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Semi-permanent surfactant coatings for inorganic anion analysis in capillary electrophoresis

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Abstract

Capillary electrophoretic separations of inorganic anions are performed using a capillary coated with a mixture of the cationic surfactant didodecyldimethylammonium bromide (DDAB) and the zwitterionic surfactant 1,2-dilauroyl-*sn*-phosphatidylcholine (DLPC). These double-chained surfactants form semi-permanent coatings on the capillary wall, which allows the excess surfactant to be removed from the buffer prior to separation. Interactions between surfactant aggregates in the buffer and analyte anions are thus eliminated. The electroosmotic flow (EOF) can be altered from fully reversed (100% DDAB) to near zero (100% DLPC) using different ratios of DDAB and DLPC. Controlling the EOF allows for improved resolution of the anions while maintaining a rapid, co-EOF separation, free from analyte–surfactant additive interactions.

Keywords: Coated capillaries; Inorganic anions; Surfactants

1. Introduction

Compared to traditional ion chromatography (IC), capillary electrophoresis (CE) offers the advantages of rapid analysis time and high efficiency for the separation of inorganic anions [1]. A CE separation is based on an ion's electrophoretic mobility, which is a function of the charge-to-mass ratio of the ion. As such, the separation mechanism of CE is different from that of anion-exchange chromatography. This makes anion separations by CE complementary to that of IC [2].

Also involved in an electrophoretic separation is the electroosmotic flow (EOF). In a bare silica capillary, anions will naturally migrate towards the anode while the EOF migrates in the opposite direction, toward the cathode. This results in counter-EOF migration, which increases the separation time. As an alternative, the EOF can be reversed so that anions co-migrate with the EOF and thus the separation time is reduced. However, sometimes a reversed EOF moves the anions too quickly to the detector for baseline resolution to be achieved. In this case tuning the EOF is necessary to obtain an optimized anion separation.

Commonly, dynamic capillary coatings are used in CE to modify the EOF [3]. In dynamic coatings, a buffer additive equilibrates with the capillary surface and alters the effective surface charge thus altering the magnitude of the EOF. The types of additives that have been employed for EOF modification include: divalent metal cations [4]; alkylammonium ions and alkylamines [5–8]; cationic polymers [9,10], and surfactants [1,11–14].

Surfactant additives, such as the single-chained tetradecyltrimethylammonium bromide (TTAB), are

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often the method of choice for EOF modification since they are inexpensive and simple to use. One drawback is that they must be present in the electrophoretic buffer at relatively high concentrations (above the critical micelle concentration, CMC) for the coating to stay intact and generate a reproducible EOF [13]. This can lead to interactions such as ion-pairing between analyte anions and buffer micelles [15] that may deteriorate the separation and/or interfere with the detection scheme. This paper presents a simple method of tuning the EOF using surfactants that form semi-permanent coatings at the capillary surface. The semi-permanent nature of the coatings allows excess surfactant to be removed from the buffer prior to separation, thereby eliminating these unwanted interactions.

2. Experimental

2.1. Apparatus

A Beckman P/ACE 5000 system (Fullerton, CA, USA) with a UV absorbance detector was used for all experiments. Untreated silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) of 47 cm (40 cm to detector)×50 μm I.D.×365 μm O.D. were used. Capillaries were always thermostated to 25 °C. Data acquisition (10 Hz) and control was performed using P/ACE Station Software for Windows 95 (Beckman) on a Pentium 120 MHz microcomputer.

2.2. Reagents

All solutions were prepared in Nanopure 18 MΩ ultrapure water (Barnstead, Chicago, IL, USA). Buffers were prepared from either Ultra Pure Tris (Schwartz/Mann Biotech, Cleveland, OH, USA) adjusted to the desired pH with hydrochloric acid (Anachemia, Montreal, Canada) or potassium chromate (BDH, Darmstadt, Germany) adjusted to the desired pH with phosphoric acid (BDH). The surfactants didodecyldimethylammonium bromide (DDAB; Aldrich, Milwaukee, WI, USA) and 1,2-dilauroyl-*sn*-phosphatidylcholine (DLPC; Sigma, St. Louis, MO, USA) were used as received. Calcium chloride dihydrate (molecular biology grade, Sigma) was used as received. Mesityl oxide (Aldrich) was used as a neutral marker for the EOF measurements. Anion solutions were prepared from sodium nitrite (BDH), potassium nitrate (BDH), potassium bromide (Fisher Scientific, Fair Lawn, NJ, USA), sodium fluoride (Fisher Scientific), sodium chloride (BDH), sodium sulfate (Fisher Scientific), potassium oxalate (Matheson, Norwood, OH, USA), sodium iodide (BDH), potassium thiocyanate (BDH), and potassium perchlorate (Fisher Scientific) without further purification.

2.3. Preparation of capillary coatings

Solutions containing DDAB and DLPC were prepared as follows. DDAB or DLPC was suspended in the appropriate buffer and stirred for 15 min. The solution was then sonicated for 10 min periods. Between each 10-min period there was a 10 min ''rest'' interval where the solution was cooled and stirred at room temperature. This sonicate/stir cycle was repeated about three times or until the solution was clear.

Employing 100% DDAB or 100% DLPC as the wall coating, a 0.1 m*M* solution of surfactant in 20 m*M* Tris–HCl and 20 m*M* $CaCl_2$, pH 7.4 buffer was rinsed through the capillary at high pressure (20 p.s.i.) for 5 min or 15 min, respectively (1 p.s.i.= 6894.76 Pa). Excess surfactant and $CaCl_2$ was removed by rinsing (20 p.s.i.) for 2 min with 20 m*M* Tris–HCl, pH 7.4 buffer. $CaCl_2$ was added to promote coating formation [26].

DLPC–DDAB mixtures were used as wall coatings in an identical fashion (15 min rinse time, 20 p.s.i.). The composition of the surfactant mixtures is expressed as % DLPC and % DDAB where the percentage refers to the mole fraction of the surfactant in the mixture.

2.4. EOF measurements

New capillaries were pretreated by rinsing at high pressure (20 p.s.i.) with 0.1 *M* NaOH for 10 min and water for 5 min. Capillaries were initially coated with surfactant and the excess surfactant was rinsed out with separation buffer (see Section 2.3). Prior to each run, the capillary was rinsed with the desired surfactant (20 p.s.i., 3 min) and the excess surfactant rinsed out with separation buffer (20 p.s.i., 2 min).

Two methods were used to calculate the electroosmotic mobility (μ_{EOF}). When μ_{EOF} was above 1.0×10^{-4} cm^2 V^{-1} s^{-1}, a single injection method was used where mesityl oxide was directly injected onto the capillary (1.0 s, 0.5 p.s.i.) and a constant voltage of −15 kV was applied. Detection was at 254 nm. The value of μ_{EOF} was calculated as described previously [16]. When μ_{EOF} was smaller than 1.0×10^{-4} cm^2 V^{-1} s^{-1}, the electroosmotic flow was measured using the three-injection method introduced by Williams and Vigh [17]. The first mesityl oxide marker was injected using low pressure (0.5 p.s.i.) for 1.0 s. This band was pushed through the capillary for 2 min using low pressure. A second mesityl oxide marker was then introduced by an identical low-pressure injection and the two bands were pushed with low pressure for 2 min. A constant voltage of +15 kV was applied for 3 min causing the two markers to move within the capillary. A third marker was then injected and all the bands were eluted by applying low pressure for 12 min. Detection was at 254 nm. The value of μ_{EOF} measured using this method was calculated as described previously [16,17].

2.5. Anion separations

New capillaries were pretreated by rinsing at high pressure (20 p.s.i.) with 0.1 *M* NaOH for 10 min and water for 5 min. Capillaries were initially coated with surfactant as described in Section 2.3. Prior to each run, the capillary was rinsed with the appropriate surfactant (20 p.s.i., 3 min) and then the excess surfactant was rinsed out with separation buffer (20 p.s.i., 2 min).

For direct detection of inorganic anions, the capillary was coated with the desired surfactant. Excess surfactant was rinsed from the capillary (20 p.s.i., 2 min) with 20 m*M* Tris–HCl, pH 7.4 buffer. A 0.5 m*M* mixture of KNO_3, $NaNO_2$, NaI, KBr, and KSCN in water was injected at low pressure (0.5 p.s.i.) for 3.0 s. The applied potential was −20 kV and the detector rise time was 0.1 s. The anions were detected using direct UV absorbance detection at 214 nm.

For the indirect detection of nine inorganic anions, the capillary was coated using a 0.1 m*M* DLPC–DDAB (95:5) solution. Excess surfactant was rinsed from the capillary with the separation buffer of 5 m*M* potassium chromate adjusted to pH 8 with phosphoric acid (20 p.s.i., 2 min). A mixture of 0.5 m*M* $NaNO_2$, KNO_3, KBr, NaI, KSCN, NaF, NaCl, Na_2SO_4, and $K_2C_2O_4$ in water was injected using low pressure (0.5 p.s.i.) for 3.0 s. The applied potential was −20 kV and the detector rise time was set to 0.1 s. The anions were detected using indirect UV absorbance detection at 254 nm. A 0.5 m*M* solution of $KClO_4$ in water was also injected and detected in the same manner as the anion mixture. All anion separations were performed in triplicate.

3. Results and discussion

3.1. Use of DDAB for anion analysis

The standard method for anion analysis in capillary electrophoresis involves adding the single-chained surfactant TTAB to the buffer to reverse the EOF [1]. With single-chained surfactants such as TTAB, it is necessary that the surfactant be present in the buffer at a concentration above the CMC to achieve a stable reversed EOF [13]. Invariably, the micelles in solution will interact with the analyte anions [15]. This can be detrimental to the separation of mixtures containing strongly interacting anions. The double-chained surfactant DDAB also reverses the EOF with the added advantage of forming a stable coating on the capillary wall. DDAB has a more cylindrical molecular geometry due its two hydrophobic chains. As a consequence, DDAB monomers aggregate to form a flat bilayer structure at the capillary wall. This has been confirmed through atomic force microscopy (AFM) imaging [18]. The more homogeneous coating and greater surface coverage provided by DDAB is thought to account for its increased stability [19]. Consequently, the excess surfactant can be removed from the buffer prior to electrophoretic separations [19].

DDAB was previously implemented as a wall coating for the ultra-rapid separation of nitrate and nitrite at low pH [20]. However it has never been used for separation of more complex anion mixtures. Herein, DDAB is employed to reverse the EOF for the separation of five inorganic anions. A 0.1 m*M*

solution of DDAB in 20 m*M* Tris–HCl, pH 7.4 buffer is used to coat the capillary wall. The excess DDAB is removed by rinsing the capillary with Tris–HCl, pH 7.4 buffer. The separation of five UV-absorbing anions: bromide, nitrite, nitrate, iodide, and thiocyanate is shown in Fig. 1. DDAB strongly reversed the EOF to a value of -7×10^{-4} cm^2 V^{-1} s^{-1}. The rapid EOF does not allow sufficient time for a separation to develop between bromide and nitrite. Further, iodide and thiocyanate strongly interact with the wall coating, leading to poor peak shapes and a long separation time (>7 min). The same behavior has been observed previously in open-tubular ion-exchange capillary electrochromatography (CEC) of inorganic anions [21]. Breadmore et al. found that polarizable anions such as iodide, thiocyanate and thiosulfate strongly interacted with a quaternary aminated stationary phase [21].

Although the excess surfactant can be removed from the separation buffer, this system is not ideal for anion analysis since the EOF produced is too fast to obtain baseline resolution of all anions and the interaction between polarizable anions and the DDAB wall coating is detrimental to peak shape.

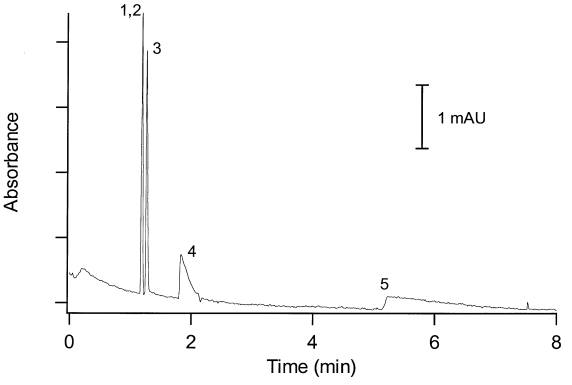

Fig. 1. Separation of five inorganic anions using a DDAB-coated capillary. Peaks: (1,2) bromide + nitrite, (3) nitrate, (4) iodide, (5) thiocyanate. Experimental conditions: 47 cm (40 cm to detector) capillary; temperature, 25 °C; separation buffer, 20 m*M* Tris–HCl at pH 7.4; sample, 0.5 m*M* anion mixture in water; −20 kV applied voltage; direct UV detection at 214 nm. Coating procedure: 5 min rinse with 0.1 m*M* DDAB in buffer followed by a 2 min rinse with separation buffer to remove excess surfactant; between runs the DDAB rinse time was shortened to 3 min and excess surfactant was rinsed out with buffer for 2 min.

3.2. EOF control using a mixed surfactant system

The ability to tune the magnitude of the reversed EOF is a necessary tool for the optimization of anion separations. Previously it has been shown that the reversed EOF can be fine-tuned using mixtures of the zwitterionic surfactant Rewoteric AM CAS U and the cationic surfactant TTAB [14]. The zwitterionic surfactant has a net charge of zero, and effectively dilutes the positive charge at the capillary wall when mixed with TTAB. When the two surfactants are mixed, the magnitude of the EOF can be modified from near zero to fully reversed. This ability to control the reversed EOF was used to improve the separation of inorganic anions [14]. The use of single-chained surfactants for EOF control demands that the surfactants be present in the buffer to maintain the wall coating. Again, this approach can lead to unwanted interactions between analytes and buffer micelles, thus degrading the separation.

Herein the mixed surfactant approach is investigated using double-chained surfactants that are known to form semi-permanent capillary coatings. As discussed above, DDAB forms a stable capillary coating [19] and is used as the cationic surfactant in the mixture. Phosphatidylcholines are zwitterionic, double-chained surfactants that are commonly employed to mimic biological membranes [22–25]. Recently we have shown in our laboratory that phosphatidylcholines are also useful in CE since they form stable, semi-permanent coatings on the capillary wall [26]. The effect of mixing the zwitterionic surfactant DLPC with DDAB on the magnitude of the reversed EOF is shown in Fig. 2. At pH 7.4, the EOF can be modified from near zero (0.2×10^{-4} cm^2 V^{-1} s^{-1}) to fully reversed (-7×10^{-4} cm^2 V^{-1} s^{-1}) where 100% DLPC produces the near zero EOF and 100% DDAB generates the fully reversed EOF.

Measurement of the EOF can be used as an indirect test of the stability of a capillary coating [10]. The stability of each mixed surfactant coating was tested by first coating the capillary with the desired mixture and then measuring the EOF after rinsing out the excess surfactant with Tris–HCl, pH 7.4 buffer. Good stability was observed for each surfactant mixture for over 10 runs (5 min per run). The EOF never decreased by more than 5% over the 50 min of the stability test. Day-to-day reproducibil-

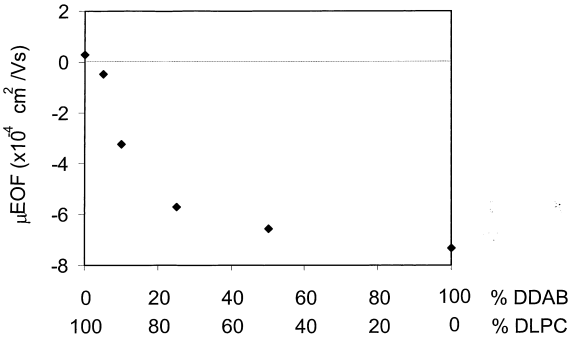

Fig. 2. Effect of DLPC–DDAB mixtures on the EOF. Experimental conditions: 47 cm (40 cm to detector) capillary; temperature, 25 °C; separation buffer, 20 m*M* Tris–HCl at pH 7.4; applied voltage, −15 kV (fast reversed EOF) or +15 kV (slow EOF, three-peak injection method used, see Section 2.4); direct UV detection at 254 nm.

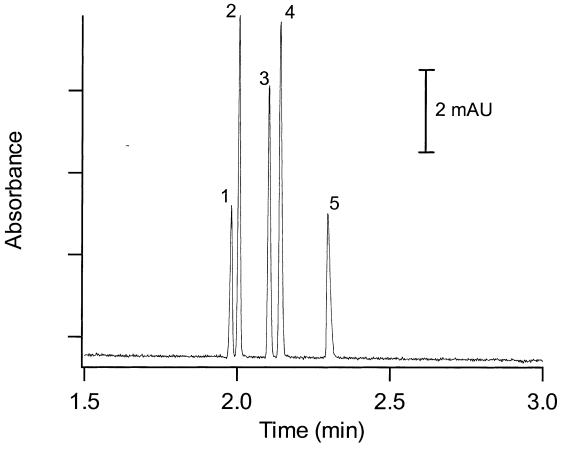

Fig. 3. Separation of five inorganic anions using a capillary coated with a DLPC–DDAB (95:5) surfactant mixture. Peaks: (1) bromide, (2) iodide, (3) nitrite, (4) nitrate, (5) thiocyanate. Experimental conditions: 47 cm (40 cm to detector) capillary; temperature, 25 °C; separation buffer, 20 m*M* Tris–HCl at pH 7.4; sample, 0.5 m*M* anion mixture in water; −20 kV applied voltage; direct UV detection at 214 nm. Coating procedure: 15 min rinse with a 0.1 m*M* DLPC–DDAB (95:5) mixture in buffer followed by a 2 min rinse with separation buffer to remove excess surfactant; between runs surfactant rinse time was shortened to 3 min and excess surfactant was rinsed out with buffer for 2 min.

ity of all EOF measurements was less than 5% RSD. However solutions were kept for no longer than 5 days.

3.3. Use of a mixed DLPC–DDAB capillary coating for anion analysis

The separation of the same five UV-absorbing anions shown in Fig. 1 was performed using a mixture of DLPC and DDAB. Fig. 3 shows the separation of the anions: bromide, nitrite, nitrate, iodide, and thiocyanate using a 0.1 m*M* DLPC–DDAB (95:5) mixture as a wall coating. The five anions are baseline resolved in a separation time of under 2.5 min. This mixed surfactant coating produced a relatively low, reversed EOF (-0.5×10^{-4} $cm^2\ V^{-1}\ s^{-1}$), ideal for obtaining baseline resolution between bromide and iodide and nitrite and nitrate while maintaining a rapid co-EOF separation. Further, the peak shapes of the polarizable anions (iodide and thiocyanate) are Gaussian, a huge improvement over the peak shapes observed in Fig. 1. The phosphatidylcholine headgroup does not show unfavorable interactions with the anions.

Since only few anions absorb UV light, indirect detection is necessary for anion analysis. To determine the compatibility of the DLPC–DDAB coating with a standard indirect UV probe, an anion separation was performed using 5 m*M* chromate adjusted to pH 8.0 as the electrophoretic buffer. A bare capillary was coated with a 0.1 m*M* DLPC–DDAB (95:5) mixture and the excess surfactant was rinsed out with chromate buffer. Fig. 4 shows the separation of nine anions using indirect UV detection at 254 nm. The separation was accomplished in less than 2.8 min. Baseline resolution is achieved between all anions with the exception of iodide and chloride, which are nearly baseline resolved. As before the DLPC–DDAB coating did not degrade peak shape. Note that for maximum reproducibility, the capillary was re-coated with surfactant between runs for 3 min. The excess surfactant was always removed by a 2 min rinse with separation buffer.

A plot of ionic equivalent conductance (conductance at zero ionic strength) as a function of migration time of the singly charged anions separated in Fig. 4 is shown in Fig. 5. There is an excellent reciprocal relationship between these two variables for all singly charged anions. Jones and Jandik previously showed that this relationship existed for most anions [1]. However the highly polarizable anions iodide, thiocyanate, and perchlorate strongly deviated from the plot in Jones and Jandik's work with TTAB.

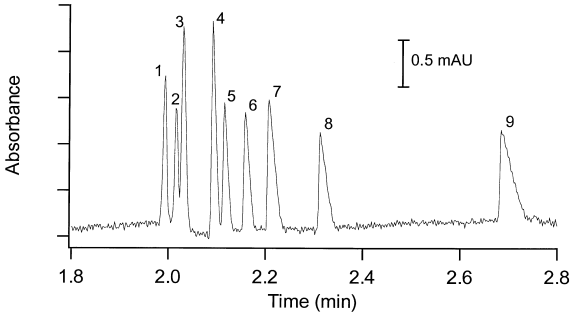

Fig. 4. Separation of nine inorganic anions using a capillary coated with a DLPC–DDAB (95:5) surfactant mixture. Peaks: (1) bromide, (2) iodide, (3) chloride, (4) sulfate, (5) nitrite, (6) nitrate, (7) oxalate, (8) thiocyanate, (9) fluoride. Experimental conditions: 47 cm (40 cm to detector) capillary; temperature, 25 °C; separation buffer, 5.0 m*M* chromate adjusted to pH 8.0 with phosphoric acid; sample, 0.5 m*M* anion mixture in water; −20 kV applied voltage; indirect UV detection at 254 nm. Coating procedure: 15 min rinse with a 0.1 m*M* DLPC–DDAB (95:5) mixture in buffer followed by a 2 min rinse with separation buffer to remove excess surfactant; between runs surfactant rinse time was shortened to 3 min and excess surfactant was rinsed out with buffer for 2 min.

These anions had a strong interaction with the TTAB micelles in the buffer (perchlorate interacted the most), which led to extremely long migration times (also seen here for iodide and thiocyanate in Fig. 1 using DDAB). Although not present in the anion separation shown in Fig. 4, perchlorate was also injected and indirectly detected using a DLPC–DDAB coated capillary (it partially overlapped with the thiocyanate peak in the mixture). Perchlorate has been added to the plot in Fig. 5 and follows the reciprocal relationship very well. Thus, interactions between the polarizable anions (iodide, thiocyanate, perchlorate) and the DLPC–DDAB wall coating are not present and separations are governed solely by the electrophoretic mobility of the anions.

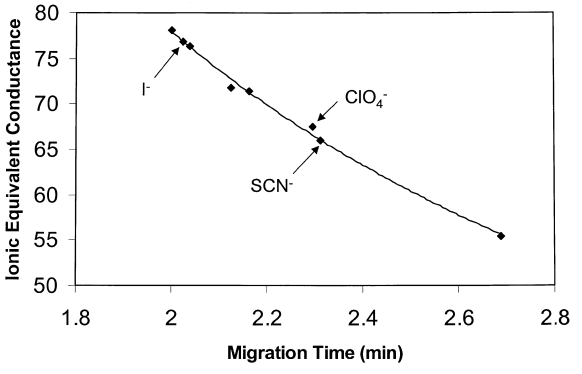

Fig. 5. Migration times of singly charged anions plotted against ionic equivalent conductance. Note that the polarizable anions (iodide, thiocyanate, and perchlorate) follow the reciprocal relationship.

4. Conclusions

Using capillary coatings comprised of mixtures of a double-chained cationic surfactant (DDAB) and a double-chained zwitterionic surfactant (DLPC), the EOF can be controlled from fully reversed to effectively zero. Tuning the EOF in this manner can optimize the separation of inorganic anions by achieving improved resolution. The coating is semi-permanent in nature, and therefore excess surfactant can be flushed from the capillary prior to the electrophoretic separation. Separations can then be performed in a simple buffer system (no surfactant) where interactions between analyte anions and the surfactant additive have been eliminated.

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