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Controlling Electroosmotic Flow in Poly(dimethylsiloxane) Separation Channels by Means of Prepolymer Additives

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The electroosmotic flow (EOF) in a poly(dimethylsiloxane) (PDMS) separation channel can be altered and controlled by adding a carboxylic acid to the prepolymer prior to curing. When the prepolymer is doped with 0.5 wt % undecylenic acid (UDA), the electroosmotic mobility in a modified PDMS channel rises to $(7.6 \pm 0.2) \times 10^{-4}$ cm^2 V^{-1} s^{-1} (in HEPES buffer at pH 8.5), which is nearly twice that in the native PDMS channel. Because this modification does not significantly change the hydrophobicity of the PDMS surface, it is possible to combine the modified PDMS with a dynamic coating of *n*-dodecyl β-D-maltoside (DDM), which prevents protein sticking (see Huang, B.; Wu, H. K.; Kim, S.; Zare, R. N. *Lab Chip* 2005, *5*, 1005−1007). The modified PDMS channel with a dynamic coating of DDM generates an electroosmotic mobility of $(5.01 \pm 0.09) \times 10^{-4}$ cm^2 V^{-1} s^{-1}, which shows excellent reproducibility both in successive runs and during storage in water. Combining this surface modification and the dynamic coating of DDM is an effective means for both providing stable EOF in the PDMS channels and preventing protein adsorption on the channel walls. To demonstrate these effects, we show that the electrophoretic separation of immunocomplexes in free solution can be readily accomplished in a microfluidic chip made of UDA-doped (0.5 wt %) PDMS with a dynamic coating of DDM.

Poly(dimethylsiloxane) (PDMS) is one of the most popular polymer substrates for microfabrication because of its transparency, ease of fabrication, capability of automatic sealing with various materials, and compatibility with biological samples.[1−3] Moreover, its elasticity allows the use of microscale valves and pumps.[3] Based on these advantages, several techniques have been realized in PDMS microfluidic devices, including two-dimensional electrophoresis and real-time polymerase chain reaction.[4,5] Many of these applications utilize electrokinetically driven flow to facilitate the transport of fluid. Nevertheless, the electroosmotic flow (EOF) in native PDMS channels is slower than that in glass channels and is highly dependent on pH.[6] To overcome this drawback, the surface of native PDMS is usually modified to increase the EOF.[7] One method is plasma oxidation of PDMS, but this procedure does not support a stable EOF over time and it usually results in the nonspecific adsorption of protein analytes.[8,9] Another method is static coating, such as covalently linking ionizable molecules to polymerizing ionizable monomers on the PDMS surface. This process usually requires carrying out a series of surface chemical reactions.[10−12] A third alternative is dynamic coating of the channel walls with a surfactant or polyelectrolyte. This requires a surfactant in the running buffer that might interfere with the analysis.[6,13,14]

The existing methods for controlling EOF in PDMS channels are focused on producing needed functional groups on the PDMS surface. It should be noted that doping additives into other polymers has been also used for the purpose of modifying conductivity, color, stability, etc.[15,16] For PDMS, there have been two such studies, the addition of a porphyrin for catalyzing epoxidation[17] and a phospholipid for immobilizing proteins on the PDMS surface.[18] To our knowledge, no previous work exists on modifying the properties of PDMS for the purpose of controlling

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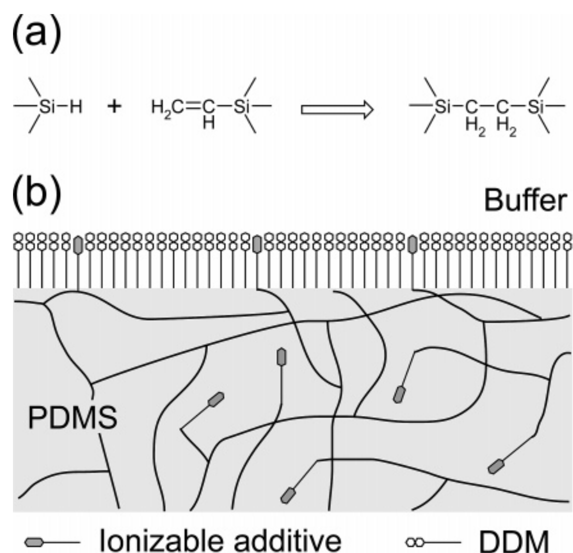

Figure 1. (a) Cross-linking reaction during the curing of PDMS prepolymer. (b) The concept of combining the additive-based surface modification of PDMS with a dynamic coating of DDM. The ionizable additive molecules are fixed on the surface to generate EOF during electrophoresis and DDM molecules form a dynamic coating layer to prevent surface adsorption.

EOF. We report here the surface modification of PDMS by adding ionizable compounds for enhancing EOF in PDMS channels.

In particular, we investigate surface modification of PDMS by means of doping with an ionizable additive containing a vinyl group to the PDMS prepolymer before curing. Because the cross-linking of PDMS is based on the reaction between ∼−Si−H and ∼−Si−CH=CH_2 (Figure 1a),[19] such an additive is expected to covalently bond with the PMDS matrix during curing. Accordingly, the cured PDMS presents ionizable functional groups on the surface, which can produce surface charge in a running buffer and generate EOF when an electric field is applied. To demonstrate this effect, we have studied the effect on EOF of doping carboxylic acid and amine compounds into the PDMS prepolymer. We also investigate combining this surface modification of PDMS with a nonionic surfactant, *n*-dodecyl β-D-maltoside (DDM), in the running buffer to coat the modified PDMS surface (see Figure 1b). Such dynamic coating almost completely eliminates the nonspecific binding of protein analytes to the hydrophobic PDMS surface.[20] To demonstrate the power of this combination, we carry out an electrophoretic immunoassay of bovine serum albumin (BSA) and anti-BSA antibody using a dynamic coating of DDM in a microfluidic chip made of PDMS channels modified by a carboxylic acid.

EXPERIMENTAL SECTION

Materials and Reagents. All reagents are obtained from commercial sources: monoclonal anti-BSA antibody (Clone BSA-33) produced in mouse (mAb), undecylenic acid (UDA), and acrylic acid (AA) from Sigma-Aldrich (St. Louis, MO); BSA tetramethylrhodamine conjugate (BSA−TMR) from Invitrogen (Carlsbad, CA); 1-amino-10-undecene (AUE) from GFS Chemicals (Powell, OH); (tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-trichlorosilane (FOTCS) from United Chemical Technologies (Bristol, PA); DDM from Anatrace (Maumee, OH); Photoresist SPR 220-7 from MicroChem (Newton, MA); silicon wafers from Silicon Sense (Nashua, NH); and PDMS prepolymers RTV 615A and 615B from General Electric (Fairfield, CT).

Fabrication of Microfluidic Devices. Photoresist masters for soft lithography are formed on silicon wafers by photolithography. The patterns are designed with the computer-aided design software Freehand 10 (Macromedia, San Francisco, CA) and printed on transparency films with a high-resolution (3600 dpi) printer (Media Morphosis, Mountain View, CA). For fabricating a master for the microchannels, a 10-μm-thick layer of positive photoresist SPR 220-7 is spin-coated on a 4-in. silicon wafer and baked on a hot plate (95 °C for 200 s). The photoresist is exposed through a transparency mask and developed. The wafer is then baked in an oven (110 °C for 30 min) to reflow the photoresist so that rounded patterns are formed. The master surface is rendered hydrophobic by exposure to FOTCS vapor in a vacuum desiccator, to prevent adhesion of the PDMS to the wafer or to the photoresist patterns during the curing step. Soft lithography is made by casting the PDMS prepolymer (mixture of RTV A and B with mass ratio of 10:1) containing a certain concentration of additive against the master and curing completely in an oven (70 °C). The patterned PDMS layer is peeled off the master, on which holes are punched as reservoirs. Then, it is placed on a glass slide coated with a thin layer of PDMS to form an enclosed microfluidic system. The two PDMS layers spontaneously form a seal.

Measurement of EOF and Contact Angle. We use the current monitoring method[21] to measure the EOF in a 6-cm-long PDMS channel (100 μm wide and ∼10 μm deep) under an electric field strength of 250 V/cm. Both HEPES buffer (denoted as HB) at pH 8.5 and 0.1 wt % DDM contained HEPES buffer (denoted as DHB) at pH 8.5 are used as running buffers. As a nonionic surfactant, DDM is capable of inserting its alkyl tail into the hydrophobic surface of PDMS, thus forming a neutral hydrophilic layer by its polar head.[20] The current change when switching the HEPES concentration between 20 and 19 mM is recorded. Contact angles reported in this article are the advancing contact angle at the water/PDMS/air interface, which is measured by an FTA 2000 contact angle analyzer (First Ten Angstroms, Portsmouth, VA).

Method of Electrophoresis. For the electrophoretic immunoassay, electrophoresis is carried out in a "double-T" microfluidic chip (Figure 3a). The running buffer is 20 mM DHB at pH 8.5 or pH 9.0. The sample is dissolved in the running buffer and placed in reservoir A, while reservoirs B−D are filled with the running buffer. Platinum electrodes are inserted into the reservoirs to apply voltages for electrophoresis. Two sets of voltages are generated by a home-built high-voltage power supply for sample loading and electrophoretic separation: (1) V_A = 1.5 kV, V_B = ground, V_C = 1.0 kV, V_D = 2.0 kV; and (2) V_A = 1.5 kV, V_B = 2.0 kV, V_C = 1.5 kV, V_D = ground. Laser-induced fluorescence detection is carried out at the detection point using the 530-nm line from an argon−krypton laser (Melles Griot, Carlsbad, CA). A photomultiplier tube (Hamamatsu, Bridgewater, NJ) that feeds a picoamperometer (Keithley Instruments, Cleveland, OH) is used

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for detection. The voltages and data collection are controlled by a PC running custom software created with LabVIEW (National Instruments, Austin, TX).

RESULTS AND DISCUSSION

Doping of PDMS. To produce ionizable functional groups on PDMS surfaces, UDA and AA are selected as additives. These compounds are liquids at room temperature, making them easy to mix uniformly with the PDMS prepolymer. The surface of UDA-doped PDMS is expected to be compatible with dynamic coating of DDM because the long alkyl chain (10 carbon atoms long) allows the carboxylic headgroup to reach the running buffer without being masked by the coating layer (Figure 1b).

It is observed that carboxylic acid doping (both UDA and AA) changes the optical and mechanical properties of PDMS. As the addition ratio increases, the doped PDMS changes from transparent to milky white, and it becomes softer and stickier. We restrict the addition ratio to the range of 0−1.0 wt % because a modified PDMS layer with a higher addition ratio breaks when peeled from the master.

At present, the nature of the bonding between the additive and the PDMS is not established. We expect that at least a portion of the unsaturated additive molecules bond covalently with the PDMS matrix because the catalyst in the prepolymer has adequate activity to facilitate the hydrosilation of olefins.[22] Unfortunately, direct measurement of the bonding state is not easy in the polymer matrix. This question might be answered by doping biotinylated olefin in PDMS and monitoring the motion of fluorescently labeled avidin molecules bound to the modified PDMS surface.[18]

The UDA-doped PDMS shows similar hydrophobicity to native PDMS by presenting closed water−PDMS contact angles. The contact angles of UDA-doped PDMS (0.10, 0.30, 0.50, 0.70, and 1.0 wt %) are in the range of 118°−129° whereas that of native PDMS is 118° ± 2°, which is consistent with the value reported by He et al.[23] Therefore, it seems reasonable to conclude that doping does not greatly alter the hydrophobicity of PDMS. It seems that the functional groups on the modified PDMS surface make only a small contribution to its wettability.

EOF in Modified PDMS Channels without Dynamic Coating of DDM. In Figure 2a, the electroosmotic mobility measured in carboxylic acid-modified PDMS channels is plotted versus the addition ratio of UDA and AA; the two compounds are added in the same molar ratio for comparison purposes. HEPES buffer (denoted as HB) at pH 8.5 is used as the running buffer because this mild alkaline buffer enhances the dissociation of the carboxylic groups of the additives and is suitable for carrying out electrophoresis. In both cases, the electroosmotic mobility is greater than that in a native PDMS channel. This observation verifies the existence of carboxylic groups on the modified PDMS surface, which ionize in the running buffer and generate EOF. It is also seen that UDA-modified PDMS channels generate slightly stronger EOF than AA-modified PDMS channels at the same molar ratios, which indicates that UDA-doped PDMS has a higher surface charge density. This difference in surface charge cannot be caused by the difference in the acidity of carboxylic groups because AA is slightly more acidic than UDA. (The difference in acidity might become even smaller if their double bonds react with PDMS prepolymer, eliminating the electron induction effect of carbon−carbon double bonds.) Therefore, this effect may have resulted from the higher exposure of the carboxylic group of UDA owing to its longer alkyl chain.

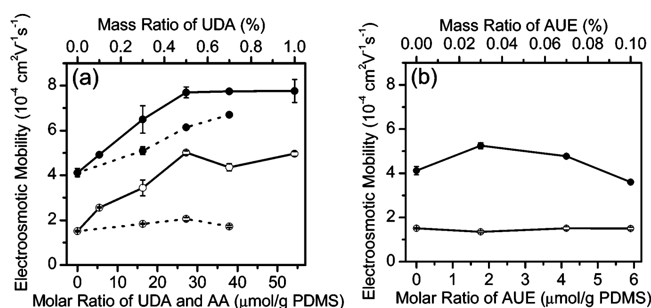

Figure 2. Electroosmotic mobility measured in channels made of modified PDMS. The current monitoring method is employed for these measurements. (a) The electroosmotic mobility measured in the channels made of carboxylic acid-doped PDMS. Solid lines, UDA; dotted lines, AA; solid symbols, 20 mM HB at pH 8.5 is used as the running buffer; blank symbols, 20 mM DHB at pH 8.5 is used as the running buffer. (b) Electroosmotic mobility measured in channels made of AUE-doped PDMS. Solid and blank symbols indicate the running buffers as in (a).

Table 1. Reproducibility of Electroosmotic Mobility Measured in Channels Made of UDA-Doped (0.5 wt %) PDMS

condition	number of measurements	electroosmotic mobility (10^{-4} cm^2 V^{-1} s^{-1})
measured in HB at pH 8.5	29	7.6 ± 0.2
measured in DHB at pH 8.5	31	4.98 ± 0.09
measured in DHB at pH 8.5 after 3 days[a]	8	5.05 ± 0.09
measured in DHB at pH 8.5 after 5 days[a]	6	5.05 ± 0.03
measured in DHB at pH 8.5 for all	45	5.01 ± 0.09

[a] The time-relevant measurements of electroosmotic mobility are taken in the same PDMS channel filled with water during storage.)

Figure 2a also shows that the EOF enhancement by UDA doping plateaus when the addition ratio exceeds 0.5 wt %. A possible explanation is that the increasing density of carboxylic groups on the modified PDMS surface facilitates the interaction between carboxylic groups in such a manner that it suppresses the ionization in aqueous running buffer. Further investigation is needed to test this hypothesis. The stability of this surface modification is verified by the excellent reproducibility of EOF measured in a channel made of UDA-doped (0.5 wt %) PDMS (Table 1).

We also investigate the surface modification achieved by amine doping. This procedure is expected to produce amino groups on the modified PDMS surface, which may generate positive charges in the running buffer and suppress EOF. In our experiments with AUE as the additive, we observe that the addition of AUE causes a greater increase of the opaqueness and stickiness of doped PDMS than UDA and AA. The maximum addition ratio is thus restricted to 0.10 wt %. Figure 2b shows the electroosmotic mobility in the channels made of AUE-doped PDMS. No significant

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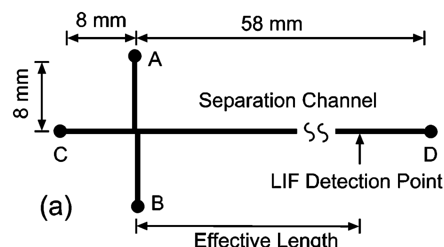
variation from native PDMS is observed using either HB or DHB (with a dynamic coating of DDM on the modified PDMS surface) as the running buffer. The expected decrease of the electroosmotic mobility is not found, probably owing to the limited addition ratios. Therefore, AUE doping appears not to be practically useful in adjusting EOF in PDMS channels.

EOF in Modified PDMS Channels with Dynamic Coating of DDM. Because the modified PDMS surfaces have hydrophobicity similar to that of the native PDMS surface, it is feasible for the nonionic surfactant DDM to adsorb on these modified PDMS surfaces and form a dynamic coating. The lines with blank symbols in Figure 2a show the electroosmotic mobility in UDA- and AA-modified PDMS channels when 0.1 wt % DDM containing HEPES buffer (denoted as DHB) is used as the running buffer. In both cases, the dynamic coating of DDM reduces the electroosmotic mobility. We find that the electroosmotic mobility in both native and UDA-modified PDMS channels decreases by a similar amount ($\sim 2.8 \times 10^{-4}$ cm^2 V^{-1} s^{-1}) regardless of addition ratio, whereas the electroosmotic mobility in AA-modified PDMS channels is comparable to that of native PDMS. An explanation for this behavior is that DDM is able to mask the charges of the native PDMS surface and of AA but cannot mask the charge of UDA because of its alkyl chain, which is composed of 10 carbon atoms. This explanation is consistent with the concept pictured in Figure 1b—that the long alkyl chain of the additive can let the carboxylic headgroup reach the running buffer and prevents it from being masked by the coating layer of DDM, and also consistent with the alkyl chain-length related decrease of EOF in silane-coated fused-silica capillaries.[24] Therefore, the length of the alkyl chain is an essential index for selecting an effective additive in this surface modification method if dynamic coating of DDM is to be simultaneously employed.

We also study the reproducibility of EOF with the dynamic coating of DDM in a channel made of UDA-doped (0.5 wt %) PDMS compared with that without the coating (Table 1). The EOF is not only stable in successive measurements, but it is also stable after water-filled storage. Such preservation of EOF facilitates the long-term use of microfluidic devices made of the doped PDMS. We found that the separation reproducibility is maintained over 10 repeats, but we did not determine how many runs would cause deterioration of the performance.

Electrophoretic Immunoassay. Previously, many electrophoretic immunoassays were done using microfluidic chips.[25−30] To demonstrate the application of our modified PDMS in electrophoretic separation of proteins, we test an electrophoretic immunoassay in microfluidic chips made of UDA-doped (0.5 wt %) PDMS. DHB is employed as the running buffer, which reduces the adsorption of proteins on the channel wall and provides stable and adequate EOF. The pH of the running buffer is varied from 7.5 to 9.0 during which the resolution of electrophoretic separation slightly increases, so the running buffer at pH 9.0 is used for studying the reproducibility of separation. BSA−TMR and mAb are selected as the model system. The microfluidic chip has the standard layout of a double-T structure for voltage-controlled sampling and separation (Figure 3a). The electrophoretic separations of both pure BSA−TMR and mixtures of BSA−TMR and mAb are presented in Figure 3b. Besides the peaks of unbound BSA−TMR, one peak attributed to a fluorescent impurity (probably hydrolyzed TMR labeling reagent) and two peaks of immunocomplexes are observed in the separations of the mixture. Their relative abundance (peak 1/peak 2) rises as the concentration of mAb increases. This finding is consistent with the trend observed in the separations of a mixture of fluorecein-labeled BSA and mAb in a glass microfluidic chip.[29]



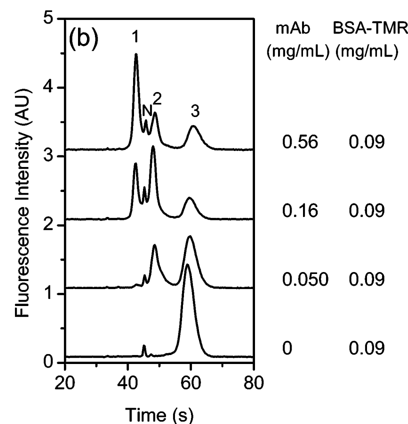


Figure 3. (a) Layout of the microfluidic chip used. (b) The separation of TMR−BSA and anti-BSA antibody mixture. The running buffer is 20 mM DHB at pH 8.5, and the effective separation distance is 5 cm. Peak assignment: (1) and (2) are immunocomplexes of BSA−TMR and mAb; (3) is BSA−TMR; and (N) is fluorescent impurity in BSA−TMR.

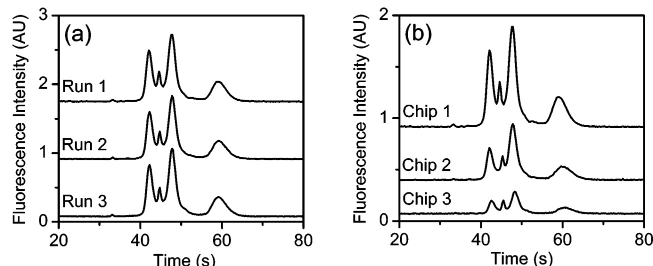


Figure 4. (a) Reproducibility of separations in the same chip. Three continuous separations are performed in 20 mM DHB at pH 9.0, and the effective separation distance is 5 cm. (b) Reproducibility of separations in three different chips. The difference of fluorescence intensity arises from both the adjustment of the excitation laser filter in the experimental setup and the variation of sample concentration. The separation condition is the same as in (a). Chip 1 is from one batch and chips 2 and 3 are from another. The electropherograms are vertically shifted for comparison.

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In a native PDMS microfluidic chip with the dynamic coating of DDM, the EOF is very slow because DDM is adsorbed on the PDMS channel wall and masks the surface charges. Consequently, the species in the mixture of BSA–TMR and mAb electrophoretically migrate in opposite directions because the low EOF rate provides inadequate fluid transport for charged analytes (data not shown). In addition, the migration times of analytes are greatly increased and the peak widths are also significantly broadened, resulting in lower separation efficiency. Thus, our modified PDMS with the dynamic coating of DDM is much superior to native PDMS with the dynamic coating of DDM. Panels a and b in Figure 4 present the single-chip and chip-to-chip repeats of the electrophoretic separations, which once again shows excellent reproducibility. We conclude that the combination of the surface modification of PDMS by prepolymer doping and the dynamic coating of DDM is a simple, fast, and reproducible method for nondenaturing electrophoretic separations of proteins in free solution in microfluidic devices.

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