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Protein separation and surfactant control of electroosmotic flow in poly(dimethylsiloxane)-coated capillaries and microchips

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Abstract

A thermally pyrolyzed poly(dimethylsiloxane) (PDMS) coating intended to prevent surface adsorption during capillary electrophoretic (CE) [Science 222 (1983) 266] separation of proteins, and to provide a substrate for surfactant adsorption for electroosmotic mobility control was prepared and evaluated. Coating fused-silica capillaries or glass microchip CE devices with a 1% solution of 100 cSt silicone oil in CH_2Cl_2, followed by forced N_2 drying and thermal curing at 400 °C for 30 min produced a cross-linked PDMS layer. Addition of 0.01 to 0.02% Brij 35 to a 0.020 *M* phosphate buffer gave separations of lysozyme, cytochrome *c*, RNase, and fluorescein-labeled goat anti-human IgG Fab fragment. Respective plates/m typically obtained at 20 kV (740 V cm^{-1}) were 2, 1.5, 1.25, and $9.4 \cdot 10^5$. In 50 m*M* ionic strength phosphate, 0.01% Brij 35 running buffer, the electroosmotic flow observed was about 25% of that in a bare capillary, and showed no pH dependence between pH 6.3–8.2. Addition of sodium dodecylsulfate (SDS) or cetyltrimethylammonium bromide (CTAB) to this running buffer allowed ready control of electroosmotic mobility, μ_{eo}. Concentrations of SDS between 0.005 to 0.1% resulted in μ_{eo} ranging from 3 to $5 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1}. Addition of 1 to $2.3 \cdot 10^{-4}$% (2.7–6.3 μ*M*) CTAB caused flow reversal. CTAB concentrations between $3.5 \cdot 10^{-4}$ and 0.05% (0.0014–1.37 m*M*) allowed control of μ_{eo} between $-1 \cdot 10^{-4}$ and $-5.0 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1}. For both surfactants the added presence of 0.01% Brij 35 provided slowly varying changes in μ_{eo} with charged surfactant concentration.

Keywords: Coated capillaries; Microfluidics; Proteins; Surfactants

1. Introduction

Capillary zone electrophoresis is a versatile technique for the separation of many analytes, however, there is often a need to reduce protein adsorption [1,2] or to control the electroosmotic flow (EOF) rate in order to obtain optimum separations. To combat protein adsorption effects a large number of surface coating methods have been developed [2–17], none of which appears to have provided a definitive solution for all sample types. Covalently bound coatings [2–9] have been extensively studied and various coated capillaries are available commercially. Adsorption of polymers [10–12] or surfactants [14–17] provides a dynamic coating approach to limiting the band broadening caused by adsorption. Still more challenging than coating conventional capillaries, is the coating of microfluidic capillary

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electrophoresis (CE) chips [18,19], due the increased difficulty of ensuring uniform coating within a multitude of intersecting channels. The hydrolytically unstable silane derivatives that are often used to form covalent linkages to a silica surface are more problematic with CE chips, since the multiple fluid paths make it hard to keep the devices dry unless working inside a glove box. Another issue of importance to CE chips is the need to retain surface charge and a zeta potential after coating, since electroosmotic flow (EOF) is frequently used as the primary pumping mechanism within the chips [18,20,21]. To this end we have explored the use of a hydrophobic coating of pyrolyzed poly(dimethylsiloxane) (PDMS), which is more easily introduced into a chip, coupled with a variety of neutral and charged surfactants to control adsorption and surface charge properties. The ability to separate proteins and control EOF so obtained should be useful for both microchip and fused-silica capillary electrophoresis.

A patent filed by Woolley [22] claims that coating a fused-silica capillary with PDMS by thermal polymerization (pyrolysis) of moderate viscosity silicone oils yields a surface coating for CE of compounds that adsorb on silica. Coating channels in a chip with such a procedure should be much easier than using the silanization procedures [1,2,19] we previously found were tedious with microchips. However, the hydrophobic character of PDMS results in adsorption of hydrophobic proteins, so we anticipated that PDMS alone was not the optimum choice. Several authors have explored the use of neutral poly(oxyethylene)-based surfactants, such as Brij 35 and Tween 20, with octadecylsilane (C_{18}) surfaces [4,23] and size-exclusion stationary phases [24] to reduce protein adsorption. Others [14,25] have shown that cationic surfactant coatings on bare capillaries give efficiencies of 10^5 plates/m for basic proteins. Using cationic surfactants Baryla and Lucy recently achieved protein separation efficiencies in the range of $1.5 \cdot 10^6$ plates/m [17]. However, cationic surfactant coatings on bare silica can be sensitive to solvent conditions, due to their relatively weak interaction with silica. This report presents the first experimental evaluation of the use of surfactant combinations with lipophilic pyrolyzed PDMS coatings to provide a non-adsorbing surface for protein separation. The PDMS–surfactant combination provides reasonably efficient protein separations within microchips and conventional capillaries.

Control of EOF can be achieved by adjusting the pH or the ionic strength when a surface is charged [26–28]. For an uncharged surface, or for cases where the above two parameters should not be varied, the adsorption of charged surfactants provides a means to control EOF [13–17]. Lucy and co-workers have discussed combining a neutral surfactant that can dynamically coat a bare capillary surface with an added charged surfactant to adjust EOF [15,29], in order to improve separation resolution, R. In the expression for R below, longitudinal diffusion is assumed to be the primary source of band broadening:

$$R = 0.177\,\Delta\mu_{ob}\left[\frac{V}{D(\bar{\mu}_{ep} + \mu_{eo})}\right]^{1/2} \quad (1)$$

In Eq. (1) V is the applied voltage, D is the diffusion coefficient for the broader peak, $\Delta\mu$ is the difference in observed mobilities, and $\bar{\mu}_{ep}$ is the average electrophoretic mobility for the two components. Lucy and colleagues were able to adjust the electroosmotic mobility, μ_{eo}, to a value close to the electrophoretic mobility, μ_{ep}, of a target analyte, showing one can maximize the resolution [15,29]. Unfortunately, the neutral/charged surfactant combinations they explored did not offer very fine control of EOF. More recently, Durkin and Foley combined cellulose coatings with a surfactant to nearly eliminate EOF on bare silica [12]. Here, we explore the use of a three-component system consisting of charged surfactants, combined with Brij 35 on PDMS-coated capillaries, in order to achieve variable control of the EOF. As illustrated, this system of permanent and dynamic coatings provides a convenient method of adjusting EOF, allowing much improved control across a large mobility range.

2. Experimental

2.1. Instrumentation

Most experiments were performed on a Beckman P/ACE 5010 CE instrument, but the earliest studies

were performed on a Waters CE instrument. Polymicro (Phoenix, AZ, USA) capillaries with an inner diameter of 50 μm, an outer diameter of 345 μm and a total length of 27 cm (20 cm to the detector) were used in the Beckman CE and a length of 40 cm (32.5 cm to the detector) in the Waters CE system. Detection was by UV absorbance at 254 nm in the Waters and Beckman systems, or by 488 nm laser-induced fluorescence (LIF) detection at 525 nm with the Beckman system. The on-chip separation was performed in a COPI device, using previously described [30] procedures, high voltage and LIF instrumentation. The signal was amplified with a 10^7 gain transimpedance amplifier and filtered with a 25 Hz cut-off filter (Krohn–Hite 3442). Electropherograms were acquired with a MacIntosh computer.

2.2. Reagents

Phosphate buffers with an ionic strength (*I*) of 50 m*M* were prepared from potassium dihydrogenphosphate (Fischer, Fair Lawn, NJ, USA) and dipotassium hydrogenphosphate (K_2HPO_4, BDH, Toronto, Canada) using the phosphate buffer nomogram [31] (approximately 26 m*M* in total phosphate). The nomogram was used to prepare the desired pH and ionic strength for the buffers, without having to adjust with acid or alkali. Values were measured with a pH meter. These are referred to as ionic strength $I=50$ phosphate buffers. Buffers were also prepared with a specified total phosphate concentration, with HCl or NaOH added to adjust the pH. The buffer used for the on-chip separation was 10 m*M* Tris, 20 m*M* boric acid at pH 9, with 20 m*M* NaCl and 0.01% Tween 20 [30], all from Sigma (St. Louis, MO, USA).

PDMS 200 (Aldrich, Milwaukee, WI, USA) was used as received for coating purposes. A 1% (v/v) PDMS solution in dichloromethane (dried and distilled before use) was prepared and used to coat the capillaries, after filtering with a 0.2 μm nylon filter. All water used in the experiments was from a Milli-Q UV plus water purifier. Protein samples used included: lysozyme (chicken egg white), cytochrome *c* (horse heart), α-chymotrypsinogen A (bovine pancreas), ribonuclease A (bovine pancreas), myoglobin (horse skeletal muscle), fluorescein isothiocyanate (FITC)-labeled goat anti-human Fab fragment (F–GFab) and FITC-labeled theophylline (FITC–Th), which were used as received from Sigma. The neutral marker used to measure EOF was 1% (v/v) benzyl alcohol (Sigma) prepared in 5 ml methanol and 95 ml $I=50$ phosphate buffer. The surfactants were sodium dodecylsulfate ($C_{12}H_{25}SO_4$–Na) (SDS) (Serva, Heidelberg, Germany), cetyltrimethylammonium bromide 95% [$CH_3(CH_2)_{15}N(CH_3)_3Br$ (CTAB)], Tween 20 and Brij 35 (Aldrich), which were used as received. All solutions were filtered through 0.2 μm nylon filters prior to use.

2.3. Coating procedures

Typically a 100 cm long capillary was coated with silicone oil using the Beckman instrument (or a syringe in our earliest studies) to provide the pressure source for conditioning and coating. Capillaries were conditioned with 0.1 *M* NaOH for 30 min, water for 45 min, then N_2 gas for 15 min. Capillaries were coated by rinsing with CH_2Cl_2 for 5 min, followed by 1% PDMS solution for 2 min, after which the PDMS sat inside the capillary for 10 min, finishing with a 10 min N_2 flush. Capillaries were then placed in a programmable oven (Model 6-525, J.M. Ney Co., Yucaipa, CA, USA) for curing. The temperature program used was: (1) 10 °C min^{-1} rise to 400 °C, then 0.5 h at 400 °C, followed by (2) cooling to room temperature inside the oven. Coating of the COPI device was done using the same procedure as above, using in-house vacuum to pull solutions through the chip. Most coating evaluations were done in capillaries, due to their lower cost compared to laboratory-made chips.

2.4. Protein separation

With both the Beckman and Waters, protein separation studies used a 2 s sample injection time (pressure injection) and a 20 kV separation voltage. On the COPI chip, the injection voltage was −1 kV between sample and sample waste reservoirs with the separation channel left floating. The separation voltage was −6 kV, with the injection channels left floating and a separation distance of 4.9 cm [30].

2.5. EOF measurement

Coated capillaries (27 cm long) were used for each trial, run on the Beckman system using varying pH, $I = 50$ phosphate buffers, with added surfactants. Capillaries were rinsed with water for 15 min, followed by the respective buffer for 30 min. The neutral marker, benzyl alcohol in appropriate buffer, was then pressure injected (0.5 p.s.i.; 1 p.s.i. = 6894.76 Pa) for 2 s and separated at 20 kV. The migration time for the neutral marker peak gave the electroosmotic mobility.

3. Results and discussion

3.1. Protein separation in poly(dimethylsiloxane)-coated capillaries

Silicone is available in a range of viscosities, and may be introduced into a capillary in neat form or dissolved in a solvent. We tested several different silicone oils with viscosities between 10 and 200 cSt and found oil with a viscosity of 100 cSt proved to give the highest quality coatings. The surface coating proved to be most effective when a 1% solution of the oil in CH_2Cl_2 was used to wet the capillary with the oil. It was necessary to use freshly distilled, dry solvent to obtain reproducible performance. Toluene could also be used as solvent, but the results were less reproducible. Excess solution was removed from the capillary by blowing N_2 through before curing at 400 °C. Attempts to coat capillaries with neat silicone, or without removing the solutions before curing usually resulted in plugging of the capillary. After curing, a PDMS coating is formed on the capillary walls [22].

Table 1 shows the effect of the PDMS coating on the electroosmotic mobility, for several pH values. The PDMS coating suppresses EOF to about 50% of the value seen for an uncoated capillary. The observed reduction in EOF is much lower than that seen for many coatings, for which little or no EOF is often reported [1,3,8]. However, the use of a coating that does not fully suppress EOF may in fact be advantageous, as EOF can still be used to pump fluids and to direct the sample ions in one direction. Towns and Regnier [4] have shown that observing residual EOF with C_{18} coatings does not preclude good protein separation performance. Those authors also reported that EOF may be further reduced when a surfactant is used with a covalent C_{18} coating. Table 1 shows that this is also the case for PDMS surfaces when 0.01% Brij 35 (83.3 μM) is added. The EOF remaining is independent of pH across the range evaluated.

Table 2
p*I* values of different proteins

Protein	p*I* value
Lysozyme	11.0
Cytochrome *c*	10.2
Ribonuclease	9.3
α-Chymotrypsinogen A	9.2
Myoglobin	7.3

The surfactant–PDMS coating was evaluated for its ability to suppress protein adsorption and so improve separation. Table 2 identifies the isoelectric point (p*I*) of the proteins used to test this coating [4]. Fig. 1 illustrates the separation of several proteins in 0.01% Brij 35 and varying pH 7.0 phosphate buffer concentrations, on a PDMS-coated surface. The buffer concentration was important in determining protein migration times and resolution. The velocity decreased with increasing ionic strength, as expected for EOF [26–28,32]. The best efficiencies typically

Table 1
The electroosmotic mobility (μ_{eo}) at different pH values

	μ_{eo} ($cm^2\ V^{-1}\ s^{-1}$)		
	Buffer[a] pH 6.3	Buffer[a] pH 7.36	Buffer[a] pH 8.2
Uncoated	$6.32 \cdot 10^{-4}$	$7.24 \cdot 10^{-4}$	$7.88 \cdot 10^{-4}$
PDMS-coated	$3.51 \cdot 10^{-4}$	$3.65 \cdot 10^{-4}$	$4.43 \cdot 10^{-4}$
PDMS/Brij 35-coated	$1.58 \cdot 10^{-4}$	$1.65 \cdot 10^{-4}$	$1.66 \cdot 10^{-4}$

[a] Phosphate buffer with the ionic strength adjusted to 50 m*M* was used.

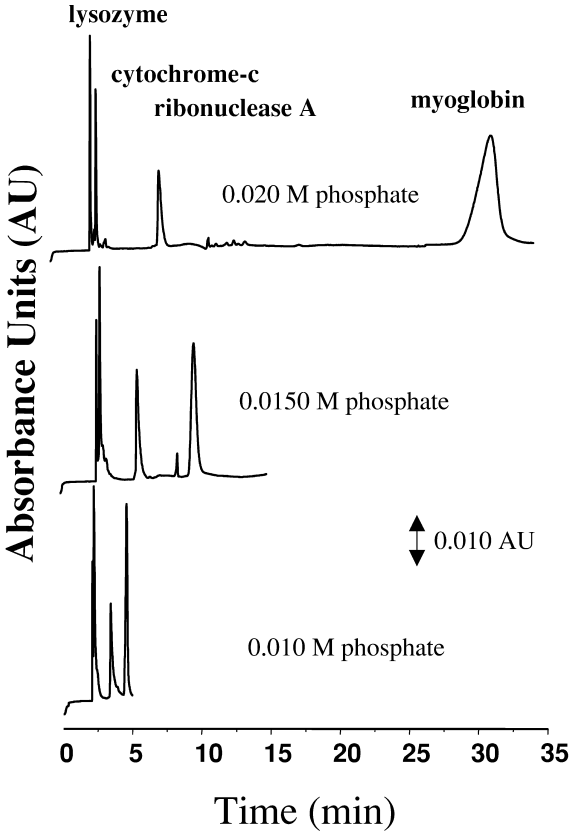

Fig. 1. Capillary electrophoresis (CE) separation of a protein mixture as a function of ionic strength, in pH 7.0 phosphate, 0.01% Brij 35 running buffer. The mixture contained 1 mg ml^{-1} each of lysozyme, cytochrome *c*, ribonuclease and myoglobin in the same ionic strength buffer. A 2 s long pressure injection was performed at 0.5 p.s.i. The migration order of the four principal peaks observed in all cases remained the same, but the early eluting peaks required 0.020 *M* phosphate buffer for better resolution in the 27 cm long capillary. Total phosphate concentrations are indicated in the diagram. Traces are offset vertically for clarity.

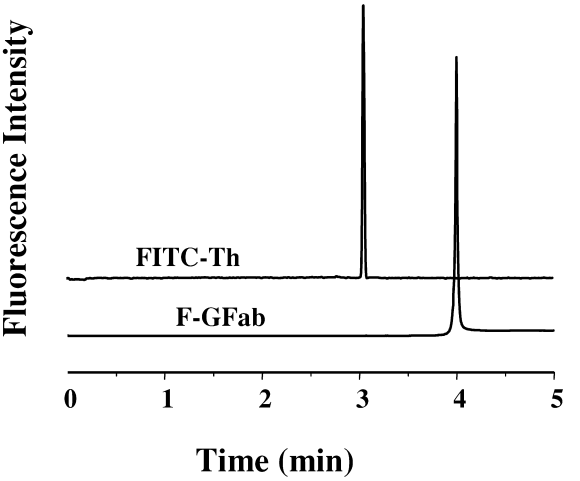

Fig. 2. CE separation of FITC-labeled goat anti-human Fab fragment (F–GFab) in a 27 cm long capillary, using 0.020 *M* phosphate buffer (pH 7) with 0.02% Brij 35. Also shown is an electropherogram for FITC-labeled theophylline (FITC–Th) in 0.01% Tween 20, 0.10 *M* Tris–borate buffer at pH 9.0. Traces are offset vertically for clarity.

obtained were 40 000 plates ($2\cdot10^5$ plates/m) for lysozyme, 30 000 ($1.5\cdot10^5$ plates/m) for cytochrome *c* and 25 000 ($1.25\cdot10^5$ plates/m) for RNase, using 0.01% Brij, 0.020 *M* phosphate at pH 7.0 and a 27 cm column. Higher plate numbers (50 000–80 000) were occasionally observed on fresh coatings. Fig. 2 shows the separation of fluorescein isothiocyanate-labeled Fab fragment of goat anti-human IgG (FGFab), for which plate numbers as high as 188 000 ($9.4\cdot10^5$ plates/m) were obtained in 0.02% Brij 35, 0.020 *M* phosphate buffer, pH 7.0. Fig. 2 also shows an electropherogram of a small molecule, fluorescein labeled theophylline, for which plate numbers of 130 000 ($6.5\cdot10^5$ plates/m) were routinely obtained in 0.01% Tween 20, 0.1 *M* Tris–borate buffer at pH 9.0. The results indicate the surfactant–PDMS coating is suitable for separating proteins with significantly differing hydrophobicity and varying p*I* values (Table 2), although the performance depends upon the protein.

In the absence of surfactant, proteins were not effectively separated on the PDMS surface and hydrophobic proteins were not observed at the detector. The concentration and type of surfactant is important, as indicated by the data in Table 3. The cationic surfactant CTAB gave the fastest migration times and anodal electroosmotic flow, but did not yield the highest plate numbers, presumably due to electrostatic interactions between CTAB and the proteins. Brij 35 gave better plate numbers than did CTAB or Tween 20 for the proteins studied (Tables 2 and 3). The plate numbers observed depended upon Brij 35 concentration, with the optimum varying from 0.010% Brij 35 (83 μM), below the critical micelle concentration (CMC) for Brij 35 in water (92 μM [33,34]), to 0.020%, depending upon the

Table 3
Effect of neutral surfactants on migration times in PDMS-coated capillaries[a]

Surfactant[b]		Migration times (min)			Theoretical plates	
Type	Concentration % (μ*M*)	Lysozyme	Cytochrome *c*	Myoglobin	Lysozyme	Cytochrome *c*
Brij 35	0.005 (42)	5.1	5.8	–	57 700	44 200
	0.010 (83)	6.1	7.1	22–32	57 400	52 000
	0.050 (420)	–	8.4	–	–	32 300
Tween 20	0.010 (80)	–	–	8.2	–	–
CTAB	0.005 (14)	–	3.4	4.4	–	22 600

[a] Performed with Waters CE system: 40 cm (32.5 cm to detector) ×50 μm I.D., at 20 kV.
[b] 0.010 *M* phosphate buffer, pH 7.0.

specific protein. Increasing concentrations of Brij 35 gave increasing migration times due to decreasing EOF, while Tween 20 gave faster migration times than did Brij 35.

The reproducibility and durability of the separation is obviously an important characteristic of a coating. The separation performance slowly degraded over time. Initially a resolution of 1.969 was observed for lysozyme and cytochrome *c*, which was reduced to 1.62 after 50 runs in 0.010% Brij 35, $I=50$ phosphate buffer, pH 7.3. The decrease resulted from decreased plate numbers, rather than changing migration times, which varied by ±1%. For protein mixtures, run to run reproducibility ($n=8$) of migration time and peak height were typically ±5% or better. Washing the column with running buffer for 1 min between runs proved beneficial for obtaining this level of reproducibility. Occasionally, very high plate numbers could be achieved, such as the 188 000 plates mentioned above for an IgG Fab fragment. These were observed on 10–20% of freshly prepared coatings, indicating that most coating preparations had some defects that limited the performance.

One of the key reasons for developing the PDMS coating was to establish a procedure that would be easy to translate to a multichannel microchip CE device. The silicone oil coating method is relatively easy to perform with a microchip. Solution is readily drawn into the channel network, then flushed out with pressurized N_2, just as in a capillary. Fig. 3 shows the peak observed for the fluorescein-labeled, goat anti-human Fab fragment, run in a 0.10% Tween 20, 0.10 *M* Tris–borate buffer at pH 9.0. A sharp, well defined peak was observed, indicating the coating in the chip performed similarly to the coating in the capillary.

3.2. Anionic surfactant control of electroosmotic flow

Adsorption of charged surfactants provides a means of controlling the surface charge and the resulting EOF, however, the magnitude of charge adsorbed on bare silica is not readily controlled.

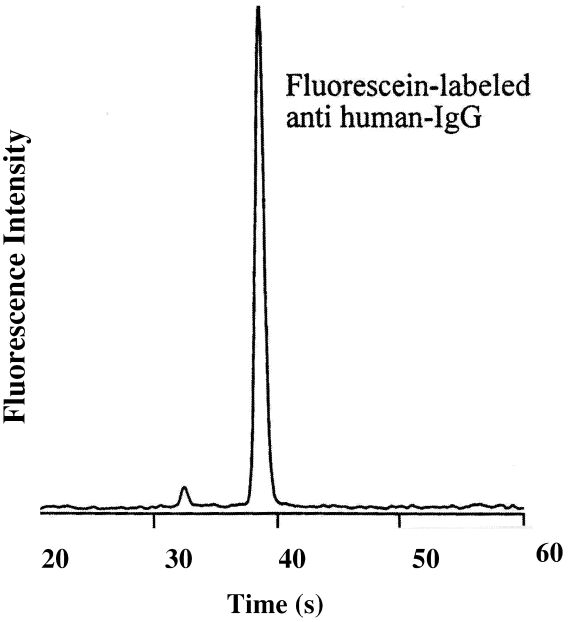

Fig. 3. On-chip CE separation in COPI device of 70 μg/ml fluorescein-labeled goat antihuman-IgG Fab fragment, in 10 m*M* Tris, 20 m*M* boric acid at pH 9, with 20 m*M* NaCl and 0.01% Tween 20. See Experimental section for operating conditions.

Table 4
μ_{eo} of bare capillaries at different SDS concentrations at pH 6.3

% SDS in buffer	0	0.0005	0.005	0.05
μ_{eo} ($\cdot 10^4$) cm^2 V^{-1} s^{-1}	6.11	6.12	6.12	6.14

Lucy and co-workers [15,29] showed that combining both neutral and charged surfactants together provided a better means to fine tune the EOF on bare silica, creating a fairly limited mobility window over which resolution could be optimized as suggested by Eq. (1). We have explored a similar mixed surfactant approach, instead using the permanent PDMS layer as a substrate for the competitive adsorption of neutral and charged surfactants. As shown below, this method provides much better control of the EOF, increasing the mobility window available for optimizing resolution in either fused-silica capillary or microchip.

SDS forms negatively charged micelles at a CMC of 8.1 m*M* in water, and an estimated CMC of 2.2 m*M* at an ionic strength of 50 m*M* [35], at which this study was performed. The data in Table 4 demonstrate that SDS does not adsorb on bare silica, which is negatively charged at pH 6.3. This observation is consistent with simple electrostatic expectations and with prior studies [5]. However, PDMS coated capillaries provide a hydrophobic surface for SDS adsorption, despite the remaining negative charge on the PDMS-coated surface. Fig. 4 shows the electroosmotic mobility, μ_{eo}, observed across an SDS concentration range of 0 to 0.08% (2.8 m*M*), for three different pH values in $I=50$ phosphate buffer. In the absence of Brij 35, μ_{eo} rises rapidly with increasing [SDS], reaching an approximate plateau at 0.02% SDS (0.7 m*M*). The plateau value of approximately $7\cdot10^{-4}$ cm^2 V^{-1} s^{-1}, is slightly higher than that of bare silica (Table 4). This SDS concentration is well below the CMC of 2.2 m*M*, and many proteins would not be denatured at this concentration [36]. Thus, SDS may be used to induce EOF in a coated capillary, without necessarily impacting the activity of proteins. This approach could be useful for assays within microfluidic devices, in which EOF is required to mobilize samples and reagents for reaction prior to performing a separation.

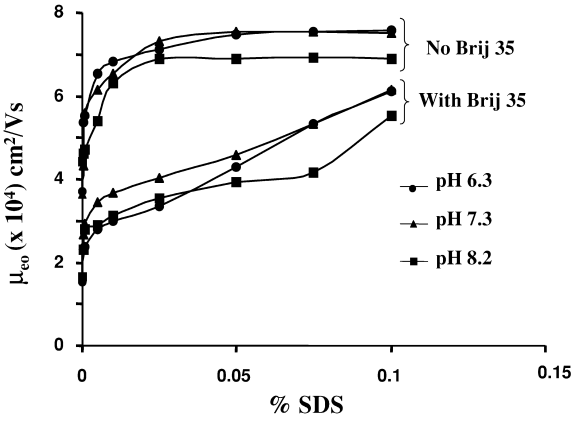

Fig. 4. Electroosmotic mobility, μ_{eo}, vs. % SDS in 50 m*M* ionic strength phosphate buffer for several pH values, with and without 0.01% Brij 35 present.

By the addition of 0.01% (83.3 μM) Brij 35 a competition between SDS and Brij 35 for the PDMS surface produced a lower slope of μ_{eo} as a function of [SDS], as illustrated in Fig. 4. After a very rapid rise in μ_{eo} at the lowest SDS concentrations, μ_{eo} varied from about 3 to $5\cdot10^{-4}$ cm^2 V^{-1} s^{-1}, across a range of 0.005 to 0.1% SDS. The combination of neutral and charged surfactant on bare silica [15,29], or the combination of SDS alone with PDMS, do not provide the same ability to target and fine tune for a specific μ_{eo} that is offered by the Brij 35–SDS–PDMS combination.

3.3. Cationic surfactant control of electroosmotic flow

Electroosmotic flow in the direction of the anode requires a cationic surface charge. The use of long chain alkylammonium salts to reverse EOF on bare silica was first reported by Reijenga et al. [37], and has been explored since for controlling flow on bare and coated capillaries. We have explored the use of CTAB to reverse EOF on PDMS and PDMS–Brij 35 coated surfaces. Key factors when using cationic surfactants include the amount of surfactant required to cause reversal, and the extent of EOF control possible, as illustrated by the slope of the mobility versus CTAB concentration curve.

Fig. 5 shows the variation in μ_{eo} with CTAB concentration in the mobility range around the flow reversal concentration. The study was performed in

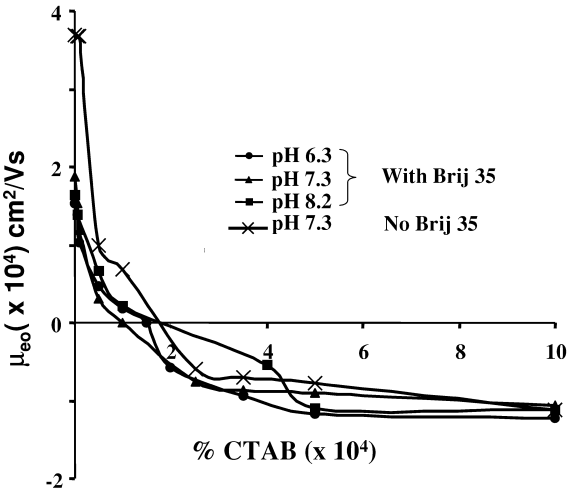

Fig. 5. Electroosmotic mobility, μ_{eo}, vs. % CTAB in 50 m*M* ionic strength phosphate buffer for several pH values, with and without 0.01% Brij 35 present. Results are shown near the flow reversal point.

$I=50$ phosphate buffer at three pH values between 6.3 and 8.2, with 0.01% Brij 35 added. The point of zero EOF lay in the range of 1 to $2.3\cdot10^{-4}$% (2.7–6.3 μ*M*) CTAB, and an apparent plateau in μ_{eo} was observed between 3.5 to $10\cdot10^{-4}$% CTAB. However, as shown in Fig. 6, the mobility continued to increase to values in the range of -3.7 to $-5.0\cdot10^{-4}$ cm^2 V^{-1} s^{-1}, as [CTAB] increased to 0.1% (2.74 m*M*). Careful evaluation of Fig. 6 shows that the rise from -1 to $-3\cdot10^{-4}$ cm^2 V^{-1} s^{-1} was considerably steeper in the absence of Brij 35 for the data obtained at pH 7.36. The results establish that the PDMS–Brij 35–CTAB coating combination provides a means of fairly accurately controlling EOF in a range of -1 to $-5\cdot10^{-4}$ cm^2 V^{-1} s^{-1}.

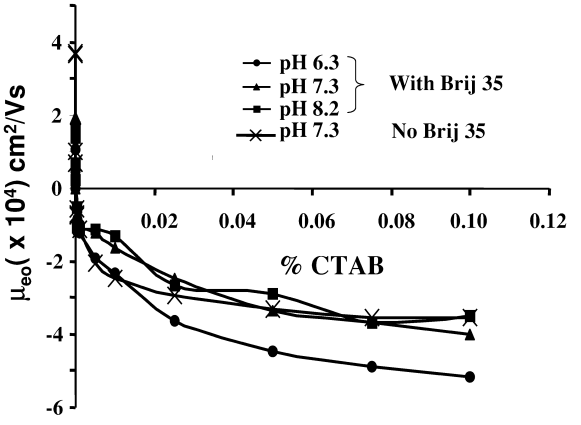

Fig. 6. Electroosmotic mobility, μ_{eo}, vs. % CTAB in 50 m*M* ionic strength phosphate buffer for several pH values, with and without 0.01% Brij 35 present. Results are shown across the entire CTAB concentration range evaluated.

Adsorption of CTAB on bare capillaries also causes flow reversal [37–39]. In $I=50$ phosphate buffer [15] flow reversal required a concentration in the range of 0.2–0.6 m*M* [15,25], and μ_{eo} plateaued at about $-0.5\cdot10^{-4}$ cm^2 V^{-1} s^{-1} across a pH range of 3.5 to 9.0. In contrast, CTAB induces flow reversal on C_8 coated surfaces [13] and on the cross-linked hydrophobic polymer PS-264 [40] at μ*M* concentration, consistent with the 3–6 μ*M* CTAB range observed here for PDMS coatings. Much higher reverse mobilities are obtained with CTAB and a PDMS surface (Fig. 6) than is seen on bare silica. Nevertheless, values between -1.5 and $-5\cdot10^{-4}$ cm^2 V^{-1} s^{-1} required [CTAB] be above the CMC of 0.1 m*M* ($36.5\cdot10^{-4}$%). Micelles are thus present over most of the useful range of CTAB concentrations, in contrast to the results obtained with SDS. A preliminary evaluation of the protein separation performance of the PDMS–CTAB coating was performed using a mix of proteins and 0.005% CTAB (0.137 m*M*), and the results are given in Table 3. The results show using a charged surfactant allows protein separation while still generating electroosmotic flow, although the separation efficiency is reduced with CTAB compared to Brij 35. Thus, this strategy should be workable within microfluidic devices intended for protein analysis.

3.4. Variation of electroosmotic flow with pH in the presence of surfactants

The PDMS–Brij 35 coating gave μ_{eo} values that were essentially pH independent. However, Fig. 4 shows that in the presence of SDS there is a modest decrease in μ_{eo} at higher pH, contrary to the anticipated increase in surface charge if acid–base chemistry alone were responsible for the effect [25–27]. The PDMS–Brij 35 coating also showed more response to pH in the presence of CTAB than it did in the absence. It is likely that either of the charged

surfactants partially disrupts the Brij 35 coating when they are present. We note that Janini and coworkers reported [13] that the maximum EOF varied slightly with pH when CTAB interacts with a C_8 coated capillary, while Underhill and Lucy reported [15] no pH dependence for CTAB adsorbed on bare capillaries. A detailed analysis of these weak pH dependencies is outside the scope of this report.

4. Conclusions

Coating capillary surfaces with pyrolyzed silicone oils provides a convenient means of preparing a hydrophobic PDMS surface in both fused-silica and microchip CE devices. The addition of a neutral, poly(oxyethylene)-based surfactant to the running buffer creates a surface that can be used effectively for separation of proteins. Residual electroosmotic flow was about 25% of that seen for bare capillaries when using the PDMS–Brij 35 combination. The plate numbers achieved for protein separations are comparable to those reported for many coatings that have been studied, although higher performance coatings for proteins exist. Importantly, the coating allows effective protein separations within microchip CE devices, and is easy to apply to these devices. The combination of Brij 35 and charged surfactants with the PDMS coating provides a means to adjust the electroosmotic mobility across a wider range of values than previously reported methods, and is suitable to both microchip and fused-silica CE. This coating approach also has advantages over the use of neutral and charged surfactants on bare capillaries due to the decreased amount of surfactant required, so that in some cases denaturing of proteins can be avoided. This use of PDMS coatings with neutral and charged surfactants will allow microchip devices to be coated to reduce adsorption, while still maintaining a surface charge to provide electroosmotic flow control when required.

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References

[1] S. Hjerten, J. Chromatogr. 347 (1985) 191.
[2] S. Hjerten, K. Kubo, Electrophoresis 14 (1993) 390.
[3] K.A. Cobb, V. Dolnik, M. Novotny, Anal. Chem. 62 (1990) 2478.
[4] J.K. Towns, F.E. Regnier, Anal. Chem. 63 (1991) 1126.
[5] M.A. Strege, A.L. Lagu, J. Chromatogr. 630 (1993) 337.
[6] M. Chiari, N. Dell'Orto, A. Gelain, Anal. Chem. 68 (1996) 2731.
[7] K. Srinivasan, C. Pohl, N. Avdalovic, Anal. Chem. 69 (1997) 2798.
[8] C. Gelfi, M. Curcio, P.G. Righetti, R. Sebastiano, A. Citterio, H. Ahmadzadeh, N.J. Dovichi, Electrophoresis 19 (1998) 1677.
[9] Y. Liu, W.G. Kuhr, Anal. Chem. 71 (1999) 1668.
[10] G.J.M. Bruin, J.P. Chang, R.H. Kulman, K. Zegers, J.C. Kraak, H. Poppe, J. Chromatogr. 471 (1989) 429.
[11] J. Preisler, E.S. Yeung, Anal. Chem. 68 (1996) 2885.
[12] D. Durkin, J.P. Foley, Electrophoresis 21 (2000) 1997.
[13] G.M. Janini, K.C. Chan, J.A. Barnes, G.M. Muschik, H.J. Issaq, J. Chromatogr. A 653 (1993) 321.
[14] A. Cifuentes, M.A. Rodriguez, F.J. Garcia-Montelengo, J. Chromatogr. A 742 (1996) 257.
[15] C.A. Lucy, R.S. Underhill, Anal. Chem. 68 (1996) 300.
[16] K.C. Yeung, C.A. Lucy, Anal. Chem. 69 (1997) 3435.
[17] N.E. Baryla, C.A. Lucy, Anal. Chem. 72 (2000) 2280.
[18] D.J. Harrison, K. Fluri, K. Seiler, Z. Fan, C.S. Effenhauser, A. Manz, Science 261 (1993) 895.
[19] J. Li, P. Thibault, N.H. Bings, C.D. Skinner, C. Wang, C. Colyer, D.J. Harrison, Anal. Chem. 71 (1999) 3036.
[20] S.C. Jacobson, R. Hergenröder, L.B. Koutny, R.J. Warwack, J.M. Ramsey, Anal. Chem. 66 (1994) 1107.
[21] C.S. Effenhauser, G.J. Bruin, A. Paulus, Electrophoresis 18 (1997) 2203.
[22] C.L. Woolley, US Patent 5 192 406 (1993).
[23] M.F. Borgerning, W.L. Hinze, Anal. Chem. 57 (1985) 2183.
[24] C.R. Desilets, M.A. Rounds, F.E. Regnier, J. Chromatgr. 544 (1991) 25.
[25] W. Ding, J. Fritz, J. High Resolut. Chromatogr. 20 (1997) 575.
[26] R. McCormick, Anal. Chem. 60 (1988) 2322.
[27] H.H. Lauer, D. McManigill, Anal. Chem. 58 (1986) 166.
[28] J.S. Green, J.W. Jorgenson, J. Chromatogr. 478 (1989) 63.
[29] K.K.-C. Yeung, C.A. Lucy, J. Chromatogr. A 804 (1998) 319.
[30] N. Chiem, D.J. Harrison, Electrophoresis 19 (1998) 3040.
[31] W.C. Boyd, J. Biol. Chem. 210 (1965) 4097.
[32] J.W. Jorgenson, K.D. Lukacs, Science 222 (1983) 266.
[33] M. Lever, Anal. Biochem. 83 (1997) 274.

[34] Y. Ashani, G.N. Catravas, Anal. Biochem. 109 (1980) 55.
[35] P.C. Hiemertz, Principles of Colloid and Surface Chemistry, Marcel Dekker, New York, 1986.
[36] C. Tanford, The Hydrophilic Effect: Formation of Micelles and Biological Membranes, Wiley, New York, 1980.
[37] J.C. Reijenga, T.P.E.M. Verheggen, F.M. Everaerts, J. Chromatogr. 260 (1983) 241.
[38] W.R. Jones, P. Jandik, J. Chromatogr. 546 (1991) 445.
[39] W.R. Jones, P. Jandik, J. Chromatogr. 608 (1992) 385.
[40] W.D. Pfeffer, E.S. Yeung, Anal. Chem. 62 (1990) 2178.