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# A modified supported bilayer/diblock polymer – Working towards a tunable coating for capillary electrophoresis

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ABSTRACT

A surfactant bilayer/diblock polymer coating was previously developed for the separation of proteins. The coating consisted of a mixture of the cationic surfactant dioctadecyldimethylammonium bromide (DODAB) and the neutral polymer poly-oxyethylene (POE) 40 stearate (Journal of Chromatography A 1130 (2006) 265–271). Herein an improved method of generating DODAB/POE stearate coatings is demonstrated, which yields more predictable EOF, more stable coatings, greater average efficiencies and easier method development. In this sequential preparation method the DODAB is first flowed through the capillary, followed by a flow of the POE stearate (sequential method). A tunable EOF ($-2.40$ to $-0.17 \times 10^{-4}$ cm$^2$/Vs) is achieved by varying the POE chain length (8, 40 and 100 oxyethylene units). Mixtures of POE 8 and POE 40 stearate enabled continuous variation in EOF from $-2.44$ to $-0.42 \times 10^{-4}$ cm$^2$/Vs. Separations of basic proteins yielded efficiencies of 760 000–940 000 plates/m. Coatings formed using the sequential method were more stable over a larger number of runs (%RSD for migration times: 0.7–1.0% over 30 runs) than those formed using the original mixed method (%RSD: 2.4–4.6% over 14 runs). The ability to tune the EOF is important in maximizing the resolution of analytes with similar electrophoretic mobilities. Histone proteins are separated on a sequentially coated capillary with resolution of nine possible subtypes. Acidic proteins are separated on a sequentially coated capillary at pH 6.4.



## 1. Introduction

A common challenge in the capillary electrophoresis (CE) of proteins and biomolecules is their adsorption onto the surface of the capillary [1,2]. Adsorption can lead to undesirable effects such as band-broadening [3], poor migration time repeatability [4], and low sample recovery [5]. The most common method of minimizing adsorption is to coat the capillary wall. Such coatings can be broadly classified as covalently bonded polymers [6–8], physically adsorbed polymers [9–11], and dynamic coatings [5,12,13]. Covalent coatings are very effective at preventing protein adsorption [14]. However, covalent coatings are more time consuming to prepare and cannot be easily regenerated. Physically adsorbed polymers and surfactant coatings are easy to prepare, regenerable, and cost effective [15].

Coatings prepared from double-chain cationic surfactants are easy to form and produce stable semi-permanent coatings [12,16–18]. However, the strong anodic EOF [12,16,19] overwhelms the mobility of most proteins, such that the resolution can be limited. A reduced EOF enables the mobility of the proteins to come to the forefront so better resolution can be achieved. The suppressed EOF of zwitterionic surfactants [20,21] and phospholipids [22,23] allows the separation of both acidic and basic proteins. However such coatings lack the strong electrostatic attraction of the cationic surfactants. As a consequence the performance of phospholipid coatings is strongly dependent on factors such as the size and lamellarity of the vesicle, the phospholipid concentration and the ionic strength and buffer type of the solution in which the vesicles are prepared [22].

Previously, we reported a coating procedure which combined the stability of a cationic bilayer coating with a suppressed EOF [24]. This coating was formed by flushing the capillary with a mixture of the surfactant dioctadecyldimethylammonium bromide (DODAB) and polyoxyethylene stearate (POE), a diblock polymer with a hydrophilic polyoxyethylene (POE) block and a hydrophobic (stearate) block. POE is also known as polyethylene glycol (PEG) or polyethyleneoxide (PEO). The DODAB forms a bilayer on the surface of the capillary. The hydrophobic portion of the diblock polymer inserts into the DODAB bilayer, leaving the hydrophilic POE moieties protruding into the surrounding solution. A schematic of this can be seen in mixed method, in Fig. 1 where the bilayer structure is adapted from Fig. 10 of Ref. [25]. The suppressed EOF allows for high resolution of either acidic or basic proteins [24]. The ability to tune this suppressed EOF is important in maximizing the resolution of analytes with similar electrophoretic mobilities. However control

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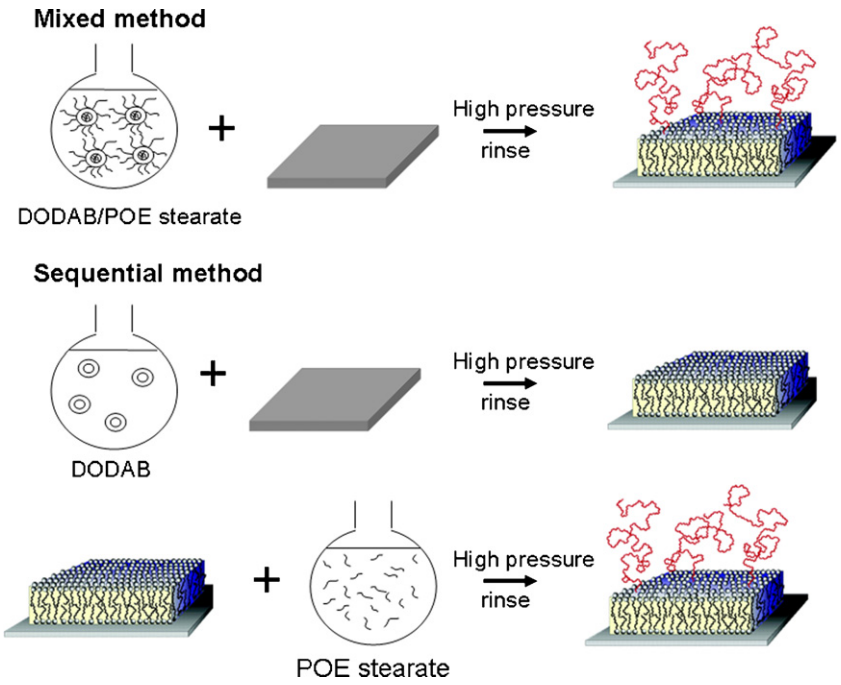


**Fig. 1.** Schematic of the mixed and sequential coating methods. For the mixed method, DODAB and POE stearate are added to a flask, sonicated and stirred, and then rinsed through the capillary. For the sequential method, a DODAB solution is first rinsed through the capillary to form a bilayer on the wall. A POE stearate solution is then rinsed through the capillary and the stearate chains intercalate into the bilayer. Bilayer structures adapted from Fig. 10 of Ref. [25].

of the EOF through variation of the DODAB to POE stearate concentration using the mixed method proved complex and unpredictable [26].

For this reason, a coating with similar properties to the one previously developed, but with an EOF that can be easily tuned is developed herein. In the Sequential method (Fig. 1) a DODAB solution is first flowed through the capillary to form the bilayer on the capillary wall through electrostatic interactions between the DODAB vesicles and the fused silica wall. This is followed by a flow of POE stearate, which forms a hydrophilic neutral coating on the surface of the cationic bilayer, through hydrophobic interactions between the surfactant hydrocarbon chains and the stearate tail, yielding a suppressed EOF.

With many tunable coatings the adjustable EOF is in response to changes in the buffer pH [27–30]. However, pH is an important variable in the optimization of a protein separation. Having both the EOF and separation selectivity governed by the same variable restricts method development. The EOF can also be tuned using additives such as diethylaminetriamine [31], hexamethonium bromide [32], polarizable anions with zwitterionic surfactants [21,33], and surfactant mixtures [20,34,35]. However addition of additives to the buffer may also alter the protein mobility and overall separation.

In this work we develop semi-permanent DODAB/POE stearate sequential coatings for which the EOF can be controlled. The stability, ease of preparation and efficiency of separations of model proteins are used to assess the coatings. The ability to control the EOF enabled the separation of histone protein subtypes.

## 2. Materials and methods

### 2.1. Apparatus

All CE experiments were performed using a P/ACE 2100 system (Beckman Instruments, Fullerton, CA, USA) equipped with a UV absorbance detector upgraded to 5000 series optics, or a P/ACE 5500 system with an on-column diode array UV absorbance detector (Beckman). Detection was performed at 214 nm. The data acquisition rate was 5 Hz (P/ACE 2100) or 4 Hz (P/ACE 5500) and the detector time constant was 0.5 s (P/ACE 2100). Instrument control and data acquisition were controlled using P/ACE station software for Windows 95 (Beckman). Untreated fused silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) with an I.D. of 50 μm, O.D. of 360 μm, and total length of 67, 47 or 27 cm (60, 40 or 20 cm to the detector, respectively) were used unless otherwise stated. The capillary was thermostated at 25 °C.

### 2.2. Chemicals and reagents

All solutions were prepared in Nanopure 18 MΩ water (Barnstead, Chicago, IL, USA). Buffers were prepared from stock solutions of sodium dihydrogen phosphate (BDH, Toronto, ON, Canada), Ultrapure tris (hydroxymethyl)aminomethane (Tris; Schwarz/Mann Biotech, Cleveland, OH, USA), acetic acid (Caledon Laboratories Ltd., ON, Canada) or formic acid (EM Science, Gibbstown, NJ, USA). Buffer pH was measured using a Corning digital pH meter model 445 (Corning, Acton, MA, USA).

The cationic surfactant dioctadecyldimethylammonium bromide (DODAB) and diblock polymers polyoxyethylene (POE) 8 stearate, POE 40 stearate, and POE 100 stearate were used as received from Aldrich (Milwaukee, WI, USA). 2 mM Benzyl alcohol (Aldrich) was used as the neutral EOF marker. The proteins lysozyme (chicken egg white), cytochrome *c* (bovine heart), ribonuclease A (bovine pancreas), α-chymotrypsinogen A (bovine pancreas), trypsin inhibitor (soybean), α-lactalbumin (bovine milk) and histone type III-S (calf thymus) were used as received from Sigma (St. Louis, MO, USA).

### 2.3. Preparation of and coating with the surfactant/polymer solution

Two different methods were used to prepare the DODAB/POE 40 stearate solutions (Fig. 1). Both are a variation of the sonicate/stir method used by Yassine and Lucy [12]. The first is essentially that

described in [24] and is referred to as the *mixed method* herein. In the mixed method, the surfactant salt and the polymer were added together in nanopure water and sonicated (Aquasonic 75 HT, VWR Scientific Products, West Chester, PA, USA) for 30 min at 75 °C and then stirred at room temperature for 20 min. This process was repeated two to three times until a clear solution was obtained. A new 47 cm capillary was rinsed with 0.1 M NaOH for 10 min using high pressure (20 psi, 1 psi = 68.95 mbar). As depicted in Fig. 1, the DODAB/POE stearate mixture was then rinsed through the capillary for 20 min (20 psi) to form the coating. Excess coating reagent was removed using a 0.5 min rinse of 50 mM sodium phosphate, pH 3 buffer (20 psi, ~1.6 capillary volumes).

In the second method, referred to as the *sequential method*, a 0.1 mM solution of DODAB was prepared using the above sonicate/stir procedure. POE 8, 40 and 100 stearate solutions of a range of concentrations (POE 8: 0.001–0.01% (w/v); POE 40: 0.0004–1% (w/v); POE 100: 0.0005–0. 1% (w/v)) were prepared separately with no DODAB, also using the sonicate/stir method. New 47 cm capillaries were preconditioned with a 10 min rinse with 0.1 M NaOH (20 psi). The coating procedure was then (Fig. 1): a 10 min flow (20 psi) of 0.1 mM DODAB; followed by a 10 min (20 psi) rinse with a POE stearate solution of the concentration of interest. A 0.5 min 50 mM sodium phosphate, pH 3 buffer rinse was performed to remove unadsorbed coating solution from the capillary.

### 2.4. EOF measurements

Fresh capillaries were used with each new buffer system to avoid hysteresis effects. The EOF was measured in two ways [36]. The direct voltage method was used to measure EOF whose magnitude was $>1 \times 10^{-4}$ cm$^2$/Vs. A 3 s hydrodynamic injection of benzyl alcohol at 0.5 psi was followed by the application of voltage across the capillary. The EOF was calculated using:

$$\mu_{eof} = \frac{L_d L_t}{V t_m} \quad (1)$$

where $L_t$ and $L_d$ are the total length of the capillary and the capillary length to the detector, respectively, $V$ is the voltage applied, and $t_m$ is the migration time of the neutral marker. Strongly suppressed EOF ($<1 \times 10^{-4}$ cm$^2$/Vs) were measured using the 3 injection method of Williams and Vigh [37].

### 2.5. Protein separations

The coating procedure for separations using the mixed or sequential coating methods is detailed in Section 2.3. Mixtures of 0.1 mg/mL each of lysozyme, cytochrome *c*, ribonuclease A and α-chymotrypsinogen A were injected for 3 s at 0.5 psi and separated using +17.5 kV. Efficiencies were calculated using the Foley–Dorsey method [38].

For histone separations a new capillary ($L_d$ = 60 cm, $L_t$ = 67 cm) was sequentially coated with 0.1 mM DODAB followed by 0.075% (w/v) POE 40 stearate. The capillary was then rinsed with 75 mM Tris formate pH 4.0. The rinse and coat times were adjusted from those in Section 2.3 based on the increased capillary length. A 0.25 mg/mL solution of the histone type III-S was injected hydrodynamically (0.5 psi) for 4 s and a voltage of +15 kV was applied. The width-at-half-height method was used for the histone separations as the baseline was not conducive to efficiency measurements using the Foley–Dorsey method.

For separation of acidic proteins, a new capillary ($L_d$ = 20 cm, $L_t$ = 27 cm) was sequentially coated with 0.1 mM DODAB followed by 0.01% POE 8 stearate. The capillary was then rinsed with 75 mM Tris-acetate with 20 mM $CaCl_2$ buffer (20 psi) to remove excess surfactant/polymer. The rinse and coat times were adjusted from those in Section 2.3 based on the decreased capillary length. A mixture of 0.2 mg/mL trypsin and 0.05 mg/mL α-lactalbumin was injected hydrodynamically for 4 s at 0.5 psi and separated using −10 kV.

## 3. Results and discussion

Coatings which prevent analyte adsorption while simultaneously allowing control of the EOF are highly desirable for protein separations in CE. A hydrophilic coating that yields a suppressed EOF can by generated by flushing a capillary with a mixture of the surfactant dioctadecyldimethylammonium bromide (DODAB) and the diblock polymer polyoxyethylene stearate (Mixed method in Fig. 1) [24]. The resultant DODAB/POE 40 stearate coating resulted in a suppressed EOF and was effective for separating both acidic and basic proteins with efficiencies of up to 1 million plates/m [24]. However, while this coating was highly effective, the magnitude and direction of the suppressed EOF depends on the rinse time and the concentration of POE stearate [24]. For instance the open squares in Fig. 2 show the effect of POE 40 stearate concentration mixed with 0.1 mM DODAB on the EOF at pH 3. Significant and unpredictable variation of the EOF is observed [26]. Coatings prepared using the mixed method showed similar unpredictable variation in EOF at pH 7.4 and pH 10. At all pH studied, POE 40 stearate concentrations of ≥0.75% resulted in strong cathodic EOF. It is hypothesized that in mixtures of POE stearate and DODAB, the POE extends from the surface of the vesicle. As the concentration of the POE stearate increases, the POE interferes with adherence of the DODAB to the walls. Regardless of the cause, systematic control of the EOF was difficult using the mixed method [26].

### 3.1. EOF of sequential DODAB/POE stearate coatings

To improve control of the EOF a sequential coating method was developed (Fig. 1). First the capillary is rinsed with a DODAB solution to form a bilayer which results in a strongly anodic EOF [12,16,19]. The DODAB bilayer forms the anchor for the hydrophilic coating. Next the capillary is flushed with a solution of POE stearate. The hydrophobic stearate anchors into the hydrophobic interior of the bilayer. The hydrophilic POE block extends into solution above the bilayer yielding a strongly suppressed EOF [24]. Formation of the DODAB/POE stearate coating in this sequential manner (solid squares, Fig. 2) results in a constant $-0.3 \times 10^{-4}$ cm$^2$/Vs (i.e., suppressed) EOF for >0.01% POE 40 stearate, independent of the concentration of POE 40 stearate.

The magnitude of the EOF from a DODAB/POE stearate coating formed in this sequential manner can be varied by changing the

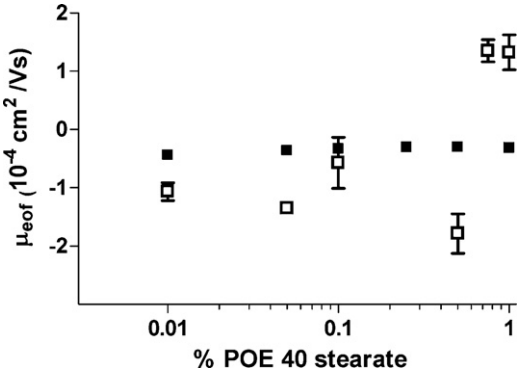


**Fig. 2.** EOF stability for mixed and sequential coating methods using POE 40 stearate/0.1 mM DODAB. Experimental conditions: applied voltage: −15 kV for sequential coating (■) and ±15 kV for the mixed coating (□); 47 cm × 50 μm I.D. capillary (40 cm to detector); 50 mM sodium phosphate buffer, pH 3.0; λ, 214 nm; 2 mM benzyl alcohol injected for 3 s at 0.5 psi; temperature, 25 °C. Error bars represent the standard deviation.

**Table 1**
EOF mobility, rate constant and correlation coefficient for sequential DODAB then POE stearate coatings.[a]

| Stearate polymer | Polymer concentration % w/v ($\times 10^{-4}$ M) | $\mu_{eof,DODAB}$ ($10^{-4}$ cm$^2$/Vs) | $\mu_{eof,DODAB+POE}$ ($10^{-4}$ cm$^2$/Vs)[b] | $k$ ($10^{-3}$ s$^{-1}$)[c] | $r^2$ |
|---|---|---|---|---|---|
| POE 8 | 0.003 (0.5) | −4.0 | −2.40 (±0.01) | 3.1 (±0.2) | 0.980 |
| POE 8 | 0.01 (1.6) | −4.4 | −2.37 (±0.01) | 16.5 (±2.4) | 0.938 |
| POE 40 | 0.0004 (0.02) | −4.4 | −0.50 (±0.01) | 1.6 (±0.1) | 0.992 |
| POE 40 | 0.002 (0.1) | −4.1 | −0.47 (±0.004) | 12.0 (±0.8) | 0.981 |
| POE 40 | 0.006 (0.3) | −3.7 | −0.37 (±0.002) | 46.9 (±1.7) | 0.998 |
| POE 40 | 0.01 (0.5) | −4.0 | −0.44 (±0.01) | 116.2 (±3.8) | 0.999 |
| POE 100 | 0.0005 (0.01) | −3.8 | −0.24 (±0.03) | 0.9 (±0.0) | 0.999 |
| POE 100 | 0.0009 (0.02) | −3.6 | −0.17 (±0.005) | 7.7 (±0.6) | 0.988 |

[a] All capillaries are first coated with 0.1 mM DODAB then POE stearate; 50 mM sodium phosphate buffer, pH 3.0, 40/47 cm capillary.
[b] Standard deviation in brackets.
[c] Standard error (i.e., standard deviation divided by square root of the number of replicates) in brackets.

length of the POE block. Increasing the POE chain length from 8 to 40 units results in a weaker anodic EOF (Table 1). This decrease in EOF is consistent with the observation that as the maximum end-to-end distance of a linear polymer chain increases, the EOF within the capillary decreases significantly [39]. Similarly, EOF in the presence of a long chain polydimethylacrylamide (PDMA) coating was 10-fold more suppressed compared to a short chain PDMA coating [40].

### 3.2. Temporal coating studies

Drummond et al. studied the interaction of a trimeric surfactant and POE 100 stearate with mica surfaces [41]. The exchange of the polymer molecules with the cationic surfactant molecules was observed to be a slow process. Therefore, the kinetics of formation of a sequential DODAB then POE stearate coating was monitored in a similar fashion to that used in previous studies of the desorption of didodecyldimethylammonium bromide (DDAB) [42] and adsorption of phospholipids [22].

A new 47 cm capillary was coated with 0.1 mM DODAB for 10 min. POE stearate solution was flowed through the DODAB coated capillary for 0.5 min, followed by a 0.5 min 50 mM sodium phosphate, pH 3 buffer rinse to remove any excess coating material. Benzyl alcohol was injected and the EOF was determined. Subsequent runs were carried out in which the capillary was rinsed for increasingly longer periods of time with polymer followed by 0.5 min with buffer.

Fig. 3(a)–(c) shows the results of the coat time studies for different concentrations of POE 8, POE 40 and POE 100 stearate sequentially coated on 0.1 mM DODAB coated capillaries. Prior to flowing POE stearate through the capillary (time = 0), the capillary possesses a DODAB bilayer coating, with a strongly anodic EOF ($\mu_{eof,DODAB}$). Upon rinsing the DODAB coated capillary with POE stearate, the EOF gradually becomes more attenuated until it reaches the suppressed EOF characteristic of a fully formed DODAB/POE stearate coating ($\mu_{eof,DODAB+POE\ stearate}$). The curves in Fig. 3 were fit to the first order kinetic expression:

$$\mu_{eof} = \mu_{eof,DODAB} + ((\mu_{eof,DODAB+POE\,stearate} - \mu_{eof,DODAB}) \times (1 - e^{-kt})) \quad (2)$$

using Prism (version 4.00, GraphPad Software Inc., San Diego, CA, USA) where $\mu_{eof}$ is the observed EOF and $k$ is the rate constant for the formation of the POE stearate coating. As shown in Table 1, in all but one case Eq. (2) fit the data with correlation coefficients ($r^2$) greater than 0.98. In Fig. 3, the EOF stabilizes (i.e., formation of the POE layer occurs) more quickly with higher polymer concentration (i.e., larger $k$ in Table 1). The trend is similar to that observed for formation of phospholipid bilayer coatings [22].

Table 1 also shows that for a given concentration, longer POE polymers form a coating more quickly than their shorter chain counterparts. For example at $0.5 \times 10^{-4}$ M POE stearate, the rate constant is almost two orders of magnitude greater for POE 40 stearate than POE 8 stearate. This is reflected in Fig. 3b by the early stabilization of the EOF compared to Fig. 3a. When comparing $0.02 \times 10^{-4}$ M POE 40 and POE 100 stearate, the coating is formed three times faster for POE 100 stearate. All further studies were conducted using POE stearate concentrations and rinse times that yielded a saturated surface.

### 3.3. Adjustable EOF with DODAB/POE stearate coatings

The ability to control the magnitude of the EOF is important in optimizing separations [21,27,29–31,33–35,43]. The EOF of the DODAB/POE stearate coating ($\mu_{eof,DODAB+POE\ stearate}$) is suppressed as the length of the POE block increases (Table 1). However, a dis-

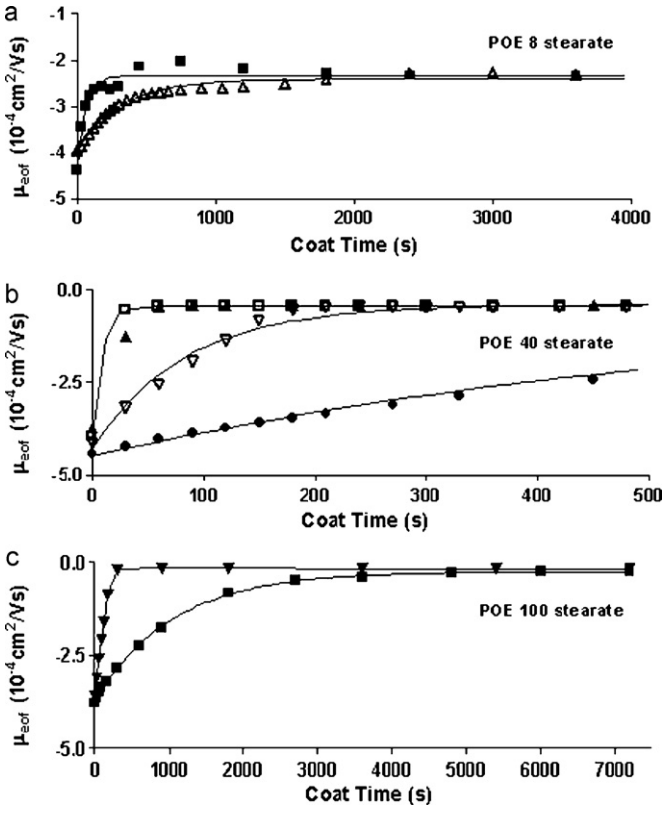


**Fig. 3.** Effect of POE stearate length and concentration on the intercalation rate into the DODAB bilayer (a) POE 8 stearate: 0.01% ($1.6 \times 10^{-4}$ M) (■) and 0.003% ($0.5 \times 10^{-4}$ M) (△) (b) POE 40 stearate: 0.01% ($0.5 \times 10^{-4}$ M) (□), 0.006% ($0.3 \times 10^{-4}$ M) (▲), 0.002% ($0.1 \times 10^{-4}$ M) (▽), and 0.0004% ($0.02 \times 10^{-4}$ M) (●) (c) POE 100 stearate: 0.0009% ($0.02 \times 10^{-4}$ M) (▼) and 0.0005% ($0.01 \times 10^{-4}$ M) (■) POE 100 stearate. Experimental conditions: DODAB concentration, 0.1 mM; applied voltage, −15 or −17.5 kV; 47 cm × 50 μm I.D. capillary (40 cm to detector); 50 mM sodium phosphate buffer, pH 3.0; λ, 214 nm; 2 mM benzyl alcohol injected for 3 s at 0.5 psi; temperature, 25 °C; data is fit to Eq. (2).

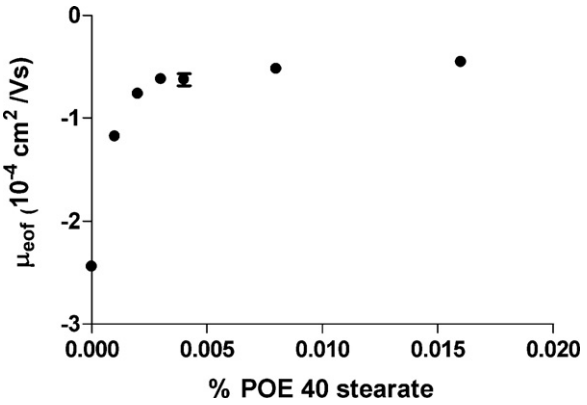


**Fig. 4.** EOF *vs.* POE 40 stearate concentration on a capillary first coated with 0.1 mM DODAB followed by coating with a mixture of 0.01% POE 8 stearate and POE 40 stearate. Experimental conditions: applied voltage, −15 kV; 47 cm × 50 μm I.D. capillary (40 cm to detector); 50 mM sodium phosphate buffer, pH 3.0; λ, 214 nm; 2 mM benzyl alcohol injected for 3 s at 0.5 psi; temperature, 25 °C. Error bars represent the standard deviation.

crete EOF is observed for each length of POE stearate. In theory very fine control of the EOF could be achieved using a series of POE stearate differing by one POE monomer unit. However, POE stearate is polydisperse. For example, the POE block of commercial POE 50 stearate ranges in size from 39 to 57 oxyethylene units [44]. Therefore, it is not feasible to vary the POE block size by one POE moiety.

Previous studies [33–35,43] have achieved EOF control by mixing two discrete additives, each of which has an intrinsic and different EOF, e.g., anionic/cationic surfactant mixtures [34,35,43] and zwitterionic/cationic surfactant mixtures [33]. In a similar manner the EOF can be fine-tuned using mixtures of two POE stearate polymers (Fig. 4). The capillary was first coated with DODAB and then rinsed with a mixture of POE 8 and POE 40 stearate. The POE 8 stearate concentration was kept constant at 0.01% (w/v) and the POE 40 stearate concentration was varied.

A similar study was carried out using POE 40 and POE 100 stearate. However since both additives individually yield a strongly suppressed EOF, the EOF could only be varied from $-0.36 \times 10^{-4}$–$cm^2$/Vs to $-0.25$–×–$10^{-4}$–$cm^2$/Vs over the POE 100 stearate range of 0.00002–0.1% (w/v) (POE 40 stearate constant at 0.001%, w/v). The narrowness of this range precluded further study.

### 3.4. Protein separations

#### 3.4.1. Separation of basic proteins

The performance of coatings prepared using the sequential method was compared with similar coatings formed using the mixed method. The coating stability was monitored by successive injection of a sample of four basic proteins with no recoating performed between successive runs.

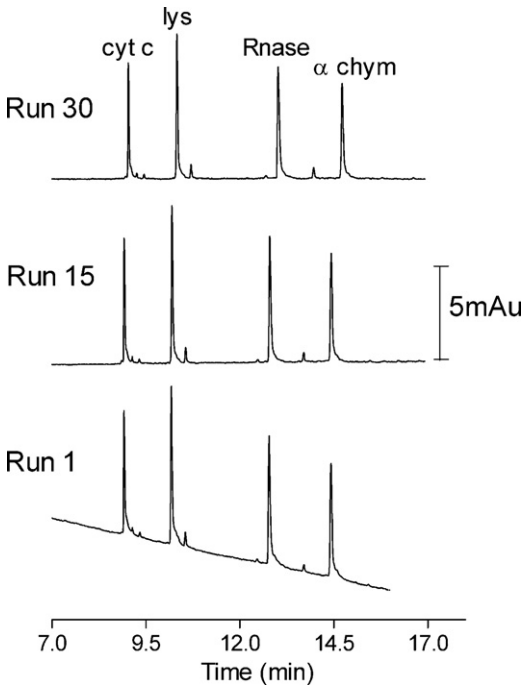


**Fig. 5.** Representative separations of basic proteins on a sequential 0.1 mM DODAB then 0.075% POE 40 stearate coating with no recoating between 30 runs. Experimental conditions: applied voltage, +17.5 kV; 47 cm × 50 μm I.D. capillary (40 cm to detector); 50 mM sodium phosphate buffer, pH 3.0; λ, 214 nm; 0.1 mg/mL protein sample injected for 3 s at 0.5 psi; temperature, 25 °C.

Fig. 5 shows the separation of four basic proteins on a sequentially coated capillary. 0.1 mM DODAB was flowed through the capillary followed by 0.075% (w/v) POE 40 stearate (Sections 2.3 and 2.5). The average efficiencies of the sequential method (Table 2) were 30% higher than for a comparable mixed coating. Also, the sequential coating method resulted in greater coating stability than that of a comparable coating formed using the mixed method. Using the mixed method, a detectable drift in migration times was observed over 14 replicate runs without recoating, leading to migration time RSDs of 2.4–4.6%. For the sequential coating, migration time RSDs of 0.7–1.0% was observed over 30 replicate runs.

After performing 30 runs on the sequentially coated capillary, the coating was removed by flushing the capillary with methanol for 10 min at 20 psi. The sequential coating was regenerated using the procedure in Section 2.3. The regenerated sequential coating yielded migration times, peak areas and efficiencies that were the same as those obtained using capillaries coated for the first time.

The separation of four standard basic proteins was carried out on coatings using a range of POE 40 stearate concentrations (0.01–1%, w/v) prepared using the sequential coating method (Table 2).

**Table 2**
Efficiency ranges and EOF values for basic proteins separated on sequential 0.1 mM DODAB then POE 40 stearate coatings.

| Coating[a] | $\mu_{eof,DODAB+POE}$ (×$10^{-4}$ $cm^2$/Vs) | N, plates/m (×$10^3$) (Foley–Dorsey) | | | |
|---|---|---|---|---|---|
| | | cyt *c* | lys | RNase A | α-chym A |
| 0.01% POE 40 stearate | −0.44 ± 0.01 | 310 | 1200 | 580 | 680 |
| 0.05% POE 40 stearate | −0.36 ± 0.006 | 360 | 1180 | 680 | 870 |
| 0.075% POE 40 stearate | | 800[b] | 760[b] | 820[b] | 940[b] |
| 0.1% POE 40 stearate | −0.33 ± 0.009 | 530[b] | 1100 | 690 | 1040 |
| 0.25% POE 40 stearate | −0.31 ± 0.004 | 430[b] | 1020 | 720 | 910 |
| 0.5% POE 40 stearate | −0.30 ± 0.003 | 420[b] | 900 | 750 | 870 |
| 1% POE 40 stearate | −0.31 ± 0.005 | 320[b] | 1040 | 680 | 850 |

[a] All capillaries are first coated with 0.1 mM DODAB then POE 40 stearate; 50 mM sodium phosphate buffer, pH 3.0, 40/47 cm capillary, 0.1 mg/mL proteins.
[b] Calculated using width-at-half-height method.

The efficiencies achieved are essentially independent of the POE stearate concentration. The efficiencies are consistently higher than those obtained from separations on a coating formed using the mixed method. These results indicate that better separations of basic proteins are obtained on the sequentially coated capillaries.

### 3.4.2. Histone separations

Histones are the major structural proteins of chromatin, which packs DNA into higher order structures to accommodate the full genome [45,46]. There are several different classes of histones, based on their lysine and arginine content. Within these classes there are several variants or subtypes, comprised of different amino acid sequences [47]. Histone type III-S from calf thymus is studied in this work. This histone is lysine rich and mainly H1 in character [48]. CE can be a useful method to separate individual histones if an effective coating is used to prevent their adsorption onto the capillary wall [45,49,50]. Aguilar et al. separated histone type III-S from calf thymus on a hydroxypropylmethylcellulose (HPMC) coated capillary and observed one broad peak [45]. Thus resolution of the histone subtypes is functional measure of the effectiveness of a CE coating.

Recently, histone type III-S from calf thymus was separated into three H1 subtypes at pH 4.0 using a zwitterionic phospholipid coating [22]. Fig. 6 shows resolution of nine possible histone subtypes using a sequential 0.1 mM DODAB then 0.075% POE 40 stearate coating (Sections 2.3 and 2.5). As noted previously, when the EOF is suppressed the protein mobilities are able to come to the forefront, enabling greater resolution. As the EOF on this coating was both suppressed and reversed, six more peaks were visible than on the phospholipid coating, which had a suppressed normal EOF.

The first run for all sets of histone separations had low peak areas. Similarly with the phospholipid coating poor resolution and low peak areas were observed on the first run of histones [22]. Subsequent runs on the sequential 0.1 mM DODAB then 0.075% POE 40 stearate coating resulted in efficiencies as high as 1.2 million plates/m. To determine whether this improvement in peak areas for the subsequent runs is due to voltage application or protein injection, voltage was applied as a pretreatment step before injection of the analytes. Peaks areas for the separated proteins in the first run after 100 min voltage application were equivalent to those without voltage application. Possibly, the first injection of the proteins blocks the few remaining active sites enabling subsequent runs to be performed with high recovery. The third, fifth and ninth runs are shown in Fig. 6 as they are representative of all of the separations. The migration time RSDs were ≤0.5% ($n$ = 9), which are comparable to those obtained on the phospholipid coating.

Comparable results were obtained for the histone separation using the mixed coating (Fig. S1). Similar to the sequential method, low peak areas were observed for proteins in the first run. Subsequent runs on the mixed 0.1 mM DODAB/0.075% POE 40 stearate coating resulted in efficiencies of 1.1 million plates/m. The migration time RSDs were ≤0.2% ($n$ = 5). While the performance of the two methods for the histones are comparable, the sequential method provides more predictable and stable EOF, and thus is more easily optimized.

### 3.4.3. Separation of acidic proteins

Fig. 7 shows the separation of two acidic proteins on a sequentially coated capillary. DODAB (0.1 mM) was flowed through the capillary followed by 0.01% POE 8 stearate at pH 6.4 ($\mu_{eof} = -1.0 \times 10^{-4}$ cm$^2$/Vs). Calcium was added to the run buffer to reduce the electrostatic interaction of the acidic proteins with any exposed DODAB [26]. Trypsin and α-lactalbumin were separated with an efficiency of 350 000 plates/m for α-lactalbumin.

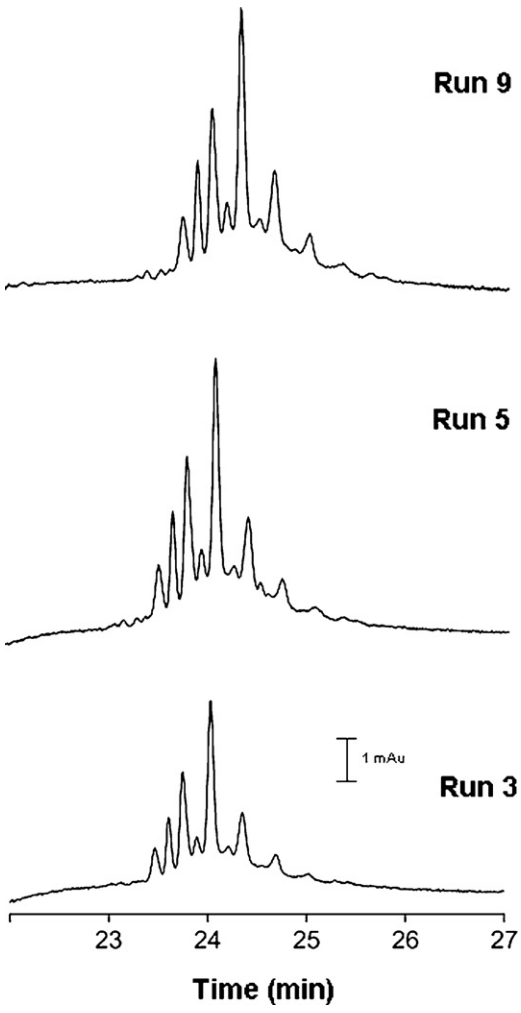


**Fig. 6.** Histone type III-S separation on a 0.1 mM DODAB then 0.075% POE 40 stearate sequentially coated capillary. Experimental conditions: applied voltage, +15 kV; 67 cm × 50 μm I.D. capillary (60 cm to detector); 75 mM Tris formate buffer, pH 4.0; λ, 200 nm; 0.25 mg/mL histone type III-S injected for 4 s at 0.5 psi; temperature, 25 °C.

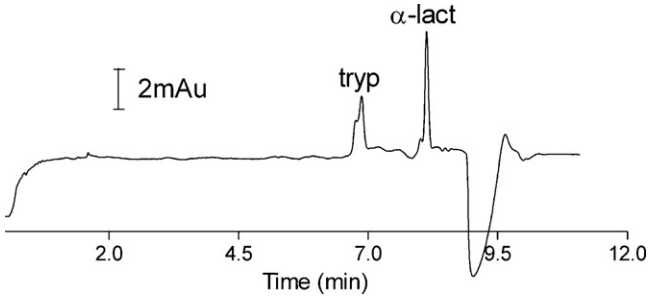


**Fig. 7.** Separation of two acidic proteins on a 0.1 mM DODAB/0.01% POE 8 stearate coated capillary. Experimental conditions: applied voltage, −10 kV; 27 cm × 50 μm I.D. capillary (20 cm to detector); 75 mM Tris–acetate with 20 mM $CaCl_2$ buffer, pH 6.4; λ, 214 nm; 0.2 mg/mL trypsin and 0.05 mg/mL α-lactalbumin injected for 4 s at 0.5 psi; temperature, 25 °C.

## 4. Concluding remarks

A coating prepared from DODAB and POE stearate was demonstrated to be tunable by adjusting the polymer chain length. A sequential rather than mixed method for coating preparation produces a more stable coating that can separate basic proteins with higher average efficiency. The EOF in the presence of this coat-

ing was tunable by varying polymer chain length, and by mixing polymer chains of different lengths. The ability of tuning EOF is important in maximizing the resolution of analytes with similar electrophoretic mobilities. Histone proteins can be separated with high efficiency into nine subtypes on coatings formed using the sequential method.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2010.10.111.

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