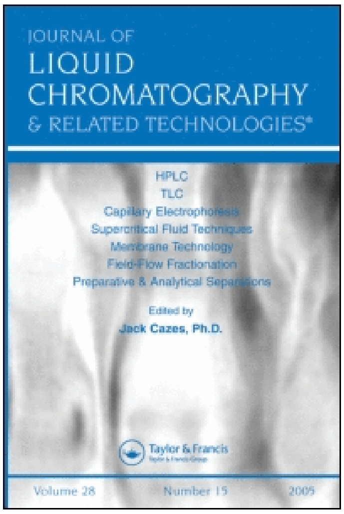

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Alkylammonium Formate Ionic Liquids as Organic Mobile Phase Replacements for Reversed-Phase Liquid Chromatography

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Abstract: Alkylammonium formate (AAF) ionic liquids, $RNH_3^+ [HCO_2^-]$, $R = C_2H_5$, C_3H_7, or C_4H_9, are prepared in high yield and purity by slowly reacting an alkylamine and formic acid in an ice bath under N_2. UV spectra of undiluted ethylammonium formate (EAF), n-propylammonium formate (PAF), n-butylammonium formate (BAF) show no appreciable background absorbance down to about 250 nm, 270 nm, and 295 nm, respectively. Physical properties such as viscosity, melting point, decomposition temperature, density, and miscibility with common organic solvents are also determined. The viscosity of EAF of 11.5 cP at 24°C is considered very low, as compared to previously reported imidazolium or alkylammonium ionic liquids. Because of the higher viscosities, van Deemter plots using the AAF ionic liquids are shifted to lower flow rate optima, as compared to that taken with methanol. The polarity index P' values for EAF, PAF, and BAF, chromatographically determined to be (6.4 ± 0.1); (5.8 ± 0.1), and (5.2 ± 0.1) ($n = 5$), are quite close to methanol (5.1) and acetonitrile (5.8). Kamlet-Taft solvatochromic parameters were also measured and the AAFs were found to have acid, base character similar to that for methanol but polarizability like acetonitrile. The solvent strength S values of 1.9, 2.2, and 2.5 for EAF, PAF, and BAF, respectively, are smaller but comparable to those for methanol (about 2.6). Application of these AAF ionic liquids in reversed-phase liquid chromatography to separate a test mixture of caffeine, p-nitroaniline, and phenol or water soluble vitamins is demonstrated using the polystyrene-divinylbenzene PRP-1 column.

Keywords: Ionic liquids, Mobile phase, Reversed phase liquid chromatography

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INTRODUCTION

Ionic liquids (ILs), composed of an organic cation such as 1-alkyl-3-methylimidazolium, N-alkylpyridinium, alkylammonium, and tetralkylphosphonium paired with an inorganic or organic anion, have unique physical and chemical properties, such as air and moisture stability, non-flammability, non-volatility, and thermal stability. The uses of ILs in analytical chemistry include liquid phase and solid phase microextraction as well as liquid–liquid extraction, sensors for a quartz crystal microbalance, solvents for matrix assisted laser desorption ionization mass spectrometry (MALDI), stationary phases for gas chromatography (GC), and mobile phase additives for capillary electrophoresis (CE) or liquid chromatography (LC).[1–5] Chromatographic and spectroscopic methods for the determination of IL solvent properties have also been reviewed.[6] A comprehensive table of ILs and their physical properties is provided, as well as a detailed discussion of IL solvation properties as measured by spectroscopy or chromatography. The application of ILs to specifically separation science has been reviewed.[7] The 1-butyl-3-methylimidazolium$^+$BF$_4^-$ IL at mM concentrations in the LC mobile phase was shown to be a suitable additive for shortening C_{18} retention and improving peak shape of basic compounds, such as the ephedrine class of aromatic amines[8] or a test mixture of aromatic amines such as anilines.[9] Separation of five nucleotides on a C_{18} silica column using 25 mM 1-butyl-3-methylimidazolium in an aqueous mobile phase was possible in about 20 min.[10]. The control of solute retention through ion-pairing and adsorption of alkyl-methyl-imidazolium ILs at the 50 mM level on a C_{18} silica column was quite thoroughly studied.[11] Masking of silanols by the imidazolium cation was proposed to be important, not only in these LC studies, but also when 1-alkyl-3-methyl imidazolium mobile phase additives were used for CE.[7]

Previously, alkylammonium nitrate and thiocyanate ILs have been characterized with respect to their physical and solvent properties.[12,13] The UV spectra for the nitrate compounds showed a strong absorbance band in the 260–320 nm region and the thiocyanate solvents were colored yellow and quite viscous, about 40–125 cP. Compatibility as solvents for fluorescence measurements is not expected, because of the electron withdrawing character of nitrate. Ethyl- and propyl-ammonium nitrates were used as liquid–liquid extraction solvents for organics from urban dust, shale oil, and urine.[14] Back-extraction of the organics from the IL into a volatile immiscible organic solvent permitted their characterization by GC. A comparison of microbore reversed phase LC of nitro aromatics using 40% n-propylammonium nitrate-60% water and 40% methanol-60% water as the mobile phases showed enhanced elution for proton-donor acceptor solutes such as nitroanilines, but enhanced retention for 2,4-dinitrophenol using the IL mobile phase.[13]

Alkylammonium ILs with a carboxylate anion are unusual. Recently, 2-hydroxyethyl ammonium formate has been synthesized and characterized as a very viscous (100 cP) high conductivity room temperature IL.[15]

Tributylammonium acetate has been tested as a sample matrix for MALDI and as a stationary phase for GC[16] but no other analytical applications are evident. This may be because of the expected high viscosity due to the bulky cation. We have shown that ethylammonium acetate (EAA) can used as a replacement for the organic solvent in reversed phase chromatography.[17] An EAA solution of about 2 M was found to have a similar polarity to methanol for the separation of an aromatic test mixture on a standard C_{18} column, and could modify the separation of a vitamin mixture on a C_8 column designed for a totally aqueous mobile phase. However, EAA is not a true room temperature IL but a hygroscopic solid turning to a liquid at about 50°C.

We are interested in using undiluted room temperature ILs as organic solvent replacements in mobile phases for reversed phase LC with spectrophotometric or fluorescence detection. This could be particularly important for minimizing the use of volatile organic solvents for quality control applications, or to improve mobile phase compatibility for proteins to remain in a more native form. In this work, we report the preparation and characterization of a new class of room temperature IL solvents, alkylammonium formates (AAF): specifically ethylammonium formate (EAF), n-propylammonium formate (PAF), and n-butylammonium formate (BAF). Important factors that went into consideration for this project include the availability of the starting materials, ease of synthesis, and no extensive purification. The viscosities of these AAF ILs were markedly lower than most other types with UV cutoff wavelengths suitable for LC with UV detection. Application of these ILs as mobile phase components for reversed-phase LC on a polymeric cross-linked PRP column to separate a test mixture of caffeine, p-nitroaniline, and phenol is demonstrated. Polarity parameter (P') and solvent strength (S) values for AAFs were determined chromatographically. The use of the poly(styrene-divinylbenzene) PRP column eliminates the possibility of the retention mechanism being controlled by the interaction of the IL with residual silanols.

EXPERIMENTAL

Instrumentation

The water content was determined by Karl Fischer titration using a Titrab radiometer (Copenhagen, Denmark). Melting points were measured with an alcohol thermometer, after freezing the IL sample in dry ice and allowing it to melt slowly by immersion in a warm water bath. Conductivity measurements were taken at 25°C using a Oakton Con 6 meter obtained from Cole-Parmer (Vernon Hills, IL). The decomposition temperature was measured using a Perkin-Elmer (Shelton, CT) TGA 7 thermogravimetric analyzer. Viscosities were determined using a Cannon-Fenske viscometer immersed in a constant temperature bath (Cannon instrument Co., State College, PA). UV spectra of the liquids were recorded in 1 cm quartz cells using a HP 8452A diode array spectrophotometer (Agilent, Wilmington, DE). IR spectra were generated from thin film samples between sodium chloride plates, using the Harrick Split Pea on a Perkin-Elmer Spectrum 2000 FTIR spectrometer. ^1H and ^{13}C NMR spectra of liquids dissolved in deuterated methanol were recorded on a Bruker Daltonics (Billerica, MA) 200 MHz NMR spectrometer. Fluorescence measurements were made on a Perkin-Elmer Model LS 55 spectrofluorometer. All the LC experiments were conducted on a Beckman (Fullerton, CA) Gold Nouveau computer controlled system equipped with solvent delivery pumps A (aqueous phase) and B (organic phase), a model 7725i Rheodyne injector (20 μL loop), and a model 166 variable-wavelength UV-VIS detector set at 254 or 280 nm. All the separations were conducted on PRP-1 columns (Hamilton, Reno, NV) with dimensions (150×4.6 mm, 5 μm particles).

Reagents

The 70% ethylamine in water solution and n-butylamine (99.5%) were obtained from Aldrich (Milwaukee, WI); n-propylamine (98%) was purchased from Lancaster (Pelham, NH). These reagents should be new and not discolored. Formic acid was of puriss grade (98% by wt., 2% water) from Fluka (St. Gallen, Switzerland). The Hydranal reagents for the Karl Fisher titrations were obtained from Aldrich. Test compounds for LC such as uracil, niacinamide, caffeine, phenol, and p-nitroaniline, as well as those for the Kamlet-Taft study, were obtained from Sigma-Aldrich (St. Louis MO). Methanol (MeOH) and acetonitrile (MeCN) were HPLC grade. EAF, PAF, and BAF were synthesized according to the procedure outlined below. Distilled, deionized water prepared in our laboratory using a Barnstead Millipore system was used throughout. Activated carbon, Darco-G60 100 mesh, was purchased from Sigma.

Synthesis of Alkylammonium Formates (AAFs)

Three AAF salts, $RNH_3^+ [HCO_2^-]$, where $R = C_2H_5$, C_3H_7, or C_4H_9, were synthesized in high yield as shown in the reaction equation below.

$$RNH_{2(liq)} + HCOOH_{(aq)} \rightarrow RNH_3^+[HCO_2^-]_{(liq)}$$

The formic acid (98 wt. %) was added slowly, drop-wise from an extraction funnel to an equimolar volume of 70% alkylamine in water for about 4 hrs to prepare about 150 mL of the AAF. Throughout the addition, nitrogen gas was bubbled through the solution and temperature was maintained at $<10°C$ in an ice bath/dry ice mixture. This was followed by water evaporation at 100°C under vacuum for about 3 days, until the liquid reached a constant viscosity. The liquids were stored in moisture tight vessels to prevent absorption of water from the air prior to Karl Fischer determination of water. The dried liquids were used as the reference standards to determine physical properties, such as melting point, viscosity, density, and thermal decomposition. The purity of the liquid organic salts was verified by NMR, IR, and UV spectroscopy as discussed later. The three liquids synthesized were all colorless, and more viscous than water. The clear liquids were assayed for ethylamine, propylamine, and butylamine, using the o-phthaldehyde-cyanide derivatization reaction as previously described.[18] For comparison to EAF as a mobile phase modifier for reversed phase LC, we also synthesized ethylamonium sulfate (EAS) from 70% ethylamine and concentrated sulfuric acid as described previously for the AAF ILs. EAS was chosen because sulfate is more compatible with stainless steel tubing than chloride and is UV transparent.

Chromatographic Conditions

The AAF ionic liquids (EAF, PAF, and BAF) were evaluated for reversed-phase liquid chromatography using the PRP column, which is stable throughout the pH range and can withstand high pressure even with a surface area of 415 m^2/g. To prepare the test mixture of p-nitroaniline, caffeine, and phenol, a solution having a concentration of 40 mgL^{-1} of each analyte prepared with 40% ionic liquid/60% water or 40% MeOH/60% water or 40% MeCN/60% water was sonicated for 10 min to ensure complete dissolution. Mobile phases were filtered and degassed by bubbling helium gas for 5–10 min before use. Triplicate injections of the mobile phase were performed to identify any solvent related peaks, followed by triplicate injections of the samples. The column was flushed with the mobile phase and equilibrated for at least 10 min before the next injection. The retention factor k′ was calculated using $[k' = (t_r - t_0)/t_0]$, where t_0 = retention time of uracil (1.2 min at 1 mL/min). All k′ values represent an average of three replicates. The resolution $Rs = 2\ (t_2 - t_1)/(w_{b1} + w_{b2})$, where t = the peak retention time and w_b = baseline peak width. The plate count N was determined as $16(t_r/w_b)^2$, where t_r = retention time and w_b = peak width at baseline.

RESULTS AND DISCUSSION

Characterization of AAF Ionic Liquids

Water Content

ILs are mildly hygroscopic and prolonged contact with the atmosphere leads to absorption of water. The amount of water absorbed by the liquids depends on the type of ionic liquid (particularly the anion), as well as the relative humidity and temperature.[19,20] The concentration of the water was easily determined by Karl-Fischer titration, using a one component Hydranal reagent, to about 2.0% for liquids dried for 3 days at 100°C and under vacuum. After storage of the AAF ILs in desiccators with molecular sieves and calcium sulfate for one month, the water content (wt %) reduced to about 0.7–0.8. Specifically, the water content values as wt. % ($n = 5$) were 0.84 ± 0.11 for EAF, 0.82 ± 0.12 for PAF, and 0.73 ± 0.12 for BAF. These values are near the range of 0.1–0.7% water, reported for preparations of dried imidazolium ILs.[21] In order to study the stability of the ILs, they were stored in open vials for 7 days and water content increased to (1.0 ± 0.1) *wt %* ($n = 3$). Previously, it has been reported by near IR spectroscopy that the anions making up butyl imidazolium ILs can interact with water through hydrogen bonding.[22] Because the formate anion would be expected to hydrogen bond with water, absorption of water by AAFs is not unexpected.

Spectroscopic Analysis

The composition and purity of the AAF ILs were determined by spectroscopic means. The fluorescence assay for primary amine showed the AAF ILs had concentrations in the 8.4–11 M range, depending on the compound. The IR spectra (not shown) show characteristic absorption peaks at 2960–2900 cm^{-1} (H-C), 3600 cm^{-1} (free O-H), and 1500–1800 cm^{-1} (C=O) for the formate anion. The presence of water was confirmed by the O-H stretching band from 3400–3800 cm^{-1}. The ammonium ion shows broad N-H stretching bands in the region 3300–3000 cm^{-1}. The ^{1}H NMR spectra (not shown) of EAF, PAF, and BAF run in deuterated methanol (CD_3OD), show characteristic chemical shifts for the ethyl, propyl, and butyl groups (carbons lettered starting with the methyl group) at 1.0–3.5 ppm (EAF: triplet, a, and quartet, b; PAF: triplet, a, sixlet, b, and triplet, c; BAF: triplet, a, sixlet, b, pentlet, c, and triplet, d), while the formate protons give a singlet at around 8.5 ppm. ^{13}C NMR spectra (not shown) exhibit the various carbons at different chemical shifts: EAF: a, 12 ppm; b, 35 ppm; PAF: a, 11 ppm; b, 21 ppm; c, 42 ppm; BAF: a, 13 ppm; b, 20 ppm; c, 30 ppm; d, 40 ppm; carbonyl carbons show a chemical shift at 166 ppm. Using activated carbon sonicated with the AAF solvents, impurities contributing to the fluorescence background (excitation 350 nm, emission 420 nm) could be reduced by a factor of 2–3. Preliminary studies monitoring the background fluorescence of EAF and PAF with time, have shown no change in the signal over a 20 day period.

The UV spectra (not shown) of the AAFs showed strong off-scale absorbance from 200–220 nm, which then decreased rapidly to 240 nm. The absorbance profiles were consistently ordered in magnitude as EAF $<$PAF $<$ BAF. EAF was found to be transparent at $\lambda \geq 250$ nm, PAF, $\lambda \geq 270$ nm, and BAF, $\lambda \geq 295$ nm, using a full-scale absorbance of 0.01. The UV cut-off, defined as the wavelength below which the solvent is opaque with an absorbance greater than 1.0, is often the parameter compared for tabulated LC solvents. These values were found to be 240, 245, and 250 nm, for EAF, PAF, and BAF, respectively. Useable detection wavelengths were also determined by LC with UV detection. A sample of each of the liquids was injected into the liquid chromatograph and eluted with a mobile phase composition consisting of 40% EAF, PAF, or BAF in water, respectively. Detection was done at 254 nm, 274 nm, and 300 nm for EAF, PAF, and BAF, respectively. No peaks were observed, indicating the UV transparency at the respective wavelengths. Thus, no chloroform extraction step was deemed necessary, unlike for EAA previously synthesized.[17] With the LC detector, use of an absorbance full scale setting of 0.01 at 254 nm with 40% EAF–60% water showed a flat stable baseline after zeroing, and even at an absorbance full scale setting of 0.001, the baseline was just a little noisy and stabilized at 0.0004 absorbance units. However, detection with a stable baseline at 254 nm was not possible with 40% PAF–60% water or 40% BAF–60% water and 0.01 absorbance units full scale, but was possible at 0.15 full scale absorbance units. This is in contrast to the imidazolium salts, which were shown to absorb strongly in the UV-visible region with only 10% (v/v) solutions in MeCN giving 0.5 AU (254 nm).[19]

Density, Thermal Stability, Aggregation, and Viscosity of AAFs

The densities of most ILs at room temperature (24°C) are typically greater than water (1.0–1.6 g/mL), and decrease approximately linearly with an increase in the cation alkyl chain length.[23] The three AAF liquids are slightly less dense than water, but do decrease in density with increasing chain length (EAF – 0.990, PAF – 0.979, BAF – 0.973). The small amount of water in the AAF solvents would not be expected to change these data based on a previous comparison of densities for dried and water saturated imidazolium ILs.[21]

The thermal operating range is defined as the temperature between the melting point and the decomposition temperature. The melting points were determined to be less than (−10°C) for all the liquids studied. Thermogravimetric analysis profiles for the AAF solvents in air show EAF, PAF, and BAF start to degrade at 165°C, 200°C, and 230°C, respectively (Figure 1). These breakpoint temperatures are somewhat lower that those of 200 and 300°C reported for ILs having a methylethylimidazolium cation with either the respective bis(methanesulfonyl)amide or bis(trifluoromethanesulfonyl)amide anion.[24] Degradation proceeds rapidly after these temperatures, reaching a minimum weight at about 300°C. Decomposed liquids were evident because of their conspicuous yellow coloration.

The conductivity profiles of the AAFs as a function of % EAF, PAF, and BAF in water were compared. The conductivity (κ) profiles increase fairly linearly from about 30–40 mS at 10% to an apparent maximum of 60–100 mS at 40–50%, before falling off to about 5–50 mS at 100%. This decrease in conductivity is due not only to a reduction of mobility due to the increase in viscosity of the AAF-water mixture, but also to the

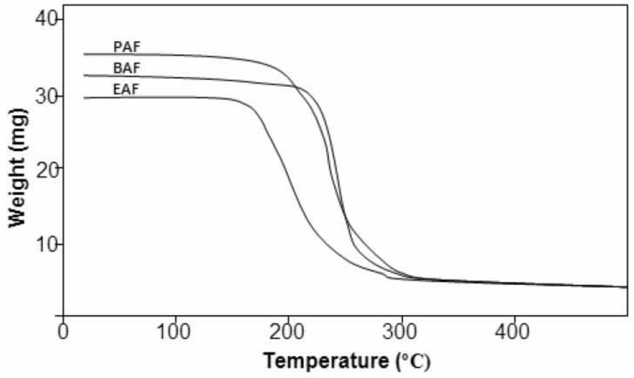

Figure 1. Thermogravimetric analysis (TGA) curves of ionic liquids EAF, PAF, and BAF, showing decomposition temperatures at the breakpoints.

reduction of charge carriers due to aggregate formation. The peak point of this profile is considered to represent the critical aggregate concentration (CAC) of the IL.[25] It can be determined more precisely[26] using the Kohlrausch relationship, which indicates the molar conductivity, $\kappa/[\mathrm{AAF}]$, is inversely related to $[\mathrm{AAF}]^{1/2}$. Extrapolation of the two linear portions of such a plot will give the CAC at the intersection point.[26] The CAC values for the AAFs are estimated to be about 5 M. This value is quite a bit higher than those (about 0.1–0.8 M) determined for C8-C4 imidazolium compounds,[26] indicating the likely less hydrophobic interaction of the cations for these short chain compounds.

The viscosity of the AAF ILs ($n = 4$), at room temperature (24°C), increase from ethyl-, (11.5 ± 0.1 *cP*), propyl-, (18.0 ± 0.1 *cP*), and butyl-, (22.2 ± 0.1 *cP)*. This trend agrees well with the results reported previously, indicating longer alkyl chains on the ionic liquid cation result in an increase in viscosity due to stronger van der Waals interactions.[27] The magnitudes of these viscosities are substantially less than those of 32 and 67 reported for ethyl- and propyl-ammonum nitrate, respectively.[13] They are considerably less than those for tetralkylammonium sulfates, which have viscosities in the thousands of cP.[14] At 40°C, the respective viscosities ($n = 4$) in cP for EAF, PAF, and BAF were 6.6, 10.0, and 14.6, all with a standard deviation of 0.1. At 100°C, the respective viscosities ($n = 4$) in cP for EAF, PAF, and BAF were 1.4, 2.0, and 2.7, all with a standard deviation of 0.1. Clearly, the viscosity of the liquids can be effectively reduced by temperature elevation closer to 1 or less, as typical for common organic solvents at room temperature.[28]

Previously, the temperature dependence of viscosity has been described in the form of the Arrhenius equation, which described the temperature dependence for non-associating electrolytes.[29] This equation is: $\ln\ \eta = \ln$

$\eta_{\mathrm{inf}} + E_\eta/RT$, where η = viscosity in mPa-s or cP, η_{inf} = viscosity at infinite temperature, E_η = activation energy in kJmol^{-1} for viscous flow, R = gas constant as 8.314×10^{-3} kJmol^{-1}K^{-1}, and T = temperature in °K. Linear regression analysis of ln η with 1/T(°K) indicated E_η values of 26 kJmol^{-1} for all three AAFs, and η_{inf} values increasing from 3, 4, and 7×10^{-4} for EAF, PAF, and BAF, respectively. E_η can be considered the measure of the energy barrier required for the IL ions to move past each other, which can be due to physical size or just a strong interaction of the ions (more order in the IL). The value of 26 for AAFs is on the high side compared to the buylimidazolium class which range from 21–26.[29] At infinite temperature, the viscosity (η_{inf}) is a measure of the structural contribution by the IL ions. The values for the butylimidazolium class are about a factor of 10 higher than those found for the AAFs.[29] One IL, 1-butyl-3-methylimidazolium $^{+}CF_3SO_3^{-}$, has values of E_η and η_{inf}, essentially the same as those for EAF.

The ln viscosity of the mobile phase, as a function of fraction of AAF in water, is shown in Figure 2. The change in viscosity of the formate liquids upon dilution with water (higher dielectric constant), is given by a third order polynomial, $y = A_1 + A_2\,(x) + A_3\,(x^2) + A_4\,(x)^3$, where A_1, A_2, A_3, and A_4 are coefficients obtained by curve fitting; A_1 is the viscosity of water at 24°C and (x) is volume percent of formate liquid. These higher order fits are similar to alkylammonium nitrates studied previously.[12] The values of A_1 obtained at 24°C (EAF = 0.84, PAF = 0.81, and BAF = 0.80 cP), are quite close to the viscosity of pure water (0.89 cP). This shows that dilution with water, which has a lower viscosity, is effective in reducing the viscosity of the liquids up to about 80% AAF composition.

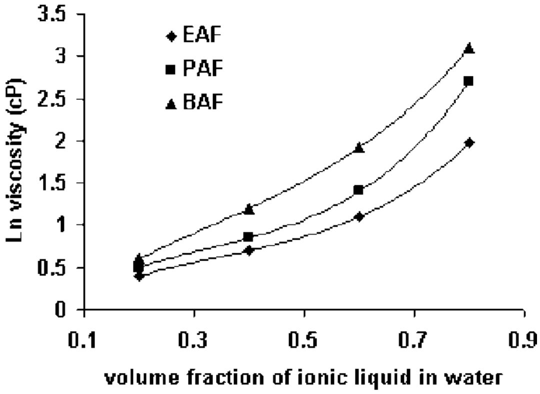

Figure 2. Plot of ln viscosity versus fraction of EAF, PAF, and BAF in water; {EAF, $y = 7.71\,(x^3) - 8\,(x^2) + 4.14\,(x) - 0.17$}, {PAF, $y = 11.46\,(x^3) - 11.25\,(x^2) + 5.29\,(x) - 0.2$}, and {BAF, $y = 7.08\,(x^3) - 7\,(x^2) + 5.22\,(x) - 0.22$}.

Chemical Properties of AAFs

The solubility of the AAF salts in organic solvents and water were similar. All the AAF organic salts were found to be insoluble in hexane, toluene, THF, ethylacetate, and dichloromethane, but miscible with a wide range of more polar organic solvents including MeOH, MeCN, ethanol, and propanol, as well as water. Volumes of about one mL of each solvent in the binary mixture were tested to ensure the presence of trace water did not affect the solubility. Because of these properties, the AAF liquids could be used in a wide range of liquid–liquid extraction processes, as well as reversed phase LC.

Using caffeine, p-nitroaniline, and phenol as test solutes, the polarity index $P'_2 - P'_1$ difference was calculated using $[k'_2/k'_1 = 10^{(P'2-P'1)/2}]$. $P'_{AAF,\ W} = P'_{AAF}\ \Phi_{EAF} + P'_W\ \Phi_W$, where P'_{AAF} and P'_W are the polarity indices of AAF and water, respectively, and Φ_{AAF} and Φ_W are the volume fractions of AAF and water.[30] The equation $P'_2 - P'_1 = \{P'_{AAF}\Phi_{AAF(2)} + P'_W\Phi_{W(2)}\} - \{P'_{AAF}\Phi_{AAF(1)} + P'_W\Phi_{W(1)}\}$ is solved for P'_{AAF}, knowing $P'w = 10.2$. The P' values for EAF, PAF, and BAF were found to be 6.4 ± 0.1, 5.8 ± 0.1, and 5.2 ± 0.1 (solute pairs, $n = 5$), respectively, which are considerably lower than that for water (10.2) and quite similar to that for MeOH (5.1).[28] This explains why the liquid organic salts were soluble in polar solvents such as MeCN and water, but insoluble in toluene ($P' = 2.9$) and hexane ($P' = 0.1$).

The polarities of the AAF ILs were also measured using a spectroscopic method based on the wavelength shift of a solvatochromic dye, 2,6-diphenyl-4-(2,4,6-triphenyl-N-pyridino) phenolate (Reichardt's dye). Because of the zwitterionic structure of ionic liquids, the solvatochromic properties of Reichardt's dye is strongly affected by the hydrogen-bond-donating solvents, which stabilize the ground state of the dye more than the excited state,[31] The solvent polarity values, $E_{T(30)}$, in kcal/mole, defined as $(28592/\lambda max)$, were determined and data are shown in Table 1. Normalized polarity values, E_T^N, were also determined for the AAF ILs (Table 1) and were all similar to that for MeOH (0.77). Analogous data reported for ethylammonium nitrate and propylammonium nitrate, which had E_T^N values of 0.95 and 0.92, respectively,[14] were similar to that for water (1.00).

The polarities of the AAF ILs were also characterized more fully using the Kamlet-Taft polarity scale.[32] This method involves determination of the spectroscopic wave number shift for multiple solutes dissolved in the solvent of interest, specifically chosen to measure dipolarity/polarizability (π^*), hydrogen bond acidity or proton donating capability of the solvent (α), and hydrogen bond basicity or ability of the solvent to accept a proton or donate an electron pair (β). The π^* parameter was averaged from data using 4-nitroanisole, N,N-dimethyl-3-nitroaniline, and *trans*-4-methoxy-β-nitrostyrene. The α value was determined using Reichardt's betaine and 4,4'-*bis*(diethylamino)benzophenone; the β value was found using probes 2-nitroaniline, 4-nitroaniline, and 4-nitrophenol. In order to do the calculations, it was discovered that the positions of v_{max} and v_0 are inverted in

Table 1. Polarity of ionic liquids as characterized by the dipolarity/polarizability (π^*), hydrogen bond acidity or proton donating capability of the solvent (α), and hydrogen bond basicity or ability of the solvent to accept a proton or donate an electron pair (β). The values of π^*, α, and β are averaged for several probe molecules as described in the text.

	Reichardt's dye		Kamlet-Taft			
Ionic liquid or solvent	$E_{T(30)}$	E_T^N	π^*	α	β	Ref.
Ethylammonium nitrate	61.6	0.95	1.24	0.85	0.46	[6]
n-Propylammonium nitrate	60.6	0.92	1.17	0.88	0.52	[6]
Ethylammonium formate[a]	56.2	0.79	0.80	0.85	0.73	
n-Propylammonium formate[a]	55.4	0.76	0.73	0.87	0.75	
n-Butylammonium formate[a]	54.8	0.74	0.68	0.89	0.78	
Methanol	55.5	0.77	0.60	0.93	0.62	[6]
Acetonitrile	46	0.47	0.75	0.19	0.31	[6]
Water	63.1	1.00	1.09	1.17	0.18	[6]

[a] Synthesized as previously described but from a 2 M solution of the alkylamine in methanol. The resultant AAF was dried for 2 hr under vacuum at 100°C and upon IR analysis, no hydroxyl peak due to water was evident.

Table 1 of reference 32. The π^*, α, and β values for water, MeOH, MeCN, ethylammonium nitrate, and propylammonium nitrate, in addition to those for the AAFs, are shown for comparison in Table 1. These AAF samples were synthesized from commercial solutions of amines in methanol and were considered dry based on the complete disappearance of the (OH) absorption band of water at around 3600 cm^{-1} in the IR spectra. Previously, IR analysis has been shown to be a sensitive measure of water content in ILs.[22] The π^* value for AAFs decrease from 0.80–0.68 with increasing amine chain length, and are quite comparable to that for MeCN ($\pi^* = 0.75$). The AAF liquids are all quite strong proton donating, with α values in the range from 0.85–0.89, similar to the nitrate ionic liquids and slightly smaller than that for MeOH (0.93). The AAF ILs are more able to donate an electron pair with β values in the range of 0.73–0.78 in comparison to the nitrate ionic liquids, with values of 0.46 and 0.52 or MeOH (0.62). In general, all three parameters for the AAFs are very different from water and α and β are very different from MeCN.

AAF Liquids as LC Mobile Phase Components

Pump Pressure and Reproducibility of Retention Factors

The pump pressure was determined using a mobile phase range of 10–80% EAF, PAF, or BAF in water at room temperature, using the PRP column.

The pressure was found to be approximately proportional to the viscosity of this solvent composition range, with a starting pressure of 5.2, 8.3, and 11.7 MPa (0.75, 1.2, and 1.7 kpsi) and ending pressures of 20.7, 24.2, and 31.0 MPa (3, 3.5, and 4.5 kpsi) for EAF, PAF, and BAF, respectively. The profiles for EAF and PAF are similar to that found previously for an EAA-water mobile phase of the same composition.[17] It is worth noting, that the viscosity trend when mixing ionic liquids with water is unlike that when common organic solvents are mixed with water. For instance, previous studies have shown that the viscosity of methanol-water mixtures increase gradually to a maximum value at about 50–50 in composition and then decrease gradually giving a rounded type curve.[33]

The retention factor (k') values for phenol using AAF-water mobile phases normalized to the same polarity ($P' = 8.7$), and the standard deviation for within the same batch of AAF and for three different batches of AAF were determined. The k' values ($n = 3$) within a batch or between batches of EAF or BAF were similar, $2.51–2.57 \pm 0.01$, with RSD values of about 0.4%. The k' values ($n = 3$) for PAF were 2.53 ± 0.08 and 2.62 ± 0.13, with RSD values of about 4%. In addition, using 40% BAF-60% water, the respective k' data ($n = 3$) for 2,4-dinitrophenol and 4-nitrophenol were 0.25 ± 0.02 and 0.67 ± 0.01 within a batch and 0.26 ± 0.08 and 0.68 ± 0.09 between batches. In general, the data show that it is possible to carry out replicate AAF synthesis and drying steps, with still reproducible chromatographic results.

Retention Comparison of AAF Solvents with Organic and Aqueous Solvents

In order to compare the performance of AAF liquids and conventional organic solvents, a test mixture consisting of a polar, nitro, and phenolic compound was chosen for analysis (caffeine, p-nitroaniline, and phenol). The pH of aqueous ionic liquid (40% ionic liquid/60% water) was below neutral, likely due to slight amine volatilization during synthesis. At this mobile phase pH, p-nitroaniline, and phenol are expected to be more hydrophobic, while caffeine is more hydrophilic. Chromatograms of this mixture are compared in Figure 3 using BAF, MeOH, and MeCN at the same composition in water. The retention order of the compounds is the same and, as expected, based on hydrophobicity for all three mobile phases. The retention factors are similar ranging from 1.1 to 1.9 for BAF, 0.7 to 2.0 for MeOH, and 0.8 to 1.9 for acetonitrile. The resolution Rs between p-nitroaniline and phenol increases from 40% MeOH–60% water (1.1), 40% MeCN–60% water (1.4) to 40% BAF–60% water (1.8). Sample throughput of less than 4 min can be noted for all three chromatograms. Figure 4 shows good resolution of this same mixture using either EAF or PAF, with a slightly longer elution time of about 4 min for phenol. Using 40% (2 M aqueous ammonium formate buffer, pH 5.3)/60% water and all other conditions as above, only peaks for

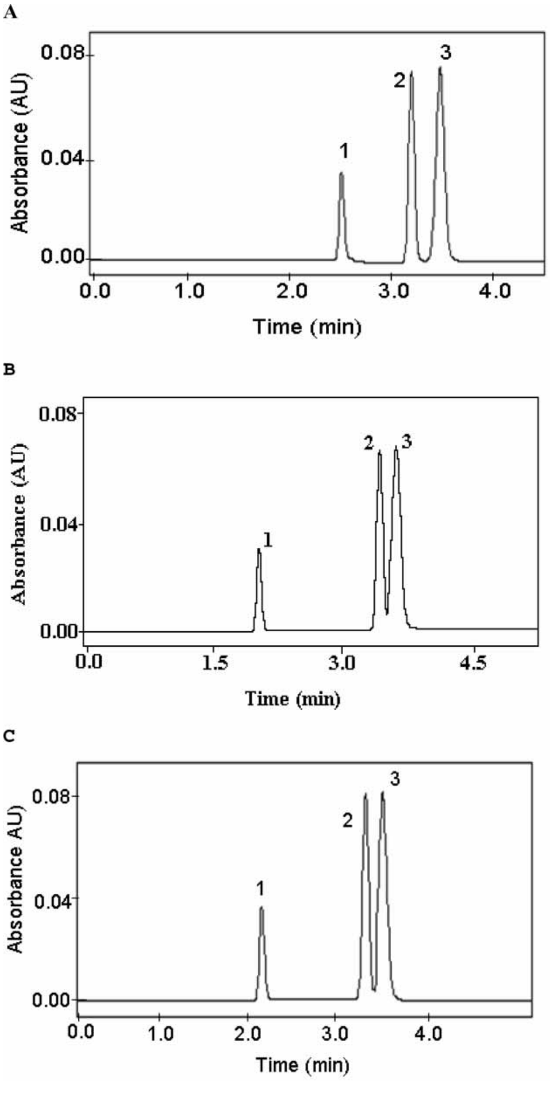

Figure 3. Chromatograms of 1. caffeine, 2. p-nitroaniline, 3. phenol; Mobile phase: (A) 40–60% BAF-water; (B) 40–60% MeOH-water; (C) 40–60% MeCN-water. Chromatographic conditions: PRP-1 column (150 × 4.1 mm); flow rate 1 mL/min; Detection wavelength: 254 nm; 24°C.

caffeine and p-nitroaniline were noted and phenol was basically adsorbed. For the separation of water soluble vitamins using 20% MeOH or EAF (Figure 5), there is a definite change in the k′ ratio (selectivity, α) for pyridoxine (peak 4) and thiamine (peak 3). Selectivity values, $\alpha_{4/3}$, for EAF, and MeOH were 1.3, and 1.1, respectively.

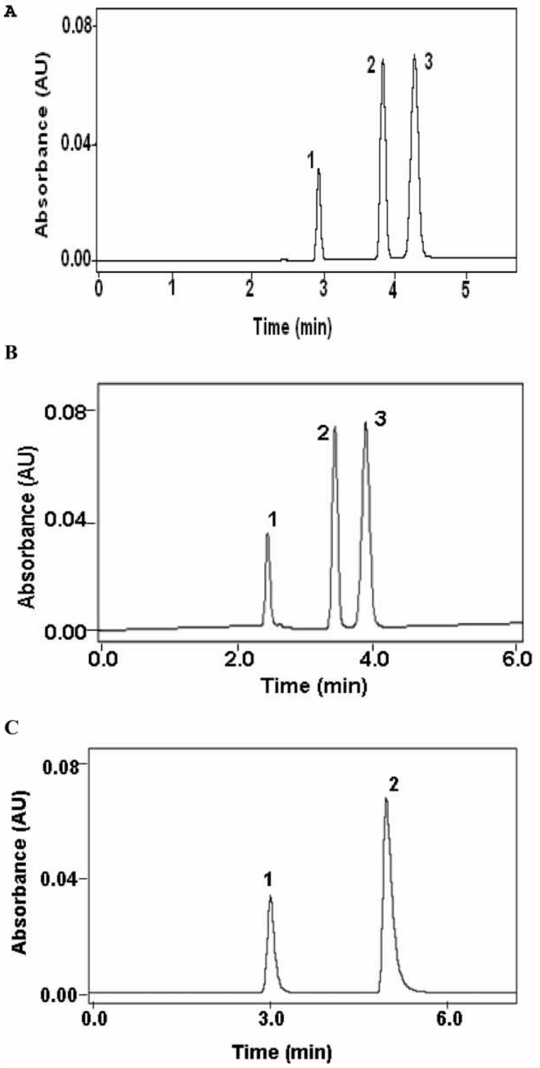

Figure 4. Chromatograms of 1. caffeine, 2. p-nitroaniline, 3. phenol; Mobile phase: (A) 40–60% EAF-water; (B) 40–60% PAF-water; (C) 40–60% 2 M ammonium formate-water. Chromatographic conditions as in Figure 3.

It is important to note, that a mobile phase of 100% 2 M ethylammonium sulfate (EAS) did not elute any of the previous Figure 3 or 4 test compounds off the column, even after 20 min. Column back-pressure or solubility limits were exceeded if solutions greater than 2 M in concentration were used. Upon switching the mobile phase to 100% MeOH, a large broad peak due to all the adsorbed compounds was noted. In an attempt to understand this

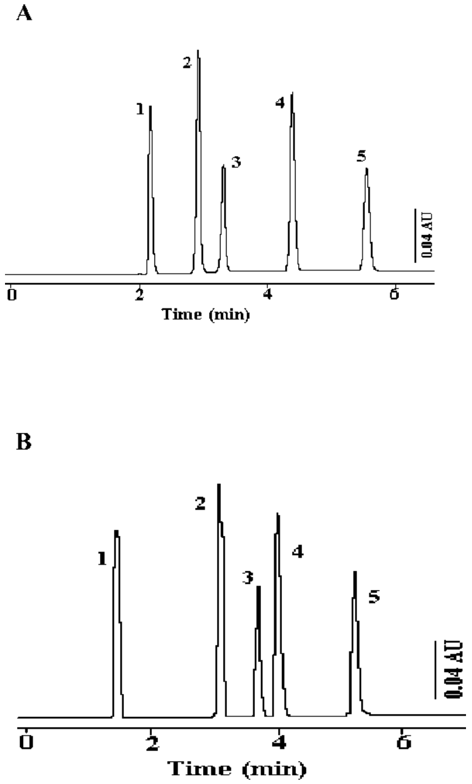

Figure 5. Comparison of chromatograms for the separation of water soluble vitamins using (A) 20% EAF, and (B) 20% MeOH, both with remaining percentage of 0.02 M acetate buffer, pH = 4.6. Peaks: 1. ascorbic acid, 2. nicotinic acid, 3. thiamine, 4. pyridoxine, 5. niacinamide. Chromatographic conditions as in Figure 3.

difference between EAF and EAS, conductivity profiles as a function of % EAF or concentrated EAS in water are compared, as shown in Figure 6. The similar profiles in the same mS conductivity range seem to indicate ionization, and aggregation of EAF and EAS are comparable. Why the AAF ionic liquids are truly different from standard salt solutions as reversed phase modifiers for standard aromatic organic molecules is not clear at this time. Possibly an ion-pair retention mechanism involving both the alkylammonium ion and the carboxylate is important.

The effect of increasing the alkyl chain length on the cation of ionic liquids from ethyl-, propyl-, to butyl-, on the retention of polar compounds was studied using three test compounds (caffeine, p-nitroaniline and phenol). Previously, it was demonstrated that increasing the cation size from ethyl- to tributyl- induces more organic solvent like behavior.[13] Retention factor values of 1.1, 1.7, and 1.9 (40% BAF–60% water), 1.1,

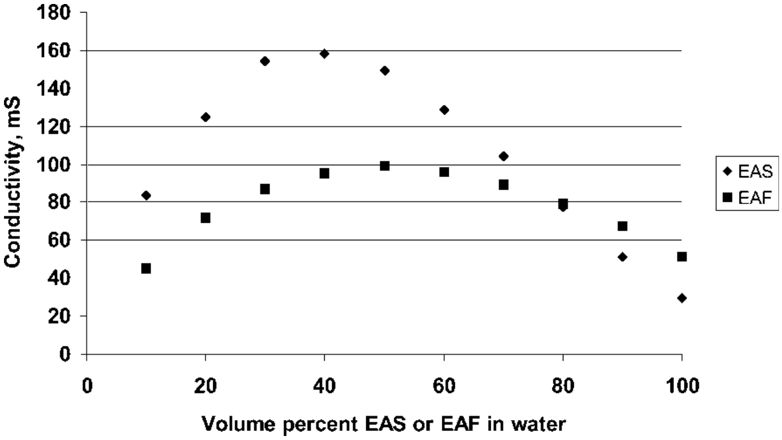

Figure 6. Conductivity in mS versus % EAF or ethylammonium sulfate (EAS) in water.

1.9, and 2.3 (40% PAF–60% water) and 1.4, 2.2, and 2.6 (40% EAF–60% water) were obtained for caffeine, p-nitroaniline, and phenol, respectively. Representative log k′ versus solvent composition profiles for p-nitroaniline are shown in Figure 7. Although, the retention did decrease with an increase of the AAF alkyl chain length, it was not a marked change and MeOH for most solutes can be considered a stronger solvent.

Comparison of AAF van Deemter plots

Figure 8 shows the van Deemter plot using phenol as the test compound. The difference in the diffusion coefficient of the solute in the mobile phase (D_m) for methanol and the AAF solvents is evident by the increased B term (proportional to D_m) for methanol and the increased C_m term (proportional to $1/D_m$) for AAF that make up the van Deemter equation. The respective plate count (N) values at the optimum flow rates for EAF, PAF, and BAF were 1,500 at 0.5 mL-min^{-1}, 1,670 at 0.4, and 1,880 at 0.3. The N value using MeOH at an optimum 0.8 mL-min^{-1} was 1,370, about twice the N values for EAF at that same flow rate. Column efficiency using EAF can also be improved to that similar to MeOH at room temperature by working at a temperature of 45 or 55°C at a higher flow rate, and this has been considered in separate studies.[34,35]

Retention Mechanism

In reverse phase, the retention mechanism can be described by the equation $log\ k' = log\ k'_w + a\Phi + B\varphi^2$, where k'_w is the retention factor using 100% water as the mobile phase, Φ is the volume fraction of the organic solvent,

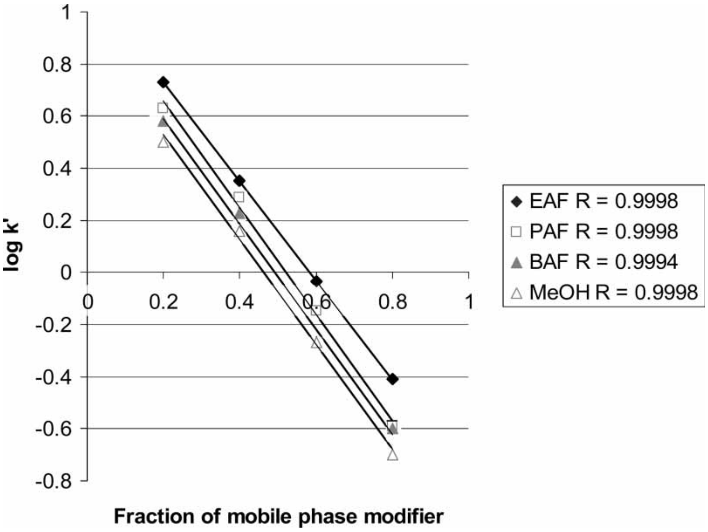

Figure 7. Plot of retention factor for p-nitroaniline as a function of % organic modifier (EAF, PAF, BAF, or MeOH).

and a and b are regression constants for the second order polynomial equation.[36] Over a narrow mobile phase composition this equation can be simplified as $log\ k' = log\ k'_w - S\Phi$, where S is a measure of organic solvent strength that is dependent on solute structure. It was shown, that S for a given solute is almost invariant with stationary phases, but does vary with

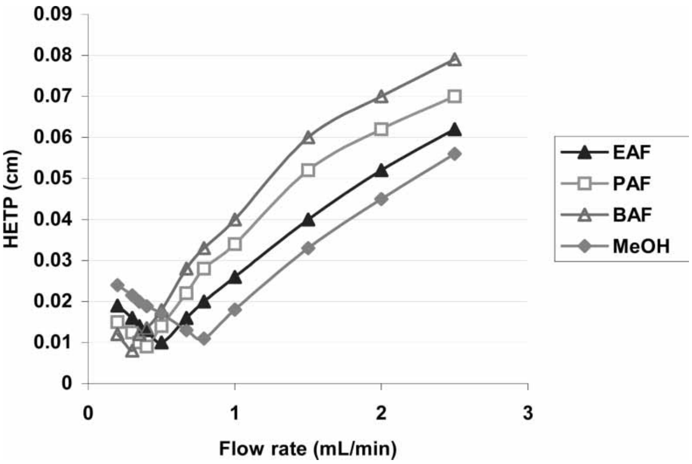

Figure 8. van Deemter plots for phenol taken at ambient temperature; mobile phase 40%-MeOH, -EAF, -PAF, or -BAF with 60% water.

Table 2. Logarithm of the capacity factor as a function of the volume fraction (Φ) of ionic liquid or methanol in the mobile phase. Solvent strength (S) for reversed phase chromatography shown for ionic liquids and methanol. The standard deviation for S ranged from 0.01–0.04 with an average of 0.02_5.

Liquid	Solute	$\text{Log k}' = \log k_w - S\Phi$	R^2
EAF-water	Caffeine	$y = 0.9 - 1.89\ (x)$	0.9997
	Phenol	$y = 1.2 - 1.92\ (x)$	0.9999
PAF-water	Caffeine	$y = 0.9 - 2.20\ (x)$	0.9999
	Phenol	$y = 1.2 - 2.20\ (x)$	0.9996
BAF-water	Caffeine	$y = 1.0 - 2.50\ (x)$	0.9989
	Phenol	$y = 1.3 - 2.50\ (x)$	0.9998
MeOH-water	Caffeine	$y = 0.8 - 2.55\ (x)$	0.9999
	Phenol	$y = 1.3 - 2.55\ (x)$	0.9998

the solvent type.[37] The binary mixture of AAF salts in water showed a linear change in the logarithm of capacity factor within the composition range of 20% to 60% *(v/v)* of AAF with water. The equations for the log k′ plots, as a function of volume fraction of AAF ionic liquid, using niacinamide (neutral hydrophilic) as the test solute were determined: (EAF), $y = -2.18\ (x) + 0.69$, $R^2 = 0.9915$; (PAF), $y = -2.20\ (x) + 0.64$, $R^2 = 0.9852$, and (BAF), $y = -2.26\ (x) + 0.63$, $R^2 = 0.9841$. The S values for the test compound (niacinamide) for the AAFs are all slightly less than methanol (2.61).

Table 2 shows the log k and solvent strength linear least square regression equations for two other compounds, caffeine and phenol, as a function of volume fraction of EAF, PAF, BAF, or MeOH. The overall curve profiles are typical for organic solvents such as MeOH.[38] The trend in the S values for caffeine and phenol increasing with increasing alkyl chain of the AAF is similar to that found previously for niacinamide, and the S values for MeOH are still higher. For the proton donor-acceptor test compound p-nitroaniline, S values of 1.90, 2.30, and 2.50 were found for EAF, PAF, and BAF, as compared to 2.55 for MeOH (Figure 7). Using a C_{18} silica column, average S values for nitro-aromatics of 1.79 and 2.12 have been reported for ethylammonium- and propylammonium- nitrate, respectively,[13] The retention mechanism using AAF solvents with a hydrophobic column is considered reversed phase, analogous to that found using standard organic solvents such as MeOH or MeCN.

CONCLUSION

We have demonstrated that AAF room temperature ILs can be easily synthesized and are reproducible replacements for organic solvents in the mobile phase for reversed-phase liquid chromatography with detection at

254 nm. The eluent strengths of EAF, PAF, and BAF as measured by P′, are slightly higher as compared to MeOH and MeCN, but much smaller than that for water. High concentrations of conventional salts like ethylammonium sulfate or ammonium formate do not seem to be effective mobile phase modifiers for reversed phase LC of small organic molecules. As expected, there is a loss in column efficiency due to the higher viscosity of the AAF solvents as compared to MeOH. However, the viscosities of the AAFs, particularly that for EAF, are considerably lower than those reported previously for most ILs. Future work is directed at reversed phase LC of pharmaceuticals using EAF at high temperature with fluorescence detection.

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