Application of dodecyl(dimethyl(2-hydroxy-3-sulfopropyl) ammonium in wall modification for capillary electrophoresis separation of proteins

A zwitterionic surfactant, dodecyl(dimethyl(2-hydroxy-3-sulfopropyl) ammonium (C_{12}H_{25}N(CH_{3})_{2}CH_{2}CHOHCH_{2}SO_{3}^{2-})-named dodecyl sulfobetaine (DSB), was used as a novel modifier to coat dynamically capillary walls for capillary electrophoresis separation of basic proteins. The DSB coating suppressed the electroosmotic flow (EOF) in the pH range of 3–12. At high DSB concentration, the EOF was suppressed by more than 8.8 times. The DSB coating also prevented successfully the adsorption of cationic proteins on the capillary wall. Anions, such as Cl^{−}, Br^{−}, I^{−}, SO_{4}^{2−}, CO_{3}^{2−}, and ClO_{4}^{−}, could be used as running buffer modifiers to adjust the EOF for better separation of analytes. Using this dynamically coated capillary, a mixture of eight inorganic anions achieved complete separation within 4.2 min with the efficiencies from 24 000 to 1 310 000 plates/m. In the presence of ClO_{4}^{−} as EOF adjustor, the separation of a mixture containing four basic proteins (lysozyme, cytochrome c, \beta-chymotrypsinogen A, and myoglobin) yielded efficiencies of 204 000–896 000 plates/m and recoveries of 88%–98%. Migration time reproducibility of these proteins was less than 0.5% relative standard deviation (RSD) from run to run and less than 3.1% RSD from day to day, showing promising application of this novel modifier in protein separation.

Keywords: Basic proteins / Capillary electrophoresis / Dodecyl sulfobetaine / Dynamic coatings
DOI 10.1002/elps.200410273

1 Introduction

Capillary electrophoresis has experienced a rapid development in the last two decades, which can be attributed to the advances in both column technology and instrumentation. The small diameter of capillary allows effective heat dissipation and, as a result, minimizes band-broadening due to the decrease of Joule heating. Thus, a high electric field up to 10 kV/cm can be used, which reduces the analytical time and increases the separation efficiencies to several million plates per meter [1]. In recent years, capillary electrophoresis has become one of the most powerful tools for the analysis of a wide variety of peptides and protein mixtures. According to the theory of Jorgenson and Lukacs [2] separation efficiencies of biomacromolecules, such as protein, peptide, and DNA, may be over one million plates per meter due to low diffusion coefficients of these molecules. However, it is difficult to obtain high separation efficiency because these biomacromolecules possess a highly order structure, which makes them apt to adsorb on the capillary wall by diverse interactions, such as hydrophobic interaction, electrostatic interaction, hydrogen bonding, and van der Waals interaction. The adsorption results in irreproducible separations, loss of efficiency, and low recoveries, and in the extreme the analytes cannot be detected.

Many approaches have been developed to prevent the adsorption of biomacromolecules on the capillary wall [3–10]. Simple methods include the use of high-ionic-strength [3] and extremely low-pH buffer [4]. However, high-ionic-strength buffers bring more Joule heating that limits the applied electric field. Extremely low pH also limits the workable pH range and results in the denaturation of proteins. The permanent coatings are usually stable, and corresponding methods also show good reproducibility [5–10]. The shortcoming of these methods is the difficult preparation of the coatings. Recent reports
reviewed the application of surface modification of the capillary wall [11, 12]. Dynamic coating possesses the advantages of low cost, ease to prepare and regenerate, and thus is a promising method to resolve these problems met during separation of biomacromolecules.

Dynamic coating can be achieved by adding compounds, such as cationic ions [13–17], polymers [18–23], and surfactants [24–32], in the running buffer to modify the capillary wall. Surfactants are becoming increasingly popular for the dynamic coating of capillary walls since they are cheap, flexible, and easy to apply. These surfactants include cationic surfactants [24] and zwitterionic surfactants [27–29]. Lucy and co-workers [27] compared the effects of three dynamic coatings prepared with dodecyldimethyl (3-sulfopropyl) ammonium hydroxide, Coco (amidopropyl)hydroxyldimethylsulfobetaine (CAS U) (RCONH(CH3)2N(CH3)2CH2CHOHCH2SO3Na, R = C9–C12), and hexadecyldimethyl (8-sulfopropyl) ammonium hydroxide on electroosmotic low (EOF). Using CAS U as a buffer additive, lysozyme and az-chymotryptsinogen A were separated completely. N-Tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate has been used to form sulfobetaine-type zwitterionic micelles for separation and direct determination of inorganic anions in human saliva [31]. In order to overcome the problem of Joule heating and peak distortion, this zwittergent has been mixed with nonionic surfactants, polyoxyethylene (20) sorbitan monolaurate (TWEEN 20), to the background electrolyte for the determination of anions in seawater [32]. The effect of zwitterionic sulfobetaine detergents with hydrocarbon tails varying from 8 to 16 carbon atoms long on the separation of hordeins has been tested by free zone capillary electrophoresis [23]. Recently, a semipermanent wall coating was prepared by adding sodium dodecyl sulfate (SDS) to cetyltrimethylammonium bromide (CTAB) to reduce the electrostatic repulsion between adjacent CTAB molecules and enhance the stability of CTAB coating [33]. The bilayer surfactant aggregate at capillary wall showed good efficiency and reproducibility for separation of five inorganic anions and four basic proteins.

In this work, a novel coating was presented by adding dodecyl sulfobetaine (DSB), a zwitterionic surfactant (Fig. 1), in running buffer to coat dynamically the capillary wall. The conveniently obtained coating improved greatly the separation efficiencies of eight anions up to 1310 000 plates/m and four basic proteins up to 896 000 plates/m, and showed good reproducibility of migration times for protein separation.

![Figure 1. Formula of DSB.](image)

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2 Materials and methods

2.1 Apparatus

Experiments were performed on an Agilent capillary electrophoresis system (Agilent Technologies Deutschland, Waldbronn, Germany) equipped with a UV-absorbance detector. Bare silica capillaries with an inner diameter of 50 µm and an outer diameter of 360 µm were purchased from Yongnian Optical Fiber Factory (Hebei, China). The capillaries with different lengths were used for studying the influences of buffer on EOF and for separation of inorganic ions and proteins (stated in the section below). Samples were injected into the capillary with 50 mbar pressure for 3 s and detected at 214 nm. The detector response time was 0.1 s. Date acquisition rate was 5 Hz.

2.2 Chemicals

DSB was a generous gift from the Institute of Jinling Petrochemical Corporation (Nanjing, China), which was synthesized with C12H25N(CH3)2 and CH2ClCHOHCH2SO3Na [34]. Lysozyme (from chicken egg white), cytochrome c (from horse heart), az-chymotryptsinogen A (from bovine pancreas), and myoglobin (from horse heart) were purchased from Sigma (St. Louis, MO, USA) and stored according to the manufacturer’s recommendations. All other reagents, such as KCl, KBr, KI, KClO4, K2SO4, Na2HPO4, NaH2PO4, KNO3, KSCN, (NH4)6Mo7O24·4H2O, KBrO3, Na2WO4, and KIO3 were of analytical-reagent grade and used without further purification. Deionized water of 18 MΩ was purified from a Milli-Q purification system and was filtered through a membrane filter of 0.25 µm pore size before use. Buffers were prepared with Na2HPO4 and NaH2PO4. Their pH values were adjusted with H2PO4 or NaOH. In view of the fact that the effect of 1.5 mM DSB on the pH of running buffer was very small, phosphate buffer was used. Buffer solutions were used to examine the effects of pH ranging from 3 to 12 on EOF in the presence and absence of DSB. A mixture containing 1 mM of KBr, KNO3, KSCN, (NH4)6Mo7O24·4H2O, KBrO3, Na2WO4, and KIO3 and another mixture containing 0.25 mg/mL lysozyme, cytochrome c, az-chymotryptsinogen A, and myoglobin were prepared in deionized water.

2.3 Conditioning of the capillary

To achieve the maximum reproducibility of migration time, the new capillary was conditioned by continuously rinsing with 1.0 M NaOH for 30 min, deionized water for 5 min, 1.0 M HCl for 20 min, and deionized water for 10 min at 1 bar pressure.
2.4 Determination of EOF

The EOF measurements were mainly conducted using a sequential injection method introduced by Williams and Vigh [35]. The total length of the capillary was 70 cm and the effective length of the capillary was 61.5 cm. The first dimethylsulfoxide marker was injected using low pressure (50 mbar) for 3 s. This band was pushed through the capillary for 0.3 min using 1 mbar pressure. The second dimethylsulfoxide marker was then introduced by an identical low pressure injection for 3 s, and both markers were pushed through the capillary for 0.3 min using a pressure of 1.0 bar. 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 three bands were pushed to the detector using 1.0 bar. Detection was performed at 214 nm. Then, EOF was calculated according to [35]. In uncoated capillaries the EOF values were obtained by determining the migration time of the dimethylsulfoxide marker under a constant voltage. Three replicates were taken for each EOF value. The capillary was rinsed with 1.0 M NaOH for 1 min, deionized water for 1 min, and running buffer for 5 min before each new buffer in order to achieve good reproducibility.

3 Results and discussion

3.1 Influence of DSB concentration on EOF

DSB is a zwitterionic surfactant. It can adsorb on the capillary wall surface when flushing the capillary with buffer containing DSB. The adsorption of DSB on the wall reduced the EOF effectively. Figure 2 shows the change of the EOF with the increasing concentration of DSB contained in the running buffer. It was obvious that more DSB molecules were adsorbed on the wall at higher DSB concentration in 10 mM phosphate buffer, pH 7.0, which led to lower EOF. It has been believed that the EOF results from the migration of the cations associated with the silanols. In analogy to the behaviors of cationic surfactants [36] and zwitterionic surfactants C16N3SO3 and CAS U [27, 28] adsorbing onto a fused-silica capillary, the zwitterionic surfactant DSB was postulated to adsorb onto the capillary. The decrease of EOF was attributed to the shielding of the silanol layer from the bulk solution by the adsorbed DSB, leading to the decrease of negative charges on the capillary wall. When the DSB concentration in the buffer was more than 1.86 mM, the critical micellar concentration of DSB in distilled water [34], the EOF tended to a constant value, indicating that the adsorption of DSB on the capillary wall reached a saturated level at high DSB concentration, at which the EOF was suppressed by more than 8.8 times, compared with the bare silica capillary.

![Figure 2](image)

Figure 2. Influence of DSB concentration in 10 mM, pH 7.0, phosphate buffer on EOF.

3.2 Influences of pH on EOF

Figure 3 shows the effect of buffer pH on EOF. With an increasing pH from pH 3.0 to 12.0, the EOF in the bare silica capillary increased and then trended to a constant value at pH > 9.0. When the pH was < 4.0, the EOF showed a slow change. At low pH the change in dissociation degree of silanol groups was little, resulting in a low magnitude and small change of the EOF [29]. When the pH was 4.0, the degree of dissociation increased greatly, leading to an increasing EOF. When the pH was > 9.0, most silanol groups dissociated and the EOF reached a maximum value. Upon addition of 1.5 mM DSB to 10 mM phosphate buffer solution, the EOF decreased greatly in the whole pH range due to the adsorption of DSB on capillary wall surface. With the increasing pH the EOF also increased and then trended to a constant value.

![Figure 3](image)

Figure 3. Effects of pH of 10 mM phosphate buffer (■) with and (●) without 1.5 mM DSB on EOF.
However, the critical pH (about 6.0) and the extent of increase were much lower than that occurring in the absence of DSB. The increasing EOF was due to the low coverage of DSB that could not shield completely the increasing negative charge of capillary wall. The suppression of EOF was more obvious at pH > 6.0 because more negative charges on capillary wall surface were shielded by the adsorbed DSB molecules at higher pH. The influence of pH on EOF was similar to that for CAS U [27], in which the EOF was suppressed to the values lower than 1.4 \times 10^{-4} \text{cm}^2/\text{Vs} in the pH range of 3–12 at the CAS U concentrations more than 1.5 mM. The EOF values in the presence of 1.5 mM DSB were lower than 1.6 \times 10^{-4} \text{cm}^2/\text{Vs}. When pH was < 4.9 a reversed EOF was observed, also in the case of the zwitterionic surfactant CAS U-coated capillary [28].

### 3.3 Influence of different anions on EOF

In view of the interaction of some anions with the quaternary ammonium of zwitterionic surfactants, various concentrations of KCl, KBr, KI, KClO₄, K₂SO₄, and K₂CO₃ were added to 10 mM, pH 7.0, phosphate buffer solution containing 1.8 mM DSB to examine the influence of anions on EOF. As shown in Fig. 4, with increasing anion concentration from 0–100 mM the EOF increased, which was similar to that reported previously with CAS U as a modifier [28]. The increase in EOF was due to the partitioning of these anions into the hemimicelle surfactant layer of adsorbed DSB to produce a negatively charged layer [28].

This phenomenon was undoubtedly beneficial to separation of analytes because sometimes it was necessary both to prevent the adsorption of proteins on the capillary wall and to remain a certain EOF for the fast separation of analytes. The extent of EOF increase depended on the anions. Among these six anions, ClO₄⁻ showed the greatest increase and CO₃²⁻ gave the lowest increase. The EOF values of anions increased according to the following series: CO₃²⁻ < SO₄²⁻ < Cl⁻ < Br⁻ < I⁻ < ClO₄⁻', which was consistent with those reported with CAS U as the modifier [28]. This behavior has been suggested to be due to the interaction of anions with the quaternary ammonium group on the zwitterionic surfactants [37].

The EOF could easily be adjusted by adding different anions with different concentrations to the running buffer. This was one important parameter for optimization of separation conditions. For the separation of proteins, the ideal anion buffer additive was that to generate the strongest cathodic EOF without absorbance in the UV range. From the results shown in Fig. 4, ClO₄⁻ was chosen to improve the EOF of dynamic DSB coating for the protein separations.

### 3.4 Separation of inorganic anions

For the determination of inorganic anions, CE offers rapid separations, high separation efficiency, and complementary selectivity to ion chromatography [38]. However, inorganic anions are usually difficult to separate with native silica capillaries because of the opposite electrophoretic movement of anions, unless very high salt concentration [32] and very low pH are used to suppress the EOF [29]. Cationic surfactants [33] and zwitterionic surfactants [31, 32, 39, 40] have been used to improve the separations of inorganic anions. Here, the separation conditions for a mixture of inorganic anions in the presence of the dynamic additive DSB were optimized. The anion mixture contained 1.0 mM Br⁻, NO₂⁻, NO₃⁻, SCN⁻, MoO₄²⁻, BrO₃⁻, WO₄²⁻, and IO₃⁻. The optimal running buffer was 25 mM, pH 7.0, phosphate buffer solution containing 1.8 mM DSB. The total length of the capillary was 50 cm and the effective length 41.5 cm. Seven of these eight anions could be separated completely in less than 2.7 min. The migration time of IO₃⁻ was 4.2 min (Fig. 5). This method was simple without presence of nonionic surfactants, Tween 20 in comparison with [32], in which a longer analysis time of Br⁻, I⁻, NO₂⁻, NO₃⁻, and MoO₄²⁻ was needed due to retention of anions on the zwitterionic surfactants, Tween 20 in comparison with [32].
surfactant pseudostationary phase when N-tetradecyl-
N,N-dimethyl-3-ammonio-1-propanesulfonate alone was
added to the background electrolyte. From the widths at
half-height and the migration times the separation effi-
ciencies for Br$^-$, NO$_2^-$, NO$_3^-$, MoO$_4^{2-}$, SCN$^-$, WO$_4^{2-}$,
BrO$_3^-$, and IO$_3^-$ were calculated to be 350 000, 510 000,
660 000, 780 000, 1 130 000, 1 310 000, 570 000, and
24 000 plates/m, respectively. All these anions possessed
high separation efficiency except for IO$_3^-$, which took a
relatively long time to reach the detector. The separation
efficiencies for Br$^-$, NO$_2^-$, NO$_3^-$, and SCN$^-$ were much
larger than those of 74 000, 82 000, 114 000, and
26 000 plates/m obtained using a capillary coated with 3:1
CTAB/SDS in [33]. These separation efficiencies were
also higher than those achieved with N,N-dimethyl-N-
methacryloxyethyl-N-(3-sulfopropyl) ammonium betaine-
grafted capillaries, in which BrO$_3^-$ showed the highest
efficiency of 333 000 plates/m using 25 mM, pH 8, phos-
phate buffer solution [29]. From $N_{max} = \frac{L_{d}^{2}}{2D_{m}}$ [41],
where $N_{max}$ is the maximum separation efficiency, $L_{d}$ is the
effective length of the capillary, $D$ is the diffusion coeffi-
cient, and $t_{m}$ is the migration time, longitudinal diffusion
of Br$^-$ and NO$_3^-$; their efficiencies could be predicted
to be 960 000 and 760 000 plates/m. These values are
slightly lower than those predicted.

3.5 Separation of proteins

A more promising application of the DSB dynamic coating
on capillary walls was in protein separations. Due to the
adsorption of proteins on capillary walls and high EOF,
protein separations with native silica capillaries were dif-
ficult. In order to get a reasonable separation on native silica capillaries, a very high buffer concentration is gen-
erally used to suppress the EOF and to prevent the
adsorption of proteins [5], which leads to more Joule
heating generated by high ionic strength and is dis-
advantageous for the separation. As an example, four basic
proteins (lysozyme, cytochrome c, α-chymotrypsinogen A, and myoglobin) were used to examine the
improvement in separation efficiency by DSB dynamic
coating. The capillary was the same as that used in inor-
ganic anion separation. Figure 6a shows the separation of these proteins in a bare silica capillary with 40 mM, pH 7.0,
phosphate buffer as running buffer. The peaks of these proteins were seriously broadened, and their separation
and CE detection were very poor, which was an indication
of irreversible adsorption onto the capillary. Upon addition
of DSB to the running buffer the peaks of these proteins
were greatly improved, indicating the DSB dynamic
coating could effectively prevent the adsorption of these proteins on the capillary wall. When the DSB concen-
tration in the buffer was 1.3 mM, the electropherogram showed minimum peak width at half-height (Fig. 6b).
In order to achieve the baseline separation of these proteins, ClO₄⁻ was added to the buffer to adjust the EOF [28]. The optimal concentration of ClO₄⁻ in running buffer was 6.5 mM. Figure 6c shows the electropherogram of these proteins with 40 mM, pH 7.0, phosphate buffer containing 1.3 mM DSB and 6.5 mM ClO₄⁻ as running buffer, which displays Gaussian peaks of the four basic proteins. From the widths at half-height and the migration times of these proteins the separation efficiencies were 396 000, 485 000, 896 000, and 204 000 plates/m, respectively, while the separation efficiencies obtained from Fig. 6d in the absence of DSB ranged from 5674 to 47 000 plates/m (Table 1). In comparison with Fig. 6d some small peaks occurred in Fig. 6c, which possibly resulted from the impurities in DSB and the separated proteins. It was also possibly related to the denaturing effect of the sulfobetaine surfactant on the proteins. The obtained separation efficiencies under optimal conditions were obviously improved and comparable to those reported in [29, 33]. However, these efficiencies, except that for α-chymotrypsinogen A, were not as good as those reported in [24, 27, 28] and [42], in which a permanent coating of dimethylacrylamide was used for protein separation. Although these basic proteins could also be separated completely in the presence of 90 mM ClO₄⁻, the high ionic strength (Fig. 6d) the separation efficiencies were much lower than those obtained with DSB dynamic coating. Thus, the zwitterionic surfactant DSB was a good modifier for dynamic coating of silica capillary walls and protein separation.

The DSB dynamic coating showed good run-to-run and day-to-day reproducibility of the migration times for these four proteins. The run-to-run precision ranged from 0.4% to 0.5% (n = 6), while the day-to-day precision was in the range of 2.3%–3.1% (3 days). These results were comparable to the reported values from 0.7% to 1.6% (within day) and 2.4%–4.6% (day-to-day) [28], and the values from 0.5% to 0.9% (within day) and 1.5%–2.6% (day-to-day) [33]. As shown in Table 1, the recoveries of these proteins, obtained using two capillaries with different lengths according to [27, 43], ranged from 88% to 98%, which were also comparable to those reported from 85% to 98% [33]. These results indicated that the DSB dynamic coating was promising for application in CE separation of proteins.

### 4 Concluding remarks

For CE separation of biomacromolecules, simple, robust, and effective wall coatings are necessary for preventing the adsorption of biomacromolecules on the capillary wall. The zwitterionic surfactant DSB can adsorb dynamically on the silica capillary surface to prevent effectively the adsorption of cationic proteins on the capillary wall. The DSB dynamic coating reduced the EOF in the pH range of 3–12 and improved greatly the separations of eight inorganic anions and four basic proteins (lysozyme, cytochrome c, α-chymotrypsinogen A, and myoglobin). The separation efficiencies for all of these analytes were high. The run-to-run and day-to-day reproducibilities of the migration times of these four proteins were excellent and the recoveries for proteins were good, which suggested that DSB coating could be suitable for the CE separation and analysis of basic proteins.

This work was supported by the Distinguished Young Scholar Fund to HX Ju (20325518), the National Natural Science Foundation of China (20275017, 90206037), the Specialized Research Fund for Excellent Young Teachers from Ministry of Education of China, the Science Foundation of Jiangsu (BS2001063), and the Key Project of Cancer Institute of Jiangsu Province.

### 5 References