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Separation of weak organic acids and bases by capillary zone electrophoresis in a sulfonated-polymer wall-modified open tubular fused-silica capillary

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Abstract

A sulfonated-polymer wall-modified open tubular fused-silica capillary has a nearly constant electroosmotic flow (EOF) over the pH range studied (pH 2–9) in capillary zone electrophoretic (CZE) applications. The EOF is less than that obtained with a bare fused-silica capillary above pH of ~6.5 but is greater at lower pH values. Altering buffer pH influences weak acid and base analyte migration time according to analyte pK_a value while maintaining a nearly constant EOF. Very weak organic bases can be separated as cations in an acidic buffer prior to neutral analyte migration while weak acid analyte migration order can be manipulated by buffer pH according to the pK_a values for the weak acid analytes. EOF can also be reduced by increasing mono- and divalent cation concentration in the buffer. Divalent cations reduce EOF to a much greater extent while hexadecyltrimethylammonium salt causes an EOF reversal that is independent of pH throughout the pH range studied. Migration order and resolution in the CZE separations of organic acids, phenols, pyridines, pyrimidines, purine bases, and nucleosides can be improved by using Mg^{2+} as a buffer additive and adjusting buffer pH depending on pK_a values of the analytes. © 1998 Elsevier Science B.V.

Keywords: Capillary columns; Buffer composition; Coated capillaries; Organic acids; Organic bases; Phenols; Pyridines; Aniline; Pyrimidines; Purines; Nucleosides

1. Introduction

The major fundamental properties that determine analyte migration in capillary zone electrophoresis (CZE) are electrophoretic mobility, μ_{ep}, and electroosmotic flow (EOF), μ_{eo}. The former is primarily influenced by analyte structure, capillary dimensions, and electrical field conditions while the latter is a function of the properties of the solution in contact with the capillary wall and the surface charge that develops at the wall [1]. Most CZE studies and applications are currently being carried out with a fused-silica capillary. However, capillaries made from other materials, for example, polytetrafluoroethylene [2–6], polypropylene [7,8], and polyethylene and polyvinyl chloride [6], have also been used.

Successful CZE separations require one to carefully control and reproduce the EOF which is present in the capillary because of surface charge on the capillary wall. Many studies have focused on establishing the scope of experimental variables that can be optimized to increase, decrease, and even reverse the EOF. Buffer pH is one of the most significant factors influencing EOF for a fused-silica capillary because of the development of charge at the silica wall surface due to dissociation of the residual silanol sites on the capillary wall. The pK_a for the

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silanol group is about 5.9 depending on the solution composition in contact with the fused-silica wall. Typically, EOF for the fused-silica capillary is high in a basic buffer, decreases significantly at about pH 6, and is low in a more acidic buffer solution. Thus, analyte migration time increases as buffer pH is reduced and the capillary inlet is positive voltage. Even with an acidic buffer, analyte migration is towards the negative electrode, but is sharply decreased because of the low EOF at the acidic conditions. Also, the change in EOF is dependent on buffer composition [1,9] and on the experimental procedure used to condition the fused-silica capillary wall [10–12].

Other experimental variables can be changed to optimize EOF and improve resolution, alter analyte migration time or direction, and/or change analysis time. Increasing the buffer ionic strength decreases the EOF while an EOF change can also be correlated to the viscosity, dielectric properties, solvent composition, and temperature of the buffer solution [13]. A correlation between EOF and viscosity or dielectric constant for nonaqueous solvents in the absence of electrolytes has also been reported [14]. Coating the fused-silica capillary wall and/or derivatization of the wall silanol sites, for example, with monomeric or polymeric hydrocarbons, alcohols, diols, glycols, carbohydrates, amides, ethers, and oxides [15], can mask or alter the influence of the residual silanol sites. While these techniques often minimize analyte–capillary wall interactions, they will also affect capillary wall surface charge and viscosity and also, therefore, the EOF. Adding ionic or nonionic surfactants to the buffer below critical micelle formation affects EOF due to interactions between the surfactant and the capillary wall surface. For example, for quaternary ammonium salts the decrease in EOF is dependent on concentration and alkyl chain length of the tetraalkylammonium cation [16–18], and for long alkyl chain lengths an EOF reversal is observed [16,19,20].

Modification of the fused-silica capillary wall with a highly ionized group, compared to the silanol group, should offer several advantages. Extensive ionization, which is less sensitive to buffer pH, whether due to anionic or cationic groups, should lead to a more constant surface charge. The dependence of the EOF on pH should be minimized, and therefore, should result in a more constant, reproducible EOF which should lead to better reproducibility in CZE separations. Furthermore, buffer pH is then a variable which can be altered to determine weak base and acid analyte dissociation rather than one to influence EOF. In addition wall–analyte interactions should be minimized depending on the analyte and the type of charged wall modification. These advantages have been realized in studies where anionic surfaces are generated by attaching a sulfonated group [15,21–24] or a boronate group [25] on the fused-silica capillary wall and where cationic surfaces are generated via the introduction of cationic groups on the wall [14,26–28].

The weakly acidic silanol sites, which are responsible for the fused-silica capillary wall charge, interact with buffer cations [11,17,18,29–42]. These interactions can be significant. For example, the silanol sites of chromatographic grade silica can be used to separate inorganic cations by cation exchange due to differences in cation exchange selectivities [43]. It has been suggested that these same interactions, namely cation exchange between buffer cations and the residual silanol sites, occur at the fused-silica capillary wall [11,17,18,33,35,36,38,39]. This cation exchange alters the fused capillary wall surface charge and causes EOF to decrease, depending on the cation. As the cation charge or its concentration increases, EOF decreases. Furthermore, the EOF change can be correlated to the cation exchange selectivities exhibited by chromatographic grade silica towards mono- and divalent cations. Evaluation of a mathematical model of EOF revealed a linear correlation between fused-silica wall charge and log cation activity indicating that the capillary surface shows cation selectivity much like an ion-selective electrode [11].

Optimization of the buffer cation and its concentration to alter EOF will significantly affect analyte migration time, resolution, and analysis time in CZE separations. Often, even resolution of analytes of similar electrophoretic mobilities is improved. For example, the CZE separation of anionic surfactants is improved with Mg^{2+} and other mono- and divalent cations as a buffer additive [17]. Mono- and dicarboxylic acid and phenol separations are enhanced with Fe^{3+}, Ca^{2+}, Mg^{2+}, or alkali metal cations in the buffer [18,35,38] while amino acids as

Dansyl derivatives [31] and proteins [29] are better resolved depending on the alkali metal cation in the buffer. Bases, nucleosides, and oligonucleotides were separated using Zn^{2+}, Cu^{2+}, or Mg^{2+} and sodium dodecyl sulfate (SDS) micelles as buffer additives [30], small organic acid analytes in sugar refinement were resolved with Ca^{2+} in the buffer [34], and the separation of diuretics containing carboxylic acid and/or sulfonamide groups was better with transition metal–ammonia complexes as buffer additives [36]. In other examples amino acids were resolved in the presence of Mg^{2+}, Zn^{2+}, or Cu^{2+} [37], metallochromic ligands were separated with Ca^{2+} or Zn^{2+} as a buffer additive [40], and the resolution of 2,4-dinitro derivatives of amino acids in the presence of Mg^{2+} was improved [41]. The chiral separation of 5-dimethylamino-1-naphthalenesulfonyl derivatives of D,L-amino acids in a buffer containing Mg^{2+} and the chiral selector Cu^{2+}–aspartame complex was improved because of EOF control by the buffer cation [42].

In this report a commercially available open tubular fused-silica capillary that has been wall modified with a sulfonated polymer is evaluated as a capillary for CZE separations. The effect of pH and buffer mono- and divalent inorganic cations on EOF is established. Optimization of these experimental conditions can be used to alter migration time, migration order, and resolution in the CZE separation of weak organic bases and organic acids of variable pK_a values, such as carboxylic acids, phenols, pyridines, anilines, pyrimidine/purine bases, and nucleosides.

2. Experimental

2.1. Chemicals

Pyrimidine, purine and nucleoside derivatives and tris(hydroxymethyl) amino methane (Tris) were obtained from Sigma. Mesityl oxide was purchased from Aldrich while carboxylic acids, phenols, anilines, inorganic salts, acids and bases were obtained from Fisher Scientific, Mallinkrodt, and Aldrich. All reagents were used as received. In-house distilled water was passed through a Milli-Q-Plus water treatment unit equipped with a final 0.2-μm filter and was used to prepare all sample and buffer solutions.

2.2. Instrumentation

The capillary was a 48 cm (40 cm effective length)×50 μm I.D., CE100-SA sulfonated-polymer wall-modified high EOF open tubular fused-silica capillary (04650-HF) obtained from Scientific Resources. All measurements were made with a Waters Quanta 4000 CE instrument equipped with a fixed wavelength filter UV detector, an automatic injection unit, an air cooled capillary compartment system, a d.c. 30 kV maximum power supply, and an automatic operation program system. A Spectra-Physics M-4270 integrator controlled by Spectra-Physics AUTOLAB software and spread sheet software was used to collect and manipulate separation data.

2.3. Procedures

The sulfonated wall-modified fused-silica capillary was preconditioned by drawing the buffer solution of interest through the capillary for about 30 min, followed by a water rinse of about 5 min, and finally an additional 10-min rinse with the buffer solution. Following studies with buffer solutions containing transition metal cations as additives the capillary was rinsed extensively with a sodium citrate solution prior to conditioning with the new buffer solution of interest. When the capillary was not in use it was stored containing deionized water. Reproducibility of the sulfonated coated capillary was determined over time by using mesityl oxide as the analyte and a 20 m*M* phosphate buffer (pH 7) solution and 20 kV. When a change in EOF of 5% or greater was indicated by the mesityl oxide migration time, the capillary was discarded in favor of a new capillary of identical dimensions. Typically, capillaries lasted over 90 h depending on the range of buffer conditions used. All buffer solutions were either aqueous 20 m*M* phosphate adjusted to the desired pH or aqueous 20 m*M* Tris mixed with 20 m*M* trichloroacetic acid to yield the desired pH that may or may not include cation additives usually as the chloride salts.

Stock solutions of purine, pyrimidine, aniline,

carboxylic acid, and phenol derivatives and their mixtures were prepared as aqueous solutions of 5 to 10 m*M* of the analyte. A mesityl oxide standard solution was used to determine EOF. Samples were introduced by hydrodynamic injection at 9.8-cm height for 10–15 s yielding a sample volume of ~3–5 nl. All measurements were carried out at +20 kV with a current of ~11–25 μA depending on the buffer composition, and the ambient temperature of 23°C. Detection was at 214 nm. Results reported here represent averages of more than three runs for a given condition and were verified by additional runs with new capillaries, buffer, and analyte solutions. Reproducibility for the marker was within ±1.0% for a given capillary and buffer condition. Identity of individual peaks in analyte mixtures was verified by comparison to individually run standards.

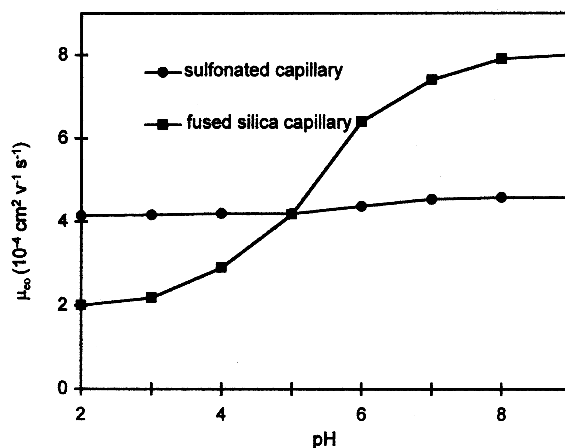

Fig. 1. Comparison of the effect of buffer pH on EOF for a bare fused-silica capillary and a sulfonated-modified capillary.

3. Results and discussion

3.1. Effect of pH

A sulfonated-polymer wall-modified open tubular fused-silica capillary should have a constant surface charge since the sulfonic acid groups are highly dissociated and are not sensitive to pH except at a very strongly acid condition. This is in contrast to the bare fused-silica capillary where weak acid silanol sites, pK_a of about 5.9, are present and pH of the buffer in contact with the capillary wall will determine the degree of silanol ionization and therefore its surface charge. Furthermore, the nature of the buffer and how the fused-silica capillary wall is preconditioned [10–12] also influence the wall charge because these parameters determine the number of silanol sites and the degree of dissociation of the silanol sites.

Fig. 1 compares how EOF, which is a reflection of the surface charge at the capillary wall, changes as a function of pH for the sulfonated modified capillary to a bare capillary. In these studies mesityl oxide was the analyte employed as the marker for EOF and the buffer was a 20 m*M* phosphate buffer adjusted to the listed pH. Over the pH range evaluated (pH 2–9) the EOF for the sulfonated coated capillary is nearly constant indicating a near constant capillary wall surface charge. For the bare fused-silica capillary the EOF undergoes a rapid decrease as the buffer pH is decreased in the pH region corresponding to the silanol pK_a. The EOF change determined in Fig. 1 for the two types of capillaries is consistent with previous studies with the bare capillary [1,10,11] and a sulfonated capillary [23,24].

The EOF for the sulfonated modified capillary is less than the EOF obtained with the bare fused-silica capillary in basic solution (see Fig. 1) but is still large enough to be of practical use in CZE separation applications in this pH range. A major advantage of the sulfonated modified capillary, however, is in the more acidic buffer pH range where the EOF for the sulfonated modified capillary is typically about twice (see Fig. 1) the EOF for the bare capillary. Since the EOF is also nearly constant for the sulfonated modified capillary over the entire pH range studied, or from pH 1.5-9.0, the ionization constant for the analyte becomes a more significant parameter in influencing migration time, resolution, and analysis time. Thus, with the sulfonated modified capillary the buffer pH can then be changed to influence ionization of the analyte, which will influence analyte migration time, rather than to influence EOF. Furthermore, since EOF is still reasonably high enough for the sulfonated modified capillary, particularly on the acidic side, other experimental variables that also influence EOF can still be critically optimized to improve migration time, resolution, and/or analysis time.

3.2. Effect of buffer cation

Several variables, in addition to buffer pH, that effect EOF and subsequently CZE separations have been identified and optimization of these parameters is essential in performing successful CZE separations. One variable that improves the quality of CZE separations is to alter the buffer cation and its concentration. While a change in ionic strength influences EOF [1] the effect on EOF is small and a much more significant EOF change is realized through control of the cation that is used for the buffer electrolyte or ionic strength electrolyte [11,17,18,29–42]. Manipulating the buffer cation and its concentration reduces EOF for the bare fused-silica capillary but the change is relatively small on the acidic side because the EOF in this pH region is already low. In contrast, when using the sulfonated modified capillary, EOF at an acidic pH is higher and changing the buffer cation and its concentration are viable parameters to optimize in order to influence analyte migration time, resolution, and analysis time. This approach of buffer cation control can be significant because cation optimization as a buffer additive often improves resolution in CZE separation of analytes that have similar electrophoretic mobilities.

An increase in buffer cation concentration causes EOF to decrease for both the fused-silica capillary [17,18] and the sulfonated modified capillary throughout the pH range studied in Fig. 1. Furthermore, the degree of EOF decrease is cation dependent. Fig. 2A and B illustrate how an increase in K^+ and Mg^{2+} concentration, respectively, decreases EOF at a buffer pH of 2.00 and 7.00. In both cases the 20 m*M* sodium phosphate buffer solution of appropriate pH is constant while the cations are added as either the K^+ or Mg^{2+} chloride electrolyte. The K^+ has little affect on EOF up to 60 m*M* K^+ and the small EOF increase at the higher K^+ concentration is probably due to excessive capillary heating since current at 60 m*M* K^+ is over 100 μA. For Mg^{2+} as the buffer additive, however, EOF is decreased significantly compared to K^+ even though the Mg^{2+} concentration is a tenth of the K^+ concentration and, as shown in Fig. 2B, decreases as Mg^{2+} buffer concentration increases. Furthermore, electrophoretic mobilities, which were determined with pyrimidine, purine, and nucleoside derivatives, are constant, as Mg^{2+} concentration increases, and

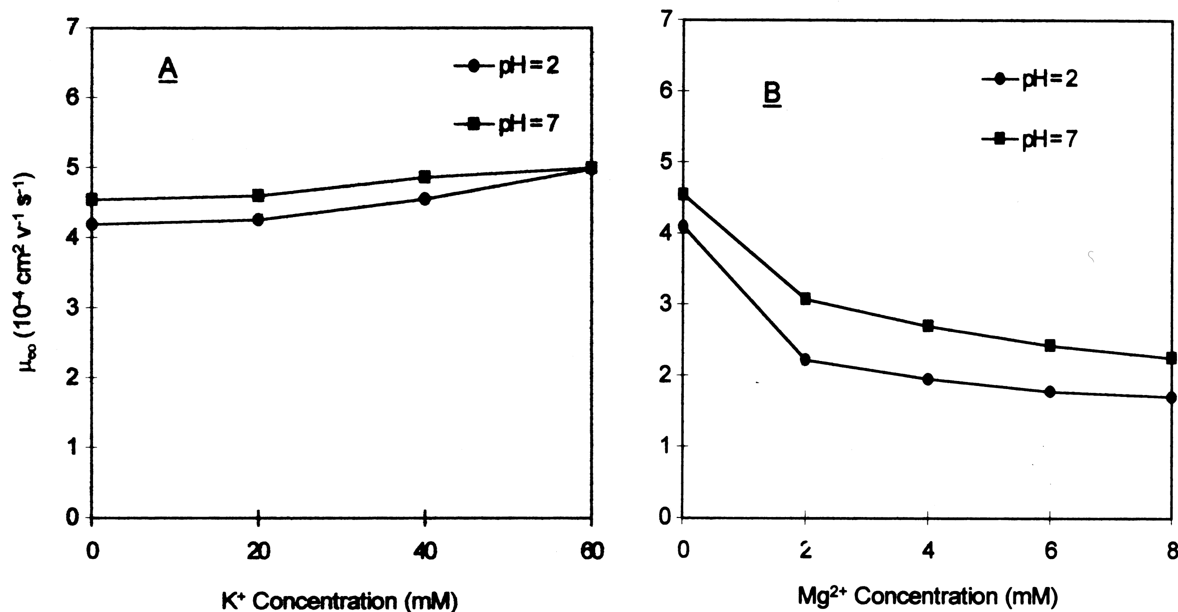

Fig. 2. Change in EOF with a sulfonated-modified capillary as a function of buffer K^+ (A) and Mg^{2+} (B) concentration at a buffer pH of 2.0 and 7.0.

the EOF change is due to the effect of Mg^{2+} as a buffer additive. As with the bare fused-silica capillary, where cation exchange occurs at the silanol sites and causes a change in surface charge [16,17], cation exchange takes place at the sulfonic acid sites for the sulfonated modified capillary.

When Li^{+}, Rb^{+}, and Cs^{+}, were studied as buffer additives as a function of cation concentration and buffer pH, their effect on EOF was similar to that found for K^{+} (see Fig. 2A). EOF differences were very small and a clear trend among the four alkali metal cations as buffer additives on EOF was not as well defined as observed previously with a bare fused-silica capillary [11,17,18]. When divalent cations, such as Cd^{2+}, Zn^{2+}, and Cu^{2+}, were studied as buffer additives from 0 to 3 m*M* in a citrate buffer (pH 3), EOF was reduced like with Mg^{2+} (see Fig. 2B) but more so. The fact that the divalent cations have a greater effect over the monovalent cations is consistent with the more favorable cation-exchange selectivities exhibited by the divalent cations towards typical strong acid sulfonated cation exchangers [44]. While there was a distinct difference between the four cations on reducing EOF with cation concentration the observed trend is also influenced by the formation of cation–citrate complexes and differences in complex formation. Although each divalent cation can be used as a buffer additive to reduce EOF and enhance resolution in CZE separations, only Mg^{2+} was used in the applications outlined in the following. The Mg^{2+} provided sharp, efficient migration peaks and did not cause precipitation over the pH range and in the presence of the buffers used in the study.

When a tetraalkylammonium salt is added to the buffer, the EOF for the sulfonated modified capillary is reduced and even reversed if the tetraalkylammonium salt contains a long chain, hydrophobic alkyl group. Fig. 3 shows how the EOF decreases and reverses as hexadecyltrimethylammonium cation (as the bromide salt) concentration increases in the buffer at pH 2.00 and 7.00. In both cases a 20 m*M* phosphate buffer is used at the appropriate pH.

The reversal is due to cation exchange between the tetraalkylammonium cations and the cations at the sulfonate sites. Because of the extensive hydrophobic character of the long chain alkyl group, interaction between the exchanged tetraalkylammonium cations

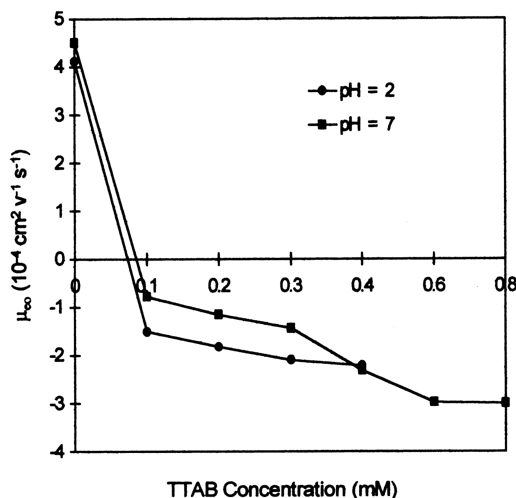

Fig. 3. Effect of hexadecyltrimethyl ammonium (TTAB) cation buffer concentration on EOF with a sulfonated-modified capillary at a buffer pH of 2.0 and 7.0.

at the sulfonated sites and the tetraalkylammonium cations in the buffer occurs via the hydrophobic groups. Thus, the capillary wall surface charge becomes positive which leads to the EOF reversal. A similar behavior is observed with the bare fused-silica capillary where tetraalkylammonium cation as a buffer additive undergoes cation exchange at the silanol sites and these subsequently interact with the tetraalkylammonium cations in the buffer to yield a positive wall surface charge [16,19,20]. Since EOF is nearly constant as a function of buffer pH in the absence of the tetraalkylammonium salt (see Fig. 1), the reversal in EOF is nearly of the same magnitude throughout a neutral to an acidic buffer condition. While an EOF reversal is also observed when using a bare fused-silica capillary and a hydrophobic tetraalkylammonium salt in the buffer [16,19,20], the degree of change in the EOF is pH dependent.

3.3. Effect of analyte pK_a

Since the EOF for the sulfonated modified capillary is constant over a broad pH range, the strategy of changing buffer pH to influence and improve migration time and resolution of the CZE separation of weak acid and base analytes can be based on the pK_a value of the analyte without concern as to how pH affects EOF. Table 1 lists the electrophoretic

Table 1
Electrophoretic mobility for several weak acids and bases as a function of buffer pH

Analyte	pK_a	Electrophoretic mobility X 10^{-4} cm^2 V^{-1} s^{-1}					
		pH					
		2	3	5	6	7	8
Phenol	9.98	0	0	0.01	0.32	0.06	0.01
2,6-Dichlorophenol	7.83	0	0	0.01	−0.03	−0.47	−1.83
p-Nitrophenol	7.15	0	0	−0.08	−0.55	−1.95	−1.83
2-Aminopyridine	6.86	3.80	3.74	3.71	2.64	0.87	0.24
Pyridine	5.23	4.35	1.89	1.82	0.32	0.06	0.09
p-Hydroxybenzoic acid	4.57	−0.05	−0.37	−2.46	−2.79	−2.85	−2.96
Benzoic acid	4.20	0.14	−0.82	−2.89	−3.17	−3.19	−3.20
p-Aminobenzoic acid	2.50 (1) 4.87 (2)	0.84	0	−2.33	−2.79	−2.94	−2.96
m-Nitrobenzoic acid	3.49	−0.57	−1.93	−2.89	−3.04	−3.04	−2.96
o-Nitrobenzoic acid	2.17	−2.34	−2.87	−2.92	−3.01	−3.02	−3.04
2,4-Dinitrobenzoic acid	1.43	−2.59	−2.74	−2.68	−2.87	−2.80	−2.78
Electroosmotic flow		4.20	4.15	4.14	4.37	4.54	4.58

mobilities determined for a series of weak acid and base analytes whose pK_a values fall within the pH 2–8 range. A phosphate buffer system, where P is constant at 20.0 m*M*, was used for all pH conditions and about 10 nl of 0.03 to 0.4 mg/ml sample was injected. The data in Table 1 show a direct correlation between analyte migration and analyte dissociation and buffer pH. As shown in Table 1, EOF (listed at the bottom of Table 1) gradually increases by only about 9% from pH 2 to 8 and analyte migration is largely determined by analyte mass and mobility of the dissociated and undissociated analyte form as pH is changed. For weak acid analytes μ_{ep} is significantly towards the positive electrode at high pH where the analytes are anions. As buffer pH is decreased μ_{ep} changes abruptly at the analyte pK_a and approaches the μ_{ep} for the corresponding undissociated, neutral form of the analyte. For the weak base analytes μ_{ep} is enhanced towards the negative electrode at low pH where the analytes are cations, abruptly changes at the pH corresponding to the analyte pK_a value, and approaches the μ_{ep} for a neutral analyte at a more basic pH.

3.4. CZE separation of weak organic acids and pyridines

Table 1 indicates that migration order for mixtures of weak acids and bases is dependent on buffer pH and analyte pK_a value since a nearly constant EOF is maintained throughout the pH 2–9 range. Fig. 4 shows the CZE separation of an eleven component mixture of carboxylic acids, phenols, and pyridines that cover a pK_a range of ~1.4–10 (see Table 1) at buffer pH conditions of pH 8.0, 6.0, 5.0 and 3.0, Fig. 4A, B, C and D, respectively. The three most important factors that determine migration time, migration order, and resolution are the pK_a values and mass for the analyte and to a lesser extent the small change in EOF that occurs as buffer pH is reduced (see Fig. 1 Table 1).

Small changes in buffer pH depending on analyte pK_a and mass have a significant effect on resolution. For example, *m*- and *o*-nitrobenzoic acid and 2,4-dinitrobenzoic acid are resolved at high pH where all three are ionized with the latter having the smallest migration time. As pH is decreased migration time slightly increases for the three because of the small decrease in EOF. At pH 5 in Fig. 4C migration time for the *m*-nitro- and *o*-nitrobenzoic acid analytes reverse with the *m*-derivative migrating the fastest because it is the least ionized. At pH 3, see Fig. 4D, the *m*-derivative migration time is further reduced while the migration times for the other two derivatives remain high because of their ionization. For the amphoteric *p*-aminobenzoic acid and the dibasic *p*-hydroxybenzoic acid only partial resolution is obtained at pH 8, see Fig. 4A. As the buffer pH is

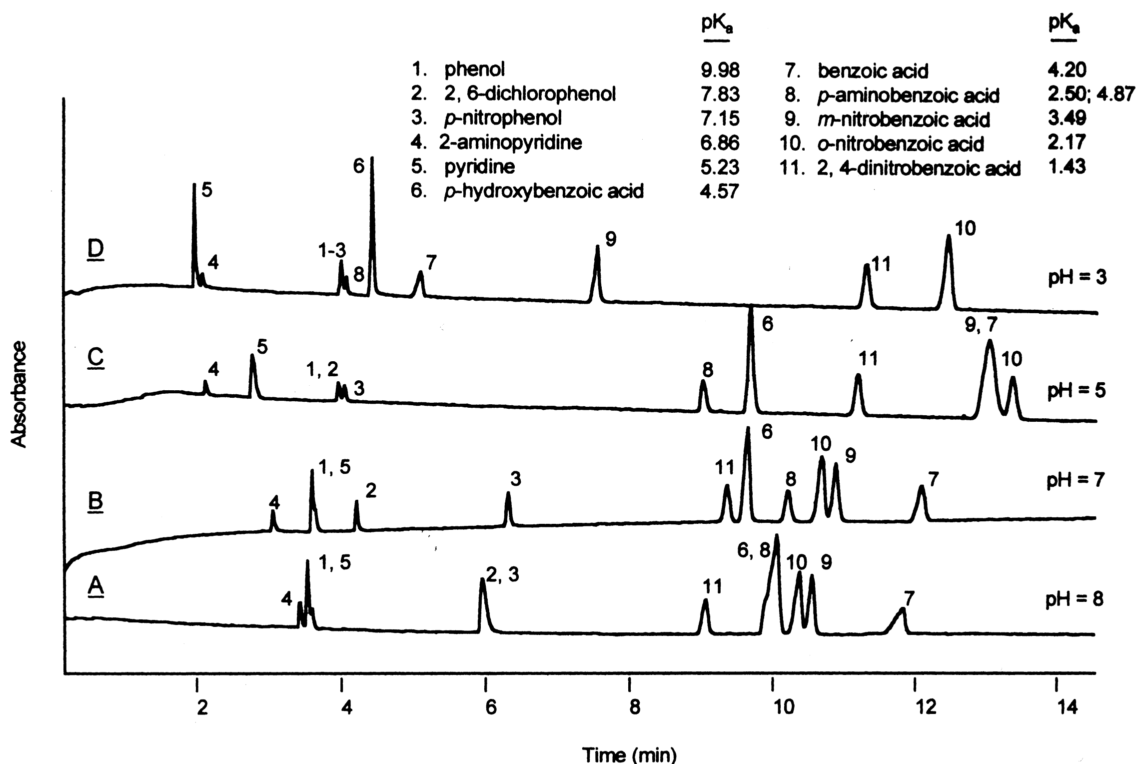

Fig. 4. Effect of buffer pH on migration time with a sulfonated modified capillary for several weak base and acid analytes that differ in pK_a values.

decreased, for example, pH 5 in Fig. 4C, the former migrates faster because of the difference in pK_a values and when the buffer is pH 3, see Fig. 4D, it migrates primarily as a neutral analyte while the *p*-hydroxy derivative is still migrating partially as a neutral analyte. Similarly, the migration of the two pyridine derivatives reverses in the cation migration region in Fig. 4A to D as buffer pH is reduced due to the difference in their pK_a values.

3.5. CZE separation of weakly basic aniline derivatives

Weakly basic aniline derivatives are protonated depending on their pK_b values at a low buffer pH. Thus, in a CZE separation the protonated weak base analytes migrate prior to the neutral marker and are resolved as cations with the sulfonated modified capillary because a sufficient EOF is still obtained at a strongly acidic condition. This is illustrated in Fig. 5 where pyridine and four different aniline derivatives with pK_a values ranging from about 3.7 to 5.2 are resolved. When Mg^{2+} is present in the buffer, migration time of the protonated weakly basic anilines is increased and resolution is improved. This is also illustrated in Fig. 5A, B, and C where Mg^{2+} concentration in the 20 m*M* phosphate buffer at pH 2.5 is increased up to 4 m*M* Mg^{2+}.

Migration order for the resolution of the amine mixture in Fig. 5 remains constant as Mg^{2+} concentration is increased in the buffer. The migration order follows molecular mass with the highest-molecular-mass aniline having the longest migration time, since at the buffer pH used all analytes are in the cationic form. However, if pH is changed migration order can be altered depending on the pK_a values for the aniline derivatives.

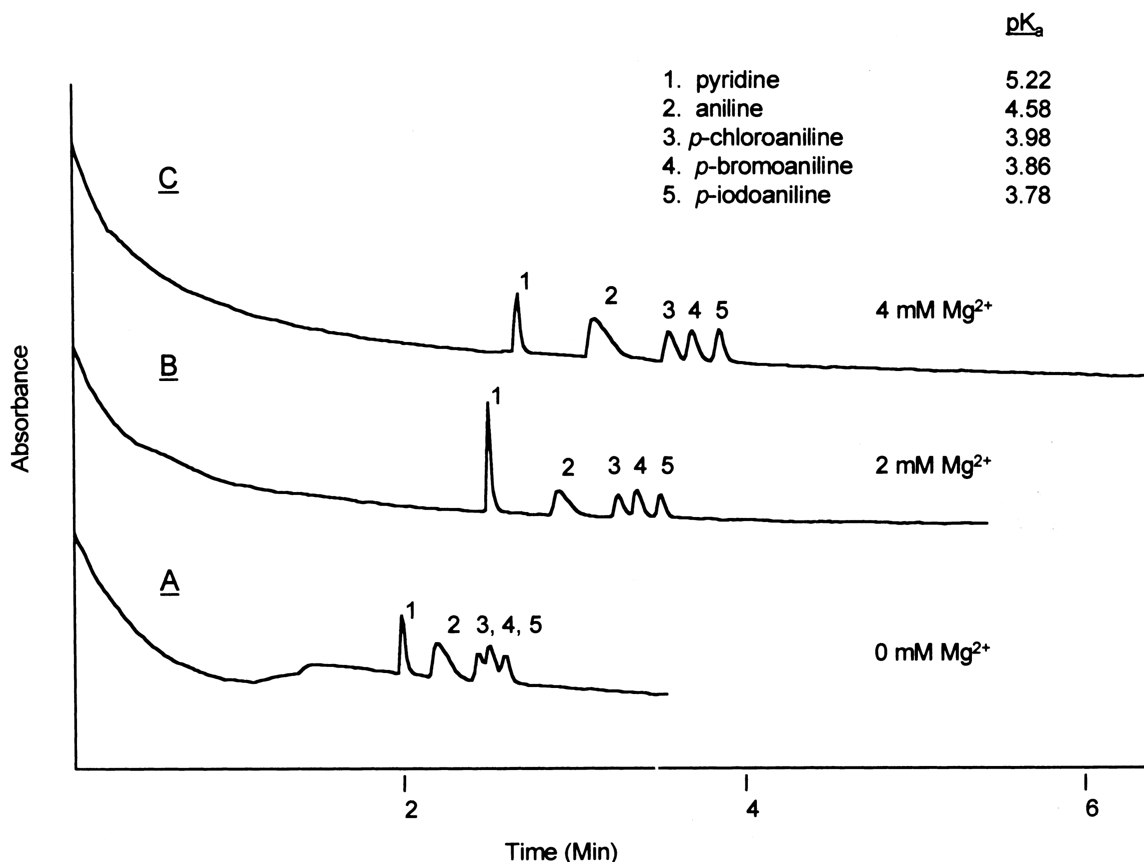

Fig. 5. Effect of Mg^{2+} buffer concentration on the resolution of several weak organic base analytes with a sulfonated-modified capillary and a buffer pH of 2.50.

3.6. CZE separation of purines, pyrimidines, and nucleosides

Several purine, pyrimidine, and nucleoside derivatives are very weak bases and protonation is possible only at low pH. These analytes like the aniline derivatives can be separated with the sulfonated modified capillary at a reasonable EOF in a an acidic buffer where the weakly basic purines, pyrimidines, and nucleosides are in their cationic forms except thymine. Fig. 6A, B and C illustrates the effect of buffer Mg^{2+} concentration on the separation of an eight-component mixture of purine, pyrimidine, and nucleoside derivatives at pH 2.00 using a 20 m*M* Tris–trichloroacetic acid buffer. Increasing Mg^{2+}concentration in the pH 2.0 buffer decreases EOF and both analyte migration time and resolution are increased, compare Fig. 6B and C to Fig. 6A.

If the buffer pH is increased, elution order changes because of differences in analyte pK_a values. This is illustrated in Fig. 7A and B where an eight-component mixture is separated at pH 2.00 and 3.00, respectively, using a Tris–trichloroacetic acid buffer. At the higher pH all the analytes have increased migration times. However, because of the change in protonation due to the different pK_a values, migration order also changes even though the buffer pH is changed by only one pH unit and is the most noticeable for analytes 3–8 where even reversals in migration order occur. Since EOF is constant at the two pH conditions in Fig. 7, the elution order is solely influenced by the pK_a of the analyte and the

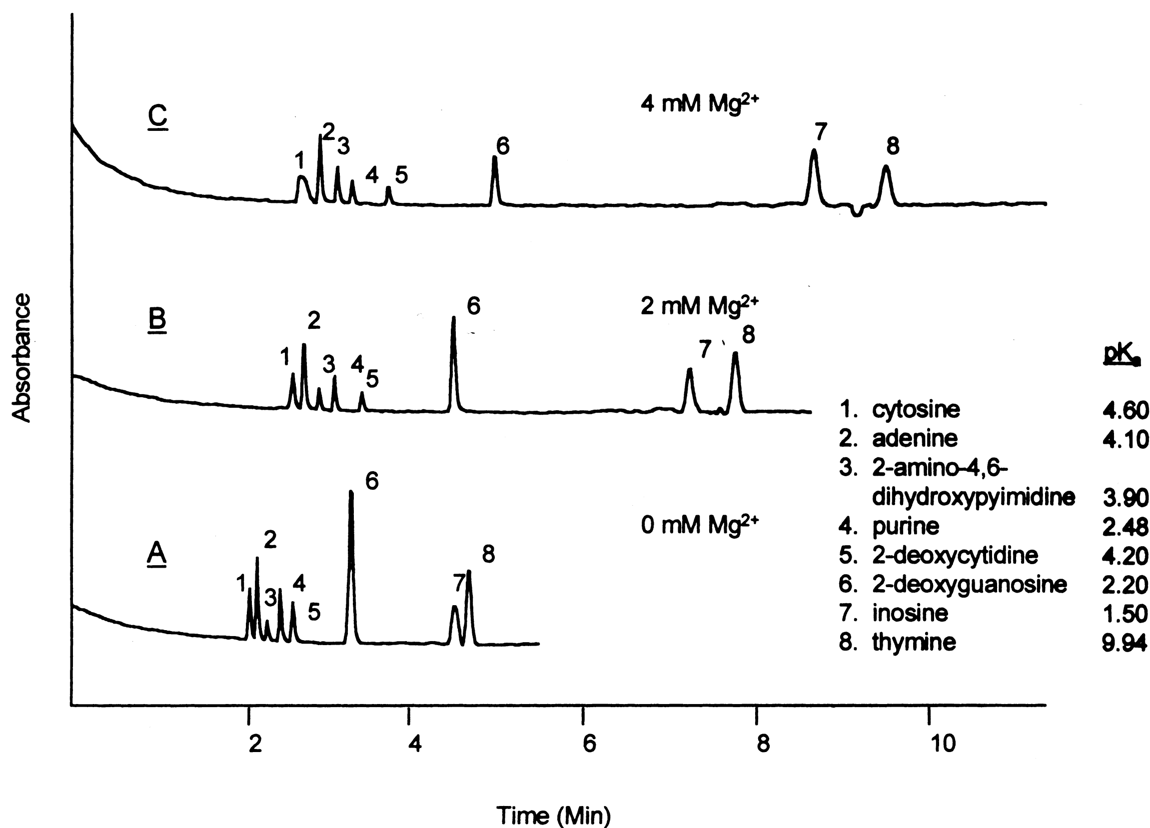

Fig. 6. Effect of Mg^{2+} buffer concentration on the resolution of several weak base purine, pyrimidine, and nucleoside derivatives with a sulfonated- modified capillary at a buffer pH of 2.0.

pH adjustment to alter protonation of the analytes. Although not shown in Fig. 7 adding Mg^{2+} to the buffer solution results in an additional increase in analyte migration time and resolution at both pH conditions because of the Mg^{2+} effect on EOF.

4. Conclusion

The EOF for a sulfonated-polymer modified open tubular fused-silica capillary is nearly constant over the pH range studied (pH 2–9), thus, buffer pH can be altered to influence analyte charge based on pK_a value rather than on its effect on EOF. The EOF is lower than the EOF found with a bare fused-silica capillary at a basic pH but is higher in the acidic range. The buffer pH and Mg^{2+} buffer concentration are variables that can be changed separately or in combination to optimize the migration time, migration order, and resolution in the CZE separation of weak organic acids and bases on the sulfonated modified capillary. Adjustment of buffer pH relative to the pK_a value of the analyte influences dissociation of the weak acids and determines their migration times as anionic analytes while for the very weak organic bases an acidic buffer allows these analytes to be separated as cations prior to the neutral marker. The Mg^{2+} in the buffer allows EOF to be changed and influences migration time since analyte electrophoretic mobility of the analyte is independent of Mg^{2+} concentration. Optimization of the two parameters will enhance the CZE separation of carboxylic acid and phenol derivatives as anions and the separation of weak base analytes, such as pyridine, aniline, pyrimidine, purine, and nucleoside derivatives, as cations.

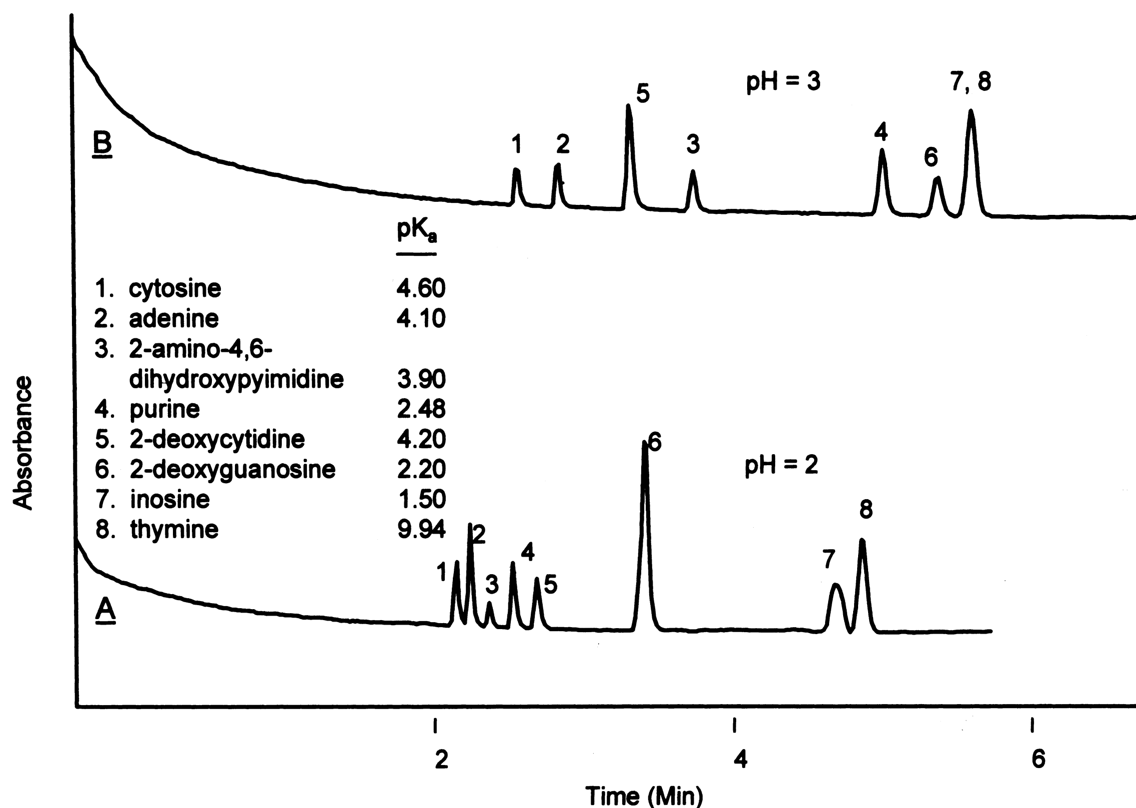

Fig. 7. Effect of an acidic buffer pH on the resolution of several purine, pyrimidine, and nucleoside derivatives with a sulfonated-modified capillary.

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