Characterization and performance of injection molded poly(methylmethacrylate) microchips for capillary electrophoresis


University of Cincinnati, Department of Chemistry, 301 Clifton Court, Cincinnati, OH 45221-0172, USA
University of Cincinnati, Department of Electrical and Computer Engineering and Computer Science, 814 Rhodes Hall, Cincinnati, OH 45221-0030, USA
Procter and Gamble Pharmaceuticals, 8700 Mason-Montgomery Road, Mason, OH 45040, USA

Received 5 December 2006; received in revised form 28 March 2007; accepted 30 March 2007
Available online 6 April 2007

Abstract

Injection molded poly(methylmethacrylate) (IM-PMMA), chips were evaluated as potential candidates for capillary electrophoresis disposable chip applications. Mass production and usage of plastic microchips depends on chip-to-chip reproducibility and on analysis accuracy. Several important properties of IM-PMMA chips were considered: fabrication quality evaluated by environmental scanning electron microscope imaging, surface quality measurements, selected thermal/electrical properties as indicated by measurement of the current versus applied voltage (I–V) characteristic and the influence of channel surface treatments. Electroosmotic flow was also evaluated for untreated and O2 reactive ion etching (RIE) treated surface microchips. The performance characteristics of single lane plastic microchip capillary electrophoresis (MCE) separations were evaluated using a mixture of two dyes—fluorescein (FL) and fluorescein isothiocyanate (FITC). To overcome non-wettability of the native IM-PMMA surface, a modifier, polyethylene oxide was added to the buffer as a dynamic coating. Chip performance reproducibility was studied for chips with and without surface modification via the process of RIE with O2 and by varying the hole position for the reservoir in the cover plate or on the pattern side of the chip. Additionally, the importance of reconditioning steps to achieve optimal performance reproducibility was also examined. It was found that more reproducible quantitative results were obtained when normalized values of migration time, peak area and peak height of FL and FITC were used instead of actual measured parameters.

Keywords: Injection molding; Poly(methylmethacrylate) (PMMA); Microchip electrophoresis; Reproducibility; Characterization

1. Introduction

There is great interest in translating standard benchtop analytical methods into ones using much smaller formats in order to increase analysis speed, decrease the use of expensive reagents and incorporate multiple functions into single platform systems. Microchip capillary electrophoresis (MCE) has received much attention recently as an attractive means for achieving many of the aims of miniaturization of CE. To date, most of the work done in MCE has been performed with glass chips but the expense of glass-based chips can be an impediment for routine use and often limits the analysis throughput due to the need to periodically recondition the chip. A number of approaches are available for the design and development of plastic-based microfabricated devices and these include plasma etching, laser ablation, imprinting and embossing, X-ray lithography and injection molding [1–7]. A switch from glass to plastic would offer more flexibility in chip design and fabrication, as well as lowering the cost of an analysis since plastic chips can be easily mass-produced. Hydrophobic surfaces, such as plastics, as well as glass surfaces, tend to adsorb hydrophobic molecules and proteins resulting in chip fouling and the need for time consuming reconditioning steps [8]. If chips were disposable the concern with surface adsorption would be lessened since the...
chip could be inexpensively discarded rather than regenerated for reuse.

It is important to carefully characterize the properties of plastic materials and the associated chip fabrication methods to evaluate their overall suitability as MCE devices. In choosing plastic materials for chips, there are a number of specific material and optical properties that must be carefully considered [9]. These properties are currently less well-understood and controlled with plastics than for borosilicate glass. Additionally, in designing a plastic chip several issues must also be addressed including the scale of the system, method of fabrication, detection scheme and, ultimately, the desired analytical performance.

Previously, different examples of separations have been reported on poly(methylmethacrylate), (PMMA), chips as well as the use of dynamic coating of chips to prevent non-specific interactions of analytes with the channel walls and also to suppress the electroosmotic flow (EOF) [10–22]. However, little attention has been devoted to examining the reproducibility of the performance of fabricated plastic chips using a dynamic coating [23,24]. In a benchmark paper on numerous plastic materials for hot-embossed CE microchips, Soper and co-workers demonstrated that several physicochemical properties and the associated operational performance must be considered to obtain reproducible electrophoretic separations [21]. We examined the detailed optical properties of several candidate plastic materials and chips for use with laser induced fluorescence detection. In that study, we concluded that PMMA was an excellent material for chip fabrication [9].

The control of the EOF is a crucial aspect in developing CE methods. The EOF values for plastic materials are generally lower than for glass materials due to fewer charged surface groups resulting in a lower zeta potential. As a consequence, hydrophobic plastic surfaces are typically difficult to wet with aqueous solutions [25,26]. These inter-related problems can be partially ameliorated by modifying the surface charge density by plasma oxidation [16,27,28], UV exposure [29] or dynamically modifying the plastic surface with surfactants [30]. Alternatively, the EOF can be eliminated or greatly suppressed by increasing the viscosity of the running buffer by adding a sufficient amount of a linear polymer, such as polyethylene oxide (PEO). In CE, the EOF can be affected by a number of different parameters including the running buffer composition and pH, the applied voltage, the temperature and the chemical nature of the separation channel surface. Small shifts in any one of these properties can potentially result in a large shift in solute migration times. In the case of capillaries, it was found that improved reproducibility could be obtained by regenerating the surface of the capillary after each run (every 4–6 injections) [31,32].

Internal standards are especially useful for MCE analyses in which the quantity of sample analyzed or the instrument response varies slightly from run to run for reasons that are difficult to control [33]. It was also found that more reproducible quantitative results could be obtained if normalized values were used instead of raw measured parameters [3,34,35]. An internal standard also corrects for some quantitative analyte losses due to other sources such as pipetting errors or instrument imprecision often caused by the injection process [36].

The goal of this work was to characterize single lane PMMA plastic microchips made by injection molding (IM-PMMA), a technique that is capable of mass production of chips. To evaluate IM-PMMA chips as a potential candidate for routine MCE disposable chip applications, it was important to characterize the chips and to find optimal physical and operational conditions. We have characterized chips in two ways, i.e., by measurements of the physical characteristics of chips and of the analytical performance of sets of uniformly fabricated chips. Analytical separation parameters were examined by evaluating the reproducibility of migration time, peak area, peak height, resolution and theoretical plates and run-to-run and chip-to-chip reproducibility. Additionally, the effect of normalizing the data via the use of an internal standard was also examined.

2. Experimental

2.1. Reagents and solutions

PMMA (GE Polymerland, Pittsfield, MA, USA) chips were fabricated by injection molding (IM). Polyethylene oxide (PEO) (MW 600,000) was from Sigma–Aldrich (Milwaukee, WI, USA) and 10 × Tris–borate–EDTA (890 mM Tris/890 mM borate/20 mM EDTA electrophoresis grade, pH 8.4) (TBE) was from Bio-Rad Laboratories (Hercules, CA, USA). A buffer composed of 3% PEO in TBE buffer was used as stock solution and was diluted 10-fold with distilled–deionized (DD)–water (Sybron/Barnstead, Boston, MA, USA) immediately prior to use to yield the running buffer (0.1 × TBE/0.3% PEO). The running buffer and DD-water were degassed offline prior to experiments by adding 4–6 mL to a 10 mL syringe, plugging the syringe hole, pulling the plunger back to create a vacuum and then tapping the syringe with a pen or an empty syringe. The process was repeated until air bubbles no longer left the solution. All solutions were then filtered through a 0.45 μm syringe filter (Millipore Corp., Bedford, MA, USA). Fluorescein sodium salt (FL) and fluorescein isothiocyanate isomer I 90% (FITC) were obtained from Sigma–Aldrich. FL and FITC stock solutions (1 mM each) were prepared fresh every time before use by dissolving appropriate amounts of each dye in methanol (Pharmco, Brookfield, CT, USA) and appropriate aliquots of each stock solution were mixed and diluted with running buffer to give a combined standard solution containing 25 μM FL and 50 μM FITC. Borate buffers, 20 mM and 10 mM at pH 9.0, for EOF measurements were prepared from sodium tetraborate (Fisher, Fair Lawn, NJ, USA).

2.2. Experimental setup

All experiments were done on an in-house constructed system described in detail previously [9]. Briefly, the system consisted of a Nikon TE 2000 epifluorescence microscope equipped with a H6780-20 PMT module (Hamamatsu, Bridgewater, NJ, USA), a CoolSnap HQ CCD camera (Roper Scientific, Trenton, NJ, USA) interfaced with a personal computer through...
a PCI card. Data were acquired using MetaMorph (Universal Imaging, Downingtown, PA, USA) software. A Lambda LS xenon arc lamp (Sutter Instrument, Novato, CA, USA) was used as the light source, voltages were applied to the microchip through platinum electrodes using an HVS 488 model 3000 (Lab Smith, Livermore, CA, USA) power supply. Current from the PMT was amplified with a low current preamplifier SR570 (Stanford Research, Sunnyvale, CA, USA) and acquired with a 16-bit PCI P6036 DAQ card (National Instruments, Austin, TX, USA) at a rate of 60 Hz. High voltage and signal acquisition were controlled by codes written in LabView (National Instruments). The experimental data was exported for further analysis into PeakFit v 4.12 (SeaSolve Software, San Jose, CA, USA).

2.3. Microchip fabrication

The microchip fabrication techniques used in this study have previously been described in detail [37]. Briefly, microstructures were fabricated using the UV–LIGA process. The master mold for microchip replication was fabricated on a nickel (Ni) mold disk (3 in. diameter, 1.6 mm thick). The Ni-disk was lapped flat and thick photoresist (SU-8 2075 negative photoresist, Microchem, Newton, MA, USA) processing was done on the disk followed by Ni-electroplating. A plating height of ~100 μm was achieved after 10 h of electroplating in a Ni-sulfamate bath with a ~10 μm/h plating rate. The electroplated pattern was mechanically polished, after removing the photoresist, resulting in greatly improved transparency and a reduced number of channel imperfections in the chips produced.

Replicate plastic microchips were made by injection molding. Injection molding was done at high pressure (19,000 psi) after insertion into a custom-designed molding block. After replication, a cleaning step composed of sequential rinses with isopropanol (IPA) (Pharmco, Brookfield, CT, USA) and DD-water was performed to remove particulate and organic material. The patterned wafer (channel width, 100 μm and depth, 39 μm) was then bonded with a cover wafer (IM-PMMA, C, 0.2 tons pressure). Usage of O₂ RIE treatment would increase the wettability of PMMA chips through generation of new functional groups that contain oxygen (–CO₂H, –OOH and –C=O) [39] and remove possible impurities from the channel surface, even if it adds an extra process for chip fabrication.

Chip fabrication studies involved varying two parameters, the O₂ reactive ion etching (RIE) process and the reservoir hole position. Usage of O₂ RIE would increase the wettability of PMMA chips through generation of new functional groups that contain oxygen (–CO₂H, –OOH and –C=O) [39] and remove possible impurities from the channel surface, even if it adds an extra process for chip fabrication.

Laplace pressure for each hydrophobic reservoir [38]. To test the effect of hydrophilic or hydrophobic properties of reservoirs on IM-PMMA chips, identical chromatographic separations were performed on chips with different reservoir tips: polypropylene pipette tips (hydrophobic) and glass tubes (hydrophilic).

2.3.2. Chip fabrication variables

Chip fabrication studies involved varying two parameters, the O₂ reactive ion etching (RIE) process and the reservoir hole position. Usage of O₂ RIE would increase the wettability of PMMA chips through generation of new functional groups that contain oxygen (–CO₂H, –OOH and –C=O) [39] and remove possible impurities from the channel surface, even if it adds an extra process for chip fabrication.

Chips were prepared in various combinations with or without O₂ RIE surface treatment and with holes drilled either in the cover plate wafer or in the patterned wafer. Therefore, four groups of chips were made: O₂ RIE treatment + holes in cover plate wafer; O₂ RIE treatment with holes in patterned wafer; no O₂ RIE treatment + holes in cover plate wafer; and no O₂ RIE treatment with holes in the patterned wafer. Each group consisted of 4 chips, making a total of 16 IM-PMMA chips. The O₂ RIE surface treatment was done by first cleaning wafers with IPA and DD-water and then applying the O₂ RIE plasma using a Technologies Miro-RIE Series 85 (Technics, Dublin, CA, USA, 20 sccm, 210 mTorr, 100 W, 30 kHz) instrument for 1 min, followed by a 30 s rest, followed by a final 1 min etch.

2.4. Microchip characterization

Chip patterned wafers were inspected for defects using an environmental scanning electron microscope XL30 (ESEM; FEI Company, Hillsboro, OR, USA). Prior to image recording,
surfaces were cleaned by sequential rinses of IPA and DD-water and 10 nm gold layers were deposited on the patterned wafers by sputtering (Cold Sputter, Denton Vacuum, Moorestown, NJ, USA). The surface roughness of the mold itself was measured using a KLA-Tencor P-10 surface profilometer (KLA-Tencor, San Jose, CA, USA) with a 10 µm/s scan rate at a 50 Hz sampling rate.

IM-PMMA patterned wafers with and without plasma treatment were investigated using X-ray photoelectron spectroscopy, XPS (Perkin-Elmer PHI ESCA 5000, Wellesley, MA, USA). Samples were irradiated with a beam from an Mg Kα X-ray source (1253.6 eV). Survey and high-resolution spectra were acquired, using a take-off angle of 45° and pass energies of 89.45 eV and 37.35 eV, respectively. FTIR–ATR spectra were recorded on a Nicolet Magna-IR-760 spectrometer (Madison, WI, USA) at incidence and reflectance angles of 85°.

2.5. Electroosmotic flow measurements

EOF values were measured using an established procedure, the current monitoring method [8,30,40–42]. Initially, channels were filled with 20 mM borate buffer by applying vacuum on the buffer waste reservoir. Then, the liquid was removed from the reservoirs and replaced by 70 µL of 10 mM borate buffer and the voltages applied. The application of the voltages causes the lower concentration buffer to fill the separation channels and as a result the current decreases until a steady state is reached once the channels are filled with the lower concentration buffer. Therefore, the time needed to obtain a constant current is considered to be the EOF rate migration time. Specifically, a high voltage was applied on the microchip and a 0.22 MΩ resistor connected in series and the potential drop over the resistor recorded. Ground was always applied on the resistor end to avoid damaging the computer and the current values were obtained by dividing acquired voltage measurements by the 0.22 MΩ resistance. Two different potential schemes were used for EOF determinations. First, voltage (1 kV) was applied to the buffer reservoir and ground on the buffer waste reservoir while the sample and sample waste reservoirs were left to float. The EOF was calculated according to \( \mu_{EOF} = \frac{L}{t} \frac{E}{t} \), where \( L \) is the channel length (cm), \( E \) the applied electric field strength (V/cm) and \( t \) is the time (s) required to reach a constant current. In the second scheme, voltage (0.5 kV) was applied to the sample, the buffer and the sample waste reservoirs with the buffer waste reservoir held at ground. The EOF was estimated using the following equation [30]:

\[
\mu_{EOF} = \frac{E_2 l_1 + E_1 l_2}{E_1 E_2 t}
\]

where \( \mu_{EOF} \) is the electroosmotic mobility, \( E_1 \) and \( E_2 \) the strengths of the electrical fields across channels from the buffer reservoir to the intersection point and from the intersection to the separation channel, respectively, \( l_1 \) and \( l_2 \) the lengths of the two corresponding channels and \( t \) is the time to fill the whole channel with buffer solution 2. In our case, \( E_1 \) and \( E_2 \), \( l_1 \) and \( l_2 \) were 44.2 V/cm, 132.6 V/cm, 0.4 cm and 3.9 cm, respectively.

2.6. Characterization of microchip separation performance

The performance characteristics of separations were evaluated using a simple model system composed of the dyes FL and FITC. To overcome the problem with wetting the PMMA surface, the electroosmotic modifier, PEO, was present in the buffer to suppress the EOF and, therefore, reverse-polarity electrophoresis conditions were used for the separations. Prior to use, the chips were conditioned by sequentially washing with DD-water (5 min) and running buffer (10 min) by filling the sample, buffer and sample waste reservoirs with the appropriate solutions and then applying vacuum to buffer waste reservoir.

If a chip was to be used for many experiments, the chip surface was reconditioned after each run by the same procedure. Following chip conditioning, the running buffer (70 µL) was added to buffer, sample waste and buffer waste reservoirs and the combined standard dye solution (70 µL) was added to sample reservoir. Platinum electrodes were placed in each reservoir and the voltage program was used for injection and separation. A reverse-polarity injection and electrophoresis scheme was used and is similar to the common pinched injection scheme [43]. Sample introduction was accomplished by applying 0.25 kV at buffer reservoir and 0.65 kV to buffer waste reservoir, while keeping sample and buffer waste reservoirs at 0 V for 25 s. Sample separation was performed by applying 0.75 kV to sample and sample waste reservoirs, 2.5 kV to buffer waste reservoir, and 0 V to buffer reservoir for 45 s. The effective separation field strength was 530 V/cm. Analyte detection was done at 30 mm from the channel cross for all chips. Additionally, the effect of adding 1–2 µL less buffer in the sample waste reservoir than in the rest of the reservoirs was examined. During injection, the level of liquid in sample reservoir decreases slightly and the level of liquid in sample waste reservoir increases slightly. Therefore, the reduced amount of liquid in sample waste reservoir partially compensates for the siphoning problem which might lead to peak height irreproducibility.

2.7. Data analysis

The separations were characterized by measuring reproducibility, as expressed by the relative standard deviation (RSD), of the peak migration time, peak area and peak height. Electropherograms obtained were fitted to Gaussian peaks using PeakFit v 4.12. The number of “theoretical plates”, \( N \) was calculated from the full-width of the peak at half-maximum.

The average and standard deviations of repeated measurements were calculated. The effect on the reproducibility of these parameters by using an internal standard to normalize the migration time, peak area and peak height results was also examined.

3. Results and discussion

3.1. Physical characterization

Several important properties of IM-PMMA chips were measured and compared: optical constants and the autofluo-
rescence of fabricated chips [9], overall fabrication quality by ESEM imaging, surface roughness measurements, certain thermal/electrical properties (measurement of the current versus applied voltage \(I–V\) characteristic to determine a voltage range at which electrical failure occurred) and the influence of channel surface treatments.

In our experiments, the intensity of PMMA autofluorescence was considerably lower than the intensity of FL and FITC signals. Thus, the base line appears constant for the results presented. If lower fluor concentrations were studied, a decaying baseline would become apparent (as shown for example in our recent publication [9] on the autofluorescence of plastic chips).

3.1.1. Surface roughness

Surface roughness is an important factor in MCE because any channel imperfection can cause erratic current or non-uniform sample flow resulting in noise and poor signal detection. Li and co-workers have done extensive modeling on how microscale surface roughness can influence the transport processes [44]. The surfaces of the microchannels typically have surface roughness values of a few nanometers to a few micrometers [45]. For example, it was found that surface roughness was from 1 to 11 nm for a fused-silica capillary [46]. Surface roughness does not affect the separation efficiency, however, it increases the relative standard deviation of the migration times and peak areas. Increasing surface roughness also produces peak broadening [47,48]. In order to prevent mixing and bubble formation, surface irregularities need to be kept to a minimum. The roughness of the Ni master was found to have a maximum height of \(\sim 80\) nm and an average roughness \((R)\) of 170 nm for the Ni disk itself, 210 nm for the electroplated pattern and 52 nm for the polished electroplated pattern. The resulting surface roughness of 52 nm is higher than the cases for wet-etched fused-silica capillary or glass CE chip shown in [46]. However, the surface roughness that we have found seems to be in an acceptable range since we did not experience any experimental difficulties.

3.1.2. Replication quality

ESEM images (for example, see Fig. 1(b)) showed that high quality replications were achieved for IM-PMMA patterned wafers and that there were no defects in the intersections or in the channels. Each process had thus resulted in a well-defined device which is important for the control of the amount of sample plug introduced during the sample loading step.

3.1.3. Electrical/thermal characterization

According to Ohm’s law, the \(I–V\) curve should follow a linear relationship when the resistance is constant. After filling channels with running buffer, a stepwise voltage increase of 0.2 kV was applied between the buffer and buffer waste reservoirs and between the sample and sample waste reservoirs. Current readings were taken after an equilibration time interval of 15 s for cooling down after each voltage change. Since the channels had uniform cross-section and the buffer filling each channel was the same, the channel resistances should be proportional to the channel lengths. The \(I–V\) response was linear \((y = 0.505x + 0.052, \text{where } y \text{ is current in } \mu A \text{ and } x \text{ is voltage in } kV)\) with a correlation coefficient of 0.999 for potentials of up to 3 kV for voltages applied across the separation channel (between the buffer and buffer waste), indicating no significant Joule-heating. The highest voltage corresponded to an electrical field strength of 700 V/cm within the separation channel. So, the maximum potential between the point of injection and detection is 2700 V for the 3.9 cm separation distance used in these studies. When the voltage was applied across the injection channel (between the sample and sample waste), a linear dependence \((y = 0.079x + 0.130, \text{where } y \text{ is current in } \mu A \text{ and } x \text{ is voltage in } kV)\) with a correlation coefficient of 0.997 was obtained up to 3 kV.

3.1.4. X-ray photoelectron spectroscopy

An XPS survey scan determined that the elemental composition of the IM-PMMA surface before treatment was 71.2% carbon and 28.8% oxygen. After O2 RIE treatment the elemental composition changed to 66.2% carbon and 33.8% oxygen.

Fig. 2. (a) A representation of the PMMA chemical structure and (b) C 1s XPS spectra of treated IM-PMMA wafers with fitted peaks associated with the structural units of the polymer.
indicating the introduction of additional surface oxygen atoms. A representation of the PMMA chemical structure is shown in Fig. 2(a). The fitted C 1s spectrum of treated IM-PMMA is shown in Fig. 2(b). The C 1s peak of the C–C bond is located at 284.6 eV and those of the C–O and the C≡O bonds are at 286.1 and 288.4 eV, respectively. The percentage composition of surface carbon atoms within C–C bonds decreased from 56.5 to 53.4% after treatment and that of C≡O bonds increased from 20.4 to 23.5%. In turn, this suggests that the –CH2– groups in the polymer backbone and the –CH3 groups in the branch chains are oxidized when the surface is exposed to the O2 plasma. For XPS of O 1s electrons, the percentage composition of oxygen atoms in C=O bonds increased from 49.6 to 57.3%, while that in C≡O bonds decreased from 50.4 to 42.7%. This suggests that C=O bonds can break more easily than C≡O bonds and one then speculates that OCH3 groups are the first oxidized. These conclusions from the analysis of IM-PMMA substrate surfaces before and after the treatment are consistent with previous reports [16,27,28].

The O2 plasma bombards the PMMA surface with high-energy gas molecules causing surface modification. It is believed that the activation mechanism starts with the creation of free radicals on the polymeric surface and then these free radicals couple with active species from the oxygen plasma environment [27,28], which create new carboxyl groups, oxides, acid anhydrides and aldehydes, leading to a more hydrophilic surface [27].

3.1.5. FTIR–ATR

The FTIR–ATR difference spectrum of treated and untreated IM-PMMA substrate surfaces is shown in Fig. 3. The peak at 2990 cm$^{-1}$ corresponds to the asymmetric stretching vibration of the methyl group ($\nu_{as}$CH3) and the peak at 2949 cm$^{-1}$ to the asymmetric stretching vibration of the methylene group ($\nu_{as}$CH2). A sharp intense difference peak at 1719 cm$^{-1}$ appeared due to the carbonyl group stretching vibration (ester bond). Peaks at 1450 and 1387 cm$^{-1}$ correspond to the scissoring-bending vibration of the methylene group ($\delta_{as}$CH2) and the symmetric bending vibration ($\delta$CH3), respectively. The broad less-defined range from 1231 to 1137 cm$^{-1}$ can be attributed to the C–O (ester bond) stretching vibration. The FTIR–ATR results agree with our XPS findings, where major changes were observed in the –CH2– groups in the main polymer chains and in the –CH3 groups in the branch chains after the surface was exposed to the O2 plasma. Vibrations that correspond to the ester group are also consistent with its oxidation.

3.2. Electroosmotic flow measurements

The EOF of IM-PMMA chips, with and without O2 RIE treatment, was determined using the current monitoring method in conjunction with simple borate buffers. Based on the XPS and FTIR data, the O2 RIE treatment would be expected to impart increased negative surface charge to the PMMA. A negative surface charge would be indicated by the EOF direction being from anode to cathode and it is expected that the magnitude of EOF will reflect the charge density of the inner channel surface, which may be changed by surface treatment. The EOF of untreated surface IM-PMMA chips at pH 9 was $(2.1 \pm 0.3; n = 12) \times 10^{-4}$ cm$^2$ V$^{-1}$ s$^{-1}$ while O2 RIE treated surface IM-PMMA chips showed an increase of EOF $(4.5 \pm 0.2; n = 12) \times 10^{-4}$ cm$^2$ V$^{-1}$ s$^{-1}$. The increase in EOF for IM-PMMA channels after plasma treatment correlates well with the introduction of new charged functional groups on the surface as confirmed by XPS and FTIR–ATR. The magnitude of the EOF for treated surface IM-PMMA chips depends on pH and experiments showed an increase in EOF with increasing pH (data not shown). At low pH (pH 3), EOF is $1.28 \times 10^{-4}$ cm$^2$ V$^{-1}$ s$^{-1}$ and non-linearly increases to $4.84 \times 10^{-4}$ cm$^2$ V$^{-1}$ s$^{-1}$ at higher pH (pH 11).

3.3. Separation performance

A typical separation for FL and FITC on the IM-PMMA chip is shown in Fig. 4, when 508.8 V/cm was applied across the separation channel; the inset shows an expanded view of

![Fig. 4. Typical separation of FL and FITC on IM-PMMA chip using glass reservoirs. Sample introduction was accomplished by applying 0.25 kV at reservoir B and 0.65 kV to reservoir C, while keeping A and D at 0 V for 25 s. Sample separation was performed by applying 0.75 kV to reservoirs A and C, 2.5 kV to reservoir D and 0 V to reservoir B for 45 s. Analyte detection was done at 30 mm from the site of injection for all chips.](image-url)
one of the separations. It can be seen that both peaks are nearly baseline resolved under the separation conditions and both have an essentially Gaussian shape.

3.4. Hydrophilic versus hydrophobic reservoirs

To verify the effect of hydrophilic or hydrophobic properties of reservoirs on the IM-PMMA chips the same separation of the combined standard (25 μM FL and 50 μM FITC) was performed on IM-PMMA chips with different reservoirs: polypropylene and glass. Chips with glass reservoirs exhibited fewer peak anomalies in terms of both peak shape and peak height. The RSD \((n = 6)\) of peak height of FL for the chip with plastic (hydrophobic) reservoir tips within one run was 22.2%, as opposed to 1.7% for the chip with glass reservoirs. The RSD \((n = 6)\) of peak height ratio (FL peak height/FITC peak height) for the chip with polypropylene reservoirs within one run was 3.2%, as opposed to 0.1% for the chip with glass tips. Therefore, it was decided that chips with glass reservoirs would be used for subsequent reproducibility studies.

3.5. Reproducibility studies

A blind study was designed to evaluate the effect of well-defined variations in the chip fabrication procedure on IM-PMMA chip performance. The study also investigated the ability to regenerate these chips for repetitive separations.

Four different groups of chips were investigated. Group 1: O2 RIE treatment and holes bored on the cover wafer; Group 2: O2 RIE treatment and holes bored on the patterned wafer; Group 3: no O2 RIE treatment and holes bored on the cover wafer; Group 4: no O2 RIE treatment and holes bored on the patterned wafer. It should be noted that only three functional chips were available for Groups 3 and 4. One of the concerns was that if holes were bored on the patterned wafer, debris from boring could potentially block channel entrances.

In order to investigate injection-to-injection reproducibility, sets of six repeated injections and separations of the combined standard (25 μM FL and 50 μM FITC) were performed. Each set of six injections was designated a run. The run-to-run reproducibility of six runs, each with six injections was then evaluated. All chips were reconditioned after each run. The chip-to-chip reproducibility was also investigated, using six runs with six injections for each chip. Between each run chips were rinsed with water and stored dry. The discussion below focuses on the migration time of FL and FITC and the migration time ratio, but the same conclusions can be made about the peak area and peak height parameters.

Variance component analysis was used to study chip reproducibility [49]. The resulting variance was a combination of the variability arising from the peak fit variance, the analytical test variance (the pooled variance based on replicates on eligible identical conditions) and the regeneration variance (variance obtained from different runs, because each chip was regenerated (conditioned) between runs) for a given chip and the fabrication variance (variance obtained from the same group of chips). The peak fit variance was determined from values obtained from the Peak Fit program and the analytical variance for each chip was calculated using the following formula:

\[
\sigma^2_s = \frac{\sum \upsilon s^2}{\sum \upsilon}
\]

where \(\upsilon\) is the number of degrees of freedom for runs with one chip and \(s\) is the standard deviation obtained from injections. Regeneration variance was calculated from standard deviations obtained from different runs. The observed regeneration variance contained contributions from the variances due to regeneration and from injections. The observed fabrication method variance contains contributions from the variance for the same group of chips, regeneration variance and the variance due to injections.

Fig. 5(a) and Table 1 show run reproducibility of FL migration time for all groups of chips. It can be seen that Groups 1 and 2 (first 8 chips) are more reproducible than Groups 3 and 4, which indicated that O2 RIE treatment contributed to more reproducible migration times. The first Group of chips had RSD values of 1.2 and 1.1% for the average migration time of FL and FITC, respectively. These results were plotted as a function of the order in which the experiments were done (data not shown). The data clustering indicated that the results were due to the fabrication parameters varied rather than some time dependent
change in the procedure and/or the instrumentation. Similar plots (not shown) were obtained for the other parameters (peak height, peak area and the ratios of these variables).

The RSD of migration times, peak area, peak height and resolution of FL and FITC for run-to-run studies for chips individually and for groups of chips fabricated differently, are shown in Table 2. The higher values of RSD for migration times can be attributed to the significant variations in the position of the detection window for each run compared to the total travel distance; slight changes in the exact point of detection can be critical. High RSD values for the peak height and the peak area can be attributed to errors introduced by injection [43].

It was found that more reproducible quantitative results were obtained if normalized values were used instead of actual measured parameters [3,34,35]. FL was used as the analyte and FITC was used as the internal standard for calculating the various ratios, therefore, reproducibility was expressed as the normalized ratio of migration time, peak area and peak height of FITC to FL. The results for the percentage of the RSD of migration time ratio, peak area ratio and peak height ratio are shown Table 2. It was found that the migration time ratio generally gave more reproducible results than the peak area and peak height ratio.

A very small RSD for migration time ratio was found where variation in the position of detection window was nearly eliminated (Table 2 and Fig. 5(b)). Fig. 5(b) shows reproducibility of average migration time for all chips represented in terms of normalized values of FL and FITC for all groups of chips (the error bars in Fig. 5(b) are small in comparison to the scale). Higher values of the variance for peak height and peak area ratios can be attributed to injection bias which comes from the pinched injection (Table 2). The first two groups (O2 RIE plasma treated) show smaller RSD values for all ratios (Table 2) than groups without plasma treatment. Increased percentage of RSD values for these two groups can be attributed to occasional chip leakage which was observed when the baseline was changing. However, the percentage of RSD for the normalized values led us to conclude that our measurements were reproducible (Table 2).

Statistical analysis was performed in order to determine whether these groups of chips were significantly different. The t-test found differences in the migration time for FL between chips with and without O2 RIE treatment at the 95% confidence level. An f-test on the variances confirmed the differences. Neither of these tests showed any significant difference between chips with the same O2 RIE treatment. Therefore, it can be concluded that the chips with O2 RIE treatment (first two groups) exhibited better reproducibility than the untreated chips. For migration time ratios of FL and FITC the t-test showed no significant difference between treated and untreated groups, but the f-test showed significant differences in the variances. This is consistent with the use of an internal standard to compensate for systematic differences. This indicates that, although the overall retention performance is the same, O2 RIE plasma treated surfaces led to chips that gave significantly more reproducible separations. The t-test found no significant differences for regeneration variance in terms of FL migration time. This suggests successful regeneration for this class of chips.

<table>
<thead>
<tr>
<th>Group</th>
<th>Chip#</th>
<th>t1</th>
<th>t2</th>
<th>a1</th>
<th>a2</th>
<th>h1</th>
<th>h2</th>
<th>R</th>
<th>t2/h1</th>
<th>a2/a1</th>
<th>h2/h1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>0.3</td>
<td>0.3</td>
<td>0.1</td>
<td>0.1</td>
<td>0.6</td>
<td>0.8</td>
<td>0.2</td>
<td>0.003</td>
<td>0.06</td>
<td>0.1</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>0.7</td>
<td>0.8</td>
<td>1.5</td>
<td>3.1</td>
<td>1.5</td>
<td>5.1</td>
<td>0.2</td>
<td>0.004</td>
<td>0.5</td>
<td>0.2</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>0.4</td>
<td>0.1</td>
<td>0.2</td>
<td>0.001</td>
<td>0.07</td>
<td>0.2</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.6</td>
<td>2.4</td>
<td>1.2</td>
<td>2.4</td>
<td>0.7</td>
<td>0.003</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>5</td>
<td>11</td>
<td>4.1</td>
<td>5.3</td>
<td>0.5</td>
<td>0.9</td>
<td>1</td>
<td>1.4</td>
<td>0.4</td>
<td>0.03</td>
<td>0.06</td>
<td>0.1</td>
</tr>
<tr>
<td>6</td>
<td>14</td>
<td>0.3</td>
<td>0.1</td>
<td>0.1</td>
<td>1.6</td>
<td>0.3</td>
<td>0.4</td>
<td>2.4</td>
<td>0.02</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>0.5</td>
<td>0.7</td>
<td>0.6</td>
<td>1.1</td>
<td>1.6</td>
<td>3.4</td>
<td>0.5</td>
<td>0.02</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>8.3</td>
<td>7.3</td>
<td>17</td>
<td>68</td>
<td>4.1</td>
<td>11</td>
<td>11</td>
<td>0.04</td>
<td>0.6</td>
<td>0.3</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>2.4</td>
<td>2.4</td>
<td>42</td>
<td>27</td>
<td>37</td>
<td>43</td>
<td>8.8</td>
<td>0.03</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>9</td>
<td>8.3</td>
<td>8.3</td>
<td>94</td>
<td>89</td>
<td>33</td>
<td>17</td>
<td>9.8</td>
<td>0.1</td>
<td>0.7</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Group 1: O2 RIE treatment and holes bored on the cover wafer; Group 2: O2 RIE treatment and holes bored on the patterned wafer; Group 3: no O2 RIE treatment and holes bored on the cover wafer; Group 4: no O2 RIE treatment and holes bored on the patterned wafer.

The percentage of RSD for normalized values was calculated as the ratio of the square root of the variance and the average value for the ratio of the individual measured parameters.
Another parameter that we evaluated was the effect of boring holes on performance. The $t$-test showed that there is no difference ($p > 0.05$) in the average values with different hole positions (pattern wafer versus cover wafer). Based on the $f$-test, there was a statistically significant difference in the scatter of the parameters around the averages between chips with different hole positions. The analytical variances are dominated by the effect of the hole position. Only in the case of holes drilled in the cover wafer was further improvement over the surface treatment discernible.

Based on the differences among various groups with and without O$_2$ RIE treatment and the "bored hole effect", Group 1 showed the best overall performance. Table 3 summarizes the results obtained from analysis of variance component analysis for Group 1. While the actual variances for chips are quite similar, it is clear that the largest contribution comes from the effect of the hole position. Only in the case of holes drilled in the cover wafer was further improvement over the surface treatment discernible.

Both untreated and treated IM-PMMA chips exhibited a similar separation performance. All groups showed similar resolution for FL and FITC. The resolutions were $1.6 \pm 0.3; 1.3 \pm 0.4; 1.1 \pm 0.4; 1.2 \pm 0.1$ for Groups 1–4, respectively. The theoretical plates for FL were $5 \times 10^4, 3 \times 10^4, 1.9 \times 10^4$ and $1.7 \times 10^4$ for Groups 1–4, respectively. Chips with O$_2$ RIE treatment showed slightly better separation efficiency.

### 4. Conclusions

The plastic material chosen for mass production of microchips for MCE separations depends significantly on chip-to-chip separation reproducibility and on the accuracy of quantification. The ability to use MCE for routine analysis also depends on the reproducibility of the migration time and on the accuracy of the quantification. Knowing this, we found it important to carefully characterize injection molded PMMA chips, both physically and analytically. Reproducible results were obtained using normalized values for migration times, peak area and peak height. Reconditioning steps are important to achieve optimal reproducibility. IM-PMMA chips show great potential for mass fabrication, since chip-to-chip reproducibility measurements showed that chips could be made and used reliably. The reduction in channel surface roughness in combination with using a PEO dynamic coating led to improved stability and reproducibility.

### Acknowledgements

Financial support of this work was provided by the National Institutes of Health (GM 69547), and the University of Cincinnati. The authors thank N. Pantelic for ESEM images.

### References