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Comparison of the performance characteristics of poly(dimethylsiloxane) and Pyrex microchip electrophoresis devices for peptide separations

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Abstract

A comparative study of electrophoretic separations of fluorescently labeled peptides and amino acids on poly(dimethylsiloxane) (PDMS) and Pyrex microchips is presented. The separation parameters for each microchip substrate were compared, including electroosmotic flow, plate numbers, resolution, and limits of detection. The effect of buffer composition on the separation was also investigated. Acceptable separations were obtained for most peptides with both substrates; however, PDMS chips exhibited much lower separation efficiencies and longer analysis times.

Keywords: Chip technology; Microfluidics; Instrumentation; Poly(dimethylsiloxane); Pyrex; Peptides

1. Introduction

The number of reports concerning microfluidic devices has increased dramatically since the initial concept was introduced approximately 13 years ago [1]. In particular, microchip capillary electrophoresis (CE) devices have been shown to offer many advantages over conventional CE systems, including improved automation, reduced amounts of reagents and waste, increased precision and accuracy, and the potential for disposable devices [2,3]. In addition, lower voltages are required with microchip CE due to the short channels that are generally employed for the separation. This makes it possible to use smaller power supplies, leading to truly portable analysis systems [4–7].

Miniaturized devices can be fabricated using well-defined lithographic procedures that have been used for many years by the electronics industry. Initially, glass and quartz were employed as microchip substrates. These substrates are optically transparent, enabling detection of the analytes by laser-induced fluorescence. In addition, the electroosmotic flow (EOF) of glass is similar to that of fused-silica, making it easy to transfer separations from one format to another [4–9]. However, while glass microchips continue to be employed successfully for the separation of many analytes, they do have some disadvantages. First, a well-equipped cleanroom is required, and the use of hydrofluoric acid (HF) for etching the substrate requires special safety precautions. Secondly, glass is fragile and expensive and can often shatter during chip processing. Lastly, the procedure for glass-to-glass bonding is not always

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straightforward. It often takes several cycles before the glass is completely bonded in the area surrounding the access holes and the separation channel.

To circumvent some of the problems with glass, alternative materials for microchips have been explored. Most of these exhibit EOF properties similar to that of glass, but are much easier and cheaper to fabricate. Some of the materials investigated thus far have been plastics [10], ceramics [11], and polymers, in particular, poly(dimethylsiloxane) (PDMS) [12].

PDMS as a chip substrate has been shown to possess several advantages over glass, including ease of fabrication and lower overall cost. PDMS microchips are fabricated by soft (contact) lithography of the polymer on a wafer with a raised microchannel design (master). The master can be produced in two ways. The first is to generate a raised photoresist structure on the silica wafer that serves as the master. Alternatively, deep reactive-ion etching (DRIE) of the wafer can be employed to produce a more durable silicon master [13].

There have been many reports of the use of polymer substrates for microchip CE [13–20]. These papers often report poor separation efficiencies. The major source of peak broadening is believed to be the adsorption of hydrophobic analytes to the polymer surface. A commonly reported solution to this less than ideal separation performance is covalent [15] or dynamic [21–24] modification of the polymer surface. Many of these methods have been shown to be successful at reducing analyte adsorption, but they can be labor-intensive and compound-specific.

Microchip analytical systems have several potential advantages for the determination of peptides. These include the potential for fast analysis times, small sample volume requirements, and the ability to integrate sample preparation steps into the analytical system. High throughput analytical methods for the determination of peptides are important in proteomics research and in the field of analytical biotechnology. Thus far, there have been only a few reports of peptide separations by CE in a microchip format [15,25–30]. Most of these have employed laser-induced fluorescence for the detection of fluorescently labeled peptides using glass chips. Electrochemical (EC) detection has also been explored [31,32] using PDMS and hybrid glass–PDMS chips.

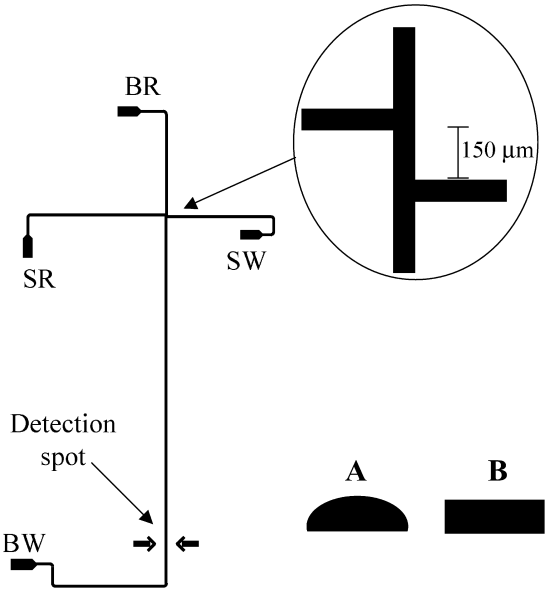

Fig. 1. Design of the microfluidic chip used for these experiments. The length of the double-T intersection in this design is 150 μm. The length from the buffer reservoir (BR) to the injection-T is 0.86 cm. The distance from the sample reservoir (SR) to the injection-T is 1.01 cm. The distance from the sample waste reservoir (SW) to the injection-T is 0.96 cm. The distance from the buffer waste (BW) reservoir to the injection-T is 3.75 cm. The distance from the injection-T to the detection spot is 2.4 cm. The final dimensions for the channels on the microchip are: 45 μm wide across the top of the channel and 12 μm deep. (A) The isotropic etch profile of the channel (Pyrex). (B) The anisotropic etch profile of the channel of the silicon wafer (PDMS).

The goal of this work is to compare the performance of PDMS with that of glass for peptide separations. Microfluidic chips of identical dimensions were fabricated in Pyrex and in PDMS (Fig. 1). Fluorescence detection was employed to rule out any losses in efficiency due to the detector configuration [20]. The chips were evaluated for the separation of a group of model peptides, including angiotensin peptides and substance P.

2. Experimental

2.1. Instrumentation

Detection was accomplished using laser-induced fluorescence (LIF) with an Ar-ion laser (Model

5400-220-00, Ion Laser Technology, Salt Lake City, UT, USA) at a power of 10 mW emitting at a wavelength of 488 nm. The laser was directed at the channel via collimating and focusing lenses at an angle of approximately 45°. The point of detection was located 2.4 cm below the injection cross. The resulting fluorescence was collected perpendicular to the microchip using a microscope objective (25×; N.A.=0.35; Leica, Glattbrugg, Switzerland) mounted on a laboratory-made microscope body. The fluorescence was transmitted through a band-pass filter at 514 nm (Melles Griot 03FIL004, 10 nm fixed width at half maximum, Irvine, CA, USA) and collected with a Hamamatsu R1477 photomultiplier tube (PMT) (Hamamatsu Photonics, Schüpfen, Switzerland). Spatial filtering with a pinhole was not performed prior to the PMT as is usually the case because improper pinhole alignment resulted in a substantially reduced signal. Two high-voltage power supplies [Models HCN 2000 (0–2 kV) and HCN 12 500 (0–12.5 kV), FUG Elektronic, Rosenheim, Germany], six relays (Günther, Nürnberg, Germany), and a control system (personal computer with LabView, National Instruments, Austin, TX, USA) were assembled to automate injection and separation and to allow data acquisition and analysis. High frequency noise was removed with an resistence–capacitance (R-C) filter (cut-off at 15 Hz) and a numerical fifth-order algorithm on the computer. The microchip holder consisted of a wafer holder mounted to an *x-y* translational stage.

2.2. Reagents

Four different buffers were employed in these studies. These were: (1) boric acid (Fluka, Buchs, Switzerland) (20 m*M*, pH 9.0); (2) boric acid, tris(hydroxymethyl)aminomethane (Tris) (Fluka) (20 m*M*, 100 m*M*, pH 9.0); (3) tricine (Fluka), Tween 20 (Sigma, St. Louis, MO, USA) (50 m*M*, 0.01%, pH 8.0); and (4) tricine, Tween 20, NaCl (Sigma) (50 m*M*, 0.01%, 10 m*M*, pH 8.0).

R-R-R-R, R-R-R-G, and R-R-G-G were purchased prelabeled with 5- (and 6-) carboxyfluorescein from New England Peptide (Fitchburg, MA, USA). Angiotensins I, II, and III and substance P were purchased from Bachem (Bubendorf, Switzerland). Arg, Phe, Gly, and Ser were purchased from Sigma. 4,4-Difluoro-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-s-indacene (BODIPY 493/503) was purchased from Molecular Probes Europe (Leiden, The Netherlands).

Stock solutions (1 m*M*) of the peptides and amino acids were prepared in water. A 1 m*M* solution of fluorescein isothiocyanate (FITC) isomer 1 (Sigma) was prepared in acetone. The amino acids and peptides were labeled with FITC by adding 100 μl of the 1 m*M* FITC solution to 1 ml of 1 m*M* amino acid or peptide stock solution. This process was repeated for all of the unlabeled compounds. After sufficient time for the reaction to occur (18 h), the samples were diluted to 10 μ*M* with respect to FITC. All aqueous solutions were filtered through 0.2 μm filters (Semadeni, Ostermunding, Switzerland) prior to use.

2.3. Microstructure fabrication

The design of the microfluidic structure used in this work is shown in Fig. 1 and is the same for both the Pyrex and PDMS devices. This chip is based on the twin-T or off-set T design.

2.3.1. Pyrex chip

The glass microchip was fabricated by lithographic procedures as depicted in Fig. 2A. A 400-nm-thick layer of polysilicon (polySi), deposited in two runs of 200 nm each, is used as a protective layer during the etching of the Pyrex 7740 wafer (Bullen-Ultrasonics, Eaton, OH, USA). Etching of glass in HF-containing solutions is an isotropic process. This simply means that etch rates in all directions are equal, so that the final dimensions will be larger than patterned. Isotropic etching also results in rounded channel profiles, which necessarily will always be wider than they are deep (Fig. 1A). A design taking into account the isotropic etching process was drawn using the program CleWin (Delta Mask, Enschede, The Netherlands). Channel widths were smaller in this design layout to compensate for widening of these features during etching. The design was transferred to a high-resolution (7 μm) transparency film to produce a negative of the design (channels transparent, background dark) (DIP Repro, Lausanne, Switzerland). Wafers were initially subjected to dehydration at 200 °C for at least 30 min and

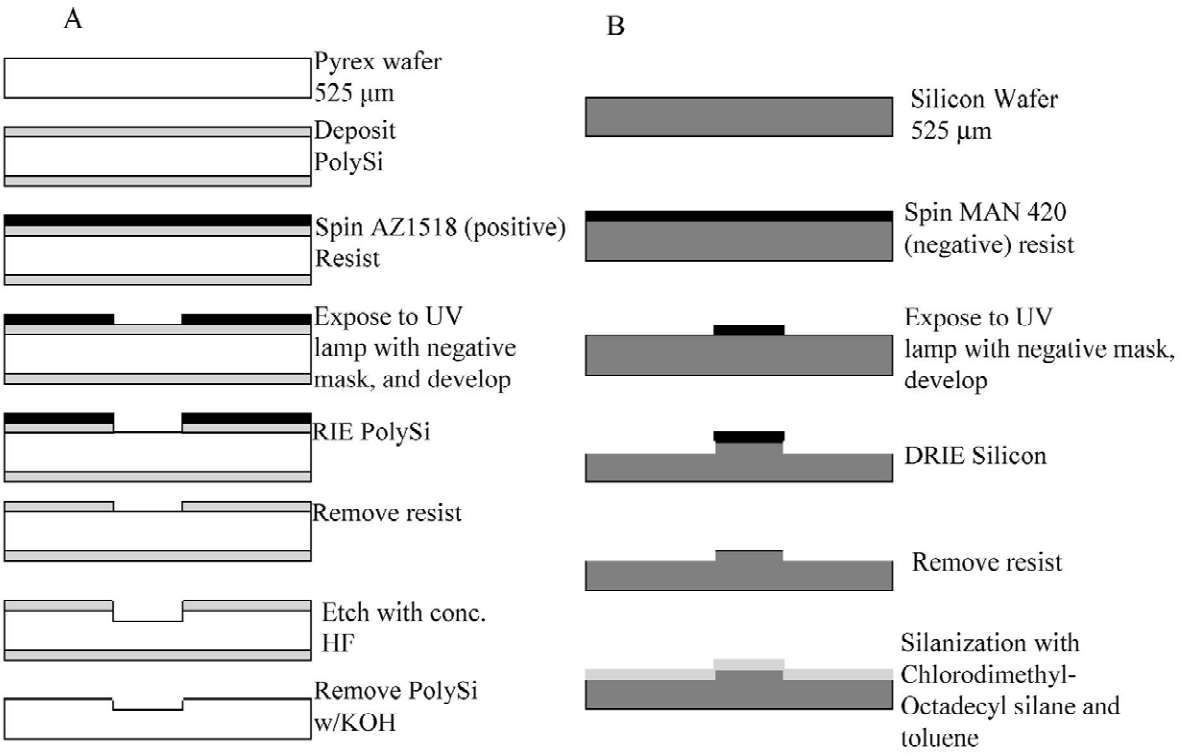

Fig. 2. Cleanroom process for fabricating microchip CE devices. (A) Process for fabricating Pyrex devices. (B) Process for fabricating a master for PDMS devices, to be used in replica molding.

subsequently exposed to hexamethyldisilazane vapor to improve the photoresist adhesion.

An AZ1518 positive photoresist (Shipley, Coventry, UK) was spun onto the Pyrex at 4000 rpm for 40 s, yielding a 1.8-μm-thick layer. After a 60 s prebake at 95 °C on a hotplate, the mask pattern was transferred to the resist using a mask aligner (Electronic Vision AL 6, Schaerding, Austria), at an exposure energy of 55 mJ. The wafer was developed in AZ 351 (Shipley) in a 1:4 mixture with water for 60 s to remove resist in the UV-exposed areas. After this, it was subjected to a postbake of 60 s at 95 °C on a hotplate.

The next step of the fabrication process was to remove polySi from the areas where the resist had been removed, using reactive-ion etching (Surface Technologies Systems, Newport, UK). The Pyrex wafer was then etched in a 50% HF solution to a depth of 12 μm and a width of 45 μm. Finally, the remaining polySi was removed by placing the wafer in a 40% KOH bath at 60 °C for 5 min. The wafer was cleaned thoroughly prior to being bonded to a second wafer containing ultrasonically drilled holes (Stecher, Thun, Switzerland).

2.3.2. PDMS chip

The PDMS microchip was fabricated with soft lithography as described previously [13,14,17–20]. The first task in producing a PDMS microchip is the fabrication of a master for the PDMS devices as shown in Fig. 2B. The master was fabricated from a 10-cm-diameter silicon wafer from Siltronix (Vernier-Geneve, Switzerland). Negative MAN 420 photoresist (Micro Resist Technology, Berlin, Germany) was spun onto the wafer at 3000 rpm for 40 s, yielding a 2.1-μm-thick layer of photoresist. A prebake of 60 s at 100 °C is required prior to exposure to remove solvents. A negative mask containing the channel design was used to carry out the lithography. The photoresist required approximately 790 mJ to complete the exposure. The wafer was developed in a MAD 336 developer water (1:1)

for 2 min. It was cleaned in a cascade bath and was subjected to a postbake of 120 s at 100 °C on a hotplate. In this case, exposed resist remained after development to define the channel layout. The wafer was then etched by DRIE to a depth of 14 μm, after which the 2.1-μm layer of MAN 420 photoresist was removed with acetone. The profile of the anisotropically etched wafer is depicted in Fig. 1B. Before replication, the wafer was silanized in 3% (v/v) chlorodimethyloctadecyl silane (Aldrich, Milwaukee, WI, USA) in dry toluene (Sigma). PDMS chips were fabricated by mixing the polymer (Sylgard 184, Dow Corning, Midland, MI, USA, obtained from Distrelec, Nanikon, Switzerland) and curing agent in a 10:1 ratio. The mixture was poured on the wafer after degassing and baked in an oven at 70 °C for 2 h. The PDMS was then peeled off the wafer and reversibly sealed to a Pyrex wafer.

2.4. Electrophoresis procedures

As previously stated, the two high-voltage power supplies [(0–2 kV) and (0–12.5 kV)] and six relays were controlled by LabView software. For these experiments, a twin-T microchip injector design was used. To inject sample, a negative potential was applied at the sample waste reservoir, effectively filling the twin-T. A separation was performed by applying a voltage of approximately 3200 V between the buffer reservoir and the buffer waste reservoir; an anti-leak voltage of 1600 V was applied to the sample and waste reservoirs to prevent sample leakage. A ratio of separation-to-anti-leak voltage equal to 2 was used for all of these experiments. Changing this ratio led to slight leaking, which resulted in a small amount of peak tailing and reduced the efficiency of the separation. Both the Pyrex and PDMS chips were conditioned at the beginning of the day by sequentially flushing for 10 min with 0.1 *M* NaOH, water, and buffer. The Pyrex microchip was flushed again with NaOH and water for 10 min at the end of each day. PDMS chips were discarded at the end of each day and a new PDMS microchip was prepared each morning.

2.5. EOF measurement

EOF was measured in the Pyrex microfluidic chips using Bodipy, a fluorescent neutral marker. The EOF and migration order of the analytes were determined at pH 9. Labeled R-R-R-G and BODIPY co-migrated, suggesting that both compounds are neutral at this pH. The R-R-R-G was then employed as a neutral marker for the PDMS studies since Bodipy absorbs strongly to the PDMS and is never detected. The EOF of the PDMS substrate was measured using the R-R-R-G peptide with buffer conditions identical to those employed with the Pyrex microchips.

3. Results and discussion

3.1. Peptide and amino acid separations using Pyrex and PDMS microchips

Analytical methods for the determination of peptides are important for proteomics research, the successful design of peptidomimetic-based drugs, and understanding the etiology of many neurological diseases. Microchip analytical systems have several potential advantages for the determination of peptides, including the potential for rapid analysis times, multichannel systems, and small sample volume requirements.

To achieve a true comparison of the performance of the glass and PDMS devices for the separation of peptides, it was critical to fabricate microchips with dimensions as similar as possible. Because different methods were utilized for fabrication of the two substrates, the same photomask (design) could not be employed. The design for the Pyrex wafer was made with narrower channels since the Pyrex is etched isotropically. Therefore, the depth and width of the channel will both be increased during the etching procedure.

DRIE was employed to produce the silicon master for the production of the PDMS chips. This is an anisotropic process, and etching takes place in a unidirectional fashion down into the bulk of the wafer. In contrast to the wet etching process used for glass, little, if any, etching occurs in other directions. The resulting relief structures are vertical-walled and have exactly the same widths as in the original layout. Because of this, a wider design was used for the PDMS master than for the Pyrex master.

Fig. 3A shows the separation and detection of

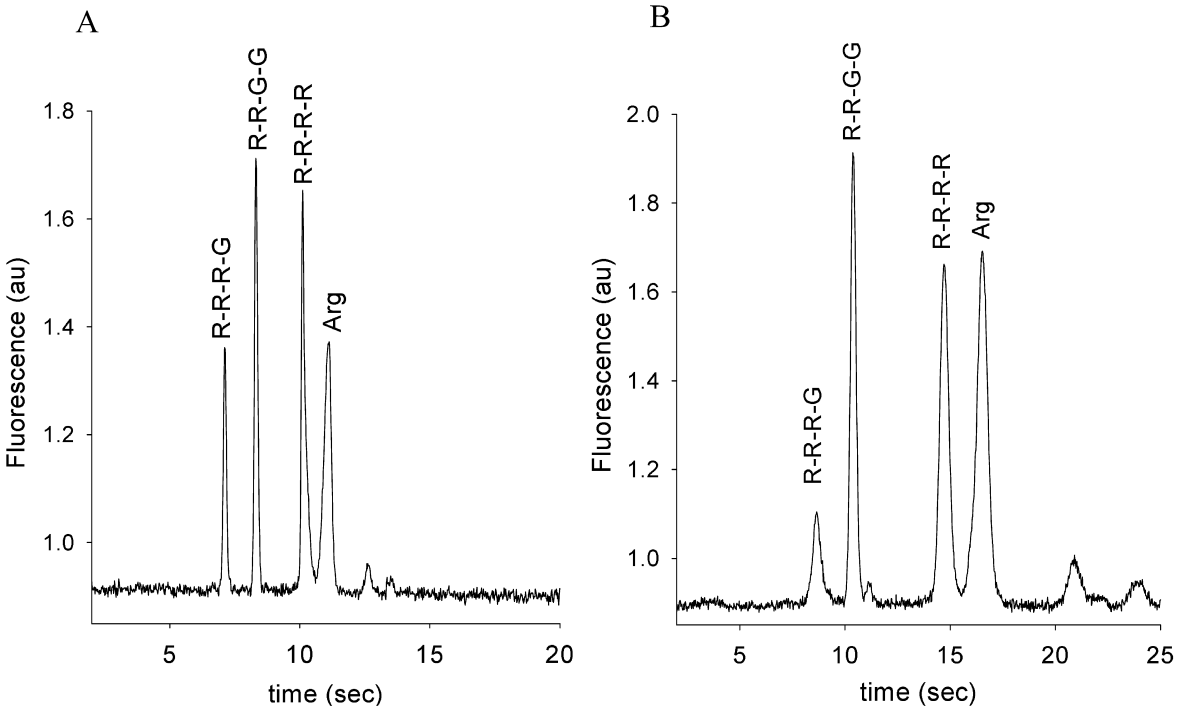

Fig. 3. Separation and LIF detection of fluorescently labeled peptides (R-R-R-R, R-R-R-G, R-R-G-G) and amino acid (Arg) at a concentration of 500 n*M*, using an injection voltage of 2000 V. The buffer consisted of boric acid, Tris (20 m*M*, 100 m*M*, pH 9.0). (A) Separation obtained using a Pyrex microchip. The separation voltage used is 3200 V with an anti-leak voltage of 1600 V. (B) Separation obtained using a PDMS microchip. The separation voltage used is 4000 V with an anti-leak voltage of 2000 V.

three arginine peptides and arginine on a Pyrex chip. The separation efficiencies and migration times for each of the peaks in the electropherogram are listed in Table 1. An excellent separation was obtained using this chip. All four peaks were resolved in approximately 12 s with separation efficiencies between 16 500 and 28 800 plates for the arginine peptides.

Fig. 3B shows the electropherogram obtained for the same compounds separated on a chip fabricated

Table 1
Migration times and efficiencies are given for the electropherograms in Figs. 3, 4, and 5

	Pyrex migration time (s)	Pyrex efficiency (plates)	Pyrex electroosmotic mobility ($cm^2/V\,s$)	PDMS migration time (s)	PDMS efficiency (plates)	PDMS electroosmotic mobility ($cm^2/V\,s$)
R-R-R-G[3]	7.1	16 500	$4.78\cdot10^{-4}$	8.6	2700	$3.17\cdot10^{-4}$
R-R-G-G[3]	8.3	22 500		10.4	6200	
R-R-R-R[3]	10.3	28 800		14.7	4900	
Arg[3]	11.1	7500		16.5	4600	
Angiotensin III[4]	11.4	13 600		15.8	5100	
Angiotensin I[4]	12.7	14 300		18.7	3000	
Angiotensin II[4]	13.4	12 300		20.7	4000	
Substance P[5]	7.7	20 400		15.2	8000	

Numbers in superscripts refer to a figure number.
Separation and injection conditions are described in the figure captions for the electropherograms.

with PDMS. A Pyrex wafer was used to seal the PDMS channel. The migration time for all of the analytes (Fig. 3B) was greater on PDMS, in spite of the fact that the separation and anti-leak voltages were increased when compared to those employed in Fig. 3A. The total analysis time was increased to 20 s. Separation efficiencies for the arginine peptides were much lower on PDMS and ranged from 2700 to 6200 theoretical plates. The EOF was approximately 1.5-fold lower in the PDMS chips.

Previous work in our laboratory has shown that small hydrophilic analytes can be analyzed relatively easily with reasonable efficiency using PDMS chips [18–20]. However, lower efficiencies were obtained on PDMS than on glass for the arginine peptides described above. In spite of the lower efficiencies, analytically useful separations of these peptides were still obtained on PDMS.

Next, the use of PDMS substrates for the separation of the biologically important peptides was investigated. Angiotensin is an important substrate of the angiotensin-converting enzyme (ACE) and is involved in blood pressure regulation [33]. In a previous study, we were able to separate angiotensin and its metabolites using conventional CE in a 75-cm long capillary with UV or EC detection [34]. Using a microchip with a 2.4-cm channel resulted in resolution equivalent to that obtained with the conventional system. Angiotensin I, II, and III were baseline resolved with both Pyrex and PDMS chips (Fig. 4) in 15 and 22 s, respectively. This is in contrast to the 18 min separation time previously reported by our group for conventional CE with UV detection. Again, the PDMS microchip separations exhibited significantly lower separation efficiencies than those obtained with the glass chips (Table 1). However, despite the lower EOF and smaller plate numbers, acceptable separations for the closely related angiotensins were still obtained on the PDMS chips.

Lastly, the separation of substance P was investi-

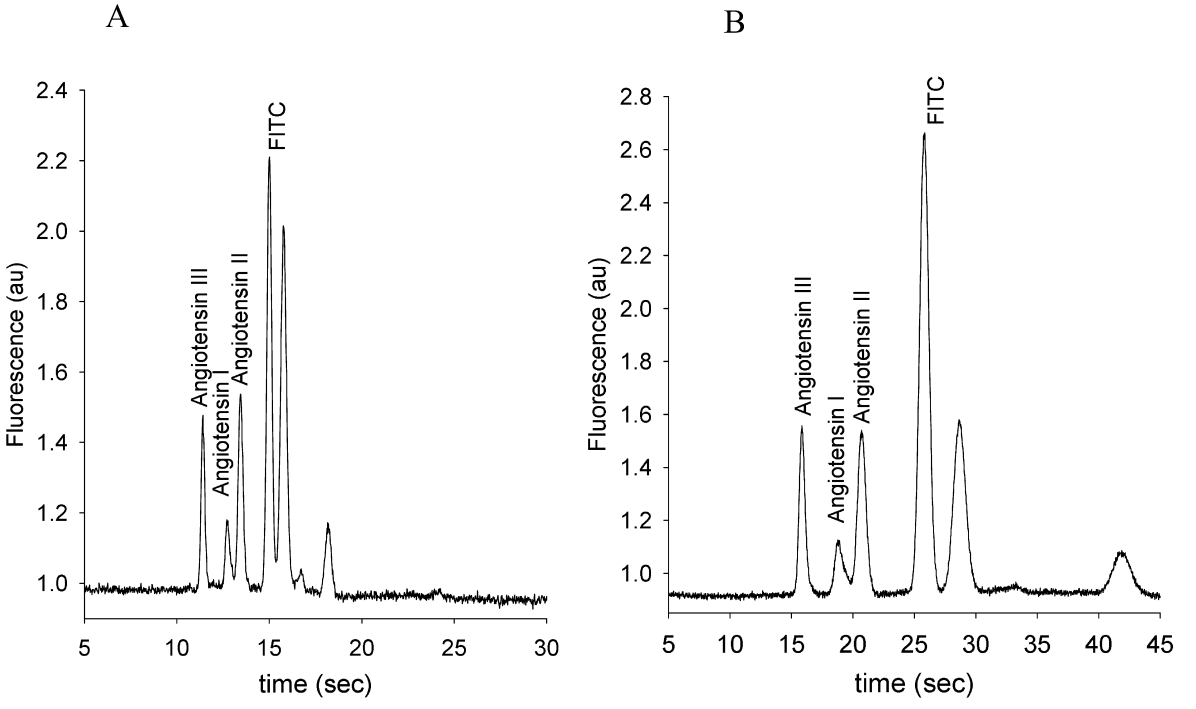

Fig. 4. Separation and LIF detection of FITC-labeled angiotensin peptides (angiotensin I, angiotensin II, and angiotensin III) at a concentration of 500 n*M* using an injection voltage of 2000 V. The buffer consisted of boric acid, Tris (20 m*M*, 100 m*M*, pH 9.0). (A) Separation obtained using a Pyrex microchip. The separation voltage used is 3200 V with an anti-leak voltage of 1600 V. (B) Separation obtained using a PDMS microchip. The separation voltage used is 4000 V with an anti-leak voltage of 2000 V.

gated. This peptide (Arg–Pro–Lys–Pro–Gln–Gln–Phe–Phe–Gly–Leu–Met–NH_2) possesses a long stretch of hydrophobic amino acids in the middle and is amidated. This is in contrast to angiotensin, in which the hydrophobic amino acids are separated by more hydrophilic ones (NH_2–Asp–Arg–Val–Tyr–Ile–His–Pro–Phe–His–Leu–COOH). Excess substance P was derivatized with a limiting amount of FITC. Based on the FITC concentration, the peak should correspond to a concentration of substance P of approximately 500 n*M*. The actual concentration is somewhat less due to incomplete labeling.

A highly efficient separation for FITC-labeled substance P was obtained on Pyrex (Fig. 5A). However, when an identical 500 n*M* substance P sample was injected into the PDMS microchip, no peaks were detected (Fig. 5B). In fact, it was necessary to inject a 10-fold-higher concentration of substance P to detect the peak shown in Fig. 5B. At this very high concentration of substance P, it appears that the surface of the PDMS may have been overloaded with sample, resulting in a drifting baseline as analyte slowly leaches from the channel surface.

The migration times for substance P with a PDMS microchip were substantially longer than those obtained using the Pyrex chip (Table 1), possibly indicating analyte adsorption. The plate numbers were also significantly lower using the PDMS substrate (8000 versus 20 400 theoretical plates). In the case of substance P, it is obvious that the performance of the Pyrex microchip is superior to that of PDMS. One possible approach for improving the performance of the PDMS microchip for the separation of hydrophobic peptides is modification of the polymer surface to make it less hydrophobic.

3.2. Buffer effects on the separation

The effect of ionic strength and buffer composition on the separation of peptides and amino acids on both PDMS and Pyrex microchips was investigated. Fig. 6A shows the effect of changing the buffer composition on the peptide and amino acid sepa-

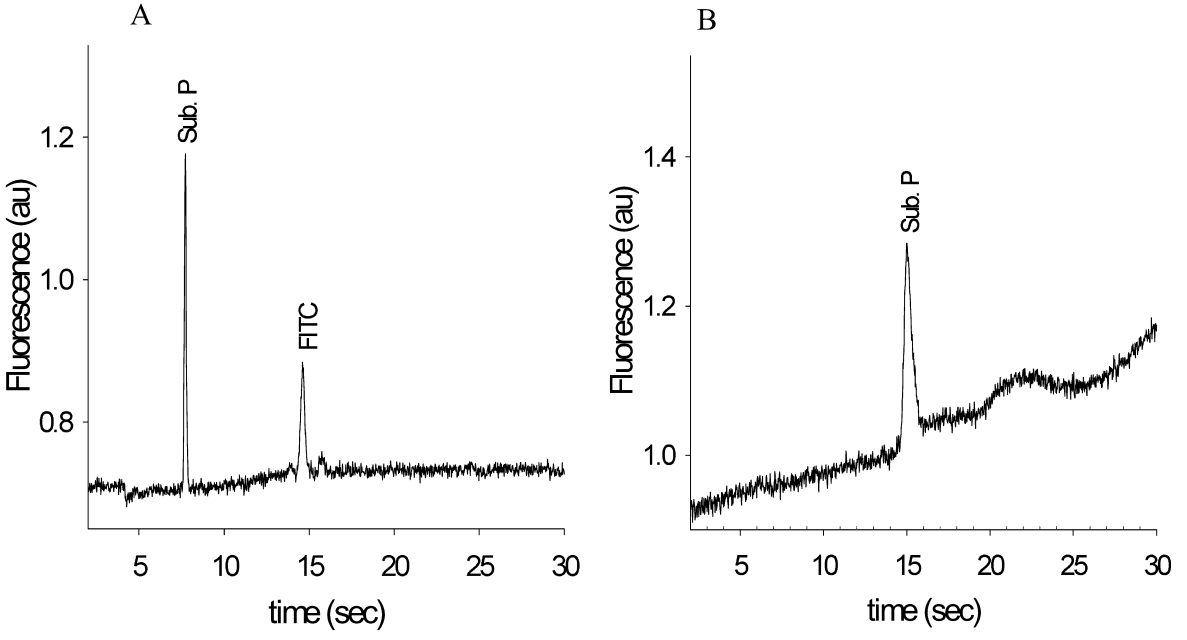

Fig. 5. Separation and LIF detection of FITC-labeled substance P using an injection voltage of 2000 V. The buffer consisted of boric acid, Tris (20 m*M*, 100 m*M*, pH 9.0). (A) Separation obtained using a Pyrex microchip with an analyte concentration of 500 n*M*. The separation voltage used is 3200 V with an anti-leak voltage of 1600 V. (B) Separation obtained using a PDMS microchip with an analyte concentration of 5 μ*M*. The separation voltage used is 3200 V with an anti-leak voltage of 1600 V.

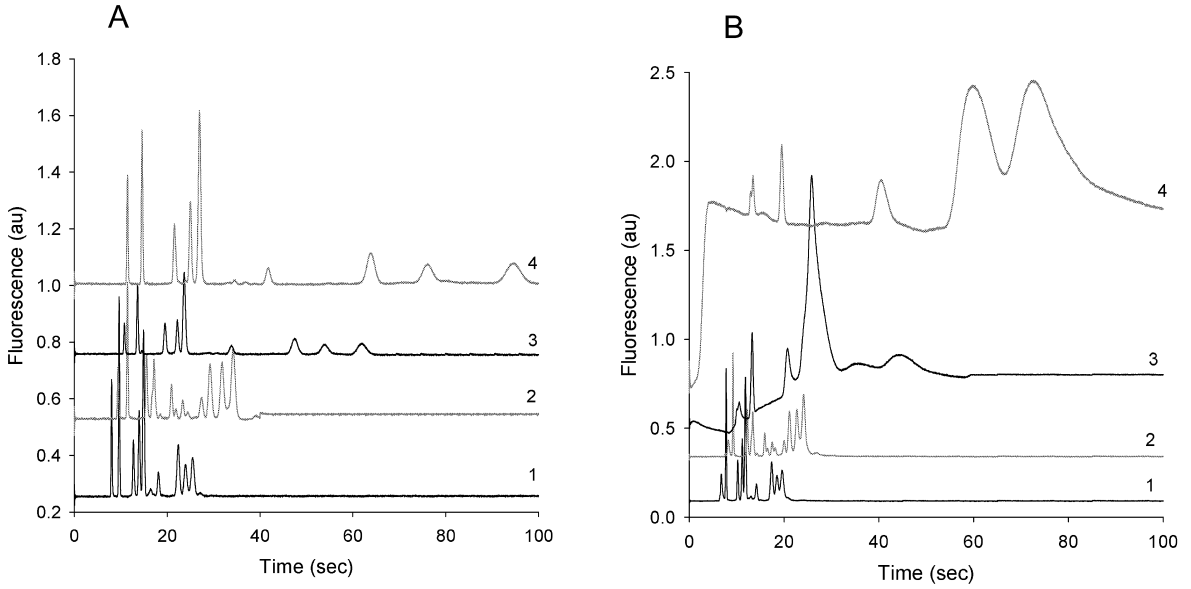

Fig. 6. Effect of buffer composition on the separation of R-R-R-R, R-R-R-G, R-R-G-G, Arg, Phe, Ser, and Gly on Pyrex (A) and PDMS (B). Analyte concentration was 500 n*M*. The voltages used are: injection 2000 V, separation 3200 V, and anti-leak voltage 1600 V. (1) Buffer: boric acid (20 m*M*, pH 9.0). (2) Buffer: boric acid, Tris (20 m*M*, 100 m*M*, pH 9.0). (3) Buffer: Tricine, Tween, (50 m*M*, 0.01%, pH 8.0). (4) Buffer: Tricine, Tween, NaCl (50 m*M*, 0.01%, 10 m*M*, pH 8.0).

ration using a Pyrex chip. Electropherogram 1 was obtained using a boric acid buffer, pH 9. The effect of Tris on the separation of the peptides and amino acids is shown in electropherogram 2. The addition of Tris caused the peaks to spread out, and the total analysis time was increased from about 30 to 40 s. Electropherograms 3 and 4 were obtained with a buffer consisting of tricine and Tween 20 at pH 8. In electropherogram 4, the buffer also contained 10 m*M* NaCl. The higher ionic strength accounts for the longer migration times observed in this electropherogram.

Tricine has been shown previously by our group to be a good buffer choice for the separation of low-density lipoproteins (LDLs) in uncoated glass microchips [35,36]. The combination of Tween 20 and tricine buffer was previously used by Chiem and Harrison for the analysis of monoclonal antibodies and theophylline [37–39]. They found that Tween eliminated protein adsorption, leading to more reproducible injections and a better separation. On the Pyrex chip, the best resolution was obtained using a run buffer consisting of 50 m*M* tricine, 0.01% Tween, and 10 m*M* NaCl; however, the total analysis time was increased to 100 s. It also appears in this case that the addition of NaCl to the buffer is responsible for peak stacking. Higher peak heights were obtained for electropherogram 4 as compared to electropherogram 3. All of these buffer systems seem to be acceptable for this peptide and amino acid separation in this substrate.

More drastic buffer effects are observed with PDMS microchips. The results are shown in Fig. 6B. First, the initial separation of the peptides obtained with PDMS chips was not quite as good as that obtained with Pyrex and the pH 9 boric acid buffer. As with the Pyrex chips, increasing the ionic strength of the buffer by adding 100 m*M* Tris resulted in longer migration times for all the peptides and amino acids. However, the most drastic difference is apparent when Tween 20 is added to the buffer as shown in separations 3 and 4 of Fig. 6B. Instead of improving the separation, the Tween appears to increase the interaction of the peptides with the PDMS surface [40,41]. The Tween concentration is well below the critical micelle concentration, so it

should not be complexing with the analytes [42,43]. The mechanism by which the separation degradation is occurring in the presence of Tween 20 is not understood at this time. However, the end result of this is an unacceptable separation for the mixture of peptides and amino acids. Since more extreme effects were witnessed with PDMS by changing the buffer composition, it shows that a simple buffer system is best for obtaining a separation of this group of analytes. Overall, the limits of detection (LODs) obtained for both of the substrates using buffer system 2 [boric acid, Tris (20 m*M*, 100 m*M*, pH 9.0] ranged from 15 to 25 n*M* for R-R-R-R, R-R-R-G, R-R-G-G, Arg, Phe, Ser, and Gly using both Pyrex and PDMS substrates.

4. Conclusions

A comparison of glass and PDMS microfluidic devices for the separation of peptides provides a meaningful reference for researchers who are interested in using polymer-based microchip CE devices. PDMS has many attractive features. Fabrication of the polymer-based chips is much less expensive, can be accomplished faster, and is amenable to mass production. PDMS has also been shown to be an ideal substrate for many of the other components of an integrated total analysis system, especially valves [44,45]. In general, glass microchip devices are more expensive, fragile, and difficult and time-consuming to produce.

This study shows that the performance of glass-based chips is superior to that of PDMS for peptide separations, although, in most cases, adequate separations can be obtained on both substrates. PDMS exhibits lower EOF and reduced plate numbers when compared to glass. All the peptides investigated in this study, with the exception of substance P, could be separated on the PDMS. Buffer additives commonly employed to reduce adsorption of hydrophobic peptides on glass did not perform well with the PDMS-based system. Currently, there are several research groups devoting significant amounts of time to finding a suitable coating for PDMS microchips. Such approaches are also currently under investigation in our laboratory.

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