Short Communication

Electroosmotic Flow in a Poly(dimethylsiloxane) Channel Does Not Depend on Percent Curing Agent

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Abbreviations: PDMS – poly(dimethylsiloxane), EOF – electroosmotic flow

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The use of microfluidic devices constructed from poly(dimethylsiloxane) (PDMS) is popular because of the ease with which such devices are fabricated. Bulk PDMS consists of a simple repeating structure ($-Si(CH_3)_2-O-$); however, its surface properties are poorly understood, especially as they relate to electroosmotic flow (EOF). In the present work, PDMS microfluidic devices were prepared from different ratios of "curing agent" (which contains silicon hydride groups) to "base" (which contains vinyl-terminated noncrosslinked PDMS), to determine the effect of this ratio on EOF. In fabricating devices for this purpose, a novel method for permanently enclosing PDMS channels was developed. As a supplement to the microfluidic method, the inner walls of capillaries were coated with PDMS formed from varying ratios of curing agent to base. EOF was found to be constant for PDMS formed with each ratio, which implies that the negative surface charges do not arise from chemical species present only in the base or curing agent.

Since microfabricated channels were first used for capillary electrophoresis [1] in the early 1990s, microfluidics has become an increasingly popular technology for chemistry and biology [2, 3]. Advantages of reducing scale and reagent use, combined with the potential for "lab-on-a-chip" integration have driven this growth. Originally, glass was the substrate of choice for microfluidics; however, the arduous efforts required to fabricate such devices have led to developing alternative fabrication methods using polymer substrates. One substrate, poly(dimethylsiloxane) (PDMS) has been the most popular polymer used for microfluidics [4-6].

Many microfluidics-based techniques rely on electroosmotic flow (EOF) for fluid transport [2, 3]. EOF is dependent on the density of charges on the surface that encloses the fluid. The acidic nature of silanol (Si-OH) groups on the surface of glass channels is widely known; however, less is known about the surface chemistry of PDMS. Bulk PDMS ($-Si(CH_3)_2-O-$) is neutral.

In the first literature report on the use of PDMS microfluidic devices for separations, Effenhauser *et al*. [7] described electrophoresis of DNA and speculated that there was no EOF. Duffy *et al*. [4] showed that plasma oxidation of PDMS created negative surface charges that could support cathodic EOF for microfluidics. Later, Ocvirk *et al*. [8] reported that native (unoxidized) PDMS devices also supported cathodic EOF and speculated that "silica fillers" were the source of the negative charges. Recently, Ren *et al*. [9] demonstrated that oxidized devices supported EOF four times stronger than native devices; also, infrared spectroscopy revealed that oxidized surfaces have large O-H stretches (suggesting silanol groups), but that unoxidized surfaces did not. The question

of why native PDMS should support EOF remains unanswered. In the present study, this question has been re-examined by taking advantage of the two-part nature of the material.

Typical PDMS formulations are polymerized by mixing a "base," consisting mainly of long PDMS monomers capped by vinyl groups, with a "curing agent," consisting of shorter PDMS monomers with silicon hydride groups [10-12]. When mixed with a platinum catalyst, the liquid polymerizes as silicon hydrides add across vinylic double bonds. The base usually contains the catalyst and other additives including various forms of silica [10] to increase tensile strength. According to the manufacturer's specifications, the base PDMS used in the present work, RTV 615 (GE Silicones, Waterford, NY), contains precipitated sodium silicate. To confirm this, RTV 615 was characterized spectroscopically.

Figure 1 presents infrared spectra of RTV 615 (neat samples prepared with NaCl plates, using a Perkin Elmer spectrometer, Wellesley, MA). The IR spectrum of the base (Fig. 1a) contains several expected peaks [13], including C-H stretches at 2900 cm^{-1}, a C=C stretch at 1480 cm^{-1}, a Si-CH_3 deformation at 1280 cm^{-1}, and two broad Si-O-Si stretches at ~1100 cm^{-1}. The IR spectrum of the curing agent (Fig. 1b) is similar except for the addition of a Si-H stretch at 2100 cm^{-1} and deformation at 850 cm^{-1}. It is notable that no O-H bands at ~3400 cm^{-1} are observed in either spectrum, suggesting that the silicate "fillers" have been reacted with silicone process aids to neutralize the reactivity of their silanol groups [10]. H^1 NMR spectra (not shown) are similar to those of related compounds [14], with peaks at 0.2 ppm (Si-CH_3) in both spectra, with the addition of a multiplet at 5.9 ppm (C=CH_2) in the base, and 4.7 ppm (Si-H) in the curing agent

(prepared by dissolving 20 mg base or curing agent in 0.7 mL deuterated chloroform using a 400 MHz Varian spectrometer, Palo Alto, CA).

The lack of an O-H stretch agrees with previous work [9], which suggests that silicate additives to the mixture are not the source of negative surface charges. Even so, we hypothesized that other additives to either the base or the curing agent (such as hydrolysis of unreacted silicon hydrides [15]) might be the source of charges for EOF. Manufacturers usually formulate two-part PDMS for a 10:1 ratio of base to curing agent; however, it is known that usable polymer can be formed from other ratios [12, 16]. In the current work, PDMS flow cells were constructed from varying ratios of base to curing agent for EOF measurements.

Microfluidic devices were fabricated using soft lithography [4-6]. Briefly, a positive mask with eight parallel, 40 mm x 100 μm lines was printed (3600 dpi) on transparency film. In the Stanford Nanofabrication Facility (SNF), silicon wafers (4 in. dia.) were spin-coated (1700 RPM, 40 s) with SPR 220 photoresist (MicroChem Corp., Newton, MA), to achieve a thickness of 11 μm. The mask pattern was photolithographically transferred onto the silicon wafers, and the resulting feature dimensions were confirmed with profiliometry. The photoresist-on-silicon master was then "silanized" with trimethyl chlorosilane (TMCS) vapor in a dessicator (atmospheric pressure, 5 min). An appropriate PDMS blend (5, 10, 20, or 40% curing agent, by weight) was mixed and degassed in an evacuated chamber (~100 torr, 1 hr); this mixture was poured onto the silanized master (in an aluminum foil lined petri dish) and cured (80° C, 25 min) in an oven (this is the first of two cure steps, see below). After cooling, the

device was peeled from the master, trimmed, and via holes were punched with a blunt 16 gauge needle.

PDMS has the unique property of forming a temporary "conformational" seal with many substrates; microchannels are sometimes sealed to cover plates in this manner. Such seals are liable to leak, and are thus difficult to fill with positive pressure. To overcome this problem, PDMS microchannels are often permanently sealed using an oxygen plasma treatment [4-6] or by chemically bonding a heterogeneous PDMS cover plate [16]. For the present experiment, the ease of filling permanently sealed devices was desired; however, each of the channel walls was required to be homogeneous for accurate EOF studies. To satisfy both of these criteria, a new method for permanently sealing PDMS devices using a two-step cure process was developed.

To maintain identical surface properties on all walls of the fluidic channels, a "cover plate" was formed, consisting of a thin layer of PDMS (spin coated, 4000 RPM, 60 s) on a glass wafer (4 in. dia.). For each device, the same mixture of PDMS (with characteristic ratio of base to curing agent) was used for the cover plate and for the microfluidic piece (described above). The cover plate was cured in an oven (80° C, 25 min). The microfluidic piece was aligned onto the cover plate, and the combined device was cured overnight in an oven at 120° C. A permanent bond between the two pieces was thus formed; all walls on the microfluidic devices formed in this manner should have identical surface properties.

After fabrication, the fluidic devices were prepared for measurement of EOF. The channels were filled with methanol followed by 20 mM sodium phosphate buffer using positive pressure from a syringe. Small glass pieces cut from microscope slides were

placed over inlet holes to limit evaporation. Platinum wires (0.25 mm dia., Goodfellow, Huntington, UK) were inserted through the walls of the PDMS to make electrical contact with buffers. High voltage was applied with a homemade power supply; a picoammeter (Keithley Instruments, Cleveland, OH) was inserted in series between the buffer outlet and ground. Channels were conditioned by applying 1000 V for 10 minutes.

EOF within the channels was measured using a 2:1 buffer dilution variation [17] of the current monitoring method [18]. After conditioning, the inlet solution was replaced with 10 mM sodium phosphate buffer and 1000 V was applied. Sometimes the process was reversed such that the channel was conditioned with 10 mM buffer, and then replaced with 20 mM buffer. For each run, a voltage trace of the current was collected into a PC and recorded as a function of time using LabView software (National Instruments, Austin, Texas). The time at which the inflection point in current occurred was observed, and electroosmotic mobility was calculated according to equation one:

$$\mu_{eof} = \frac{L^2}{Vt} \tag{1}$$

where L is the length of the channel, V is the applied voltage, and t is the time at which the current change is observed.

Figure 2 shows a sample current trace. The EOF of PDMS microchannels was observed to vary widely between devices, regardless of cross-linker concentration (data not shown). The irreproducible EOF in PDMS microchannels has been widely reported [9, 19]. Several explanations have been proposed, including subtle variations in mixing/curing conditions, and batch-to-batch variations in the two-part formulations. PDMS has low thermal conductivity [5] (0.2 W·m/K) that makes high-voltage dependent methodology especially susceptible to Joule heating and ambient temperature changes.

Also, PDMS is gas permeable, which means that evaporation can change the electrolyte concentration in the run buffer.

For the reasons listed above, we conclude that untreated PDMS is a poor material for microfluidic-based methods relying on reproducible EOF. Although the common method of plasma oxidation of PDMS surfaces has been demonstrated to have unstable EOF after drying [4, 9], perhaps other methods, such as grafted polymeric coatings [20], or "dynamic" coatings with surfactants [21, 22] or polyelectrolytes [4, 17] would prove to be useful. Regardless, it is anticipated that the novel method of creating permanently sealed microchannels might be useful for future work (for example, if an oxygen plasma etcher is not available).

In an attempt to conduct reliable EOF measurements on untreated PDMS surfaces, coated capillaries were used with a commercial capillary electrophoresis instrument (PACE 5000, Beckman Coulter, Fullerton, CA). This approach offered several advantages, including easier wetting and filling of channels, autosampling apparatus for more reproducible injections, and active temperature control.

Capillary walls were coated using the static method [23]. Separate 5.3% solutions of PDMS base (part A) or curing agent (part B) in diethyl ether were prepared. Before coating, solutions were mixed with curing agent concentrations of 5, 10, 20, and 40%. Mixed solutions were degassed with ultrasonication and pressure filled into fused silica capillaries (150 μm o.d., 75 μm i.d., Polymicro Technologies, Phoenix, AZ). Each capillary was closed at one end with silicone sealant (Dow Corning, Midland, MI), and the other end was connected to a mechanical pump (Alcatel, Reston, VA) to evaporate

the solvent. Evaporation was confirmed visually, and each coated capillary was cured in an oven (80° C, 1.5 hr).

Each capillary was conditioned with pressure-driven rinses of 100 mM NaOH (0.1 min), and 20 mM sodium phosphate buffer (10 min), followed by an EOF-driven rinse with 20 mM phosphate buffer (3 kV, 15 min) before EOF measurements. Temperature was controlled at 20° C, and the linearity of Ohm's Law (current vs voltage) plots (from 1-10 kV) for 10 and 20 mM phosphate buffers (not shown) confirmed that heat was dissipated effectively.

Two types of EOF measurements were conducted for each capillary: current monitoring and injection of a neutral peak. For current-monitoring studies, after conditioning with 20 mM buffer, the inlet buffer solution was changed to 10 mM buffer, and a current trace as a function of time was recorded. Electroosmotic mobility was calculated according to equation one. For neutral peak measurements, thiourea was injected (2.5 mM in D.I. water, 3 s pressure injection), and detected with absorbance, running in 10 mM phosphate buffer (same conditions as above). For these data, windows were formed in the polyamide outer-coating of the capillaries for absorbance detection. Elution time was recorded, and equation one (corrected for effective length of capillary) was used to calculate electroosmotic mobility. Representative data from the current monitoring and neutral peak injection measurements are shown in Figure 3.

For each ratio of base to cross-linker, at least two separate capillaries were evaluated, with at least five measurements for each capillary. As a control, the EOF of bare capillaries was also evaluated. The calculated electroosmotic mobilities [$cm^2/(V \cdot s)$] for uncoated capillaries using the current monitoring method ($5.73 \times 10^{-4} \pm 0.11 \times 10^{-4}$)

and neutral injection ($6.22 \times 10^{-4} \pm 0.23 \times 10^{-4}$) were similar, and consistent with previous observations [24] that the second method yields larger absolute EOF measurements. Regardless, in the present work, only relative EOF measurements were important to determine the influence of different concentrations of curing agent in the PDMS coating.

Figure 4 depicts the cumulative results of measurements in capillaries coated with PDMS polymerized with four different concentrations of curing agent. As has been reported [9, 21], PDMS surfaces exhibited reduced EOF compared to that of bare silica. Both measurement techniques resulted in similar values for coated capillaries. Somewhat surprisingly, there was no trend in EOF on curing agent concentration.

Because cathodic EOF was observed in all cases (for coated capillaries and microfluidic devices), it is apparent that the surface of native PDMS is negatively charged. The data in Figure 4 imply that the surface charges on untreated PDMS do not arise from silicates, silicon hydrides, or other chemical species present only in the base or curing agent. It is known that bulk PDMS is vulnerable to nucleophillic or electrophillic attack at extreme pH values, resulting in hydrolysis to form silanol groups [11]. Others [25] have shown that exposure to water for long periods (*i.e.,* more than 20 hr) can cause PDMS surfaces to become more hydrophilic, but that hydrophobicity recovers quickly after exposure to air. It is possible that EOF in unoxidized PDMS microfluidic devices is caused by this phenomenon; however, it is difficult to understand why this process would take place so quickly using the moderate pH buffers that most microfluidic techniques employ. This question clearly requires more study. In any case, the present study shows that the source of the EOF in unoxidized PDMS channels is not to be found in the amount of curing agent used in fabricating these structures.

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Figure Captions

Figure 1: Infrared spectra of (a) base and (b) curing agent used in forming PDMS channels.

Figure 2: Sample EOF measurement using PDMS flow cell. At $t = 0$, 20 mM sodium phosphate buffer was replaced with 10 mM buffer. Buffer exchange was observed at $t = 43$ s.

Figure 3: EOF measurements in PDMS-coated capillaries using (a) the current monitoring method and (b) injection of a neutral analyte. For (a), the buffer was changed from 10 mM to 20 mM sodium phosphate at $t = 15$ min; buffer exchange was observed at $t = 9.5$ min. For (b), 2.5 mM thiourea was injected at $t = 0$ min and was detected at ~6.4 min. These measurements agree well; the shorter distance to the detection window caused the time difference in (b). The data shown were collected in capillaries coated with PDMS prepared with 10 % curing agent.

Figure 4: Electroosmotic mobility measured for capillaries coated with PDMS prepared from varying ratios of curing agent to base. EOF measured with the current monitoring method (■) and by detection of a neutral analyte(○). Error bars are ± 1 SD (at least five replicate measurements for each condition).

Figure 1

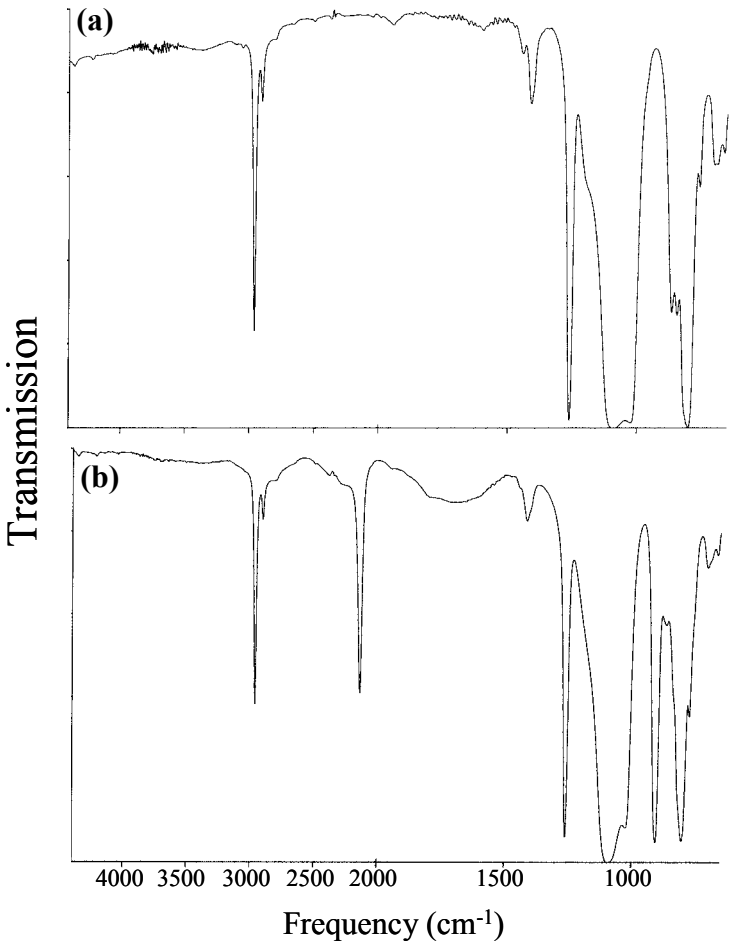

(a)
(b)
Transmission
4000 3500 3000 2500 2000 1500 1000
Frequency (cm-1)

Figure 2

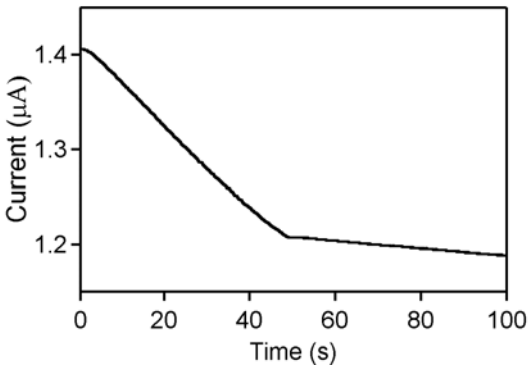
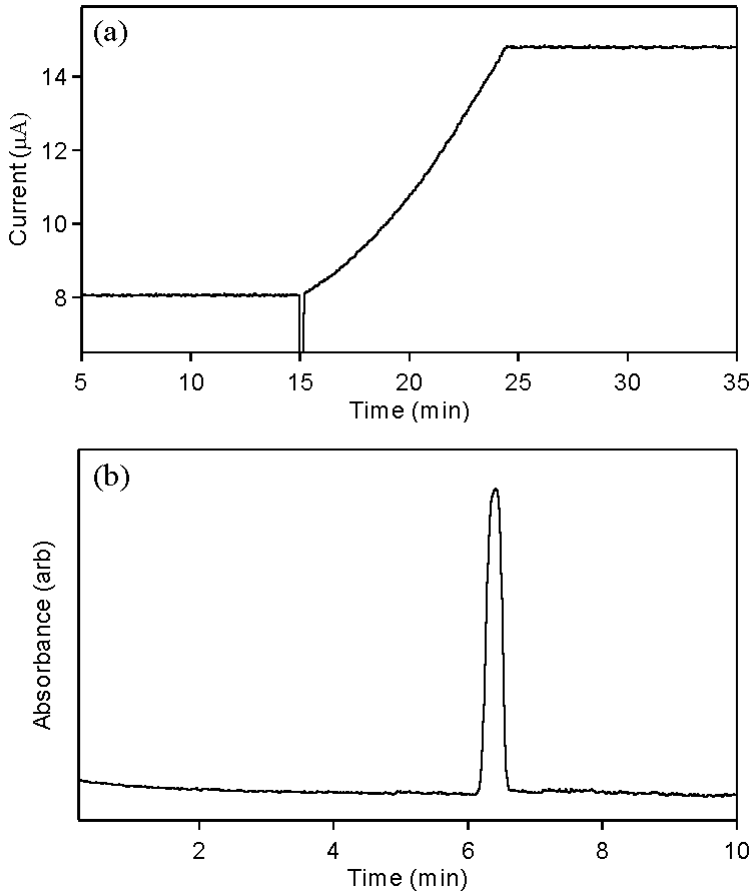


Figure 3


(a)
Current (μA)
14
12
10
8
5
10
15
20
25
30
35
Time (min)
(b)
Absorbance (arb)
2
4
6
8
10
Time (min)

.

Figure 4