Ethylammonium Formate as an Organic Solvent Replacement for Ion-Pair Reversed-Phase Liquid Chromatography

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Abstract

Ethylammonium formate (EAF), an inexpensive and easily synthesized room-temperature ionic liquid, acts like a conventional organic solvent for reversed-phase liquid chromatography (LC). In this report, the use of standard ion-pair reagents with this ionic liquid LC mobile phase and a polystyrene-divinylbenzene PRP-1 column is explored. Starting with the column equilibrated with a methanol mobile phase, the required equilibration time of the column by the EAF ion-pair mobile phase is determined by the plate number profile. Chromatograms of six aromatic carboxylic acids, with either methanol or EAF as the mobile phase, at room temperature (in the absence of an ion-pairing agent) lack resolution with significant peak overlap of nitro-substituted benzoic acids. The addition of 30mM tetrabutylammonium ion to the EAF or methanol mobile phase provides baseline resolution for all peaks in approximately 10 min. Analogous studies using a mixture of four aromatic amines, including protonated tyramine, diphenhydramine, and neutral nitroanilines in the absence or presence of 30mM sodium dodecylsulfate (SDS) in the mobile phase are similar to those for the aromatic acids, indicating baseline resolution with only the ion-pair reagent. Raising the column temperature to 55°C improves the plate count by a factor of approximately 1.2 when using the EAF mobile phase. The retention factor profiles for either the carboxylic acids or the amines, as a function of the organic modifier percentage or ion-pair reagent concentration, are similar for both EAF and methanol. The polymerized acyl monoglycinate surfactant, poly(sodium-*N*-undecenoyl glycinate), is used for the first time as an LC ion-interaction reagent and is about as effective as SDS for the resolution of organic amines.

Introduction

Ionic liquids in capillary electrophoresis (CE) and liquid chromatograpy (LC) have predominantly been applied as mobile phase additives at mM levels. As mobile phase additives, imidazolium-based ionic liquids can act as a dynamic capillary coating for CE and shield residual silanols present in a silica-based stationary phase for LC. Although these topics have been described in recent review articles (1–4), it has been emphasized that imidazolium ionic liquids can also affect solute retention as ion-pairing agents through adsorption on the C18 stationary phase (5).

The tetrabutylammonium (TBA) ion-pair reagent and 1-butyl-3-methylimidazolium tetrafluoroborate, both varied from 0–50mM in a methanol (MeOH)–water mobile phase, were compared separately for the LC separation of phthalic acids and amines (6). The retention of o-, m-, and p-phthalic acids increased by factors of 5, 1.5, and 1.2, respectively, after TBA was added, though the retention of the four bases gradually decreased by a factor of 1.3. In contrast to TBA, the retention of both phthalic acids and amines decreased when the concentration of the imidazolium ionic liquid increased. A retention mechanism involving ion-pairing and hydrogen bonding between the imidazolium cation and its counter-ion with the solutes is proposed.

Short-chain alkylammonium nitrates have been shown to be room-temperature ionic liquids that are useful organic solvent replacements for reversed-phase (RP) LC. The potential of alkylammonium nitrates for the separation of proton donor and acceptor compounds was demonstrated by Poole et al. (7–9). The RP retention of 2,4-dinitrophenol was particularly enhanced using n-propylammonium nitrates instead of MeOH in the aqueous mobile phase with a C18 column. Chromatographic and spectroscopic methods for the determination of solvent properties of room-temperature ionic liquids have been reviewed by Poole (10). Emphasis is placed on physicochemical properties, using solvatochromic methods, and the determination of the gas–liquid partition coefficients is made.

Previously, alkylammonium carboxylate ionic liquids have been shown to be viable nonvolatile substitutes for MeOH for RPLC with detection in the moderate UV region (11–13). As expected, elution of salicylate or phenol was not possible even when using 100% of a conventional salt solution, such as 2M ammonium sulfate or 2M ammonium formate. A concentrated (2.3M) solution of the low-melting point ionic liquid ethylammonium acetate (EAA) acted like an organic solvent to control

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the retention of a salicylate, nitrofurantoin, and phenol test mixture in that order. It also provided enhanced retention for nitrofurantoin, which eluted first when using a MeOH–water mobile phase. Retention control by EAA of the water-soluble vitamins on LC columns designed for totally aqueous mobile phases is facile (11). Ethyl-, n-propyl-, and n-butylammonium formate have comparable polarity (P') values in the 5.8–6.3 range, and solvent strength (S) values in the 1.8–3.2 range, both parameters were similar to those for MeOH (12). In comparison to other room-temperature ionic liquids, the viscosity and UV cutoff for ethylammonium formate (EAF) were particularly favorable at 11 cpoise and 250 nm, respectively. The retention of ionic compounds, such as salicylate and benzenesulfonate, using the EAF mobile phases in the absence of ion-pair reagents was slight. The retention for salicylate decreased with increasing pH, as expected for only RP retention (13). In contrast, the retention factor (k') for salicylate, using a MeOH–2M ammonium sulfate or MeOH–2M ammonium formate mobile phase, increased by approximately a factor of 1.7–1.8 as compared with a MeOH–water mobile phase, indicating some apparent ion-pair retention. There has also been a report of the apparent ion-pair enhancement of the retention of sulfonated steroids when using ammonium sulfate (14). The addition of a conventional ion-pair reagent to the EAF mobile phase would be expected to enhance the retention of ionic compounds. To the best of our knowledge, there is no report of using ionic liquids as organic solvent replacements together with conventional ion-pair reagents in LC mobile phases to facilitate the separation of charged organic compounds.

In the present work, ion-pair RPLC methods are compared for the separation of both aromatic amines and aromatic carboxylic acids, using either an ionic liquid or an organic solvent as the mobile phase modifier. The retention behavior of the compounds was evaluated both in the presence and absence of ion-pair reagents, tetrabutylammonium (TBA) and sodium dodecyl sulfate (SDS), with either EAF or MeOH as the solvent modifier. In addition, the poly(sodium N-undecenoyl glycinate) polymerized surfactant is compared with SDS as an ion-interaction agent. Polymerized surfactants as pseudostationary phases for micellar electrokinetic chromatography have recently been reviewed (15), but they have yet to be explored as ion-interaction agents for LC. Column temperatures above ambient were also evaluated in order to improve the separation efficiency; therefore, a polymeric PRP-1 column is used for all separations. This column has previously been shown to be effective for ion-pair LC (16). The interaction of the ionic liquid mobile phases with residual silanols should also not be a factor.

Experimental

Instrumentation

All LC experiments were conducted on a Beckman Gold Nouveau computer controlled system (Fullerton, CA) equipped with solvent delivery pumps (A was the aqueous phase and B was the organic phase), a model 7725i Rheodyne injector (20 µL loop) (Rohnert Park, CA), a Waters TCM column heater (Milford MA), and a Model 166 variable-wavelength UV–vis detector set at 254 nm. A Hamilton PRP-1 column (Reno, NV), 100Å RP-HPLC column (150 × 4.1 mm, 5 µm), was used. The PRP-1 column was packed with crosslinked poly(styrene-divinylbenzene) particles and was stable throughout the pH range, even at elevated temperatures.

Reagents

The ethylamine, as a 70% solution, was obtained from Aldrich (Milwaukee, WI) and used as received. Formic acid (98%, high purity) was from Fluka (St. Gallen, Switzerland). The EAF was synthesized by mixing, at sub-ambient temperature, formic acid with a stoichiometric equivalent of ethylamine over a 4 h period in the presence of bubbling N_2, as previously described (12). All of the following sample components were of at least analytical grade. p-Hydroxybenzoic acid, acetylsalicylic acid, 4-nitrobenzoic acid, tetrabutylammonium, sodium dodecyl sulfate, tyramine, and diphenhydramine were obtained from Sigma-Aldrich. Salicylic acid and benzoic acid were purchased from Fisher Scientific (Pittsburgh, PA). 3,5-Dinitrobenzoic acid came from J.T. Baker (Phillipsburg, NJ) and p-nitroaniline was obtained from Matheson, Coleman, and Bell (Cincinnati, OH). The acyl

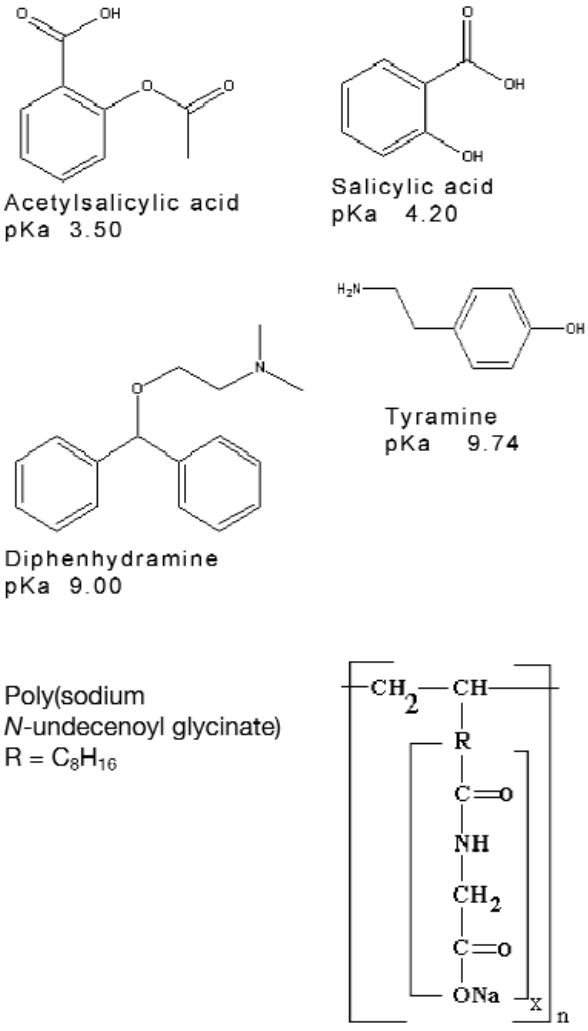

Figure 1. Structures of selected test compounds and the acyl glycinate ion-pairing agent, poly(sodium *N*-undecenoyl glycinate).

monoglycinate poly(sodium N-undecenoyl glycinate), with n = 45, was synthesized as previously described (15). Structures for this N-undecenoyl glycinate, as well as selected test solutes, are shown in Figure 1. Trace LC-grade MeOH was from Pharmco (Brookfield, CT). Redistilled, deionized water prepared in the laboratory using a Barnstead Model D4641 E-pure system (Dubuque, IA) was used throughout.

Chromatographic conditions

Two classes of compounds were studied: aromatic carboxylic acids and amines. A solution of 40 mg/L for each analyte was prepared by dissolving each compound in a 40% EAF–60% water or phosphate buffer or 40% MeOH–60% phosphate buffer and sonicating for 10 min to ensure complete dissolution. The column, flushed first with 100% MeOH and then with 50% MeOH–50% water, was allowed to equilibrate with the mobile phase for at least 20 min. Triplicate injections of the mobile phase were performed to identify any solvent-related peaks, followed by triplicate injections of the samples. Chromatograms were obtained at either room temperature, at a flow rate of 1.0 mL/min, or at 55°C, at a flow rate of 1.8 mL/min. All retention factor (k') values represent an average of three replicates. Plate count numbers were determined from expanded chromatograms and calculated using peak widths measured at the baseline.

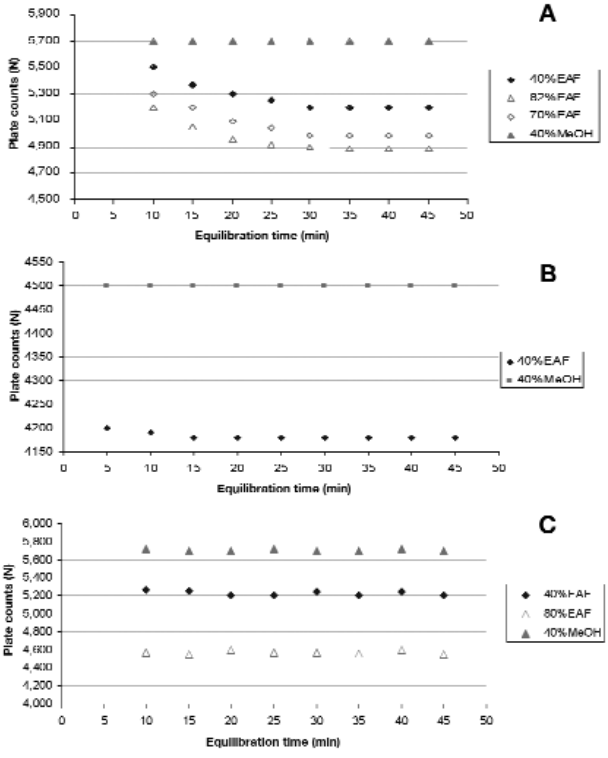

Figure 2. Reproducibility of plate counts (N) with change in equilibration time (min) from a starting mobile phase of 100% MeOH using: 40% EAF–phosphate buffer, 80% EAF–phosphate buffer, 70% EAF–phosphate buffer, or 40% MeOH–phosphate buffer, pH 6, 30mM TBA, 25°C for the test compound 4-nitrobenzoate (A); 40% EAF–phosphate buffer or 40%MeOH–phosphate buffer, pH 6, 30mM SDS, 25°C, for the test compound diphenhydramine (B). After a starting mobile phase of 50% MeOH–50% water, 40% EAF–phosphate buffer, 80% EAF–phosphate buffer, or 40%MeOH–phosphate buffer, pH 6, 30mM TBA, 25°C, was equlibrated for the test compound 4-nitrobenzoate (C).

Results and Discussion

Column equilibration studies with ion-pair mobile phases

One problem encountered in ion-pair chromatography was that column equilibration can be especially slow when the ion-pair reagent is quite hydrophobic, such as TBA or SDS (17). Another study (18) also showed that the equilibration time for the adsorption of perfluorocarboxylate (C3–C7) ion-pair reagents on various C18 columns increased with chain length. However, it did not change with a concentration ranging from 0.25–1mM. Although it would seem imperative to confirm reproducibility of chromatographic retention when the eluent used contained an ion-pair reagent, in general, equilibration time of the ion-pair mobile phases has not been widely studied. In addition, because of higher mobile phase viscosity when using EAF, the low diffusion coefficient of the analyte in the mobile phase may affect retention reproducibility. Column regeneration using 100% MeOH is not recommended because of a fairly long equilibration time of 30 min when switching from MeOH to the EAF mobile phase with TBA (Figure 2A). Figure 2B shows that the plate count change that occurs when switching from 100% MeOH to EAF with an SDS mobile phase is more modest with time. In order to ensure that no irreversible surface adsorption occurred, the column was regenerated, first with 100% MeOH and then with 50% MeOH–50% water, before equilibration with the ion-pair mobile phase. Figure 2C shows the change in the plate count for 4-nitrobenzoate with time, going from 50% MeOH–50% water to 40% or 80% EAF in a phosphate buffer with TBA now being minimal. Fast equilibration times on the order of 10 min were now possible. It was suspected that the low viscosity of MeOH compared with EAF was the cause; thus, increasing the viscosity by using 50% MeOH% water proved necessary.

Chromatographic comparison using EAF and MeOH with and without the ion-pairing agent

The chromatogram of five aromatic carboxylic acids with EAF in the absence of an ion-pairing agent lacked resolution and had significant peak overlap of the nitro-substituted benzoates (peaks 5 and 6) (Figure 3A). Using a mobile phase with similar polarity, but with MeOH as the modifier solvent, the chromatogram showed an improvement, but it showed no baseline resolution for the nitrobenzoate solutes and approximately the same analysis time of 6 min (Figure 3B) was needed. The retention order of p-hydroxybenzoate, benzoate, and 4-nitrobenzoate was the same as previously reported at high pH on a PRP-1 column (19). The average value and standard deviation (n = 3) of the retention factor (k') for BA, SA, and 4-NBA using EAF and TBA as seen in Figure 3A was 2.00 ± 0.10, 2.51 ± 0.11, and 5.12 ± 0.09, respectively. These standard deviations were similar to those found for analogous retention factor (k') data using the MeOH and TBA mobile phase (Figure 3B).

The addition of a 30mM TBA ion to the EAF or MeOH mobile phase showed baseline resolution for all peaks (Figures 4A and

4B). Analysis times were now about double, in the 10–12 min range. Although the chromatograms looked similar, the MeOH chromatogram, as expected, indicated plate numbers approximately 1.5–2.5 times better. A potential application was the hydrolysis of aspirin tablets in 0.1M HCl to form the salicylate product that could be followed as a function of time.

The amount of ion-pair reagent adsorbed onto the hydrophobic stationary phase was controlled by varying the percent of organic solvent in the mobile phase (20). The change in retention factor (k') of 4-nitrobenzoate, with EAF from 20–80% in the phosphate buffer and TBA-containing mobile phase, was compared with a similar percentage range for MeOH. Typically, a plot of log k' versus % organic modifier in RPLC showed a linear decrease in retention factor, with an increase in % organic modifier (ϕ) in accordance with the equation:

$$\log k' = \log k_W - S\phi \qquad \text{Eq. 1}$$

where k'_W is the capacity factor for pure water or aqueous buffer solution, and S is constant, representing the solvent modifier strength. This linear relationship has been previously reported for the ion-pair LC of some classes of solutes, such as carboxylic acids (21), phenols (21), and polythionates (22). For EAF, the linear least squares regression equation was:

$$\log k' = -0.72(\phi) + 0.97 \qquad \text{Eq. 2}$$

with $R^2 = 0.9908$. The analogous equation for MeOH was:

$$\log k' = -0.89(\phi) + 0.91 \qquad \text{Eq. 3}$$

with $R^2 = 0.9850$. The retention factor (k') values for EAF were consistently about 1 unit higher than the corresponding ones for MeOH because of the higher polarity (P') of EAF (P' = 6.4) than MeOH (P' = 5.1), as measured previously (12). The presence of the ion-pairing agent TBA does not seem to affect the RP modifier capability of EAF.

Using a test mixture of aromatic amines, chromatographic resolution was particularly better for the nitroaniline compounds when using EAF as the mobile phase modifier without an ion-pairing agent, as oppose to MeOH (Figures 5A and 5B). This enhanced retention of nitro aromatics, when using alkylammonium-based ionic liquids, has previously been observed with n-propylammonium nitrate and alkylammonium carboxylates (8,11). The addition of 30mM SDS to either MeOH or the EAF mobile phases provided a baseline separation of the mixture in 9–10 min (Figures 6A and 6B). As expected, the retention of the protonated amines (peaks 3 and 4) was improved, markedly, to approximately 4 min for diphenhydramine. Although the chromatograms look similar, the MeOH chromatogram indicated plate numbers approximately 1.1–1.4 times better.

Although the nitroaniline compounds have low pKa values (< 3) and should not ion-pair at pH 6, enhanced retention for these compounds was observed using either EAF or MeOH in the presence of SDS. One explanation was that the electron-withdrawing

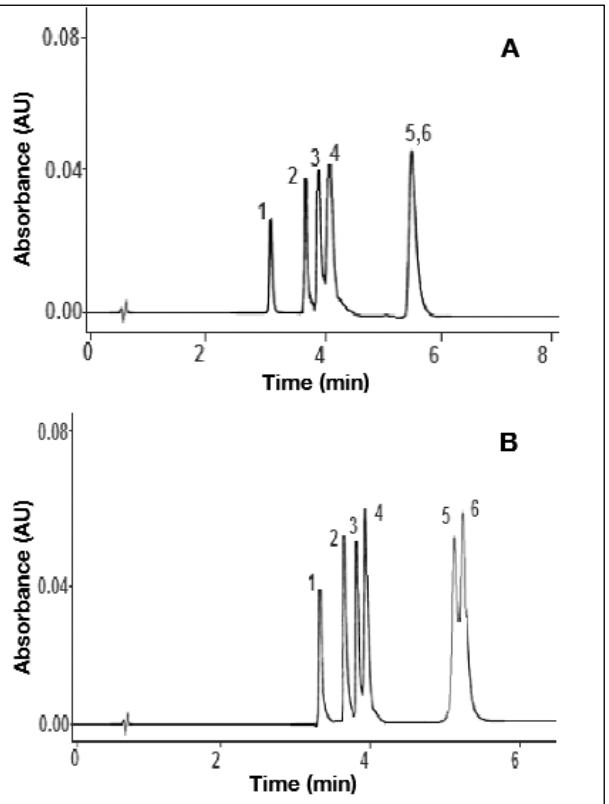

Figure 3. Chromatograms of aromatic carboxylic acids. 40% EAF–60% 0.05M phosphate buffer, pH 6.0 (A) and 40% MeOH–60% 0.05M phosphate buffer, pH 6.0 (B). The peak numbers are: *p*-hydroxybenzoate, 1; acetylsalicylate, 2; benzoate, 3; salicylate, 4; 3,5-dinitrobenzoate, 5; 4-nitrobenzoate, 6.

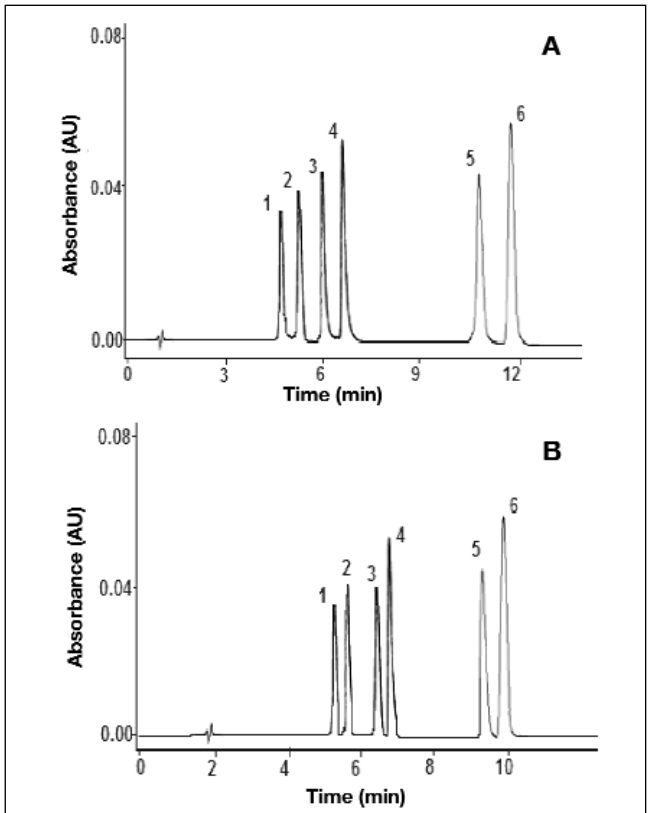

Figure 4. Chromatograms of aromatic carboxylic acids with the ion-pairing agent. 40% EAF–60% 0.05M phosphate buffer, pH 6.0, 0.03M TBA (A) and 40% MeOH–60% 0.05M phosphate buffer, pH 6.0, 0.03M TBA (B). Peak numbers are: *p*-hydroxybenzoate, 1; acetylsalicylate, 2; benzoate, 3; salicylate, 4; 3,5-dinitrobenzoate, 5; 4-dinitrobenzoate, 6.

nitro group intensified the positive charge of the aromatic ring carbonium ion, which could interact with SDS. The electron release by the amine group through resonance may not counteract effectively. However, in contrast, retention control of nitroanilines (using an octanesulfonate ion-pairing reagent on a silica based C8 column) was not effective in improving peak resolution (23).

Figure 6C shows the chromatogram of amines using the poly(sodium N-undecenoyl glycinate) polymeric surfactant as the ion-interaction agent, instead of using SDS with EAF as the modifier. Poly(sodium N-undecenoyl glycinate) has a similar structure to an SDS micelle, but there is no critical micelle concentration for a polymerized surfactant. Thus, based on previous work with CE (15), 17mM was chosen as an effective concentration. The polymerized surfactant exhibited weaker interactions with the nitroaniline analytes than SDS, as shown by a comparison of retention times for Figures 6A and 6C. Using the EAF mobile phase, the plate number (N) was improved for the SDS additive when compared with poly(sodium N-undecenoyl glycinate) by a factor of approximately 1.3–1.7, except for peak 2, which was about 3 times better.

Effect of temperature on retention factor and plate count

The mass transfer resistance from the mobile phase to the stationary phase can be lessened by conducting the separations at a temperature above ambient. An increase in temperature lowers the viscosity of the mobile phase, thus, improving the diffusion coefficient (D_m) of the analyte. Previously, using the polymeric PLRP column, an improvement of column efficiency of a factor of 1.4 was noted at 90°C as compared with 30°C for methyl-p-hydroxybenzoate using an acetonitrile–water mobile phase (24). Using a C18 silica-based column and a predominately aqueous mobile phase, a plate count improvement of about 1.2–1.5 times was noted using a column temperature of 75°C as compared with 54°C (25). Similar improvements in plate count up to approximately 11,000–12,000 have been noted for organic bases, such as quinine and nortriptyline, when the column temperature was increased from 20–60°C (26).

The chromatogram, at 55°C, of benzoate, salicylicate, and 4-nitrobenzoate (4-NBA) using 40% EAF and 30mM TBA in the phosphate-buffered mobile phase was baseline resolved, with 4-NBA eluting in 6 min instead of 12 min as it did at 25°C (chro-

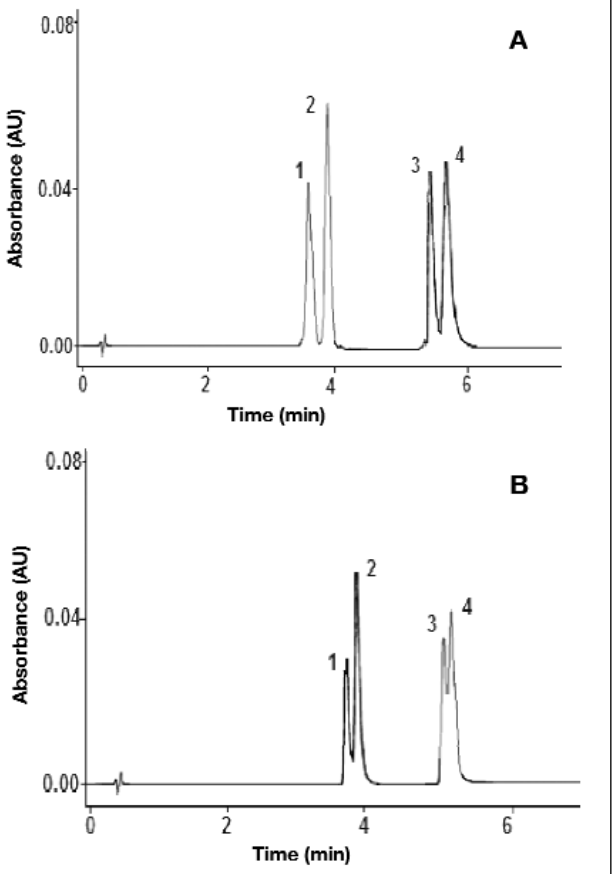

Figure 5. Chromatograms of aromatic amines. 40% EAF–60% 0.05M phosphate buffer, pH 6.0 (A) and 40% MeOH–60% 0.05M phosphate buffer, pH 6.0 (B). Peak numbers are: 4-nitroaniline, 1; *N,N*-dimethyl-3-nitroaniline, 2; tyramine, 3; and diphenhydramine, 4.

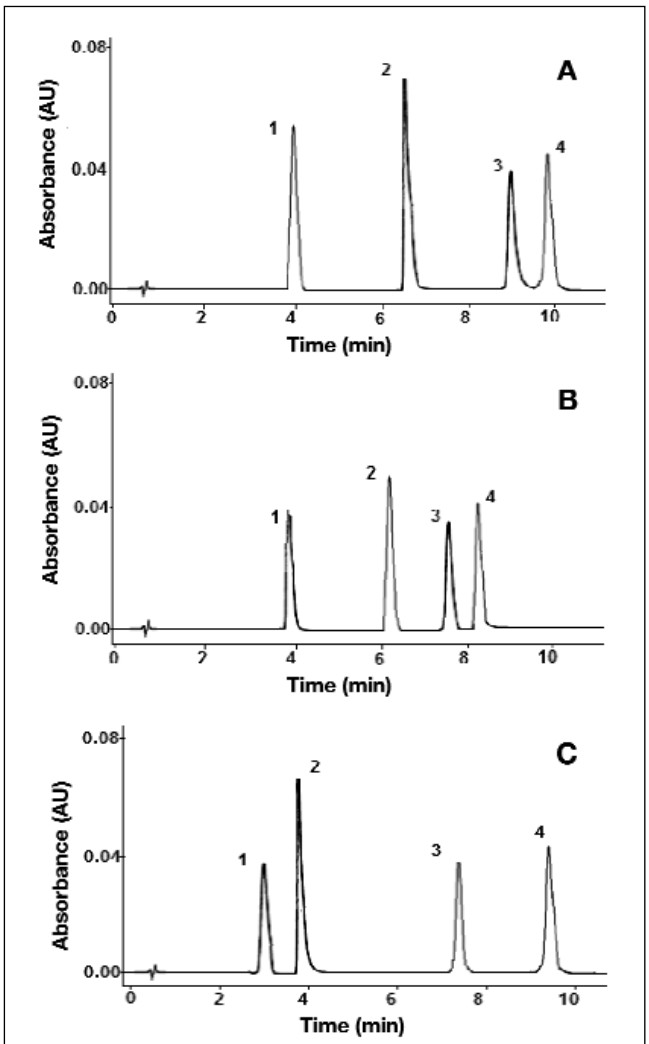

Figure 6. Chromatograms of aromatic amines with the ion-pairing agent. Peak numbers are: 4-nitroaniline, 1; *N,N*-dimethyl-3-nitroaniline, 2; tyramine, 3; and diphenhydramine, 4. 40% EAF-60% 0.05M phosphate buffer, pH 6.0, 0.03M SDS (A); 40% MeOH–60% 0.05M phosphate buffer, pH 6.0, 0.03M SDS (B); and 40% EAF–60% 0.05M phosphate buffer, pH 6.0,17mM poly(sodium *N*-undecenoyl glycinate) (C).

matogram not shown). A pressure reduction was observed from 3.0 kPsi at 25°C to 1.8 kPsi at 55°C, permitting the use of a faster flow rate. This test mixture at 55°C, using the EAF mobile phase indicated an improvement of plate count of about 1.1–1.4 times, when compared with the same mobile phase with TBA at room temperature. Using the same mobile phase, but 40% MeOH, the plate count for 4-nitrobenzoate increased about 1.2 times at 55°C compared with ambient temperature. The chromatogram at 55°C of 4-nitroaniline, N, N-dimethyl-3-nitroaniline, and tyramine using 40% EAF and 30mM SDS in the phosphate buffered mobile phase also showed baseline resolution with tyramine eluting about half the time at 5 min (Figure 7A). The separation of the test mixture at 55°C using the EAF mobile phase indicated a plate count improvement of about 1.2 times as compared with the same percentage of EAF with SDS at room temperature. A marked increase in plate number (of approximately 2 times) for the later eluting peaks using the same mobile phase (but with MeOH at 55°C) is shown in Figure 7B.

Change of retention factor with increasing TBA and SDS concentration in either EAF or MeOH mobile phases

In order to evaluate the effect of counter-ion concentration on the retention of acidic compounds, mobile phases consisting of either EAF or MeOH and varying concentrations of TBA were used. Quantitative evidence for the ion-pairing mechanism was attributed to the linear dependence of the solute retention factor with the ion-pair concentration in the mobile phase for the range studied (10–40mM) (17,27). Whatever the concentration of TBA, the retention order of the organic acids remained the same in both EAF (Figure 8A) and MeOH (Figure 8B). Moreover, the lin-

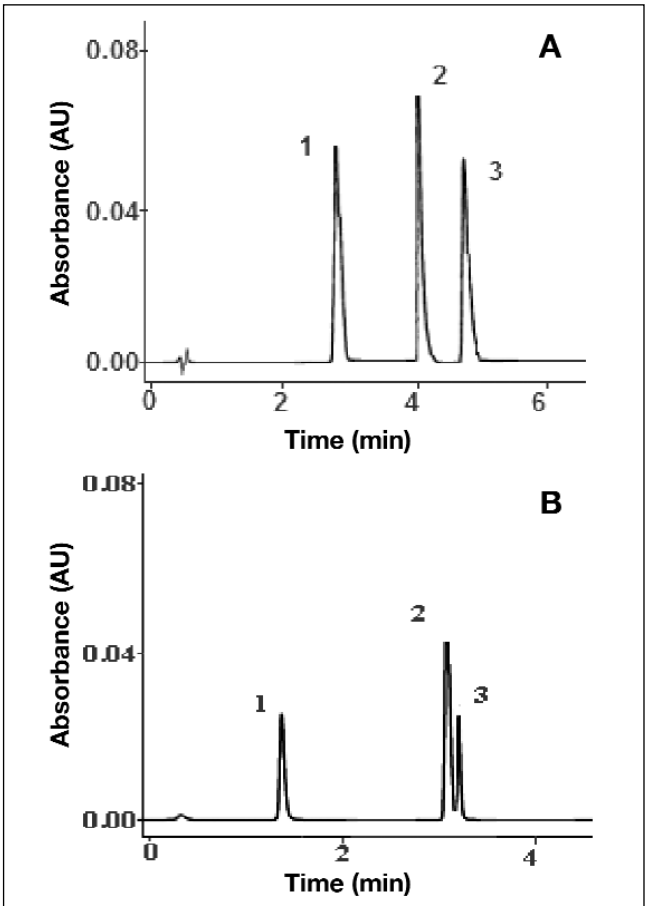

Figure 7. Chromatograms of aromatic amines taken above ambient temperature (55°C). Mobile phase: 40% EAF–60% 0.05M phosphate buffer, pH 6.0, 0.03M SDS (A). Peaks numbers are: 4-nitroaniline, 1; *N*,*N*-dimethyl-3-nitroaniline, 2; tyramine, 3. Mobile phase: 40% MeOH–60% 0.05M phosphate buffer, pH 6, 0.03M SDS (B). Peak numbers are: 4-nitroaniline, 1; *N*,*N*-dimethyl-3-nitroaniline, 2; and tyramine, 3.

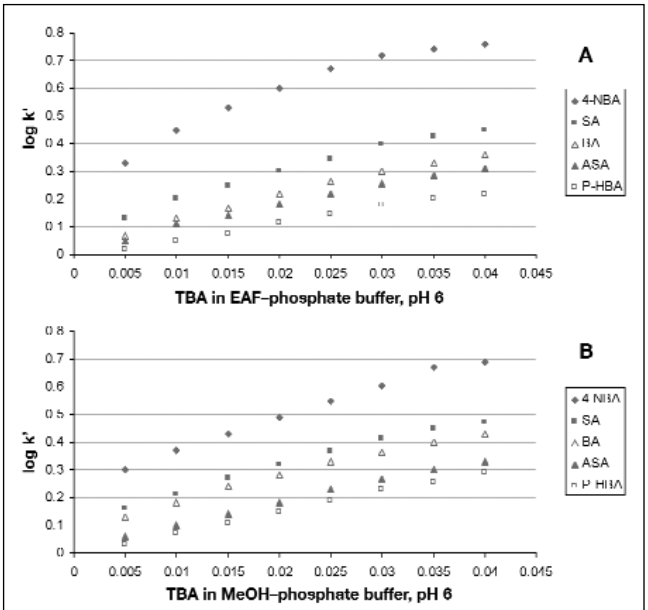

Figure 8. Retention factor (k') as a function of TBA concentration in mobile phase of 40% EAF-phosphate buffer pH 6.0, (A) and 40% MeOH-phosphate buffer pH 6.0 (B). Solutes: 4-nitrobenzoate (4-NBA); salicylate (SA); benzoate (BA); acetylsalicylate (ASA); and *p*-hydroxybenzoate, (*p*-HBA).

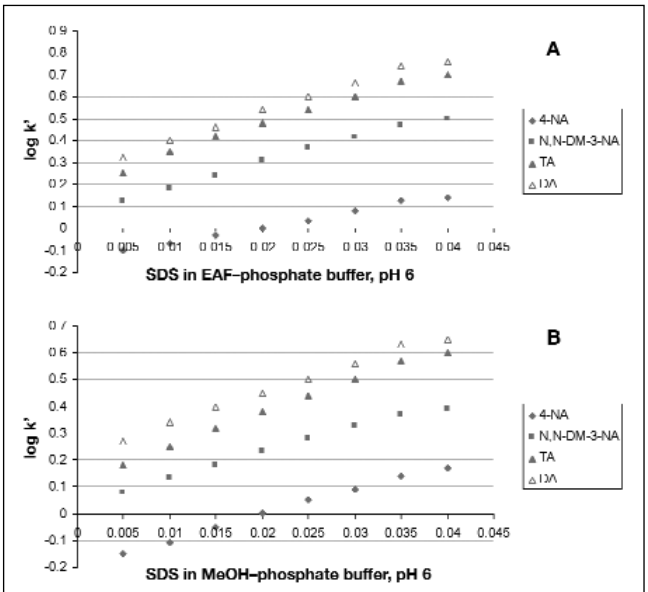

Figure 9. Retention factor (k') as a function of SDS concentration in mobile phase of 40% EAF–60% 0.05M phosphate buffer, pH 3.0 (A) and 40% MeOH–60% 0.05M phosphate buffer, pH 6.0 (B). Solutes: 4-nitroaniline (4-NA), *N*,*N*-dimethyl-3-nitroaniline (*N*,*N*-DM-3-NA); tyramine (TA); and diphenhydramine (DA).

earity was primarily limited to low concentrations and began to flatten at high TBA concentrations (e.g. > 30mM), particularly for the EAF mobile phase. Because the alkyl chain length for TBA was short and the organic modifier percentage of 40% was moderately high, aggregation was not expected.

The effect of ion-pair concentration (SDS) on retention of protonated and neutral amine compounds was also studied. The retention of solutes was observed to increase with an increase in the concentration of SDS, within the range 10–40mM. Whatever the concentration of SDS, the retention order of the organic acids remained the same using either EAF (Figure 9A) or MeOH (Figure 9B). Similarly, the linearity was only limited to low concentrations and began to flatten with higher SDS concentrations (e.g., > 35mM) for both EAF and MeOH mobile phases. Despite the critical micelle concentration of about 8mM for SDS, formation of micelles was not expected because the organic modifier percentage of 40% was moderately high.

Because the log k' profiles versus the surfactant concentration in the mobile phase are so similar for both MeOH and EAF, it was believed that the retention mechanisms using both organic modifiers were similar. That mechanism was a combination of both the solute–surfactant ion-pair or other nonelectrostatic associations and solute ion-exchange in a complex dynamic equilibrium (28). Because of the mass transfer differences between EAF and MeOH mobile phases and because EAF is more polar than MeOH, it was possible that the ion exchange mechanism may dominant more using EAF when compared with MeOH. However, other interactions, such as more extensive hydrogen bonding of the solute with EAF in the mobile phase, can be envisioned.

Conclusion

This study demonstrated that EAF acts as an organic modifier, not as an ion-pair reagent for ion-pair liquid chromatography using a polymeric column. A linear decrease in retention factor (k') as a function of % EAF in an ion-pair mobile phase and a linear increase in retention factor (k') as a function of ion-pair concentration in an EAF mobile phase were consistent, with similar trends using a MeOH mobile phase. Similar baseline resolution and analysis times for aromatic carboxylates and amines using either EAF or MeOH based ion-pair mobile phases were evident in the chromatograms. Although the higher viscosity of EAF as compared with MeOH will lower column efficiency, the plate count difference between EAF and MeOH ion-pair mobile phases was less pronounced for the separation of the aromatic amines than the organic acids.

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