm depth by DRIE process. Then hydrophobic material, fluorocarbon, was coated on the master mold, which made it possible to construct the better shape of PMMA microchannel in hot embossing process by reducing surface energy of the master mold. The bottom PMMA plate was exposed to the silicon master with heat (130°C) and pressure (2000N) for embossing for 90 sec. Top plate was drilled to construct the reservoir of 2mm diameter. After cleaning both PMMA plate, they were brought together aligned and contacted under pressure then transferred into an oven at 110°C for 10 min, then annealed to room temperature.

### 2.2.4 Microchip using PDMS molding

PDMS microchip was fabricated by soft lithographic method. The microchannel was constructed on a PDMS substrate using rapid prototyping and replica molding. A thick photoresist, N-PMER (PMER N-CA3000PM, TOK, Kanagawa, Japan) was used as a PDMS (Sylgard 184 silicone elastomer, Dow Corning Co. Midland, MI, USA) replication mold. A photolithographic process only was used to generate channel mold for the PDMS channels without any wet etching procedures. A curing agent and PDMS prepolymer were thoroughly mixed in a 1:10 weight ratio. The prepolymer mixture was degassed in a beaker with a vacuum pump oven for 1 h to remove any air bubbles in the mixture and to insure complete mixing between the two materials. The prepolymer mixture was poured onto the master, and cured for 4 h at 65°C in an oven. After curing, the thin PDMS replicas were peeled off from masters. The replicated PDMS channel was bonded to a PDMS substrate. Due to the excellent adhesive properties of PDMS, the closed PDMS channel system was readily formed by mere adhesion without applying an external force.

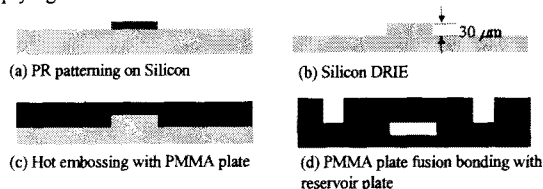


Fig.3 Sequence of PMMA chip fabrication

### 2.3 Microchip electrophoresis

Microchip electrophoresis was performed using laboratory-constructed capillary electrophoresis equipment (Fig 4). 10  $\mu\text{M}$  fluorescein and 10  $\mu\text{M}$  dichloro-fluorescein (DCF) were separated in microchips to compare the performance in each microchip.

Fluorescein and DCF was monitored by a laser-induced fluorescence detection system using Ar ion laser operating at 488 nm. The laser beam impinged upon the microchip at a 45° angle from the microchip surface and at a 90° angle from the separation channel.

The fluorescent light was collected by a 10× microscope objective (Edmund Optics, Barrington, NJ, USA) through a 405 nm bandpass filter (Melles Griot, Irvine, CA, USA) and was detected with an integrated photomultiplier tube (PMT; HC 120-01, Hamamatsu, Bridgewater, NJ, USA). A pinhole of a 1 mm aperture was located between the band pass filter and the PMT to reduce noise induced by stray light. Data acquisition and analysis were performed using a MIO-16-XE DAQ board (National Instruments, Austin, TX, USA) with LabVIEW 5.0.1 (National Instruments).

A high-voltage power supply (Model 1000/3125, Canberra, Meriden, CT, USA), capable of delivering 0-5 kV, was used to generate the electrophoretic voltages necessary for sample injection and separation in the microchip. Platinum electrodes were inserted into the reservoirs for electrical contact. EOF was measured using 1% acetone as a neutral marker by indirect detection method. The run buffer was 10mM borate buffer (pH 9.2) containing 1  $\mu\text{M}$  fluorescein, which was added as a chromophore for indirect detection of EOF. The microchip was operated under a gated sample loading mode. That is, the electrokinetic injection was performed by floating a potential at the buffer reservoir for 1 s, while holding the potential fixed at the other reservoirs. The voltages applied to the buffer, sample, sample waste, and waste reservoirs were 1, 0.9, 0.81, and 0 kV, respectively. The electric field strength applied to the separation channel during the separation process was 200 V/cm.

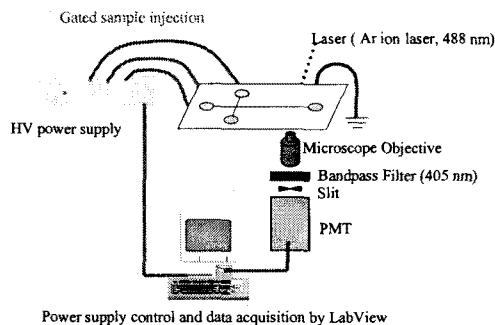
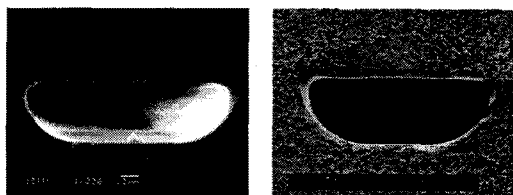
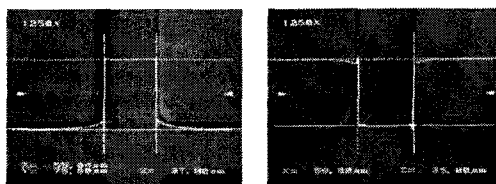


Fig.4 Experimental setup for microchip electrophoresis



(a) Cross section of quartz chip (b) Cross section of glass chip  
Fig.5 SEM image of microchannels

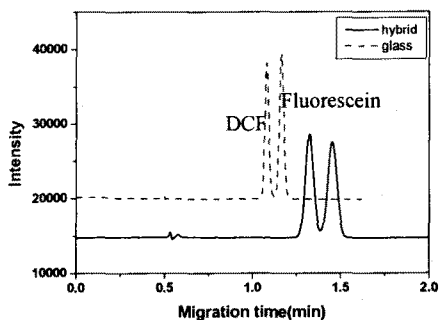
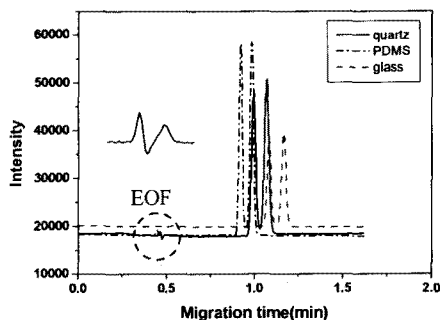


(a) Silicon mold after DRIE process (b) PMMA replica  
Fig.6 Fabrication result of hot embossing process

## 3. Results and Discussion

### 3.1 Fabrication results

Cross section of microchannel in quartz and glass microchip was observed by scanning electron microscope (Fig. 5). Both channels were trapezoidal due to the isotropic etch and two substrates were bonded successfully in both microchip. It is proven that anodic bonding method can be applicable in fabrication glass microchip. In fabrication of PMMA electrophoresis microchip, microchannel was constructed by hot embossing process and silicon deep reactive ion etching process was employed to create a master mold. The profile of silicon mold and PMMA replica was measured by surface profiler (Alpha-Step 500, KLA-Tencor Co., San Jose, CA, USA). Compared with silicon mold, channel width of PMMA replica was enlarged after imprinting process from 55.6  $\mu\text{m}$  to 59.8  $\mu\text{m}$  and depth of the channel was reduced from 37.8  $\mu\text{m}$  to 35.8  $\mu\text{m}$  (Fig. 6).



(a) Electropherogram obtained in each chip  
(b) Band broadening due to hybrid microchannel  
Fig.7 Electropherogram for the separation of DCF, and fluorescein

**Table 1.** EOF mobility in microchip electrophoresis

	migration time(min)	EOF velocity (cm/s)	EOF mobility ( $10^{-4}$ cm <sup>2</sup> /V*s)
quartz	0.462 ± 0.001	0.115 ± 0.001	5.75 ± 0.06
glass	0.492 ± 0.001	0.109 ± 0.001	5.43 ± 0.01
PDMS	0.473 ± 0.30	0.114 ± 0.008	5.70 ± 0.40

### 3.2 Comparison of the separation efficiency

The separation efficiency can be compared by measuring migration time and bandwidth of EOF and analytes in each microchip. 1% acetone was used as a neutral marker to detect EOF by indirect detection method. Because the run buffer has fluorescence as containing 1 μM fluorescein, which was added as a chromophore, EOF can be detected by negative peak when acetone passes by the detection point. The peak of EOF was observed in electropherogram for the separation of DCF, and fluorescein (Fig. 7(a)). It shows that EOF varies according to substrate material in spite of same separation length and electric field. It means that characteristics of material can influence the EOF and separation efficiency. EOF velocity was obtained from separation length divided by migration time and EOF mobility was estimated using following equation.

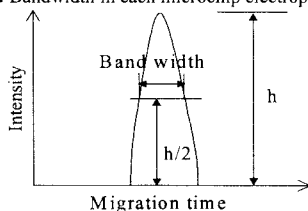
$$\mu_{EOF} = v_{EOF} / E$$

(where  $v_{EOF} (= l / t_{m})$  : EOF velocity,  $E$  : electric field,  $l$  : separation length,  $t_m$  : migration time)

EOF mobility in each microchip was indicated in table 1. It shows that EOF in microchip using quartz substrate was larger than any other microchip and EOF in PDMS chip has large deviation comparing with that of silica substrate. It means that more silanoate group is generated in quartz substrate and microchip electrophoresis in PDMS substrate has poor reproducibility. It shows the same tendency in analysis of sample separation of DCF and fluorescein.

In Fig. 7(b) and table 2, electropherogram for comparing band width of hybrid microchips and the microchips used same material, was shown. Glass(hybrid) chip was fabricated by silicon dioxide deposition on microchannel in bottom substrate, while silicon dioxide was patterned in glass chip for sealing with same material. Less band broadening was observed in the microchip composed of single material than in the microchip of hybrid material such as glass and oxide membrane due to non-uniformity of surface charge density at the walls of the channel.

**Table 2.** Bandwidth in each microchip electrophoresis



	DCF	Fluorescein
quartz	1.60 sec	1.80 sec
Glass	1.55 sec	1.75 sec
Glass(hybrid)	3.03 sec	3.43 sec
PDMS	1.53 sec	1.73 sec

## 4. Conclusions

The influence of the properties of substrate material on the separation efficiency in microchip electrophoresis was investigated. We fabricated the various microchips and studied separation efficiency in microchannels composed of a single material such as quartz, glass, PDMS, and PMMA, as well as hybrid microchannels composed of different materials. In this study the EOF velocity and EOF mobility were measured using 1% acetone as a neutral marker by indirect detection method and broadening effect was detected. EOF was more reproducible in silica chips than that of PDMS chip at the same conditions. More band broadening was observed in the microchip composed of hybrid material such as glass and oxide membrane than in the microchip of single material due to non-uniformity of surface charge density at the walls of the channel. From the results of measuring migration time and bandwidth of EOF and analytes in each microchip, separation efficiency was highly dependent upon the substrate materials and channel surfaces uniformity in microchip electrophoresis.

### Acknowledgements

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### [References]

- [1] R. Scott Martin, A. J. Gawron and S. M. Lunte, "Dual-Electrode Electrochemical Detection for Poly(dimethyl-siloxane)-Fabricated Capillary Electro-phoresis Microchips," *Anal. Chem.*, Vol. 72, pp. 3196-3202, 2000
- [2] Shuichi Shoji, "Micro Total Analysis System (mTAS)," *Electronics and Communications in Japan, Part 2*, Vol. 82, No. 2, 1999
- [3] N. Lacher, K. Garrison, R. Martin, S. Lunte. "Microchip capillary electrophoresis/electrochemistry," *electrophoresis*, Vol. 22, pp. 2526-2536, 2001
- [4] V. Dolnik, S. Liu, S. Jovanovich, "Capillary elecphoresis on microchip," *Electrophoresis 2000*, Vol. 21, pp.41-54, 2000
- [5] D. Ross, T. J. Johnson, and L. E. Locascio, "Imaging of Electroosmotic Flow in Plastic Microchannels," *Anal.Chem.*, Vol. 73, pp.2509-2515, 2001
- [6] J. C. Mcdonald, D.C. Duffy and G. Whitesides, "Fabrication of microfluidic systems in poly(dimethylsiloxane)," *Electrophoresis*, Vol. 21, pp.27-40, 2000
- [7] K. Seiler, D.J. Harrison and A.Manz, "Planar glass chips for capillary electrophoresis: repetitive sample injection, quantition, and separation efficiency," *Anal. Chem.*, Vol. 65, pp. 1481-1488, 1993
- [8] D. Harrison, A. Manz, Z. Fan, H. Ludi, and H. Widmer, "Capillary electrophoresis and sample injection systems integrated on a planar glass chip," *Anal. Chem.*, Vol. 64, pp. 1926-1932, 1992
- [9] S. Jacobson, A. Moore, and J. Ramsey, "Fused quartz substrates for microchip electrophoresis," *Anal. Chem.*, Vol. 67, pp. 2059-2063, 1995
- [10] Zhonghui H.Fan and D.Jed Harrison, "Micromachining of capillary electrophoresis injectors and separators on glass chips and evaluation of flow at capillary intersections," *Anal. Chem.*, Vol. 66, pp. 177-184, 1994