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# Applications of a sulfonated-polymer wall-modified open-tubular fused-silica capillary in capillary zone electrophoretic separations

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**Abstract**

A fused-silica capillary that is wall-modified via chemically bonding a sulfonated polymer to the capillary wall has a uniform negative charge density on its surface and produces an electroosmotic flow (EOF) greater than $4 \cdot 10^{-4}$ $cm^2$ $V^{-1}$ $s^{-1}$. The EOF is nearly independent of buffer pH over the pH range of 2 to 10 and is lower than the EOF obtained for the bare fused-silica capillary at the more basic pH but is higher at the more acidic buffer pH. Optimization of buffer pH can be based on analyte p$K_a$ values to improve the overall quality of the capillary zone electrophoresis (CZE) separation of complex mixtures of weak acid and base analytes. Because of the high EOF in an acidic buffer, the capillary is useful for the separation of weak organic bases which are in their cation forms in the acidic buffer. EOF for the sulfonic acid bonded phase capillary can be adjusted via buffer additives such as organic solvent, tetraalkylammonium salts, multivalent cations and alkylsulfonic acids. The advantages of utilizing buffer pH and the EOF buffer modifiers to enhance migration time, selectivity, and resolution in CZE separations with this capillary are illustrated using a series of test analyte mixtures of inorganic anions, carboxylic acids, alkylsulfonic acids, benzenesulfonic acids, sulfas, pyridines, anilines or small-chain peptides. 

*Keywords:* Capillary columns; Buffer composition; Sulfonic acid bonded phase capillaries; Inorganic anions; Organic acids; Organic bases; Alkylsulfonic acids; Benzenesulfonic acids; Sulfa drugs; Pyridines; Anilines; Peptides

## 1. Introduction

An electroosmotic flow (EOF) develops in capillary zone electrophoresis (CZE) when a voltage is applied to a buffer contained in the capillary. Optimization of the EOF is one of the most important properties that characterize separations by CZE. The EOF is often large enough to overcome the electrophoretic mobility of ions and thus move both anions and cations as well as neutral analytes in the same direction. The ability to generate an EOF is therefore a key feature in determining migration time, peak shape, resolution and analysis time for the separation of a complex mixture of analytes. Once the voltage is applied the generation of the EOF depends on both the composition of the capillary and the properties of its inner surface and on the properties of the buffer and its composition. Although other capillary materials can be used, the fused-silica capillary is still the most versatile capillary and is used in most applications [1]. The fused-silica capillary offers many advantages for CZE with one of them being high EOF, particularly at a high buffer pH due to ionization of the free silanol sites on the fused-silica surface. The role of buffer pH in determining the magnitude of EOF was quickly recognized [1–5] and

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optimization of EOF through buffer pH control is often one of the most critical steps in obtaining a favorable CZE separation and analysis time when using a fused-silica capillary.

As CZE and related capillary electrophoretic methodologies were developed other experimental factors were subsequently recognized as important variables for controlling EOF and subsequently improving the quality of the separation. As buffer pH is increased the dissociation of the weak acid silanol sites on the fused-silica surface increases, thus, the charge density on the fused-silica capillary wall increases and EOF increases with a sharp rise in the vicinity of the silanol p$K_a$. Other controllable experimental parameters will also influence the charge density at the fused-silica capillary wall surface and these can also be altered systematically to increase, decrease, and even reverse EOF. For a fused-silica capillary increasing buffer ionic strength or adding organic solvent to the buffer decreases EOF. The type of cation and its oxidation state in the ionic strength electrolyte also significantly influences EOF; as buffer cation concentration and its oxidation state increases EOF decreases [4,6–9]. These studies suggest that the major effect on fused-silica wall charge density, which influences the EOF, is due to cation-exchange between the buffer cation and the weak acid silanol sites on the wall. Many studies have been reported that demonstrate, and which are reviewed elsewhere [8,10–13], that coating the fused-silica capillary wall with polar and nonpolar polymeric materials or with charged polymeric materials will influence EOF. Buffer additives, such as alkylsulfonic acids and their salts ($RSO_3^-$) [12–15], zwitterionic surfactants [16–18], tetraalkylammonium salts ($R_4N^+$) [12,13,19–23] and amines [24,25], reduce EOF as their concentration increases in the buffer. The $R_4N^+$ salts and the amines depending on $R_4N^+$ concentration and R group structure produce a positive charge density on the fused-silica glass surface and reverse the EOF direction.

Instead of coating the fused-silica capillary wall surface materials can be chemically bonded to the silica surface via the silanol sites to bring about a more permanent change in the EOF. Substrates containing polar and nonpolar groups [26–30] and polymers containing sulfonic acid groups [8,26,31–33], tetraalkyammonium groups [33–37], or amine groups [30,38–44] have been chemically bonded to the fused-silica wall surface and have an appreciable effect on EOF, depending on the group. For a sulfonic acid modified bonded surface, the surface has a negative charge while for the $R_4N^+$ and amine modified surface, the surface is positive. For the modified $R_4N^+$ and amine chemically bonded fused-silica capillaries the EOF direction is often reversed depending on the group's chemical structure.

One of the key features of the chemically bonded wall modified fused-silica capillaries containing either the $SO_3^-$ or the $R_4N^+$ groups is that these groups are highly dissociated except for a very acidic or basic condition, respectively. Thus, the charge density at the wall surface is nearly independent of pH and the EOF generated in this type of modified fused-silica capillary undergoes little change with buffer pH. For the $SO_3^-$ modified capillary the EOF is nearly constant over a broad buffer pH range of 2 to 10 [8,26,31–33]. For the $R_4N^+$ modified capillaries the EOF, which is reversed in direction, undergoes a gradual, modest change over the pH 2 to 10 range [30,33–45]. This near constant EOF property is an advantage in applications of these capillaries in the CZE separation of mixtures of weak acid or base analytes. In these applications the major objective of adjusting the buffer pH is to influence dissociation of the analytes in the mixture, and therefore analyte migration time and resolution, while still maintaining a suitable, constant EOF.

This report focuses on the applications of a commercially available fused-silica capillary that has been wall-modified by chemically bonding a polymer that also contains a high level of $SO_3^-$ groups to the inner wall surface of the capillary. For this capillary EOF is nearly independent of buffer pH but other buffer additives can be used to influence EOF. These include organic solvent, multivalent cations, quaternary ammonium salts and $RSO_3^-$ salts. Mixtures of test analytes used to illustrate the scope of applications of this capillary and the buffer additives to improve CZE resolution include simple inorganic anions and small-chain peptide, sulfa, alkyl- and arylsulfonic acid, carboxylic acid, and organic weak base derivatives.

## 2. Experimental

### 2.1. Instrumentation

A Waters Quanta 4000 CE instrument equipped with a 30 kV d.c. power supply and platinum electrodes, an automatic hydrodynamic/electrokinetic injection system, an air-cooled unit, and a fixed-wavelength UV absorbance detector was used for all measurements. The high EOF sulfonated bonded phase wall modified fused-silica capillary CE-SA (04650-HF) (sulfonic acid bonded phase capillary) is commercially available from Scientific Resources. The 50 μm I.D. capillary was 48.5 cm in length with an effective length of 32.5 or 40.0 cm. The detector window of about 0.5 cm in length was formed by removal of the exterior polyimide coating at about 8.5 cm from the outlet end of the capillary. A fused-silica capillary of similar dimensions was obtained from Polymicro Technologies. A Spectra-Physics M-4270 integrator controlled by Spectra-Physics AUTOLAB software and spreadsheet software or a Hewlett-Packard HP 3396 series II integrator were used to collect and process the CZE separation data.

### 2.2. Chemicals

Mesityl oxide and acrylamide, used as markers for the determination of EOF, were purchased from Aldrich and Sigma, respectively. Inorganic salts, acids and bases used for buffers, aniline, sulfa, amino acid, peptide and arylsulfonic acid derivatives used as analytes, and alkylsulfonic acids, acetonitrile and tetraalkylammonium salts used as buffer additives were obtained from Fisher Scientific, Mallinkrodt, Eastman and Aldrich. All reagents were used as received. Laboratory-distilled water, which was also treated with a Milli-Q Plus water treatment system with 0.2 μm final filtration, was employed in the preparation of all capillary conditioning, buffer and analyte sample and mixture solutions.

### 2.3. Procedures

A new sulfonic acid bonded phase capillary was pretreated in the following manner. A 10 min water rinse was pulled through the capillary by vacuum followed by a 30 min rinse with the buffer of interest, a 10 min rinse with pure water, and finally a 10 min rinse with the buffer solution. When not in use the capillary was stored containing water. When salts of multivalent cations were used as buffer additives the capillary was rinsed for 20 to 30 min with 0.5 *M* sodium citrate solution prior to using the capillary in other applications. A test sample of benzenesulfonic acid derivatives (2,4-dimethylbenzenesulfonic acid, 2,5-dichlorobenzenesulfonic acid, *p*-phenolsulfonic acid, *p*-toluenesulfonic acid, *p*-chlorobenzenesulfonic acid, *p*-nitrobenzenesulfonic acid and benzenesulfonic acid) was separated periodically to verify capillary reproducibility. The conditions were an aqueous pH 7.0, 10 m*M* phosphate buffer, applied voltage of 25 kV, and detection at 254 nm. Whenever the EOF, migration times for the test analytes, and/or resolution of the test mixture changed by more than 10%, the capillary was replaced by a new one. EOF was determined at the different buffer conditions from the migration times obtained for either mesityl oxide or acrylamide as the test EOF marker analyte. The procedure used for pretreatment and conditioning of the fused-silica capillary is described elsewhere [8].

Buffer solutions depending on pH and typically 10 or 20 m*M* were made by dilution of known aliquots of more concentrated standard stock solutions of known concentration of either HCl and KCl or of sodium mono-, di- and tribasic phosphate stock solutions to a known, fixed volume. When required, acetonitrile and/or known aliquots of stock solutions of additives such as $MgCl_2$, alkylsulfonic acids, or tetraalkylammonium salts were included in the aqueous buffer as EOF modifiers. If indirect absorbance detection (254 or 280 nm) was necessary, a known aliquot of a standard stock solution of 1,5-naphthalenedisulfonic acid was included in the buffer preparation. All buffer pH values were verified by measurement with a pH meter.

Individual and mixed analyte standard solutions, which were typically 0.5 to 1.0 m*M* in each analyte, were prepared by dilution of more concentrated aqueous standard stock solutions of the analyte. Sample injection was by the hydrodynamic injection procedure, typically about a 10 cm height for 10 to

15 s to yield about 3 to 5 nl or about 1 pmol of each analyte. Applied voltage depended on the CZE separation but typically was in the 7 to 25 kV range while currents were not over 10 to 20 μA depending on the applied voltage and buffer composition. All measurements were done at an ambient temperature of 23°C and were verified by multiple runs. Reproducibility for the EOF markers was within ±1% for a given capillary and buffer condition. Capillary efficiency depended on the experimental conditions; for example, for the sulfa derivative separation in 4 m*M* $Mg^{2+}$ (see Fig. 5) efficiency ranged from 60 for peak 1 to 49 for peak 7 plates per column ($\cdot 10^3$). In other cases over $100 \cdot 10^3$ plates per column were obtained. Individual peaks in the electropherograms of mixtures were verified by comparison to migration times for individual standards and by analyte spiking techniques.

## 3. Results and discussion

### *3.1. Buffer variables*

For an ordinary open tubular fused-silica capillary the EOF changes from about $2.0 \cdot 10^{-4}$ $cm^2$ $V^{-1}$ $s^{-1}$ at pH 2.0 to about $8.0 \cdot 10^{-4}$ $cm^2$ $V^{-1}$ $s^{-1}$ at pH 9.0 [1–4,8]. The EOF obtained, however, will also depend on capillary pretreatment and conditioning. In contrast the commercially available open tubular sulfonated bonded phase wall-modified fused-silica capillary, hereafter referred to as the sulfonic acid bonded phase capillary, has an EOF of about $4.1 \cdot 10^{-4}$ $cm^2$ $V^{-1}$ $s^{-1}$ at pH 2.0 and a value of about $4.6 \cdot 10^{-4}$ $cm^2$ $V^{-1}$ $s^{-1}$ at pH 9.0 [8]. The near constant EOF for the sulfonic acid bonded phase capillary in the pH 2 to 10 range is due to the strong acid property of the sulfonic acid group and its high degree of dissociation which produces a nearly constant negative surface charge over this pH range. In contrast the surface charge of the fused-silica capillary is dependent on buffer pH and increases with pH because of the increased dissociation of the weak acid silanol sites. Thus, its EOF also increases with buffer pH particularly as the buffer pH passes over the silanol $pK_a$ value. Because of the near constant EOF for the sulfonic acid bonded phase capillary it can be concluded that the remaining silanol sites, if not all consumed in the surface bonding, have little effect on the capillary's EOF.

Two approaches can be used to reduce the EOF for the sulfonic acid bonded phase capillary. First, fewer sulfonic acid groups can be synthetically introduced into the chemically bonded capillary. Capillaries with fewer sulfonic acid sites, and therefore lower EOF, are commercially available but only the high EOF capillary was used in the studies reported here. Second, buffer additives can be used to influence EOF. This is particularly an advantage with a more acidic buffer because of the higher EOF for the sulfonic acid bonded phase capillary at this buffer condition.

The sulfonic acid bonded phase capillary has two major advantages over a fused-silica capillary in CZE applications. First, EOF for the sulfonic acid bonded phase capillary is virtually independent of pH over a wide buffer pH range. Thus, buffer pH can be optimized to enhance analyte migration time and resolution in CZE separations of complex mixtures of weak acids and bases based on analyte $pK_a$ values and dissociation rather than on the affect of pH on EOF. And second, sulfonic acid bonded phase capillary EOF is about twice the EOF for the ordinary fused-silica capillary when using an acidic buffer. Thus, very weak organic base analytes can be separated as cations (for example at pH 2) and migration times can be reduced, analysis times can be halved, and in certain cases resolution can be better.

While buffer pH is primarily used to influence analyte dissociation and/or solubility other buffer additives can be employed to improve CZE separations via a change in EOF or as the result of analyte–additive interactions when using the sulfonic acid bonded phase capillary. For example, for a 2 m*M* $Mg^{2+}$ buffer concentration EOF for the sulfonic acid bonded phase capillary is about halved compared to the absence of the $Mg^{2+}$. A further increase in the $Mg^{2+}$ concentration only gradually decreases the EOF. Similarly, tetradecyltrimethylammonium bromide (TTAB) as a buffer additive decreases EOF and causes it to reverse in direction which is also observed when using TTAB with a fused-silica capillary [45]. At 4 m*M* TTAB and an aqueous buffer from pH 2 to 7, EOF for the sulfonic acid bonded phase capillary is about $-2 \cdot 10^{-4}$ $cm^2$ $V^{-1}$

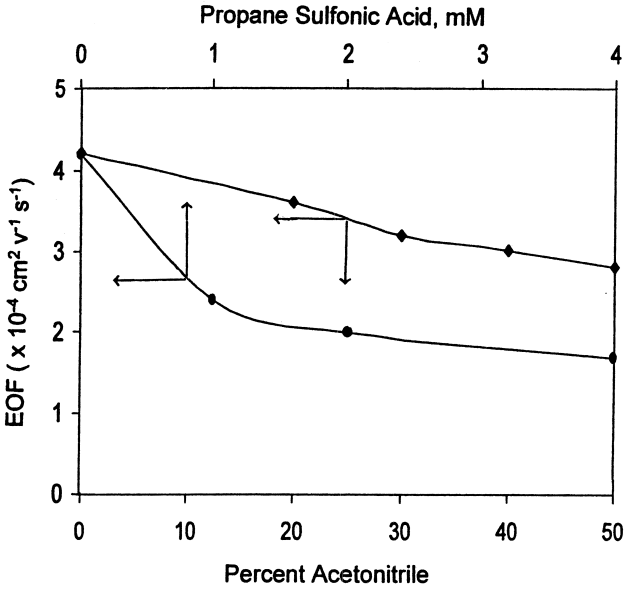


Fig. 1. Effect of acetonitrile and propanesulfonic acid on EOF for the sulfonic acid bonded phase capillary.

$s^{-1}$ when the applied voltage is in the positive mode [8]. Fig. 1 illustrates the effect of two other buffer parameters; increasing $CH_3CN$ buffer concentration decreases EOF and increasing propanesulfonic acid concentration (also a potential ion pairing reagent for organic basic analytes in their cation form) causes a small decrease in EOF. In both studies described in Fig. 1 acrylamide was the analyte marker for the EOF determination and the buffer was 10 m*M* phosphate, pH 5.0 for the $CH_3CN$–water study and 10 m*M* phosphate, pH 2.3 for the propanesulfonic acid study.

Optimization of each buffer variable, namely pH, multivalent cation, $R_4N^+$, $CH_3CN$ and propanesulfonic acid concentration, enhances CZE separations with the sulfonic acid bonded phase capillary. Analyte migration time can be altered, selectivity can be increased, resolution can be improved, and analysis time can often be decreased. Several examples illustrating the scope of these variables with different types of test analytes are described in the following sections.

### *3.2. Separation of simple inorganic anions*

Simple inorganic anions can be separated by CZE with either a $R_4N^+$ salt as a buffer additive to reverse the EOF direction [45] or by electrophoretic migration [45,46] coupled with applied voltage polarity switching. Fig. 2 compares the resolution obtained for the electrophoretic CZE separation of a test mixture of six common inorganic anions, pH 2.4 and 6.0 for a fused-silica capillary (Fig. 2A) to the separation obtained with the sulfonic acid bonded phase capillary (Fig. 2B). The anion migration order is the same for both capillaries. Also, analyte migration times and selectivity for adjacent analytes increases with pH for both capillaries. If pH increases, for example from pH 6.0 in Fig. 2 to pH 8, the separation times for the anion mixture in Fig. 2 will increase particularly for the fused-silica capillary. At this pH the EOF for the bare fused-silica capillary is much greater than for the sulfonic acid bonded phase capillary and this causes a greater effect on the electrophoretic migration of the anion analytes. Thus, at pH 8 migration times in the fused-silica capillary are larger. Resolution at pH 6.0 is only modestly improved with the sulfonic acid bonded phase capillary but significantly improved at a buffer pH 2.4 compared to the bare silica capillary. At pH 2.4 in Fig. 2 the EOF for the bare fused-silica capillary is about half that of the sulfonic acid bonded phase capillary due to low dissociation of the silanols of the bare fused-silica capillary. Electro-

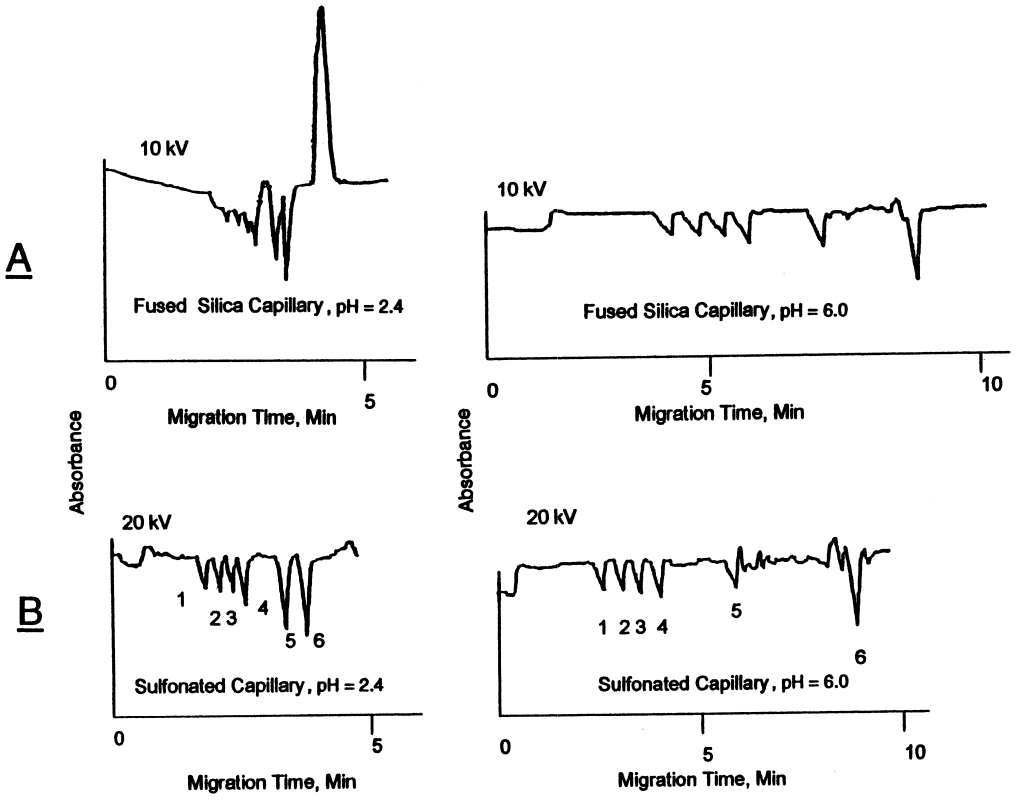


Fig. 2. Comparison of the electrophoretic separation of a mixture of six inorganic anions with (A) a bare fused-silica capillary and (B) a sulfonic acid bonded phase capillary. An aqueous 25 m*M* phosphate, pH 2.4 or 6.0, 2.0 m*M* 1,5-naphthalenedisulfonic acid buffer at −10 or −20 kV applied voltage and indirect detection at 280 nm. 1=Chloride; 2=nitrite; 3=chlorate; 4=perchlorate; 5=bromate; 6=periodate.

phoretic migration time for the anion analytes is reduced in the sulfonic acid bonded phase capillary, and thus, resolution of the inorganic analyte anions at pH 2.4 is significantly improved. The optimum applied voltage differs for the two capillaries. For the sulfonic acid bonded phase −20 kV is optimum while for the bare fused-silica capillary it is −10 kV. For both capillaries 1,5-naphthalenedisulfonic acid was present in the buffer as the probe molecule for indirect detection.

### *3.3. Sulfonic acid analytes*

Table 1 lists migration times obtained with the sulfonic acid bonded phase capillary for alkanesulfonic acid ($RSO_3^-$, R=linear $C_4$ to $C_{10}$) analytes as a function of $CH_3CN$ buffer concentration. The buffer was pH 5.0, 10 m*M* phosphate, indirect detection was used, and the applied voltage was 12 kV. As $CH_3CN$ concentration increases analyte migration time increases, selectivity of adjacent analytes in-

Table 1
Migration times for linear alkanesulfonic acid derivatives ($RSO_3^-$) as a function of buffer acetonitrile concentration[a]

| $RSO_3^-$ (R=) | Migration time (min) | | | | |
|---|---|---|---|---|---|
| | Percent acetonitrile | | | | |
| | 10 | 20 | 30 | 40 | 50 |
| $C_4$ | 12.5 | | | | |
| $C_6$ | 11.7 | 18.7 | 28.2 | | |
| $C_7$ | 10.8 | 17.0 | 23.4 | | |
| $C_8$ | 9.97 | 15.5 | 20.3 | 29.2 | 51.3 |
| $C_{10}$ | 8.97 | 13.1 | 16.2 | 21.2 | 31.7 |

[a] A sulfonic acid bonded phase capillary and a 10 m*M* phosphate, pH 5.0, 1,5-naphthalenedisulfonic acid in a $CH_3CN$–water buffer mixture at 12 kV applied voltage with indirect detection at 214 nm.

creases, and resolution increases due to the EOF decrease.

For mixtures of benzenesulfonic acid derivatives resolution was favorable from an aqueous buffer solution over the studied pH range of 2 to 9. Fig. 3 lists an electropherogram for a separation of a eight-component mixture of benzenesulfonic acid derivatives at pH 2.0, 25 kV applied voltage, and direct UV detection. When buffer pH is increased above pH 2.0 migration times for the benzenesulfonic acid derivatives decrease only slightly because analyte dissociation does not change and EOF increases slightly. In contrast migration times for the analytes will sharply decrease for a fused-silica capillary because of the sharp increase in EOF that occurs with this capillary when buffer pH is increased.

If benzoic acid derivatives were in the analyte mixture in Fig. 3 their location in the electropherogram would shift with pH depending on their p$K_a$ value. For example, migration time for benzoic acid (p$K_a$=4.2) is less than 5 min at pH 3.0, while for *o*-nitrobenzoic acid (p$K_a$=2.17) it would be over 12 min. When pH is increased dissociation for the two derivatives increases, particularly for benzoic acid, and migration times change. For example, at pH 8 migration order, which follows mass-to-charge, is *o*-nitrobenzoic acid at just over 10 min and benzoic acid at just under 12 min. The migration times for the benzensulfonic acid derivatives on the other hand would only decrease by 1 to 5 min, depending on the substituent, for a buffer pH change of 3 to 8. This is due to the near constant EOF for the sulfonic acid bonded phase capillary and high level of dissociation of the benzenesulfonic acid derivatives in this pH range. Thus, for a successful separation of this mixture buffer pH would be optimized to influence resolution based on analyte p$K_a$ rather than on the magnitude of the EOF.

### *3.4. Effect of buffer on the CZE separation of sulfas*

Sulfa derivatives are cations in a strong acid buffer, uncharged neutral species at an intermediate pH, and anions at a basic pH. The pH range, where the neutral form of the sulfa derivative is the major species, depends on the substituent on the sulfa derivative and its influence on the p$K_a$ values, particularly p$K_{a2}$. Consequently, migration times for the sulfa derivatives are strongly affected by both buffer pH and derivative substituent. When using a fused-silica capillary, sulfa derivative migration time is also very sensitive to EOF because of the capillary's EOF dependence on pH [47,48].

Fig. 4 illustrates the CZE separation of a seven-component mixture of sulfa derivatives with the sulfonic acid bonded phase capillary as a function of buffer pH. Resolution for a 20 m*M* phosphate buffer and a 10 kV applied voltage is favorable at a high buffer pH where the sulfa derivatives are anions, poor at intermediate pH where the sulfas are neutral depending on p$K_a$ values, and modest at low pH where the sulfas are cations. Sulfa migration order

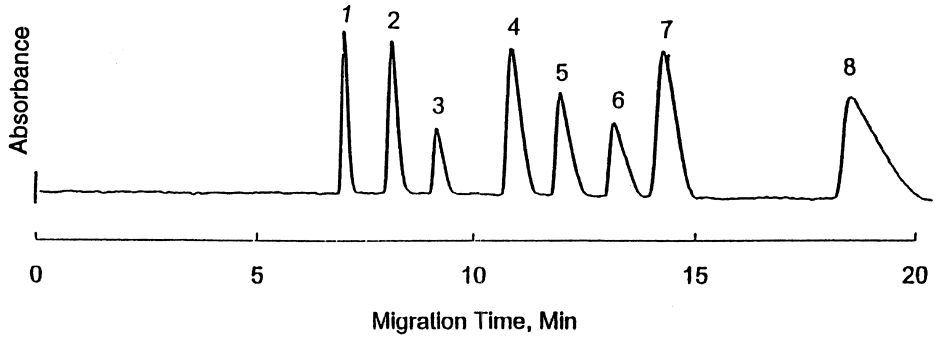


Fig. 3. CZE separation of a complex mixture of benzenesulfonic acid derivatives. A sulfonic acid bonded phase capillary and an aqueous 10 m*M* phosphate, pH 2.0 buffer at 25 kV applied voltage and detection at 214 nm. 1=2,4-Dinitrobenzenesulfonic acid; 2=2,5-dinitrobenzenesulfonic acid; 3=4-hydroxybenzenesulfonic acid; 4=4-methylbenzenesulfonic acid; 5=4-chlorobenzenesulfonic acid; 6=4-nitrobenzenesulfonic acid; 7=benzaldehyde-2-sulfonic acid; 8=benzenesulfonic acid.

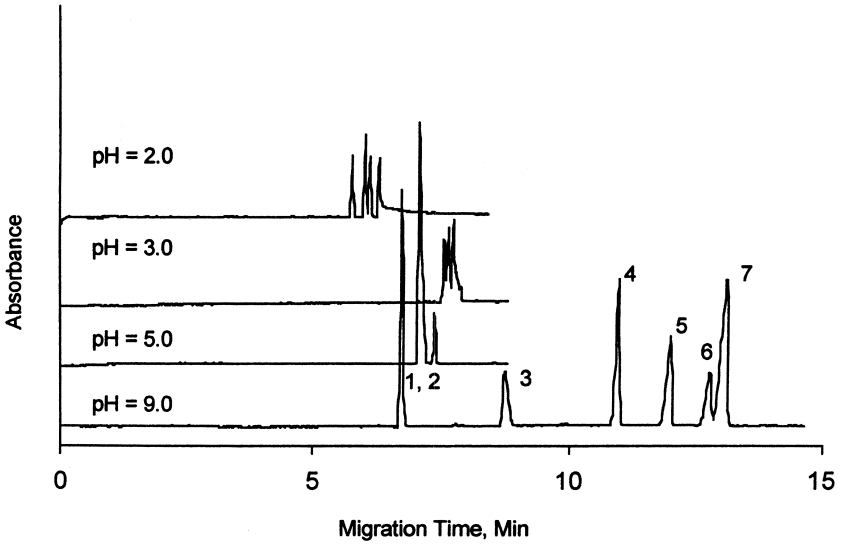


Fig. 4. CZE separation of seven sulfa derivatives as a function of buffer pH. A sulfonic acid bonded phase capillary (effective length of 40.0 cm) and an aqueous 20 m*M* phosphate, pH 2.0, 3.0, 5.0, or 9.0 buffer at 10 kV applied voltage with detection at 254 nm. 1=Sulfanilamide; 2=sulfaguanidine; 3=sulfapyridine; 4=sulfamethazine; 5=sulfamerazine; 6=sulfadiazine; 7=sulfathiazole.

undergoes several changes as buffer pH is decreased from pH 9.0 because of differences in p$K_a$. The migration order is listed for pH 9 in Fig. 4. In the neutral pH region the migration time for sulfanilamide is greater relative to sulfas 2, 3 and 4 (see Fig. 4) while migration time for sulfathiazole is less than sulfas 5 and 6 (see Fig. 4). However, resolution is poor at pH 5 and the differences are small. Resolution improves at a lower pH and the migration oder is again altered. For example, at pH 2.0 sulfathiazole appears between sulfas 4 and 5 (see Fig. 4) rather than last as is the case when the buffer is pH 9.0. If the applied voltage is reduced, resolution at pH 2.0 can be improved and the option of optimizing the applied voltage is possible because of the higher EOF for the sulfonic acid bonded phase capillary in acidic conditions compared to the fused-silica capillary. Sulfa derivative migration order on the sulfonic acid bonded phase capillary at pH 9.0 or 2.0 is the same as that obtained with the fused-silica capillary [47,48].

For an acidic buffer EOF for the sulfonic acid bonded phase capillary is high enough that an EOF modifier can be added to the buffer to improve resolution. This is illustrated in Fig. 5, where the $Mg^{2+}$ concentration in an aqueous, 20 m*M* phosphate, pH 2.5 buffer is increased up to 4 m*M* $Mg^{2+}$. For pH 5 the migration order for sulfanilamide and sulfathiazole is again different from the order observed at lower and higher buffer pH conditions (compare Fig. 5 to 4). As $Mg^{2+}$ concentration increases, EOF decreases, sulfa derivative migration time increases, sulfa migration order is constant as $Mg^{2+}$ concentration increases, and resolution improves. Using a simple inorganic cation buffer additive, particularly a multivalent cation, reduces EOF and this markedly improves resolution in the CZE separation of other organic analyte anions and cations [4,6,7,9,49] on a bare fused-silica capillary. The CZE separation of mixtures of weak base analytes, such as purines, pyrimidines, anilines, and nucleosides, has also been markedly improved when using the sulfonic acid bonded phase capillary and $Mg^{2+}$ as the buffer additive [8]. Although not shown in Fig. 5, increasing the $Mg^{2+}$ concentration (other multivalent cations can also be used providing precipitation does not occur) in a basic buffer where the sulfa derivatives are anions reduces EOF and increases sulfa derivative migration time and resolution of mixtures of the sulfa derivatives.

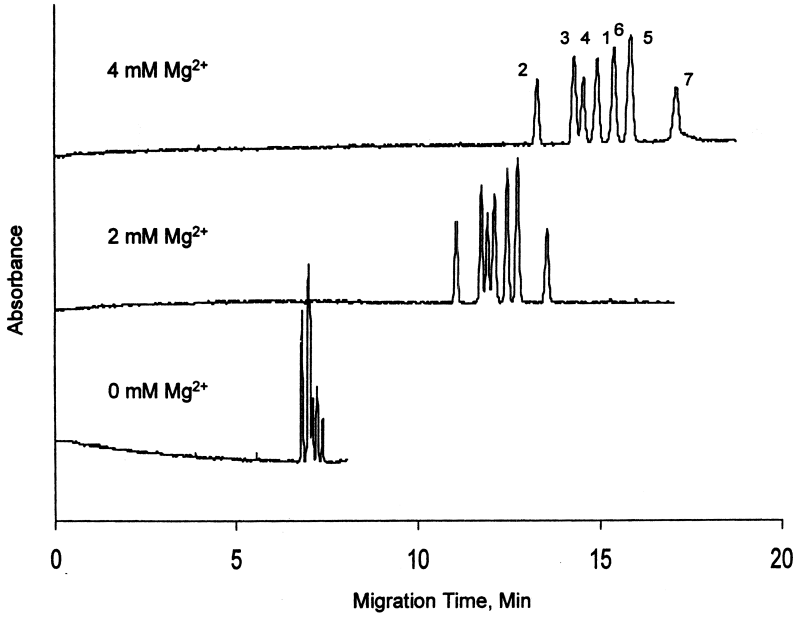


Fig. 5. Effect of $Mg^{2+}$ as a buffer additive on the CZE separation of seven sulfa derivatives. A sulfonic acid bonded phase capillary (effective length of 40.0 cm) and an aqueous 20 m*M* phosphate, pH 2.5 $Mg^{2+}$ buffer at 10 kV applied voltage with detection at 254 nm. Peak numbering as in Fig. 4.

### *3.5. Propanesulfonic acid as a buffer additive*

The EOF for a sulfonic acid bonded phase capillary decreases gradually as the propanesulfonic acid concentration increases in an acidic buffer, see Fig. 1. In previously reported work *n*-alkanesulfonic acids from 1-ethane- to 1-octanesulfonic acids were studied as buffer additives for the CZE separation of free amino acids with a fused-silica capillary at low buffer pH where the amino acids are in their cation form [13]. The alkanesulfonic acid buffer additive causes the EOF to decrease sharply as the buffer pH decreases from pH 5.0 to pH 2.0; at pH 2.8, EOF also increases as linear alkane chain length increases. The effect is particularly pronounced as the alkyl chain length approaches $C_8$. It was proposed [13] that the fused-silica wall is altered to produce a negative charge surface by a double layer dynamic coating of the alkanesulfonic acid additive on the fused-silica capillary wall surface. This decreases the EOF and has a strong influence on the migration time and resolution of amino acid analytes as cations for an acidic buffer. It was also suggested that an ion pair interaction can occur between the alkanesulfonic acid buffer additive and the amino acid cation, which would affect electrophoretic migration and thus influence amino acid migration time and resolution. When octanesulfonic acid is the buffer additive and pH 2.4 the mobilities for the amino acid analytes decrease as the concentration of the octanesulfonic acid increases in the buffer. For a buffer pH 2.4 containing 35 m*M* octanesulfonic acid, a fused-silica capillary, and a 30 kV applied voltage 20 amino acids were separated in about 38 min; for ethanesulfonic acid as the buffer additive separation time was about 20 min [13]. While the effect of EOF was important, it was concluded that the change in electrophoretic migration for the amino acid analytes in the presence of the additive was the more significant factor in influencing the overall separation performance.

When a sulfonic acid bonded phase capillary is used with an acidic buffer, for example, pH 2.5, containing an alkanesulfonic acid as an additive, EOF decreases only modestly (see Fig. 1) and does not reverse as was observed for the fused-silica

capillary [13]. In addition to the small effect on EOF, propanesulfonic acid can also participate in ion pairing with cationic or basic analytes that are in their cation form due to the low buffer pH. Since the sulfonic acid bonded phase capillary is compatible with a low buffer pH and has a higher EOF compared to a fused-silica capillary at this condition, a low pH buffer can be used to convert weak organic bases into cations to enhance their interaction with the propanesulfonate anion buffer additive. Thus, the migration times for organic weak base analytes as cations should be increased and selectivity and resolution for mixtures of weak base analytes should be enhanced depending on the association constants for these interactions.

Fig. 6 compares the CZE separation of pyridine and aniline derivatives with the sulfonic acid bonded phase capillary in the absence and presence of propanesulfonic acid in the buffer at three applied voltages. The aqueous buffer is 10 m*M* phosphate, pH 2.50, and 50 m*M* propanesulfonic acid. The CZE separation is markedly improved in the presence of the propanesulfonic acid. Since EOF is reasonably high for the sulfonic acid bonded phase capillary at an acidic buffer pH, a lower applied voltage, which reduces EOF, can also be used to improve resolution for the separation of the mixture of weak base analytes.

Fig. 7 illustrates the CZE separation of a eight-component mixture of short chain peptides with the sulfonic acid bonded phase capillary and an acidic buffer in the absence and presence of propanesulfonic acid as a buffer additive. At pH 2.3 the peptides are predominately in their cation form and potentially can undergo interactions with the propanesulfonate anions. In the absence of the propanesulfonic acid (see Fig. 7A) resolution of peptide analytes 2 to 7 is incomplete even at the lowest

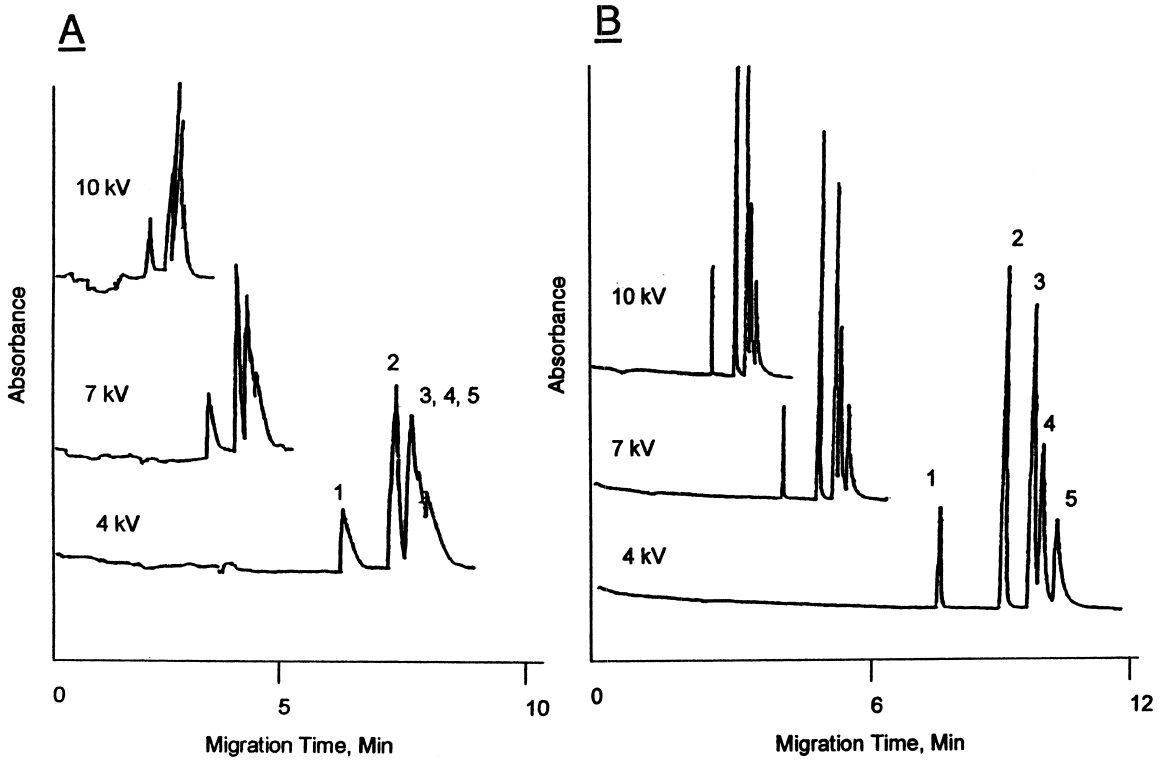


Fig. 6. The effect of propanesulfonic acid on the CZE separation of pyridine and aniline derivatives. (A) A sulfonic acid bonded phase capillary and an aqueous 10 m*M* phosphate, pH 2.5 buffer at 4, 7 and 10 kV applied voltage and detection at 185 nm and (B) same as (A) except the buffer also contains 50 m*M* propanesulfonic acid. 1=Pyridine; 2=aniline; 3=*p*-chloroaniline; 4=*p*-bromoaniline; 5=*p*-iodoaniline.

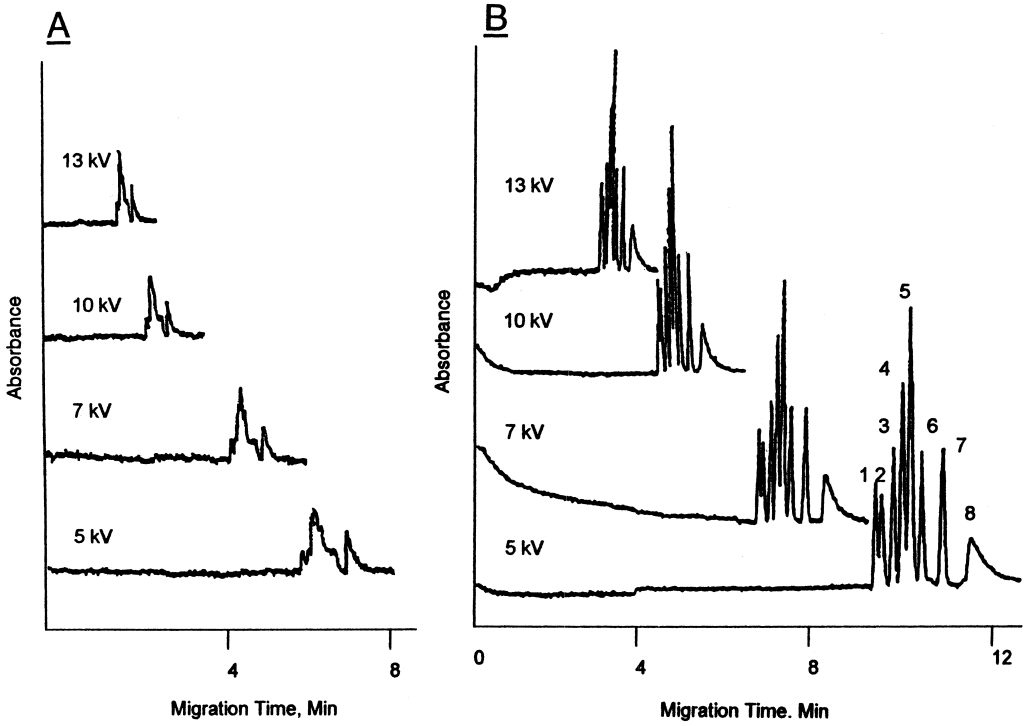


Fig. 7. The effect of propanesulfonic acid on the CZE separation of an eight-component mixture of small-chain peptides. (A) A sulfonic acid bonded phase capillary and an aqueous 10 m*M* phosphate, pH 2.3 buffer at 5, 7, 10 and 13 kV applied voltage with detection at 214 nm and (B) same as (A) except the buffer also contains 50 m*M* propanesulfonic acid. 1=L-Ala–L-Ser; 2=L-Ser–L-Ser; 3=Gly–L-Phe; 4=Gly–L-Tyr; 5=Gly–Gly–L-Phe; 6=L-Tyr–Gly–Gly; 7=L-Leu–Gly–L-Phe; 8=L-Phe–L-Phe–L-Phe.

applied voltage. When the pH 2.3 buffer contains 50 m*M* propanesulfonic acid the migration times for all the small chain peptides increase, selectivity for adjacent peptide analytes increase, and resolution (see Fig. 7B) is baseline for most small chain peptides. A lower applied voltage also aids the resolution of the peptide mixture.

For both the pyridine and aniline derivative (Fig. 6) and the small chain peptide (Fig. 7) CZE separation examples, decreasing propanesulfonic acid in the buffer decreases analyte migration time with an accompanying systematic gradual decrease in resolution. When either hexane- or octanesulfonic acid is the buffer additive at a 50 m*M* concentration, migration times for all the analytes are systematically increased (increases with alkyl chain length) but resolution is only slightly improved.

A modest increase in the buffer pH affects analyte migration time in the presence of the propanesulfonic acid buffer additive depending on the analyte weak base $pK_b$ value. For example, for the pyridine and aniline derivatives in Fig. 6 increasing buffer pH from 2.5 to 2.8 does not appreciably change migration time or resolution of the pyridine and aniline derivatives. Based on their $pK_b$ values the concentration of these analytes as cations does not change appreciably over this modest pH range. In contrast for the short chain peptides in Fig. 7 increasing the buffer pH from 2.3, which was used in Fig. 7, to pH 2.8 causes the migration times for all the peptide analytes to decrease. However, resolution for peptides 2 to 7 is lost due to the appreciable decrease in cation character of the peptides because their $pK_{a1}$ values coincide with this pH change. This loss of resolution is consistent with the reduction of association between the peptide analytes and the propanesulfonate anion as the buffer pH increases.

The small decrease in EOF caused by the propanesulfonic acid is not large enough to account for the increase in migration times for the pyridine, aniline,

and peptide derivatives. A more likely factor is that association between the alkanesulfonate anion and the weak base analyte cation is responsible for the increases in migration times, selectivity, and resolution. The fact that there is little difference between propane-, hexane- and octanesulfonic acid on analyte migration time, and resolution is, however, not consistent with this conclusion since it would be expected that of the three sulfonic acid derivatives, octanesulfonic acid would have the largest effect of the three.

## 4. Conclusion

The sulfonic acid bonded phase capillary has two major advantages compared to a bare fused-silica capillary. First, the EOF is nearly pH independent for the sulfonic acid bonded phase capillary. Thus, optimization of buffer pH to affect CZE separation can be based on analyte p$K_a$ value. Even very weak organic bases can be separated by CZE as cations by using a low pH buffer. The second advantage is that the EOF for the sulfonic acid bonded phase capillary at low pH is over twice that of the bare fused-silica capillary. Thus, separation time in an acidic buffer is more favorable for the sulfonic acid bonded phase capillary. The sulfonic acid bonded phase capillary is compatible with other mobile phase additives which can be optimized to increase analyte migration time, increase selectivity, and improve resolution for the CZE separation of complex mixtures while still maintaining favorable separation times. Increasing acetonitrile concentration in the buffer reduces EOF while adding $R_4N^+$ salts causes the capillary surface charge to be converted to a positive surface and reverses the direction of the EOF. Addition of a multivalent cation, such as $Mg^{2+}$, to the buffer decreases EOF, increases analyte migration time, and improves selectivity and resolution. Propanesulfonic acid as a buffer additive modestly reduces EOF and appears to act as a pairing ion to enhance the CZE separation of weak organic base analytes that are in their cationic form. Optimization of buffer pH relative to analyte p$K_a$ and buffer additives to adjust EOF improves the CZE separation of inorganic anions and carboxylic acids, alkylsulfonic acid, benzenesulfonic acid, sulfa, aniline, pyridine and small-chain peptide derivatives with the sulfonic acid bonded phase capillary.

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