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Capillary-electrochromatographic separations with copolymeric reversed-stationary phase and ion-exchanger-packed columns

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Abstract

A macroporous, spherical, 7 μm, polystyrene–divinylbenzene (PS–DVB), reversed-phase adsorbent (PRP-1) was evaluated as a stationary phase for the capillary electrochromatographic (CEC) separation of neutral, acidic, and basic analytes of pharmaceutical interest. Electroosmotic flow (EOF) for a PRP-1 packed capillary is nearly constant over the pH 2 to 10 range and is higher than for a silica-based C_{18} packed capillary on the acidic side. EOF increases with an increase in buffer acetonitrile concentration or as applied potential increases. As analyte hydrophobicity increases, analyte retention and migration time increases. Increasing buffer acetonitrile concentration reduces analyte partitioning with the PS–DVB stationary phase and analyte retention and migration time decreases. When exchange sites are present on the PS–DVB copolymer, EOF (EOF is reversed for the anion-exchanger) increases as the exchange capacity increases. An increased exchange capacity also reduces partitioning of the analyte with the PS–DVB matrix and analyte retention and migration time decrease. Because of excellent stability in an acid environment, the PRP-1 packed capillary can be used in strong acid buffer solution and weak acid and base analytes depending on pK_a values can be separated as neutral species and cations, respectively. CEC separations on a PRP-1 capillary of neutral steroids, weak base pharmaceuticals (separation as cations), purines and pyrimidines (as cations), fatty acids (as undissociated species), and sulfa derivatives (as cations) are described. Efficiency for the PRP-1 packed capillary for acetone or thiourea as the analyte is about $6 \cdot 10^4$ plates m^{-1}.

Keywords: Electrochromatography; Stationary phases, electrochromatography; Mixed-mode chromatography; Polystyrene–divinylbenzene stationary phases; Steroids; Fatty acids; Purines; Pyrimidines; Sulfa compounds; Drugs

1. Introduction

A microparticle, macroporous, polystyrene–divinylbenzene (PS–DVB) copolymeric reversed-phase adsorbent has been used very successfully as a stationary phase in high-performance liquid chromatography (HPLC) [1]. In establishing the parameters that influence retention of neutral and charged analytes on a PS–DVB LC column several advantages of the PS–DVB column versus a typical silica-based C_{18} reversed-phase column were recognized. The PS–DVB column, which exhibits typical reversed-phase properties towards neutral analytes, has excellent stability in both low and high pH mobile phases and thus is particularly useful for the separation of weak organic acids or bases in either their neutral or charged forms. It has high analyte loading capacity and usually provides a linear isotherm over a wide concentration range. The principal disadvantage of a macroporous PS–DVB LC column, however, is that

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column efficiency is lower than for a typical C_{18} column due to a slower mass transfer. If the PS–DVB copolymeric matrix contains ion-exchange groups, the resulting ion-exchangers are also very useful in ion chromatographic strategies for the separation of inorganic and simple organic anions and cations [2,3].

A recent study [4] demonstrated that a macroporous PS–DVB copolymeric reversed stationary phase adsorbent and PS–DVB-based cation and anion-exchangers are suitable stationary phases for electrochromatography (CEC). Electroosmotic flow (EOF) and analyte migration time and resolution were found to be influenced by buffer concentration, pH, solvent composition, and inert electrolyte (cation) concentration and by applied voltage and ion-exchange capacity. Several of these experimental variables are also the ones that must be controlled and optimized in LC separations with these stationary phases.

Previously, most polymer-based stationary phases packed in capillaries were polyacrylamide-based copolymers. For example, soft crosslinked and non-crosslinked polyacrylamide gels formed in fused-silica capillaries are now commonly used in gel electrophoresis [5] and are particularly valuable in DNA sequencing studies [6]. Acrylamide monomers containing sulfonic acid groups and alkyl groups and a crosslinking agent have been copolymerized in fused-silica capillaries and used for the CEC separation of polycyclic aromatic hydrocarbons [7,8] and several alkylphenones, short chain peptides, and oligosaccharides [9]. A more rigid coploymer derived from butyl methacrylate, ethylene dimethacrylate, and 2-acrylamido-2-methyl-1-propanesulfonic acid was prepared in a fused-silica capillary and used for an efficient CEC separation of benzene derivatives with a phosphate, pH 7.0 buffer [10]. The porosity of the resulting monolithic column can be varied via the polymerization procedure. Molecular imprinted particles have also been packed in a fused-silica capillary and used for chiral CEC separation [11].

A PS–DVB reversed stationary phase adsorbent and PS–DVB-based ion-exchangers offer several advantages as stationary phases in CEC when compared to silica-based C_{18} reversed-phases and ion-exchangers [4]. EOF for the PS–DVB CEC column (acetone as the marker) is nearly independent of pH and is similar to a CEC silica-based C_{18} packed capillary in a basic buffer, depending on inlet and outlet silica frit thickness and length of the open part of the fused-silica capillary. In a strong acid environment the EOF is about twice as large. Increasing the ion-exchange capacity increases EOF (reversed for an anion-exchanger) and reduces analyte partitioning with the PS–DVB stationary phase matrix and thus, plays a major role in determining analyte migration time and analysis time. The exchange sites themselves can participate in analyte interaction. Thus, these stationary phases are capable of participating in a multimode type interaction mechanism. Residual silanol sites, which can be present with silica based stationary phases and can influence analyte partitioning, particularly for basic analytes in a basic buffer, are absent in the PS–DVB stationary phases. Since the PS–DVB stationary phases have good stability in a low pH buffer and have appreciable EOF at this condition, these packed capillaries are useful for the separation of weak acid analytes in their undissociated form and for weak basic analytes as cations.

Applications of silica-based C_{18} and other bonded phase CEC columns for the separation of organic and biological analytes of interest, which are reviewed in detail elsewhere [4,5,12–19], are numerous while silica-based ion-exchanger CEC column applications for the separation of neutral and charged analytes, including inorganic analytes, for example anions on a CEC strong base anion-exchanger packed capillary [20–22], are less in number. In contrast macroporous, micro particles of a PS–DVB-based reversed stationary phase or ion-exchangers packed in capillaries have been used in only a few cases. Neutral analytes, including simple organic structures that are often used as EOF markers, aromatic compounds, substituted benzene derivatives, aliphatic methyl ketones and aldehydes, and linear alkyl chain carboxylic acids have been separated [4]. A separation of two tetrapeptides in an acetonitrile–aqueous 25 m*M* phosphate, pH=3.5 (2:3, v/v) buffer was also reported for a PS–DVB packed capillary [23]. For the few CEC separation examples that have been reported for capillaries packed with PS–DVB particles [4,23,24] analyte peak shape, column efficiency (efficiencies are less than those obtained with silica-based C_{18} packed capillaries), and resolution are favorable.

A major advantage of PS–DVB-based stationary

phases in CEC applications is stability and favorable EOF in a strong acid buffer. These advantages are also demonstrated in this study where weak acid and base analytes of pharmaceutical and biological interest are separated with a fused-silica capillary packed with a PS–DVB (PRP-1) reversed stationary phase. The strong acid buffer allows weak acid analytes to be separated as neutral, undissociated species and weak bases to be separated as cations. A basic buffer allows the acid and base analytes to be separated as anions and neutral, undissociated species, respectively.

2. Experimental

2.1. Instrumentation and materials

The unit used for all CEC studies was a Spellman high-voltage d.c. power supply, Model UHR, 0–30 kV (Spellman High Voltage Electronics), a Spectra-Physics 100 UV–visible variable-wavelength detector equipped with an on-column capillary accessory, Model 9550-0155, and a packed fused-silica capillary. Buffer reservoirs containing the capillary inlet and outlet were connected to the power supply by platinum electrodes. The capillary/reservoir setup was encased in a Plexiglas box to prevent electrical shock. All packed capillaries were prepared with 75 μm I.D.×365 μm O.D. polyimide coated fused-silica capillary obtained from Polymicro Technologies. The reversed-phase adsorbent PRP-1, strong acid ($-SO_3^-$) cation-exchangers PRP-X300 (low exchange capacity) and PRP-X400 (high exchange capacity), strong base ($-R_4N^+$) anion-exchangers PRP-X100, RCX-10, RCX-30 (low to high anion-exchange capacity), and weak base ($-RNH_2$) anion-exchanger PRP-X700 (high anion-exchange capacity) used to pack the fused-silica capillaries were obtained in bulk form from Hamilton. All stationary phases are derived from a PS–DVB copolymeric matrix and are macroporous, spherical, microparticles of either 5 or 7 μm. Details of the stationary phases and the procedure used to pack the fused-silica capillary are available elsewhere [4]. Total capillary length for anion-exchanger packed capillaries was 27 cm and all others were 30 cm. The effective or packed length of all capillaries was 20 cm. The Data were collected and processed with a Spectra-Physics M-4270 integrator controlled by Spectra Physics Autolab Software.

LC studies were done with a Beckman 110B solvent delivery module, NECPC8300 gradient controller, sample injector, and Model 160 UV detector. Data were collected and processed with the Spectra-Physics Integrator/Software system. The LC reversed-phase PS–DVB (PRP-1) column obtained from Hamilton was a 150 mm×4.1 mm I.D. stainless steel column packed with 10 μm macroporous, spherical, micro particles.

Tris(hydroxymethyl)aminomethane (Tris) was obtained from Sigma. Inorganic reagents for buffer solutions were obtained from Fisher Scientific, Mallinkrodt, and Aldrich as analytical-reagent grade when possible. Pyrimidine, purine, and sulfa derivatives and basic drugs were purchased from Laboratory-Sigma and Aldrich. HPLC-grade acetonitrile was purchased from Fisher Scientific. Distilled water was passed through a Milli-Q-Plus water treatment unit with a final 0.2 μm filtration and was used for all sample and buffer solutions.

2.2. Procedures

All aqueous and mixed solvent buffer solutions were prepared with degassed solvents. Aliquots of aqueous solutions of phosphate (5 to 10 m*M*), tris and its hydrochloride salt (10–30 m*M*), or H_2SO_4 (5–10 m*M*) were mixed with acetonitrile to prepare the buffer solutions and are expressed as v/v. Buffer concentrations were low enough so that current passed was below 10 μA (usually 1–2 μA at 10 kV) but concentrated enough to have appreciable buffer capacity. Analyte stock solutions of 1 to 10 mg ml^{-1} were prepared in degassed acetonitrile:aqueous buffer solutions. Known aliquots of the stock solution was taken and diluted with the buffer solution to yield analyte stock solutions of about 0.1 to 1 mg ml^{-1}.

The packed capillary was filled with the desired buffer solution and then placed in the Plexiglas container with the inlet and outlet connected to the power supply. For the PRP-1 packed capillary the detector end is the cathode and the inlet end the anode. The capillary was subjected to 10 kV and allowed to equilibrate with the buffer solution for about 1 h prior to initiating the study. Packed capillary performance was monitored initially and

during each study using acetone as the EOF marker. Detection was either at 214 or 254 nm depending on the analytes and the buffer solution. Electrokinetic injection, usually for 5 to 10 s at 1 kV, was used for introducing the sample into the packed capillary. Sample sizes depending on the analyte were about 1 to 50 pmole. All measurements were done at ambient temperature or 23°C. Capillary lifetime depended on the range of buffer solutions employed and varied from a few days to several months. Data reported here represent the results for repetitive experiments; reproducibility of the acetone marker was typically less than ±2%. When a packed capillary was not in use it was stored containing an acetonitile:aqueous 20 m*M* Tris–Tris·HCl, pH 7.0 (4:1, v/v) with the capillary outlets also immersed in the buffer solution. Analyte peaks were verified by comparison to standards and by a spiking strategy. Peak efficiency, which is analyte, applied voltage, and buffer condition and pH dependent was $>5\cdot10^4$ plates per m (10 kV, pyrimidine/purine analytes; acetonitrile–aqueous 10 m*M* H_2SO_4, 1:9, v/v).

3. Results and discussion

3.1. Properties of polymer and exchanger packed capillaries

EOF (acetone as the marker) is appreciable for a fused-silica capillary packed with microparticle, macroporous, PRP-1, which is a reversed-phase adsorbent for hydrophobic neutral and charged analytes, even though the PRP-1 surface is neutral. The resulting EOF is largely due to the fused-silica wall and silica frits used in the packed capillary preparation [25]. Multimode processes influence analyte migration through the packed capillary and serve as the driving forces for the migration of neutral and charged analytes through the capillary. These include EOF, electrophoretic effects, and partitioning/adsorption of the analyte with the stationary phase. A key feature of the PRP-1 packed capillary is that EOF is nearly constant over the pH=2 to 10 range. For acetone as the marker EOF changes from 1.56 to 1.69 ($\times10^{-4}$ cm^2 v^{-1} s^{-1}) for a pH change from 2.5 to 7.0 (acetonitrile–aqueous 10 to 30 m*M* Tris–Tris·HCl, 4:1, v/v, buffer solution) [4] versus a change of 1.0 to 1.9 ($\times10^{-4}$ cm^2 v^{-1} s^{-1}) for a typical silica-based C_{18} packed capillary (acetonitrile–aqueous NaH_2PO_4/Na_2HPO_4 U, 7:3, v/v, buffer solution) [26]. An advantage of the PRP-1 capillary, therefore, is that buffer pH can be varied to influence weak acid and base analyte dissociation with little affect on EOF. This in turn influences analyte partitioning with the PRP-1 stationary phase and determines analyte migration time, resolution, and analysis time. Also, because the PS–DVB is acid and base stable, the entire pH range is available for adjusting analyte migration time and resolution. If ion-exchange sites are present on the PRP-1 stationary phase surface, EOF increases as the exchange capacity increases [4]; a similar observation of higher EOF with the presence of exchange sites also appears to be the case with silica-based ion-exchangers [4,16–22]. However, increasing the exchange capacity for the PS–DVB-based stationary phases also decreases the partitioning of the analyte with the PS–DVB matrix and causes analyte migration time to decrease. Thus, at high exchange capacity EOF, electrophoretic effects, and interactions at the exchange sites become the major driving forces affecting analyte travel through the packed capillary and analyte interaction with the PS–DVB matrix is a minor effect.

Fig. 1 compares resolution for a mixture of three neutral analytes of simple chemical structure on PRP-1, low and high capacity PS–DVB-based cation and anion-exchangers, and a PS–DVB-based weak base anion-exchanger. All three analytes undergo partitioning with the stationary phases including acetone and thiourea, which are frequently used as EOF markers in CE and CEC studies. The lower Tris concentration was used with the higher capacity exchangers to maintain a lower current through the packed capillary.

For PRP-1 and the low capacity cation-exchanger, PRP-X300, both EOF (applied voltage of +20 kV) and analyte partitioning with the stationary phase affect migration. Analyte migration follows hydrophobicity of the neutral analytes and the most polar, thiourea, is the least retained and the most nonpolar, benzene, is the most retained. When comparing PRP-1 and the low capacity cation-exchanger PRP-X300 in Fig. 1 analyte partitioning with the PS–DVB matrix of the latter stationary phase is reduced, EOF

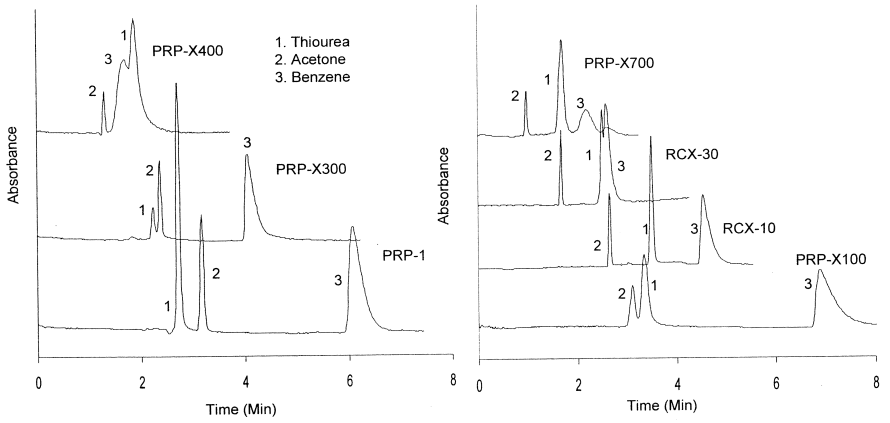

Fig. 1. Migration of analytes used as EOF markers with PRP-1 reversed-phase, PRP-X400 and PRP-X300 cation-exchangers, and PRP-X700, RCX-30, RCX-10, and PRP-X100 anion-exchanger packed capillaries. The buffer solution is 4:1 acetonitrile–aqueous 10 or 30 m*M* Tris–Tris·HCL, pH 7.0 U (4:1 v/v); the applied voltage is +20 kV for PRP-1 and the cation-exchangers and −20 kV for the anion-exchangers. The cation-exchanger capacities are: PRP-X300 (0.17 mequiv g^{-1}) and PRP-X400 (5.0 mequiv g^{-1}); the anion-exchanger capacities are: PRP-X100 (0.19 mequiv g^{-1}), RCX-10 (0.35 mequiv g^{-1}), RCX-30 (1.0 mequiv g^{-1}), and PRPX-700 (1.4 mequiv g^{-1}).

is increased, and migration time decreases. For the high capacity cation-exchanger, PRP-X400, in Fig. 1 analyte migration time is reduced further, EOF is increased, and the partitioning with the PS–DVB matrix changes significantly. Not only is the migration time reduced but the order is also changed and the high cation-exchange capacity stationary phase appears to be exhibiting some normal-phase partitioning.

The strong base anion-exchangers, PRP-X100, RCX-10, and RCX-30, and the weak base anion-exchanger, PRP-X700, in Fig. 1 have positive surfaces and reverses the EOF (applied voltage is −20 kV). Similar to the cation-exchangers increasing the anion-exchange capacity increases EOF and reduces partitioning of the analyte with the PS–DVB matrix. In all cases acetone for the three-component mixtures undergoes the least partitioning and has the shortest migration time while the benzene analyte partitions to the greatest extent and has the longest migration time.

3.2. Steroids

When mixtures of neutral analytes are separated on a PRP-1 packed capillary migration is influenced by both EOF and analyte stationary phase interactions and elution order tends to follow analyte hydrophobicity. This was previously demonstrated with mixtures of alkyl aldehydes, alkyl ketones, aromatic compounds, and substituted benzene derivatives [4]. Fig. 2 compares the CEC and LC separation of a mixture of neutral steroids and thiourea as the EOF marker with a PRP-1 packed capillary and a PRP-1 LC column, respectively. The same buffer composition was used in both cases. The analyte migration and elution order is the same for both CEC and LC and migration time and retention

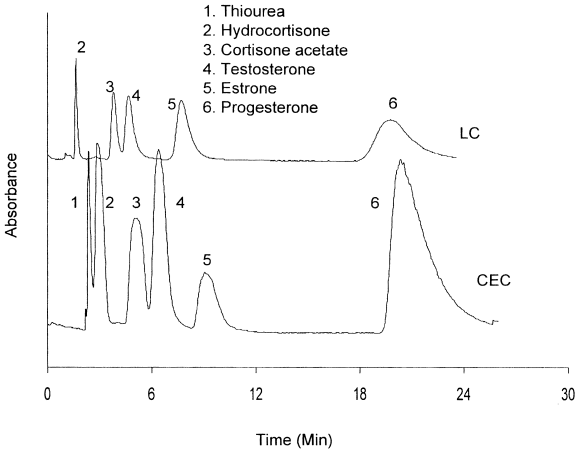

Fig. 2. The CEC and LC separation of a steroid mixture on a PRP-1 packed capillary and PRP-1 LC column. For CEC the buffer was acetonitrile–water, 30 m*M* H Tris–Tris·HCl, pH 8.0, (1:1 v/v) and an applied voltage of +30 kV; the eluent was the same at 1.0 ml min^{-1}.

time decrease as acetonitrile concentration increases. Thus, in both CEC and LC the differential migration of the neutral steroid analytes is consistent with a partitioning of the analytes with the PS–DVB stationary phase matrix. If the low cation-exchange capacity PRP-X300 is used for the CEC separation EOF is increased, partitioning with the PS–DVB matrix is reduced but not eliminated, and migration times for the steroids are reduced.

3.3. Basic pharmaceuticals

Fig. 3 shows the baseline resolution for the separation of four basic drugs, procainamide, procaine, tricane, and tetracaine, with a PRP-1 packed capillary as a function of buffer pH and acetonitrile concentration. All four drugs are weak bases with tricane having only an aniline-N group while the other three have both aniline-N and tertiary alkyl-N groups. At pH 10.5 the analytes are predominantly in their neutral form and the difference in analyte migration through the capillary is due to the difference in hydrophobicity of the neutral form of the analytes and the resulting partitioning with the PS–DVB matrix. It should be noted that resolution is possible even though the difference in structure is small. For example, procainamide and procaine differ structurally only in that the former is an amide while the latter is an ester. If the buffer acetonitrile concentration is increased from 60 to 70% (see Fig. 3) analyte partitioning with the PRP-1 decreases and analyte migration time is less. When the analytes are protonated by a more acidic buffer, they undergo less partitioning as cations with the PRP-1 stationary phase, and their migration through the column is accelerated. For example, when the buffer pH was lowered from pH=10.5 to 7.0 (see Fig. 3) the weak basic drugs are partially protonated, resolution is lost, and they migrate as a single peak close to the marker peak. Even with a lower applied voltage, which decreases the EOF, than used in Fig. 3, resolution is not improved significantly.

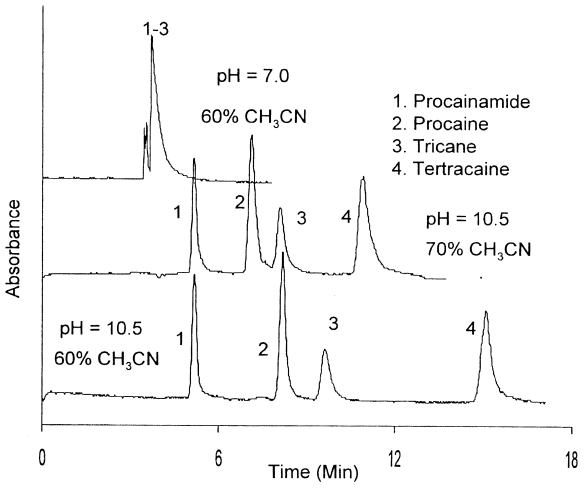

Fig. 3. The separation of four basic pharmaceuticals on a PRP-1 capillary and the effect of buffer pH and acetonitrile concentration on resolution. The buffer was acetonitrile–aqueous 5.0 m*M* phosphate, pH=7.0 or 10.5 buffer (6:4, v/v) and (7:3, v/v); +20 kV applied voltage at pH 10.5 and +10 kV at pH 7.0.

3.4. Fatty acids

The PS–DVB based reversed and ion-exchange stationary phases have extraordinary stability in strong acid and base solution and this can be an important advantage in LC separation applications of these columns [1]. This property is also useful in CEC with capillaries packed with the PS–DVB stationary phases, particularly in acidic solution, a condition which can be damaging to silica-based C_{18} and ion-exchangers. Fig. 4 shows the separation of several fatty acids with alkyl chain lengths from C_2 to C_{10} using a low capacity cation-exchanger PRP-

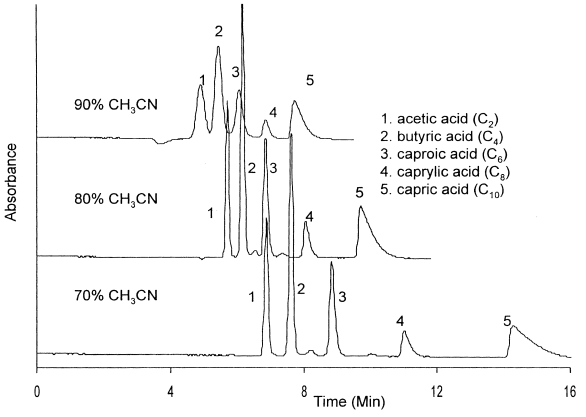

Fig. 4. The effect of acetonitrile concentration on the separation of several fatty acids on a low cation-exchange capacity (PRP-X300) packed capillary in a highly acidic environment. The buffer is acetonitrile–aqueous 10 m*M* H_2SO_4, (6:4, v/v) to (7:3, v/v), at +10 kV.

X300 packed capillary and an acidic 10 m*M* H_2SO_4 solution as a function of acetonitrile concentration. In the strong acid buffer fatty acid dissociation is suppressed and the acids are separated as neutral species. Discrimination among the fatty acid analytes occurs because of interaction of the hydrophobic portion of the acids with the PRP-X300 PS–DVB matrix. The undissociated acid mixture is baseline resolved, the fatty acid analyte peaks are well defined, capillary efficiency is $6.0 \cdot 10^4$ plates m^{-1} for acetic acid in the 70% acetonitrile buffer solution, and the analytes have migration times that increase with acid alkyl chain length (hydrophobicity). Interaction between the analytes and the PRP-X300 matrix is solvent dependent, just as in LC, and retention and migration time decreases as the buffer acetonitrile concentration increases. The small number of cation-exchange groups on the PRP-X300 increases EOF and enhances peak shapes. The higher EOF and modest reduction in the availability of the PS–DVB matrix for partitioning decreases migration time for the acid analytes in their undissociated form compared to the same separation done with a PRP-1 packed capillary [27]. When the PRP-1 packed capillary is used, EOF is less, analyte partitioning is greater, and analyte migration time is increased.

3.5. Purine and pyrimidine derivatives

Fig. 5 compares the CEC and LC separation of five purine and pyrimidine bases using a PRP-1 packed capillary and a PRP-1 LC column, respectively, as a function of acetonitrile concentration and a highly acidic environment. The background electrolyte and eluent are 1:9 and 3:7 acetonitrile:aqueous 5.0 m*M* H_2SO_4 U (1:9, v/v) and (3:7, v/v), respectvely. Electrophoretic migration, EOF, and partitioning with the stationary phase are major factors in the CEC separation. Migration time for acetone as the analyte EOF marker would be 6 to 6. 5 min (EOF at 10% and 30% acetonitrile corresponds to 1.53 and $1.70 \cdot 10^{-4}$ cm^2 v^{-1} s^{-1}, respectively, or an increase of about 10%). Cytosine, adenine, and purine, are predominately in their cation form in the H_2SO_4 background solution and migrate as cations undergoing partitioning with the PRP-1 stationary phase. The strongest base, purine, migrates last of the three because it has the more hydrophobic

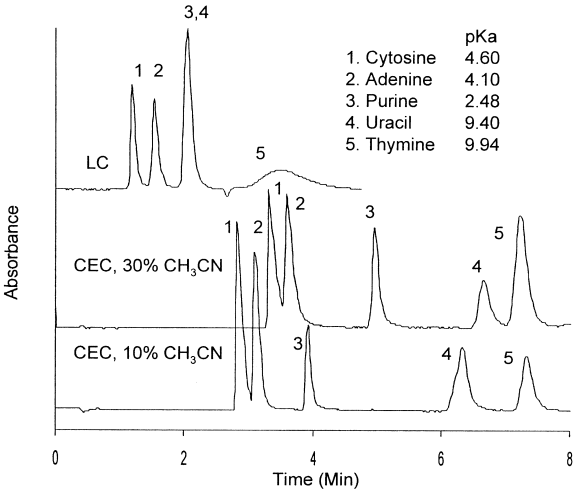

Fig. 5. The CEC and LC separation of purines and pyrimidines on a PRP-1 packed capillary and LC column. The buffer was acetonitrile–aqueous 5.0 m*M* H_2SO_4 (1:9, v/v) and (3:7, v/v) and an applied voltage of +10 kV; the LC eluent was aqueous 5.0 m*M* H_2SO_4 at 1.0 ml min^{-1}.

center. In contrast cytosine migrates first even though it is the weakest base of the three because it partitions the least due to its hydrophobic center is the least hydrophobic. Uracil and thymine are not in their cation form under the CEC conditions in Fig. 5 and their migration is influenced by EOF and partitioning. The increase in acetonitrile concentration from 10 to 30% in Fig. 5 is not sufficient to decrease the partitioning of the analytes with the PRP-1 stationary phase. The change in migration time between 10 and 30% acetonitrile in Fig. 5 is probably due to the effect of solvent change on analyte dissociation.

Fig. 5 also shows the LC separation of the five purine and pyrimidine analytes on a PRP-1 LC column using an aqueous 5 m*M* H_2SO_4 mobile phase at 1.0 ml min^{-1}. LC elution order is the same as the migration order for the PRP-1 CEC separation and is consistent with partitioning being a significant factor causing differential migration of the purine and pyrimidine derivatives in the PRP-1 CEC separation. When comparing the LC and CEC separation at these acidic conditions, resolution for the CEC separation is much better, particularly for purine and uracil. Peaks in the CEC separation are well-defined and efficiencies for cytosine, adenine, and purine are

about $6.0 \cdot 10^4$, $5.3 \cdot 10^4$, and $5.0 \cdot 10^4$ plates m^{-1} while for the LC separation efficiencies are $6 \cdot 10^3$, $5 \cdot 10^3$, and $4.5 \cdot 10^3$ plates m^{-1}, respectively.

3.6. Sulfa derivatives

Sulfa derivatives exhibit acidic and basic properties and exist as cations in strong acid, anions in strong base, and neutral species at an intermediate pH depending on their pK_a values. Migration of sulfa derivatives in an open tube fused-silica capillary has been studied from pH 2 to 9 [28,29]. The best resolution for complex mixtures of the sulfas in the open tube fused-silica capillary is at pH 6.9 where differences in ionization of the sulfas are the most significant. If a strong acid or base buffer is used resolution is very poor and many sulfa derivatives co-migrate. When a PRP-1 capillary and a strong acid background solution is used for the CEC separation of the sulfa derivatives resolution, which is acetonitrile concentration dependent, is markedly improved as shown in Fig. 6. Migration of the sulfa derivatives as cations is retarded because of partitioning of the charged derivative via the sulfa derivative hydrophobic center and the PS–DVB matrix of the stationary phase. The migration order also differs from the order obtained with the open tube fused-silica capillary. For the acidic CEC conditions used in Fig. 6, the migration time for acetone as the analyte EOF marker is 6 to 6.5 min and the EOF increases by about 6 to 7% when the acetonitrile concentration is increased from 20 to 30%.

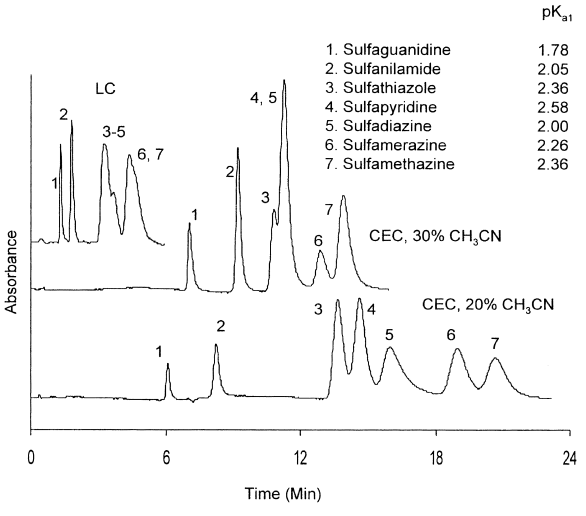

Fig. 6. The CEC and LC separation of sulfa derivatives on a PRP-1 packed capillary and LC column. The buffer was acetonitrile–aqueous 5.0 m*M* H_2SO_4 (1:5, v/v) and (3:7, v/v) at +10 kV; the LC eluent was 1:5 acetonitrile–5.0 m*M* H_2SO_4 (1:5, v/v), at 1.0 ml min^{-1}.

Sulfaguanidine and sulfanilamide migrate through the PRP-1 capillary ahead of an EOF analyte marker largely as cations with little partitioning. In contrast the other five sulfas, while also still cations, migrate more slowly (and after the analyte marker) because of increased partitioning with the stationary phase. Thus, their migration order is consistent with hydrophobic properties of the sulfa derivative. For an open tube fused-silica capillary, where pH 6.9 is used, pK_a values for the sulfa derivatives are the deciding factor in determining migration order and resolution. For the PRP-1 capillary and an H_2SO_4 environment, partitioning rather than pK_a value is the dominant factor in determining migration order and resolution.

When acetonitrile concentration in Fig. 6 is increased from 20 to 30%, migration of the last five sulfas is affected the most and their migration times decrease significantly. Migration times for the first two sulfa derivatives increase modestly because EOF is slightly increased and the solvent change has a small effect on dissociation. When the seven sulfa derivatives are separated on a LC PRP-1 column (also shown in Fig. 6) using an H_2SO_4 mobile phase, the sulfa derivative retention order is the same as the migration order for the CEC separation. Thus, partitioning of the sulfa derivatives with the PRP-1 matrix is the major factor determining the rate of travel through the PRP-1 capillary. A major difference, however, is that resolution for the LC separation is incomplete for the strong acid conditions used and several of the sulfa derivatives co-elute.

4. Conclusion

A PS–DVB adsorbent is a useful stationary phase for the CEC separation of neutral, acidic, and basic analytes. EOF for PRP-1 and the PS–DVB-based ion-exchangers is nearly independent of buffer pH. Thus, the focus of optimizing buffer pH can be on influencing weak acid or base dissociation rather

than EOF. EOF increases as exchange capacity or applied voltage increases and is reversed for anion-exchangers. Increasing acetonitrile concentration in the buffer decreases partitioning of the analyte with the PS–DVB stationary phase causing analyte migration times to decrease. When exchange sites are present the PS–DVB matrix is less accessible and analyte partitioning and migration times are reduced. The PS–DVB based adsorbent and ion-exchangers are stable in an acid environment and have an EOF that is greater than the EOF for a silica-based C_{18} stationary phase in an acidic buffer. Thus, an acidic buffer can be used to suppress dissociation of weak acid analytes so they can be separated as neutral species or to enhance dissociation of weak organic base analytes so they can be separated as cations. Mixtures of neutral steroids, weak basic pharmaceuticals as cations, fatty acids as neutral species, and purines, pyrimidines, and sulfa derivatives as cations are separated with good separation times, resolution, and efficiency. Efficiencies for the EOF markers, acetone and thiourea, and most fast moving analytes depending on the conditions are typically $6 \cdot 10^4$ plates m^{-1}.

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References

[1] D.J. Pietrzyk, in: P.R. Brown, R.A. Hartwick (Eds.), High Performance Liquid Chromatography, Wiley-Interscience, New York, 1989, pp. 223–276.
[2] P.R. Haddad, P.E. Jackson, Ion Chromatography: Principles and Applications, Elsevier, Amsterdam, 1990.
[3] J. Weiss, Ion Chromatography, VCH, Weinheim, 1995.
[4] Y. Liu, D.J. Pietrzyk, Anal. Chem. 72 (2000) 5930.
[5] S.C. Beale, Anal. Chem. 70 (1998) 279R.
[6] M.C. Ruiz-Martinez, J. Berka, A. Belenkii, F. Foret, A.W. Miller, B.L. Karger, Anal. Chem. 65 (1993) 2851.
[7] J.L. Liao, N. Chen, C. Ericson, S. Hjertén, Anal. Chem. 68 (1996) 3468.
[8] C. Fujimoto, Y. Fujise, E. Matsuzawa, Anal. Chem. 68 (1996) 2753.
[9] A. Palm, M.V. Novotny, Anal. Chem. 69 (1997) 4499.
[10] E.C. Peters, M. Petro, F. Svec, J.M.J. Fréchet, Anal. Chem. 69 (1997) 3646.
[11] L. Schweitz, L.I. Anderson, S. Nilsson, Anal. Chem. 69 (1997) 1179.
[12] A.L. Crego, A. González, M.L. Marina, Crit. Revs. Anal. Chem. 26 (1996) 261.
[13] M.M. Robson, M.G. Cikalo, P. Myers, M.R. Euerby, K.D. Bartle, J. Microcol. Sep. 9 (1997) 357.
[14] L.A. Colón, Y. Guo, A. Fermier, Anal. Chem. 69 (1997) 461A.
[15] T. de Boer, R.A. de Zeeuw, G.J. de Jong, K. Ensing, Electrophoresis 2220 (1999) 2989.
[16] K. Walhagen, K.K. Unger, A.M. Olsson, M.T.W. Hearn, J. Chromatogr. A 853 (1999) 263.
[17] M. Ye, H. Zou, Z. Liu, J. Ni, J. Chromatogr. A 869 (2000) 385.
[18] P. Huang, X. Jin, Y. Chen, J.R. Srinivasan, D.M. Lubman, Anal. Chem. 71 (1999) 1786.
[19] J. Zhang, X. Huang, S. Zhang, C. Horváth, Anal. Chem. 72 (2000) 3022.
[20] D. Li, H.H. Knobel, V.T. Remcho, J. Chromatogr. B 695 (1997) 169.
[21] M. Ye, H. Zou, Z. Liu, J. Ni, Y. Zhang, Anal. Chem. 72 (2000) 616.
[22] E.F. Hilder, M. Macka, P.R. Haddad, Anal. Commun. 36 (1999) 299.
[23] G. Choudry, Cs. Horváth, J. Chromatogr. A 781 (1997) 161.
[24] M. Hugener, A.P. Tinker, W.M.A. Niessen, U.R. Tjaden, J. van der Greef, J. Chromatogr. 647 (1993) 375.
[25] E.F. Hilder, C.W. Klampfl, M. Macka, P.R. Haddad, P. Myers, Analyst 125 (2000) 1.
[26] N.W. Smith, M.B. Evans, Chromatographia 41 (1995) 197.
[27] Y. Liu, Ph.D. Thesis, University of Iowa, Iowa City, IA, 2001.
[28] C.E. Lin, C.C. Chang, W.C. Lin, J. Chromatogr. A 768 (1997) 105.
[29] C.E. Lin, W.C. Lin, Y.C. Chen, S.W. Yang, J. Chromatogr. A 792 (1997) 37.