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Electrophoretic separations in poly(dimethylsiloxane) microchips using a mixture of ionic and zwitterionic surfactants

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Abstract

The use of mixtures of ionic and zwitterionic surfactants in poly(dimethylsiloxane) (PDMS) microchips is reported. The effect of surfactant concentration on EOF was studied for a single anionic surfactant (sodium dodecyl sulfate, SDS), a single zwitterionic surfactant (N-tetradecylammonium-N,N-dimethyl-3-ammonio-1-propanesulfonate, TDAPS), and a mixed SDS/TDAPS surfactant system. SDS increased the EOF as reported previously while TDAPS showed an initial increase in EOF followed by a reduction at higher concentrations. When TDAPS was added to a solution containing SDS, the EOF decreased in a concentration dependent manner. The EOF for all three surfactant systems followed expected pH trends, with increasing EOF at higher pH. The mixed surfactant system allowed tuning of the EOF across a range of pH and concentration conditions. After establishing the EOF behavior, the adsorption/desorption kinetics were measured and showed a slower adsorption/desorption rate for TDAPS than SDS. Finally, the separation and electrochemical detection of model catecholamines in buffer and reduced glutathione (GSH) in red blood cell lysate using the mixed surfactant system were explored. The mixed surfactant system provided shorter analysis times and/or improved resolution when compared to the single surfactant systems.

Keywords

EOF control; Electrochemical detection; Microchip capillary electrophoresis; Mixed surfactants (ionic + zwitterionic); PDMS microchip

1 Introduction

As the dominant bulk flow mechanism in CE and microchip CE, EOF plays an important role in the analyte separations and microfluidic transport [1, 2]. Given the importance of EOF, it is not surprising that this subject has been studied extensively [3, 4]. EOF control in microchip electrophoresis has proven more challenging than in traditional CE in part because of the variety of materials used to make these devices. While glass microchips have similar EOF characteristics to fused silica capillaries, polymer microchips are becoming more popular, and each polymer has a characteristic surface chemistry and therefore EOF. EOF within microfluidic devices made from polymers such as poly(dimethylsiloxane) (PDMS) [5], poly(methyl methacrylate) (PMMA) [6, 7], polycarbonate [8], and polystyrene (PS) [8, 9] can vary widely due to the diversity of the surface-exposed functional groups. In addition, the zeta potential of these materials that gives rise to the EOF is often less than optimal for a specific separation. As a result, a number of surface modification strategies

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have been developed to alter the EOF and prevent analyte-wall interactions. Demonstrated modifiers include inorganic anions [10], divalent metals [11], polyamines [12], polyelectrolytes [13–17], covalent modifications [18–20], variations in the bonding method [21], and the addition of surfactants to the background electrolyte (BGE) [22–24].

Surface coatings can be divided into three categories, permanent coatings based on covalent reactions between the modifying agent and the surface, adsorbed coatings based typically on ionic interactions between a polymer and the surface, and dynamic coatings which rely on the equilibrium between the modifier in solution and the surface [25, 26]. While permanent coatings have demonstrated remarkable stability and migration time reproducibility, they also require longer capillary preparation times and can be susceptible to chemical degradation, particularly at alkaline pH [27]. Adsorbed coatings are attractive because they do not require the modifier be present in the solution and tend to be more stable at alkaline pH relative to covalent coatings. A successive multiple-ionic-layer approach [28–30] for adsorbed coatings has been applied to both microfluidic PDMS [14] and thermoset polyester (TPE) [31] channels. In these studies, polyelectrolyte multilayers are created by exposing the surface to the cationic polymer Polybrene (PB) followed by a layer of dextran sulfate (DS) as the anionic polymer. Unfortunately, most adsorbed coatings provide little adjustability in the EOF. Dynamic coatings formed by BGE additives have been widely used for EOF modification for both conventional and microchip CE because of their low cost, simplicity, and versatility [32, 33]. Surface-active compounds are added to the BGE or are applied within a rinsing step prior to analysis, which interact with the surface, changing the zeta potential [1, 2] and therefore affect the EOF and the separation. The most common dynamic coatings rely on ionic surfactants such as sodium dodecyl sulfate (SDS) to alter the surface charge of polymeric microchips [34, 35]. In particular, the addition of SDS may increase the EOF [34, 35] and improve the electrochemical signal of various molecules, such as carbohydrates [36, 37], metabolites [38], and catechols [39]. The improved electrochemical response for some species in the presence of surfactants can be attributed to increased solubility of the oxidation product of the electrochemical reaction [39]. In addition, cationic surfactants such as cetyltrimethylammonium bromide (CTAB) [40, 41], tetradecyltrimethylammonium bromide (TTAB) [42], and didodecyltrimethylammonium bromide (DDAB) [43] have been added to reverse the EOF direction in microchip CE. Zwitterionic surfactants have also been used in both traditional and microchip CE. Lucy's group first investigated the use of low concentrations of zwitterionic surfactants to suppress the EOF and prevent adsorption of cationic proteins on the walls of fused silica capillaries for traditional CE [44, 45]. In particular, the separation of both cationic and anionic proteins with efficiencies as high as 1.4 million plates/m was achieved using a capillary coated with a double-chained zwitterionic phospholipid, 1, 2-dilauroyl-*sn*-phosphatidylcholine (DLPC) [45]. Another zwitterionic surfactant, dodecylmethyl (2-hydroxy-3-sulfopropyl) ammonium (DSB) was also used to suppress EOF and prevent analyte absorption for the separation of basic proteins and a mixture of eight inorganic anions. The later separations were achieved in 4.2 min with efficiencies of 24 000 to 1 310 000 plates/m [46]. Recently, Henry's group reported the application of sulfobetaine zwitterionic surfactants for the determination of inorganic anions in atmospheric aerosols [47] and perchlorate in drinking water [48] in PDMS microchips, in which these surfactants suppressed the EOF and influenced separation selectivity.

Mixed surfactant systems represent an interesting alternative to single surfactant systems for both better control of EOF and separation chemistry. Mixtures of zwitterionic and cationic surfactants have been investigated by Lucy's group [49, 50] for traditional CE. The zwitterionic surfactant CAS U (coco (amidopropyl)hydroxydimethylsulfobetaine) alone yielded essentially a zero EOF since it has no net charge. On the other hand, TTAB alone yielded reversed EOF because of its positively charged quaternary amine functional group.

When these two surfactants were mixed, the EOF could be modified from nearly zero to fully reversed ($-5 \times 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$) [49]. This ability to control EOF was used to fine-tune the separation of inorganic anions [49] and to separate ammonium isotopes through EOF counterbalance [50]. Similar work has been done using a capillary semi-permanently coated with a cationic/zwitterionic mixture of DDAB/DLPC, which allowed the excess surfactant to be removed from the buffer prior to separation [51]. Furthermore, capillary electrophoretic separations of basic proteins were achieved using mixed cationic/anionic surfactants of CTAB/SDS with enhanced EOF stability relative to CTAB alone [52]. Alternatively, Roeraade's group reported the capillary electrophoretic separation of proteins and peptides by using different combinations of nonionic, cationic, anionic and zwitterionic fluorosurfactants [53] again on fused silica capillaries. Furthermore, increased variability and improved control of the EOF across a large EOF range was achieved in Harrison's group, which employed a mixture of charged surfactants and the non-ionic surfactant Brij 35 for the protein separation on PDMS-coated fused silica capillaries and glass microchips [23].

In this paper, the use of a mixed surfactant system to control EOF and modify separation selectivity in PDMS microchips is reported. EOF measurements as a function of surfactant concentration and pH were made for a single anionic surfactant (SDS), a single zwitterionic surfactant (TDAPS), and a mixed SDS/TDAPS surfactant system. Next, the adsorption/desorption kinetics were measured for both SDS and TDAPS at different surfactant concentrations. Finally, separation and electrochemical detection of model analytes using surfactants were explored for their ability to adjust the EOF and improve the separation.

2 Material and methods

2.1 Reagents and solutions

Reagents used for the fabrication of microchips include SU-8 2035 photoresist (Microchem, Newton, MA), Sylgard 184 elastomer and curing agent (PDMS) (Dow Corning, Midland, MI), 4-in. silicon wafers (University Wafer, South Boston, MA), and microwires made of 99.99% Pd (25 μm) and 99.99% Au (25 μm) (Goodfellow, Huntingdon, England). Aqueous solutions were prepared in 18.2 M Ω ·cm water from a Millipore Milli-Q purification system (Millipore Corp., Billerica, MA). The BGEs were prepared by weighing the desired amount of N-tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid (TES; Sigma-Aldrich, St. Louis, MO) or sodium phosphate (either Na_2HPO_4 or NaH_2PO_4 ; Fisher, Pittsburgh, PA) and adjusting the pH with either 2 M NaOH (Fisher) or 2 M HCl (Fisher). Following the pH adjustment, the desired amount of surfactant was added to the running buffer. SDS (Aldrich, Milwaukee, WI, USA), TDAPS (Fluka, Buchs, Switzerland), and N-dodecyl-N,N-dimethyl-3-ammonio-1-propansulfonate (DDAPS; Sigma-Aldrich) were selected for the present study (structures are shown in supporting information). 10-mM stock solutions of dopamine, catechol, ascorbic acid, DL-homocysteine (Hcy), reduced glutathione (GSH) (Sigma-Aldrich), and L-cysteine (Cys) (Fluka) were prepared daily in 10 mM HCl, while L-tyrosine (Tyr) (Sigma-Aldrich) was prepared daily in 10 mM NaOH. Samples were prepared by dilution of the stock with background electrolyte. All chemicals were used as received.

2.2 Sample preparation and analysis

The red blood cell (RBC) sample for the detection of reduced GSH using pulsed amperometric detection (PAD) was treated according to the literature [54]. Briefly, 10 mL of whole blood was collected from a volunteer (between 24 and 32 years old) in heparinized tubes. The sample was centrifuged 1,000 $\times$ g for 15 min at 4 $^\circ\text{C}$, and the plasma discarded. The remaining erythrocytes were washed three times with phosphate buffer solution (0.2 M, NaCl (Mallinckrodt, Phillipsburg, NJ) 0.9%, pH 7.4) and aliquots of the erythrocyte were hemolysed (1:1 v/v) in 1 mmol L^{-1} $\text{Na}_2\text{H}_2\text{EDTA}$ (Mallinckrodt) solution. After preparing

the hemolysate, 10% (w/v) 5-sulfosalicylic acid (Sigma) was added, followed by vigorous shaking and centrifugation at $1,000\times g$ for 10 min at 4 °C. The supernatant was collected as hemolysate sample and was 4x diluted in 20 mM boric acid buffer (pH 9.2) with an appropriate composition of mixed SDS/TDAPS surfactants. The solution containing reduced GSH was analyzed by microchip CE immediately after removing the precipitated protein with a 3K microcentrifuge filter.

2.3 Fabrication of the PDMS microchip

Depending on the purpose, two different PDMS microchips were fabricated using a previously described method [55, 56]. EOF measurements were performed in single straight channels ($50\text{ }\mu\text{m} \times 50\text{ }\mu\text{m} \times 4.75\text{ cm}$) using the current monitoring method [57]. For the experiments involving separation and electrochemical detection of model analytes, a previously reported design [58] consisting of a straight T injector and a bubble cell with its width 4x of the separation channel width in the detection zone for the purpose of enhanced electrochemical detection [58] was used and had channel dimensions of $50\text{ }\mu\text{m} \times 50\text{ }\mu\text{m}$. The sample, sample waste, and buffer channel lengths were 0.8 cm, and the separation channel length was 5.0 cm. A Pd decoupler and a Au working electrode (WE) were inserted to pass through the bubble cell and the electrode channels. Each electrode channel is $50\text{ }\mu\text{m}$ wide with $125\text{ }\mu\text{m}$ spacing between the channels.

2.4 Instrumentation

A 3-channel (two positives and one negative) laboratory built high-voltage power supply was used for all the experiments involving an injection/separation step [59]. For separations, a 1-s gated injection [60] was used for the separation of dopamine, catechol, and ascorbic acid, while a 3-s gated injection was employed for the separation of Tyr, Hcy, Cys and GSH. During the injection, sample introduction was achieved by applying a positive potential of 1051 V (99 V cm^{-1}) to the sample reservoir while floating the buffer reservoir. The separation was performed by applying 895 V to the buffer reservoir and 1051 V to the sample reservoir, resulting in a separation field strength of 125 V cm^{-1} . Sample waste and buffer waste reservoirs were held at ground.

2.5 EOF measurements

Current monitoring was used to measure EOF as described previously [57]. Briefly, the first reservoir and the channel were filled with 20 mM buffer and the second reservoir was filled with 18-mM buffer. Upon application of 880 V in the first reservoir, EOF caused the lower concentration electrolyte solution to displace the higher concentration buffer in the channel, resulting in an increase in the electrical resistance of the channel. The change in separation current under a constant applied voltage difference was monitored using a Fluke multimeter (Everett, WA). Once a constant current was obtained, the potential was reversed and the above procedure repeated. The time required to reach a current plateau was used to calculate EOF. EOF measurements were calculated as the average from four microchips, with six replicates performed on each microchip. Reported uncertainties are the standard deviations obtained from the four microchips.

2.6 Adsorption/desorption kinetics study

Adsorption/desorption kinetics studies were performed in the following way. The initial EOF was measured using pH 7.0 phosphate as the BGE. After 30 min of consecutive runs, the solutions were replaced with 18 mM and 20 mM phosphate buffers at pH 7.0 containing the corresponding surfactant and the analysis was repeated until a stable EOF was reached. Once a stable EOF was reached, the solutions were replaced with the initial solutions without surfactant and the EOF was measured again until a stable value was observed.

2.7 Electrochemical detection

Amperometric detection of dopamine, catechol, and ascorbic acid and PAD of Tyr, Hcy, Cys, and GSH was performed using a CHI 1010A Electrochemical Analyzer (CH Instruments, Austin, TX) in a two-electrode configuration. The Pd decoupler was held at ground in all injection and separation phases to prevent exposing the detector electronics to high voltage. A gold wire (25- μ m diameter) was used as the working electrode and the corresponding detection potential was optimized for each compound. A platinum wire (1-mm diameter) in the waste reservoir was acted as both auxiliary and pseudo-reference electrode.

3 Results and discussion

3.1 Effect of the concentration of surfactants

3.1.1 Single SDS surfactant system—Modification of PDMS surface chemistry to alter the EOF using a single surfactant has been reported by several research groups [61, 62]. In these studies, it was proposed that the surfactant molecules interact with the PDMS surface by their hydrophobic tails, while exposing negatively charged head groups to the solution, leading to an increase in the zeta potential and a higher EOF [63]. In order to systematically study the effect of mixed surfactants on EOF in PDMS microchips, we initially measured the concentration dependent effect of SDS on EOF. EOF measurements were performed using pH 7.0 TES buffers as shown in Fig. 1 (inverted triangles). As the SDS concentration increased, EOF increased, reaching a plateau above 3.0 mM, with its value changing from 3.56 ± 0.10 to $7.13 \pm 0.13 \cdot 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ ($n = 4$) in the range of 0 ~ 6 mM SDS. The plateau in EOF at SDS concentrations above 3.0 mM suggests the surface of PDMS microchip was fully coated. EOF was also measured in phosphate buffer at pH 4.0, 7.0, and 10.0 for SDS concentrations of 0 ~ 20 mM to explore pH effects. The EOF shows a similar trend as a function of SDS concentration as that measured for the pH 7.0 TES buffer. Furthermore, the EOF increases as pH increases, consistent with an increased negative charge density on the PDMS surface at the higher pH [34]. It should be noted, however, that the differences in EOF as a function of pH might be affected by differences in the ionic strength of the BGEs because nothing was done to keep these constant between pHs. The results are also in agreement with previous work presented by Harrison's and Culbertson's groups [34, 35].

3.1.2 Single TDAPS surfactant system—Zwitterionic surfactants represent an interesting alternative for surface modification in polymeric microchips as well as for alternative agents for micellar electrokinetic chromatography (MEKC). The impact of these surfactants on EOF in fused silica capillaries has been studied by Lucy's group [44, 45] but their impact on EOF in polymeric microchips has not been studied. Here, we measured the impact of 0 to 2 mM TDAPS (CMC: 0.1 ~ 0.4 mM at 20 ~ 25 °C [62]) on EOF for pH 7.0 TES and pH 4.0, 7.0, and 10.0 phosphate buffers. Fig. 2 shows a similar EOF trend for all four buffers, in which EOF rapidly increases at low concentrations, and after reaching a highest EOF value at 0.1 mM TDAPS, gradually decreases to a nearly constant value. Differences in the starting EOF values are the result of differences in the ionic strength of the BGEs, the pH effects on surface charge, and the inherent variability of the EOF of PDMS. The unique behavior of TDAPS on PDMS can be explained by considering existing models for the interaction of surfactants with surfaces. Lucy's group showed a decreasing EOF trend for three sulfobetaine zwitterionic surfactants, DDAPS, HDAPS, and CAS U in fused silica capillaries, and a hemimicelle model was proposed for this behavior [44]. It was believed that the EOF results from the migration of the cations associated with the silanols on the surface of fused silica capillary, and the decrease of EOF can be attributed to the shielding of the silanols layer from the bulk solution by the adsorbed zwitterionic

surfactants, leading to the decrease in zeta potential. This model can explain the decrease in EOF at higher TDAPS concentrations but does not explain the initial increase observed with a PDMS substrate. To verify that the behavior was not unique to TDAPS, DDAPS (CMC: 2.2 mM [44]), which has two less carbon chains than TDAPS, was tested as well. A similar EOF trend was obtained for DDAPS in PDMS microchips as compared to TDAPS, while the highest EOF value was obtained at 2 mM DDAPS (see supporting information Fig. S2 for details). The mechanism for the decrease in EOF could be from one of several possible behaviors, including bilayer formation, hemimicelle or full micelle. As hypothesized in the inserted panel in Fig. 2, the molecules of zwitterionic surfactants at their low concentrations may preferentially interact with the PDMS surface by their hydrophobic tails rather than their charged headgroups. Two potential results could cause an increase in EOF. First, the outermost exposed group of the surfactant is the anionic sulfonate functionality which would cause formation of the cationic double layer. Second, the larger head group of the zwitterionic surfactant will extend further from the surface resulting in a thicker double layer, a larger zeta potential and thus higher EOF. As the TDAPS concentration increases, a variety of mechanisms are again possible, ranging from bilayer formation to hemimicelle layer formation. The gradual decrease in EOF could also be attributed to charge neutralization of adsorbed surfactant molecules in the diffuse part of the double layer. The highest EOF was achieved at 0.1 mM of TDAPS which might be evidence of micelle formation of TDAPS at this buffer condition. More experiments using zwitterionic surfactants with different headgroup functionalities are needed to validate this hypothesis. However, some of necessary surfactants are currently not commercially available and synthesis of these molecules was outside the scope of the present work.

3.1.3 Mixed SDS and TDAPS surfactant system—Mixtures of anionic surfactant (SDS) and zwitterionic surfactant (TDAPS) were investigated next for their effect on EOF. EOF was measured at 0 to 20 mM SDS concentrations from while the concentration of TDAPS remained constant at 0.1 mM, 0.5 mM, 1.0 mM, or 2.0 mM (Fig. 3). In the absence of SDS, the EOF values measured for varying concentrations of TDAPS matched the values shown in Figure 2. The addition of SDS caused an increase in EOF for all four TDAPS concentrations with a trend similar to that seen with SDS alone. However, the overall magnitude of the EOF was dependent on the TDAPS concentration with 0.1 mM TDAPS giving the highest average EOF and 2.0 mM giving the lowest. EOF values were similar to or higher than values measured for SDS alone. For example, an EOF value of $8.37 \pm 0.15 \cdot 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ was produced using a mixture of 3.0 mM SDS and 0.1 mM TDAPS, which is 19% higher than that using 3.0 mM SDS alone. Hence, it would be advantageous to use these mixed surfactants for separations that require high EOF. The SDS:TDAPS ratio was also plotted to show the relative effect (Fig. S3). Except for data obtained at 0.1 mM TDAPS, the observed EOF closely correlates with the SDS:TDAPS ratio, irrespective of absolute surfactant concentrations. Monomeric SDS adsorbs to the PDMS surface and increases the EOF, whereas TDAPS suppresses the EOF above the critical micelle concentration. These results suggest that a desired EOF can be achieved through the range presented by simply varying the ratio of anionic and zwitterionic surfactants. Additionally, pH effects on the EOF for mixed surfactants in phosphate buffer at pH 4.0, 7.0, and 10.0 were studied, and the results are shown in supporting information. (Fig. S4, S5, and S6) Similar EOF behaviors as those in TES buffer at pH 7.0 can be observed for all three phosphate buffer conditions with different pH values.

3.2 Adsorption/desorption kinetics of surfactants

After establishing the EOF behavior, measurements were also performed using the current monitoring method to evaluate the adsorption and desorption kinetics of SDS and TDAPS. Similar studies have been made on PDMS using the separation of a neutral marker for EOF

measurements [65]. Fig. 5 shows the experimental results of adsorption/desorption kinetics of SDS (0.8 mM) and TDAPS at concentrations of 0.1 mM, 0.5 mM, and 2.0 mM. The differences observed in the initial EOF values likely arise from variability in the native surface charge of PDMS and/or variations in ionic strength. As can be observed in Fig. 4, a significant increase in the EOF was observed once SDS was added to the BGE, with EOF changing from the initial value of $3.5 \cdot 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ for native PDMS to $5.0 \cdot 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ with 0.8 mM SDS. The equilibrium was reached in less than 10 min. When BGEs without SDS were reloaded, the EOF rapidly decreased to a value of $4.0 \cdot 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$, resulting from the desorption of SDS from the surface. However, the initial EOF value was never reached even after 40 min, suggesting a significant amount of SDS was retained on the surface. Similar behavior was observed when 0.1 mM TDAPS was added to the BGE. As can be observed in Fig. 4, the initial EOF increased from $3.2 \cdot 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ to $3.9 \cdot 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$, and decreased to a final EOF value of $3.7 \cdot 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$, showing slower adsorption and desorption kinetics than those in 0.8 mM SDS. After 40 min, ~69% of the total EOF change due to TDAPS remained due to semi-permanently attached TDAPS on the PDMS surface. When 0.5 mM TDAPS was added to the BGE, EOF decreased from $2.9 \cdot 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ to $2.3 \cdot 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$, while in the case of 2.0 mM TDAPS, EOF changed from $3.4 \cdot 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ to $2.6 \cdot 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$. Likewise, after removing TDAPS from the BGE, EOF did not recover to its initial value. Hence, the PDMS channels should be in contact with each solution prior to EOF measurements for at least 40 min to minimize kinetic contributions in the adsorption stage.

3.3 Separations using mixed SDS and TDAPS surfactant system

The advantages of the mixed SDS/TDAPS surfactant system on PDMS microchips were demonstrated with the separation of dopamine, catechol, and ascorbic acid as model analytes. Separations were performed with different mixtures of SDS and TDAPS surfactants and compared to the individual surfactant systems. Electropherograms for 50 μM analytes under these buffer conditions are shown in Fig. 5, with calculated EOF values of $7.22 \pm 0.31 \cdot 10^{-4}$, $8.20 \pm 0.32 \cdot 10^{-4}$, $6.17 \pm 0.26 \cdot 10^{-4}$, $8.75 \pm 0.37 \cdot 10^{-4}$, $8.00 \pm 0.31 \cdot 10^{-4}$, $7.74 \pm 0.32 \cdot 10^{-4}$ and $7.17 \pm 0.33 \cdot 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ corresponding to the electropherograms from bottom to top, respectively. Shorter migration times (37.4 ± 1.3 to 39.7 ± 1.4 , 46.9 ± 2.0 to 58.2 ± 2.1 , and 60.5 ± 2.4 to $77.2 \text{ s} \pm 3.5$) and higher peak heights (8.6 ± 0.8 to 12.0 ± 0.8 , 4.6 ± 0.4 to 5.0 ± 0.5 , and 1.1 ± 0.2 to $1.3 \pm 0.3 \text{ nA}$) were obtained for dopamine, catechol, and ascorbic acid, respectively, in four mixed SDS/TDAPS surfactant systems as compared to the TES buffer with 0.1 mM TDAPS (Migration time of 46.9 ± 1.8 , 62.3 ± 2.6 , and $88.1 \pm 2.6 \text{ s}$ and peak heights of 8.0 ± 0.7 , 2.5 ± 0.3 , and $0.6 \pm 0.1 \text{ nA}$ for three analytes, respectively). Differences in the relative migration times here with respect to the EOF results discussed above are likely due to differences in the interaction of the analytes with the surfactant. Furthermore, differences in peak height may be the result of variation in injection volume. A significant improvement in the resolution ($R = 4.16 \pm 0.08$, 4.32 ± 0.09 , 5.88 ± 0.11 , and 9.39 ± 0.15 for 0.5 mM SDS mixed with 0.1, 0.5, 1.0, and 2.0 mM TDAPS, respectively) between dopamine and catechol was also achieved by using mixed surfactants as compared to the resolution ($R = 1.37 \pm 0.05$) of these two analytes in TES buffer with 1.0 mM SDS, while the resolution of all analytes obtained using 0.1 mM TDAPS were still higher than those in mixed surfactant systems due to the much slower EOF in single TDAPS surfactant than mixed surfactant systems. Although clear separation can be obtained using TES buffer with SDS concentrations lower than 1.0 mM, mixed SDS/TDAPS surfactant system may provide a faster analysis time than single surfactant system with the same SDS concentration, while still keeping comparable or even better analyte resolutions. As TDAPS concentration increases in the surfactant mixtures, analytes resolution was enhanced, and the best results were achieved using a mixture of 0.5 mM SDS and 2.0 mM TDAPS among four tested mixed surfactant systems. In addition, the highest

separation efficiencies of 150000 ± 8100 , 200000 ± 12000 , and 170000 ± 20000 plates/m (corresponding to 7300 ± 380 , 9700 ± 550 , and 8000 ± 950 plates) were also obtained for dopamine, catechol and ascorbic acid, respectively, using the same SDS and TDAPS ratio. Comparisons of resolution and separation efficiency calculated from peak information in electropherograms under different conditions is shown in Fig S7 and Table S1. Furthermore, comparable or lower LODs were observed in mixed surfactant systems relative to individual surfactants, with an example LODs of dopamine, catechol and ascorbic acid at 60 ± 15 nM, 150 ± 42 nM, and 600 ± 180 nM, using a mixture of 0.5 mM SDS and 2.0 mM TDAPS, as compared to LODs of these analytes at 80 ± 19 nM, 150 ± 30 nM, and 550 ± 150 nM using 0.5 mM SDS or 120 ± 25 nM, 250 ± 50 nM, and 1000 ± 360 nM using 2.0 mM TDAPS, respectively.

Next, the mixed SDS/TDAPS surfactant system was used for the separation of Tyr and three amino thiols (Hcy, Cys and GSH). Electropherograms for 200 μ M Tyr, Hcy, and Cys as well as 600 μ M GSH as a function of SDS/TDAPS ratio are shown in Fig. 6. The calculated EOF values for the electropherograms from bottom to top under different buffer conditions are $7.87 \pm 0.32 \cdot 10^{-4}$, $6.73 \pm 0.27 \cdot 10^{-4}$, $6.27 \pm 0.28 \cdot 10^{-4}$, $5.72 \pm 0.23 \cdot 10^{-4}$, $4.86 \pm 0.19 \cdot 10^{-4}$, and $3.95 \pm 0.20 \cdot 10^{-4}$ $\text{cm}^2 \text{V}^{-1} \text{s}^{-1}$, respectively. Longer migration times (79.3 ± 2.8 to 124.5 ± 4.1 , 88.5 ± 2.9 to 149.5 ± 7.2 , 107.8 ± 3.3 to 226.8 ± 10.2 , and 116.3 ± 4.1 to 268.8 ± 11.5 s) were obtained for Tyr, Hcy, and Cys and GSH, respectively, using mixed SDS/TDAPS surfactant system than single SDS surfactant (0.1 mM) system (75.3 ± 2.4 , 86.3 ± 2.6 , 100.3 ± 3.4 , and 108.0 ± 3.9 s for four analytes, respectively) due to the decrease in SDS/TDAPS ratio in the BGE. The separation of four analytes using a single TDAPS surfactant (2 mM) was also performed but produced much longer migration times (160.3 ± 7.7 , 210.6 ± 9.1 , 280.3 ± 13.7 , and 340.8 ± 18.1 s for four analytes, respectively) and broader analyte peaks relative to the mixed surfactant system. A clear baseline resolution was achieved for four analytes using the same BGE with 0.1 mM SDS and 0.5 mM TDAPS while separations using only SDS resulted in co-migration. Under this surfactant condition, the separation efficiencies and LODs of Tyr, Hcy, Cys, and GSH are 56000 ± 2600 , 82000 ± 4400 , 120000 ± 7100 , and 92000 ± 6500 plates/m (corresponding to 2700 ± 120 , 4000 ± 210 , 5800 ± 340 , 4400 ± 310 plates) and 4.5 ± 1.2 μ M, 4.3 ± 1.4 μ M, 5.6 ± 1.9 μ M, and 15.8 ± 4.8 μ M, respectively. Additionally, analyte resolution improved with the increase of relative TDAPS concentration. Comparisons of resolution and separation efficiency for these four analytes under different conditions are shown in Fig S8 and Table S2.

3.4 Separation and detection of reduced GSH in human red blood cells

GSH is the most abundant intracellular non-enzymatic component of the antioxidant system found in healthy RBCs, existing mainly in its reduced form, and is important as a diagnostic marker of oxidative stress [66, 67]. To demonstrate the ability of the mixed SDS/TDAPS surfactant system to improve separations in real samples, the determination of GSH in human RBCs was performed using PAD with 0.1 mM SDS and 0.5 mM TDAPS in 20 mM boric acid buffer at pH 9.2. Electropherograms of this analysis are given in Fig. 7. A main analyte peak, eluting at 131.2 s, corresponded to the reduced GSH in the hemolysate was confirmed by spiking with 100 μ M reduced GSH. Here, the analyte peak of Cys was not observed since it is present at low levels in erythrocyte hemolysate samples [68, 69]. The concentration of reduced GSH in the 4x diluted hemolysate sample was determined by standard addition to be 379 ± 52 μ M ($n=3$), which corresponded to a concentration of 1.52 ± 0.21 mM GSH in RBCs. The recovery under this condition for GSH in the RBCs was between 90.2% and 96.4%, and the measured GSH concentration was within the normal reference interval [68–71].

4 Concluding remarks

The use of mixed ionic and zwitterionic surfactants on PDMS microchips to control EOF and improve separation was reported here. EOF measurements as a function of the surfactant concentration were performed using the current monitoring method with SDS, TDAPS, and a mixed SDS/TDAPS surfactant system. SDS increased the EOF as reported previously while TDAPS showed an initial increase in EOF followed by a reduction above the CMC. pH effects were also studied in these single and mixed surfactant systems, exhibiting the expected pH dependences of EOF. With use of these mixed surfactants, higher EOF values and expanded EOF windows were obtained as compared to a single surfactant. Also, the correlation between EOF and surfactant concentration demonstrated that EOF could be tuned over a range of values based on the surfactant ratio. SDS exhibited faster adsorption/desorption kinetics than TDAPS. In all cases, the initial EOF was not fully recovered after removal of the surfactant, showing a residual amount of surfactant remaining on the PDMS surface. Furthermore, separation and electrochemical detection of model analytes using mixtures of these two types of surfactants were performed. Faster analysis times and/or improved resolutions for some model analytes were achieved in mixed surfactants relative to the single surfactant system. Additional experiments are currently in progress to explore these phenomena as a function of different types of mixed surfactant systems. Finally, by using the optimal mixed SDS/TDAPS surfactant composition, the concentration of GSH in RBCs was determined to be 1.45 ± 0.08 mM, which is in the normal reference interval for this analyte.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

Brij-35	polyoxyethylene ether
CAS U	coco (amidopropyl)hydroxydimethylsulfobetaine
DDAB	didodecyldimethylammonium bromide
DDAPS	N-dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate
DLPC	1,2-dilauroyl- <i>sn</i> -phosphatidylcholine
DS	dextran sulfate
DSB	dodecyldimethyl (2-hydroxy-3-sulfopropyl) ammonium
ECD	electrochemical detection
HDAPS	N-hexadecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate
PAD	pulsed amperometric detection
PB	Polybrene

PS	polystyrene
RBC	red blood cell
TES	N-tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid
TDAPS	N-tetradecyl-N,N-dimethyl-3-ammonio-1-propansulfonate
TPE	thermoset polyester
TTAB	tetradecyltrimethylammonium bromide
Tween 20	polyoxyethylene (20) sorbitan monolaurate

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Figure 1.
EOF trends as a function of the concentration of anionic surfactant SDS.

Figure 2. EOF trends as a function of the concentration of zwitterionic surfactant TDAPS. The inserted panel describes the hypothesized surfactant molecule interaction with the PDMS surface.

Figure 3. EOF as a function of SDS concentration using various TDAPS concentrations in TES buffer at pH 7.0.

Figure 4.

Adsorption/desorption experiments of surfactants. The mark* denotes the point when the surfactant was added and the arrow denotes the point when the surfactant was removed from the solution reservoirs.

Figure 5. Example electropherograms for 50 μ M dopamine, catechol, and ascorbic acid in 20 mM TES buffer at pH 7.0 with various surfactant conditions. Field strength: 125 V/cm, 1-s gated injection; Detection: DC Amp., $E_{\text{det}} = 1.2$ V.

Figure 6. Example electropherograms for 200 μ M Tyr, Hcy, Cys and 600 μ M GSH in 20 mM boric acid buffer at pH 9.2 with various surfactant conditions. Conditions are given in the experimental section of the main text. Field strength: 125 V/cm, 3-s gated injection; Detection: PAD, $E_{\text{det}} = 1.6$ V.

Figure 7.

Electropherograms of 4x diluted hemolysate samples of RBCs without and with the standard solution containing 100 μM GSH. BGE: 20 mM Boric acid, 0.1 mM SDS, 0.5 mM TDAPS, pH 9.2; Field strength: 125 V/cm, 3-s gated injection; Detection: PAD, $E_{\text{det}} = 1.6$ V.