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Dynamic Coating Using Polyelectrolyte Multilayers for Chemical Control of Electroosmotic Flow in Capillary Electrophoresis Microchips

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Poly(dimethylsiloxane) (PDMS) capillary electrophoresis (CE) microchips were modified by a dynamic coating method that provided stable electroosmotic flow (EOF) with respect to pH. The separation channel was coated with a polymer bilayer consisting of a cationic layer of Polybrene (PB) and an anionic layer of dextran sulfate (DS). According to the difference in charge, PB- and PB/DS-coated channels supported EOF in different directions; however, both methods of channel coating exhibited a pH-independent EOF in the pH range of 5−10 due to chemical control of the effective ζ-potential. The endurance of the PB-coated layer was determined to be 50 runs at pH 3.0, while PB/DS-coated chips had a stable EOF for more than 100 runs. The effect of substrate composition and chip-sealing methodology was also evaluated. All tested chips showed the same EOF on the PB/DS-coated channels, as compared to uncoated chips, which varied significantly. No significant variation for separation and electrochemical detection of dopamine and hydroquinone between coated and uncoated channels was observed.

In the past decade, microchip capillary electrophoresis (CE) has risen from an academic concept to a commercial product.[1] Numerous methods have been reported for the fabrication of CE microchips. Initially, work focused on microfabrication in glass and quartz because of the mature micromachining technology available for these materials.[2−8] In addition, glass and quartz, being chemically similar to fused silica, maintained many of the properties already developed for conventional CE. The optical clarity of these substrates was also significant because early work relied solely on laser-induced fluorescence (LIF) for detection.[2−4] There are several disadvantages to the use of glass. The devices require extensive use of clean-room facilities, which are expensive both to set up and to maintain. Furthermore, the fabrication process produces one chip, which, when fouled or broken, is subsequently useless. Optical-quality glass and quartz are expensive as compared to plastics and polymers, which raises the cost of each device. Finally, the fabrication process produces a permanent seal between the two plates that make up the device. If a channel clogs, the device is ruined.

The disadvantages of glass microchips have led to the investigation of alternative substrate materials for the construction of CE microchips. Several types of polymer substrates have been used successfully for CE.[9−14] Polymer substrates are advantageous because they are much less expensive than glass, are not as fragile as glass, and a wide range of material properties can be explored. The primary advantage of polymer substrates, however, lies in the ability to mass-produce devices using either embossing or molding technology outside a clean-room environment.[11] This methodology begins with the production of a molding master containing a positive relief of the pattern to be transferred onto a solid substrate.[11] The polymer material is then molded on top of the master slide, leaving an impression of the channels and other features. Sealing a second layer of material over the channels forms the final structure. Unlike glass substrates, which use a second piece of glass to form the completed channel, polymer substrates can be bonded to many different materials, including other polymers and glass, which increases the potential application.[11,14] Finally, the overall process is faster than conventional micromachining, which allows numerous devices to be produced in a short time period.

One of the most successful polymer substrates used is poly(dimethylsiloxane) (PDMS).[11−13] PDMS was first used for microchip CE by Effenhauser et al.[15] for the separation of DNA fragments in a gel-filled capillary. Duffy et al.[13] reported the first use of PDMS for open-tube microchip CE. The primary advantage

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of PDMS is the ability to rapidly prototype very complex devices. Typical fabrication times from idea to chip completion can be less than 12 h. PDMS has the added advantage of being optically transparent in the visible range, facilitating LIF detection in that spectral region. There are also several disadvantages to the use of PDMS. Analyte absorption into PDMS has been well-documented for nonpolar hydrophobic species.[11,13,16] In addition, PDMS contains significant light absorbance in the UV spectral range, limiting the applications in that spectral region. Finally, PDMS is known to absorb organic solvents, which limits buffer systems to water and some alcohols.

One common problem with polymer devices, including PDMS, is poorly defined electroosmotic flow (EOF).[16] This is a significant problem because the EOF typically dominates the linear flow velocity of both the run buffer and the analytes being separated. In PDMS, the nature of EOF is dependent on the process used for sealing chips, with some reporting[13] that an oxidation step is required to produce EOF while others[16] claim that no such step is necessary. In addition, the use of multiple substrate materials in a single device, each with a unique ζ-potential, may create problems for consistency of flow velocity and diminish separation efficiency as the result of nonuniform flow in the capillary channels. The discrepancies in both PDMS devices and other types of polymer substrates are the result of minimal characterization of surface ionizable groups under typical CE conditions. Finally, the EOF decreases rapidly with pH, making rapid separations of mixtures of anions and cations at low pH difficult.[16]

Control of EOF in CE has been addressed through both chemical and electrical manipulation of the ζ-potential.[17−20] Conventional CE frequently relies on chemical modification of the inner walls through silanization to control EOF.[17] Anionic, neutral, and cationic surfaces can be generated in this way and are useful for minimizing analyte adsorption as well as controlling EOF direction and magnitude. A second method of chemical modification is based on dynamic coating of the inner wall with a material that strongly adsorbs and alters the effective ζ-potential.[19,20] Dynamic coating typically relies upon ionic interactions. A comparison of chemical modification methods finds that dynamic coatings are easier to apply and withstand higher pH values than covalent ones; however, they are not as stable as covalent modification.[21] Hayes et al.[22] reported the first use of an external voltage to control EOF. In this technique, a high voltage was applied to the exterior of the capillary. The result was a change in the effective ζ-potential on the inner wall of the capillary. They were able to control EOF velocity and direction independently of the separation voltage. More recently, Polson et al.[23] and van den Berg et al.[24] independently applied this same technique to the modification of EOF in microchip systems for EOF control as well as microfluidic pumping and preconcentration steps.

Despite the tremendous progress that has been made in microchip CE, several areas still need to be addressed. Detection is accomplished in the vast majority of systems using LIF.[25,26] Although LIF is one of the most sensitive forms of detection, it frequently requires derivatization of the analytes to form a fluorescent species. This adds time, complexity, and cost to the analysis. These shortcomings have led to the development of alternative detection methods such as mass spectrometry (MS)[27,28] and electrochemistry (EC).[29,30] Martin et al.[12] recently reported the development of a new hybrid microchip CE−EC system that utilizes the rapid prototyping method developed by Duffy et al.[13] in PDMS in combination with microfabricated microelectrodes on glass. This method is promising because it allows reuse of the microfabricated electrodes with interchangeable channels and increases selectivity through dual-electrode detection. In the development of these devices, a concern is raised over the effect of two different ζ-potentials, one for the PDMS and one for the glass, on EOF and peak efficiency.

We report a simple method for coating microchip capillary channels using the successive multiple-ionic-layer approach reported by Katayama et al.[21,31] This method utilizes a cationic polymer, Polybrene (PB), coating, followed by a layer of anionic dextran sulfate (DS) to generate and control EOF. Native PDMS, oxidized PDMS, and hybrid PDMS/glass devices have been coated and show similar EOF values. Furthermore, the pH stability with both PB and PB/DS layers is significantly improved between pH 3 and 10 as compared to native PDMS. The overall stability of the coatings varies. PB is stable for approximately 50 runs at pH 3.0, while PB/DS is stable for well over 100 runs. Finally, coating does not appear to diminish the signal intensity for the electrochemical detection of dopamine and hydroquinone separated by CE in coated glass/PDMS devices as compared to uncoated devices.

EXPERIMENTAL SECTION

Chemicals. Sylgard 184 silicone elastomer and curing agent were obtained from Dow Corning (Midland, MI). Dextran sulfate (MW_{av} 5000; DS), hexadimethrine bromide (Polybrene, PB), and 3-hydroxytyramine (dopamine) were obtained from Sigma (St. Louis, MO). Sodium dihydrogen phosphate (dihydrate) was purchased from Acros Organics and *o*-phosphoric acid (85%), methanol, hydroquinone, 2-propanol, hydrofluoric acid, and nitric acid were obtained from Fisher Scientific (Fair Lawn, NJ). Platinum wire (diameter 0.5 mm) was obtained from Goodfellow

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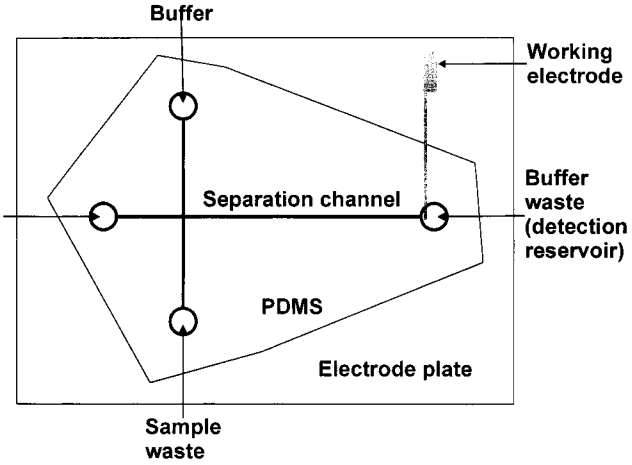

Figure 1. Schematic representation of the microchip showing the external working electrode.

(Huntingdon, England). SU-8 50 negative photoresist and XP SU-8 developer were obtained from Microchem Corp (Newton, MA). SC 1827 positive photoresist and 351 developer were obtained from Shipley (Marlborough, MA). Hydrochloric acid and hydrogen peroxide were obtained from EM Science (Gibbstown, NJ), and sulfuric acid was purchased from LabChem (Pittsburgh, PA). All chemicals were used as received.

Fabrication of PDMS Devices. A 3-in. silicon wafer was cleaned and oxidized with piranha solution (2:1 H_2SO_4:H_2O_2). (Caution! Piranha solution is a powerful oxidizing agent that reacts violently with organic compounds; it should be handled with extreme care.) The wafer was then coated with SU-8 50 negative photoresist using a spin coater (Laurell Technologies, North Wales, PA) operating at 2200 rpm for 30 s. A digitally produced mask containing the channel pattern was placed on the coated wafer, and the sandwich was exposed to light via a near-UV flood source for 5 min. The wafer was then developed in propylene glycol methyl ether acetate for 15 min, during which time the unexposed photoresist was removed, leaving a positive relief of the intended channel pattern as seen in Figure 1. The dimensions of the positive pattern, which are equal to channel dimensions created in the PDMS, were measured with a profilometer to be 83 μm wide and 17 μm deep. When fabrication of a master was completed, replica molding was used to create the channel pattern in the PDMS. A degassed mixture of Sylgard 184 silicone elastomer and curing agent (10:1) was poured onto a silicon master that had been cleaned sequentially with water and methanol and dried with a nitrogen stream. After at least 3 h of curing at 65 °C, the PDMS replica was peeled from the mold, resulting in a pattern of negative relief channels and reservoirs in the PDMS. Buffer reservoirs were then opened with a hole punch and the PDMS was trimmed to size with a scalpel. Bare PDMS replicas were formed by casting the PDMS mixture on a clean dry silicon wafer.

Fabrication of Electrode Plates. A 2.5-in. square glass plate was cleaned with piranha solution and subsequently placed in a 2-propanol bath for two minutes. The glass plate was then rinsed thoroughly with deionized water, dried with N_2 gas, and baked at 105 °C for 5 min to remove any additional water. A thermal evaporator (Denton Vacuum, Cherry Hill, NJ) was then used to sequentially deposit 50 Å of titanium and 1000 Å of gold (99.99% pure, Kurt Lesker, Clairton, PA) onto the glass plate. After the coated plate was piranha-cleaned as described above, it was coated with positive photoresist using a spin coater at 4000 rpm for 30 s. The desired positive pattern was then placed on the coated plate and exposed to near-UV light for 45 s via a UV flood source. The plate was then developed and the photoresist patterns were cured by postexposure baking. The gold and titanium were etched using aqua regia (3:1 HCl:HNO_3) and titanium etch (2% HF/0.5% HNO_3), respectively. Finally, acetone was used to strip the photoresist from the plate, which was then rinsed, dried, and stored. Prior to use, the electrode plates were cleaned with piranha solution for 10 min. The width of the working electrode in the detection zone was 150 μm.

Microchip Sealing. Modifications of previously published reversible and irreversible sealing methods were used to assemble the microchips.[12,13] Reversible sealing involved thoroughly rinsing a PDMS replica and a glass plate with methanol and bringing the two surfaces into contact with one another prior to drying. The assembled microchip was then dried in an oven at 65 °C for 10 minutes. This method of reversible sealing gave the most consistent sealing and did not require the use of clean room/hood facilities. Irreversible sealing was accomplished by first thoroughly rinsing a PDMS replica and a glass plate with methanol and then drying them separately under a stream of nitrogen. The two pieces were then placed in an air plasma cleaner (Harrick plasma cleaner/sterilizer PDC-32G) and oxidized at medium power for 45 s. The substrates were brought into conformal contact immediately after removal from the plasma cleaner and an irreversible seal formed spontaneously. This seal was sufficiently strong that the two surfaces could not be separated without destroying the assembled microchip.

Dynamic Coating of Separation Channel. Capillaries were coated with Polybrene (PB) and dextran sulfate (DS) according to the conventional CE procedures developed by Katayama et al.[21] Briefly described, the separation channel was rinsed with 0.1 M NaOH and deionized water, respectively, for 4 min each. Once preconditioned, the channel was sequentially filled with 5% PB solution and 3% DS solution (both in water) for 2 min each, with a 15-minute waiting period after each rinse. This procedure of successive coating (Figure 2) resulted in a bilayer of PB/DS on the channel walls. When only a single PB-coated layer was needed, the channel was preconditioned in the same manner and then filled with 5% PB solution for 2 min and allowed to set for 15 min before use. All rinsing was performed by applying a vacuum to the buffer waste reservoir with the other three reservoirs filled with the respective rinsing solution.

EOF Detection. The running electrolyte for electrophoresis experiments was pH 3−10 phosphate buffer. The pH was established by titrating a solution of either *o*-phosphoric acid or sodium dihydrogen phosphate with sodium hydroxide. All buffers were prepared in deionized water, passed through a 0.20-μm pore size syringe filter (Whatman), and degassed for 5 min in a sonicator (Fisher Scientific, FS 20) before use. The buffer was introduced into the reservoirs and subsequently flushed through the separation channel via a vacuum until no air bubbles were observed. Only the sample and buffer waste reservoirs were used for EOF detection (see Figure 1). The sample reservoir was connected to a high-voltage power supply (Stanford Research

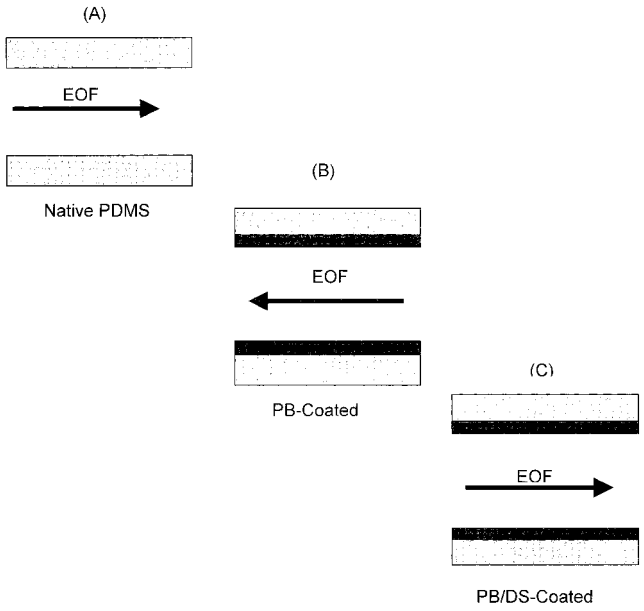

Figure 2. Successive multilayer coating procedure: (A) preconditioned channel containing negative surface groups, (B) first layer coating with a 5% PB water solution rinsing under vacuum, (C) second layer coating with a 3% DS water solution rinsing under vacuum. Arrows indicate the relative direction of EOF.

Systems, PS350/5000V-25W) through a platinum electrode while the buffer waste reservoir was grounded. This setup was used for all EOF experiments except those involving the PB coating, in which the relative location of the high voltage and ground were switched due to the reverse EOF supported by the PB coating.

A modification of a previously published current monitoring method was used to determine the EOF.[32,33] All reservoirs were filled with dilute buffer (2:1 buffer:water), and the channels were subsequently conditioned at a potential of 1200 V for 15 min. The increased dilution factor as compared to standard protocol (19:1 buffer:water)[16] was used to ease end point detection. No statistical differences in the absolute values were noted between the two protocols. The sample reservoir was then filled with concentrated buffer, and the potential was reapplied. The time required for the current plateau was measured for each run and was indicative of the concentrated buffer's filling the separation channel. The sample reservoir was then filled with dilute buffer and the above procedure repeated. Six to eight consecutive measurements were obtained for each experiment. The time required for the current to reach this plateau was used as the migration rate of a neutral marker, and the EOF is determined by

$$\mu_{\mathrm{EOF}} = L^2/(Vt) \qquad (1)$$

where L is the length of the separation channel (4.2 cm), V is the total applied voltage (1200 V), and t is the time in s required to reach the new current plateau. This is a modification of the traditional mobility equation that takes into account that the total and effective capillary lengths are identical.

Microchip CE−EC. The gated injection method developed by Jacobson et al.[34] was used for injection in microchip CE−EC experiments. The channels and reservoirs were first filled with buffer solution under vacuum. The buffer solution in the sample reservoir was then replaced by sample solution. A potential was applied to the buffer and sample reservoirs while both waste reservoirs were grounded. This allowed only buffer to flow down the separation channel, while the sample flowed to its respective waste reservoir. Gated injections were accomplished by floating the potential at the buffer reservoir. This allowed the sample reservoir to be additionally grounded through the buffer waste reservoir,which caused the sample to flow down the separation channel. When the potential was reapplied to the buffer reservoir, a plug of sample remained in the separation channel, which allowed the separation and detection of the analytes. Approximately 30 MΩ of resistors was needed between the sample waste reservoir and its ground to prevent leakage of sample down the separation channel during pre-injection and separation modes.

Electrochemical detection was performed in the amperometric mode using an electrochemical detector (CHI 812, CH Instruments, Inc.) operating in a three-electrode configuration. A Ag/AgCl (3 M KCl) reference electrode, a platinum wire counter-electrode, and a microfabricated gold band electrode at 0.75 V were used for all experiments. Alignment of the working electrode was accomplished during the reversible sealing process. Immediately after the microchip was assembled, the working electrode was aligned at the exit of the separation channel into the buffer reservoir using a stereomicroscope (M2 Associates) and then allowed to dry as previously described. In situ cleaning of the working electrode was accomplished via cyclic voltammetry with twenty consecutive sweep segments from −1.8 V to 1.2 V (scan rate, 0.5 V/s) while buffer was electrokinetically pumped over the working electrode.

RESULTS AND DISCUSSION

Dynamic Coating of PDMS/Glass Microchips. The pH dependence of EOF in both fused silica and glass and PDMS microchips is well-known.[16,31] At low pH, EOF is at a minimum, while at high pH, it is at a maximum. This behavior is the result of titration of the surface-bound silanol groups. One method for modifying this behavior is to dynamically coat the inner walls of the capillary with molecules containing different acid/base functional groups.[21,22,31] We applied the method to microchip CE in order to stabilize EOF with respect to pH. Therefore, the EOF with respect to pH in PB- and PB/DS-coated channels was investigated and compared to the EOF of an uncoated natively sealed microchip. The results are shown in Figure 3. The EOF of the native chip ranged from 0 to $4.89 \pm 0.36 \times 10^{-4}$ cm^2 V^{-1} s^{-1} from pH 3−10, respectively, and exhibited similar behavior as previously published for natively sealed microchips.[16] Coating with PB generates a cationic surface once it becomes ionically adsorbed onto the capillary wall, which supports a reverse EOF (cathode to anode). The change of EOF from pH values of 3 to 10, which varied from $-4.29 \times 10^{-4} \pm 0.04$ to $-1.95 \times 10^{-4} \pm 0.06$ cm^2 V^{-1} s^{-1}, was less than that observed in the uncoated channel. The EOF was significantly stabilized between pH 5 and 10, with a variation of 17.7%, and good stability of EOF showed between pH

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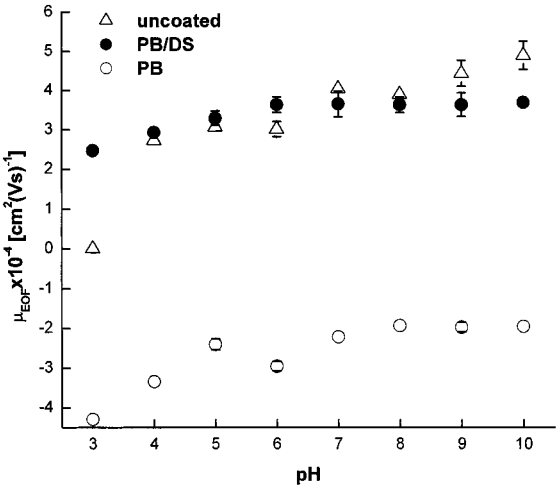

Figure 3. EOF comparison of uncoated (△), PB-coated (○), and PB/DS-coated (●) PDMS/glass chips. Applied voltage, 1200V; buffer, 10 mM phosphate buffer; pH, 3.0−10.0; channel length, 4.2 cm. Error bars are within symbols unless shown.

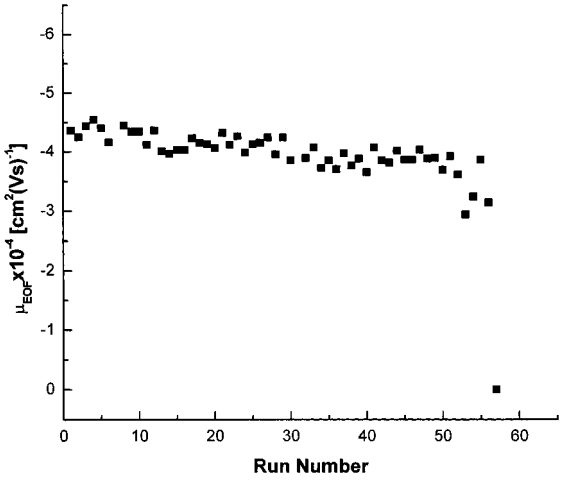

Figure 4. Stability of PB coating layer as a function of the number of runs. Conditions were as described in Figure 3.

7 and 10, with a 6.5% difference. After the addition of DS, the EOF is again reversed (anode to cathode) as a result of the anionic nature of DS. The EOF varied from $2.47 \times 10^{-4} \pm 0.10$ to $3.69 \times 10^{-4} \pm 0.09$ cm^2 V^{-1} s^{-1} over the pH range from 3 to 10. The stability between pH 6 and 10 was extremely good, with only a 1.6% difference. Additionally, EOF at pH 3 for the PB/DS-coated channel was $2.47 \times 10^{-4} \pm 0.10$ cm^2 V^{-1} s^{-1}, as compared to 0 for an uncoated microchip. This should allow for the exploration on microchips of simultaneous separations of cationic, neutral, and anionic species requiring strongly acidic conditions.

The ability of both PB and DS to stabilize EOF with respect to pH is a result of the pK_a's of these two polymers. For PB, the functional group is a quaternary amine. This structure is easily protonated and retains this charge throughout the pH range studied in these experiments. The functional group responsible for EOF with DS coatings is a simple sulfate. These groups are easily deprotonated throughout the pH range studied and, as a result, stabilize EOF with respect to pH while giving flows that are similar to the native silanol groups. The ability to rapidly alter the surface chemistry of microfluidic channels is significant, especially for plastic and polymer substrates for which the surface functional groups are unknown. Furthermore, this approach may be used to add functionality to the surface through the incorporation of enzymes or other functional molecules. Finally, because EOF is the result of the surface chemistry and does not rely on the bulk properties of the substrate, this method offers the promise of providing a constant EOF regardless of substrate material. One limitation with any coating method, and especially dynamic coating, is the stability of the resulting layers.

Stability and Reproducibility of Coated Layer. One concern with the noncovalent dynamic coating procedure is the stability of the coating. A pH of 3 was chosen for evaluation of the PB lifetime because at this pH, uncoated channels do not support EOF, due to their uncharged nature. The PB layer should be the least stable at this pH as a result of the lack of charges on the PDMS. As the coating becomes detached from the channel wall, the EOF will approach zero until the coating becomes completely detached, at which point the EOF direction will be reversed, and no current change will be detected. Ten consecutive runs were performed in ten-minute intervals, and the results are shown in Figure 4. The PB layer endured a total of 56 runs, with a noticeable falloff in EOF occurring from runs 50 to 56. For subsequent experiments, the channel was recoated every 50 runs to replenish the PB interaction with the channel walls. Loss of the PB coating is most likely the result of the reprotonation of silanol groups and the loss of ionic interaction between the channel wall and the PB. PB/DS channels have a longer lifetime than that of a single PB layer. No significant variation was noticed after more than 100 runs. This is possibly because the DS layer prevents the PB layer from detaching from the channel walls.

The reproducibility of the coated layer from chip to chip was also investigated. The EOF was detected in six PB/DS-coated chips at three different pH values, and the results are shown in Figure 5. The relative standard deviation (RSD) of the EOF for 6 PB/DS-coated PDMS/glass chips was 2.40%, 1.60%, and 3.23% respectively, for pH 5.0, 7.0, and 9.0. Excellent EOF consistency at differing pHs was observed among all chips. This is indicative of the complete and effective covering of the channel walls by the PB and DS layers. If only partial covering of the native functional groups were obtained, it would be unlikely that this level of consistency could be observed.

Influence of Substrate and Sealing on PB/DS Coating. The coating's ability to generate a consistent EOF regardless of substrate and sealing technique was investigated. PDMS and glass are reported as having similar surface functional groups and, therefore, should exhibit similar behavior as substrates in CE microchips.[13] The EOF differed significantly between uncoated oxidized and native PDMS/glass microchips (Figure 5a). Measured EOF values for both sealing methods were consistent with those previously published.[16] An expected increase in EOF with pH was observed on both chips, with the oxidized chip supporting a greater EOF at all three pHs. The direction of EOF (toward the cathode) indicates that both of these chips contain a negative surface charge. Furthermore, PDMS/PDMS chips have already been shown to contain negative charges.[11] Because all three kinds of chips supported a negative surface charge, it was hypothesized that the PB/DS coating layer would compensate for the differences in type and density of anionic groups as well as the

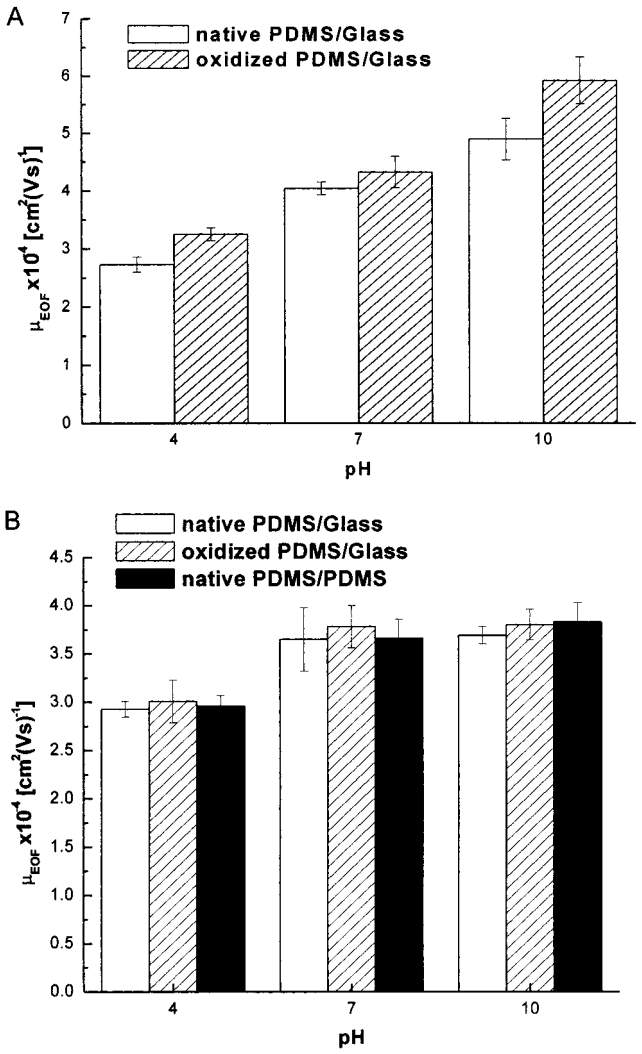

Figure 5. (A) EOF of uncoated native and oxidized PDMS/glass microchips; (B) EOF comparison of native PDMS/glass, oxidized PDMS/glass, and native PDMS/PDMS with all chips coated with PB/DS. Conditions were as described in Figure 3.

difference between chip sealing techniques. Experiments were run for PB/DS-coated channels of native and oxidized PDMS/glass microchips and natively sealed PDMS/PDMS microchips to explore this possibility. The results for pH values of 4, 7, and 10 are shown in Figure 5b. Similar EOF values were observed for the three types of microchips after coating. This result is significant because it implies that this coating procedure may be used to establish a consistent EOF regardless of the substrate material as long as the initial ionic interaction can be preserved. This supports the hypothesis that the coating is solely responsible for the generation and control of EOF in the microchannels.

Effect of Coating on Electrochemical Detection. The coating's influence on electrochemical detection (EC) was explored. EC is advantageous for microchip CE for several reasons. First, many compounds can be detected electrochemically without derivatization while maintaining high levels of sensitivity.[12] Second, miniaturization of the working electrode is accomplished via microfabrication methods that are similar to those used to fabricate the master for PDMS channel formation. Finally, EC does not require large and expensive off-chip optical sources. Adversely,

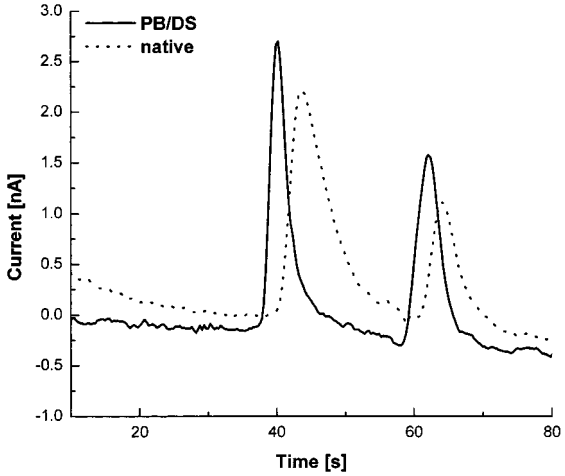

Figure 6. Electropherogram showing the separation of 20 μM dopamine and 40 μM hydroquinone in PB/DS-coated and uncoated microchips. Separation conditions: applied potential, 600 V (142 V/cm); buffer, 10 mM phosphate buffer; pH, 7.0; injection, 3 s at 600 V.

one concern with EC detection is electrode fouling, which can occur as a result of either analyte or polymer adsorption onto the working electrode surface.[12] This is of particular interest when using a dynamically coated layer in which any detached coating could potentially adsorb onto the electrode surface. This would cause a decrease in the detection signal as a result of the analyte's inability to reach the electrode surface and undergo its respective redox reaction.

Separations were performed using both a PB/DS-coated and an uncoated microchip. Three-second injections of 20 μM dopamine and 40 μM hydroquinone mixtures were achieved by the gated injection method described previously.[34] An overlay of the separation on both a PB/DS-coated and an uncoated microchip is shown in Figure 6. Both analytes are oxidized at the working electrode and are, therefore, represented by positive peaks for dopamine (positively charged) and hydroquinone (neutral), respectively. No significant difference in detection sensitivity between the coated and uncoated channel was observed. This suggests an insignificant amount of adsorption of the coated layer onto the working electrode surface. This was expected because the electrode surfaces are uncharged and, therefore, would have no ionic attraction for the polymers. The EOF value, as determined from the migration time of hydroquinone, was consistent with that reported by the current monitoring technique.

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