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Rapid quantification of imidazolium-based ionic liquids by hydrophilic interaction liquid chromatography: Methodology and an investigation of the retention mechanisms

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Abstract

The separation of nine *N,N'*-dialkylimidazolium-based ionic liquids (ILs) by an isocratic hydrophilic interaction high performance liquid chromatographic method using an unmodified silica column was investigated. The chosen analytical conditions, using a 90:10 acetonitrile-ammonium formate buffer mobile phase on a high purity, unmodified silica column were found to be efficient, robust, and sensitive for the determination of ILs in a variety of solutions. The retention window ($k' = 2$ to 11) was narrower than that of previous methods, resulting in a 7-minute runtime for the nine IL homologues. The lower limit of quantification of the method, $2\text{--}3\ \mu\text{mol L}^{-1}$, was significantly lower than those reported previously for HPLC-UV methods. The effects of systematically modifying the IL cation alkyl chain length, column temperature, and mobile phase water and buffer content on solute retention were examined. Cation-exchange was identified as the dominant retention mechanism for most of the solutes, with a distinct (single methylene group) transition to a dominant partitioning mode at the highest solute polarity.

Keywords: ionic liquid determination; HPLC; aqueous normal-phase; rapid separation; mixed-mode retention

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38 **1. Introduction**

39 Ionic liquids (salts with melting points $\leq 100^\circ\text{C}$) have recently been the object of
40 increasingly intense scientific investigation. Many of these compounds, especially those
41 containing disubstituted imidazolium cations, have been shown to be useful in an ever-
42 growing variety of chemical processes, including media for catalysis [1], electrolytes in
43 batteries [2], and solvents and stationary phases in chemical separations [3-9]. ILs exhibit
44 several advantages over molecular solvents typically employed in these processes;
45 among them ~~are their~~ relatively low vapor pressures, wide liquid ranges (often
46 encompassing ambient conditions), unique solvation environments, and ~~and~~ ease of
47 functionalization [10]. Vast numbers of cation/anion combinations form ILs, affording an
48 extensive library of physicochemical properties [11]. Given the range of structures
49 comprising ILs, acquisition of information regarding their presence, transport, and
50 speciation in the environment, and for their quantification in various chemical separation
51 systems (*e.g.*, liquid-liquid extraction), necessitates rapid, facile, and flexible analytical
52 methodologies to quantify them in a variety of media.

53 The determination of IL concentrations in solution has generally been carried out by
54 mass spectrometry, NMR spectroscopy [12, 13], or UV-visible spectrophotometry, the
55 latter by exploiting the presence of a chromophore in the cation [14], or less frequently,
56 in the anion (*e.g.*, aromatic acids) [12, 15]. Because of their expense and complexity,
57 however, mass spectrometry and NMR are not commonly available for such routine
58 analyses. While far less complicated, direct UV spectrophotometry is plagued by
59 interferences, particularly with organic solvents at low wavelengths. In such cases, more
60 labor-intensive sample pretreatment (*e.g.*, solid phase extraction) or standard preparation
61 (*i.e.*, matrix matching [14]) must be undertaken, which can be time-consuming and
62 generate significant solvent waste. To perform routine analysis for ILs (*e.g.*, in studies of
63 their liquid phase partitioning behavior, biodegradability, and ecotoxicity [14, 16-19]),
64 investigators have generally relied upon the traditional dual-beam cuvette technique,
65 which can be both laborious and sample intensive. A better approach to the determination
66 of imidazolium-based ILs in a variety of matrices (including those involving liquid-liquid

partitioning measurements) would, in addition to simplicity and low sample consumption, be capable of handling samples with multiple analytes in UV-absorbing, water-immiscible solvents (*e.g.*, octanol), provide a wider dynamic range than UV analysis, involve minimal sample pretreatment, and comprise a flexible platform amenable to detection by mass spectrometry. Toward this end, we have explored an approach using dilute-and-inject pretreatment with HPLC. A number of previous studies have examined the separation of ILs by various liquid chromatographic methods [17-30]. Due to the variety of solute-stationary phase interactions, the efficient separation of ILs using reversed-phase HPLC has thus far proven difficult. To achieve a separation of polar or charged analytes in reversed-phase systems, where interactions with exposed silanols can lead to poor band shape, either ion-pairing agents or high salt concentrations have been employed [20-23]. The unsatisfactory peak shape and resolution in the analysis of ILs by reversed-phase HPLC reinforces the necessity for strong ion-pairing agents on hydrophobic bonded phase, which precludes the use of various detection methods, including mass spectrometry.

Exploiting the strong ionic interactions observed with IL solutes, several researchers have explored ion chromatography (IC) with direct conductivity detection (most eluent suppressors cannot withstand organic-modified eluents) as an approach to the determination of IL cations and anions [24-27]. Excellent results have been obtained by employing low conductivity buffers (*e.g.*, phthalate) and an acetonitrile modifier in the eluent [23]. The advantage of IC methods is that both the IL cations and anions can be determined simultaneously, often with excellent sensitivity and linearity (1-300 μM). However, IC systems utilizing tandem orthogonal separations and gradient elution programs can be costly and difficult to maintain. In addition, the use of organic eluent modifiers for more hydrophobic ILs requires that the system not include eluent background suppression, and employing aromatic buffers like phthalate salts may contribute to high background in UV detection systems.

Although historically they have been less frequently used than reversed-phase systems, normal-phase methodologies are currently undergoing a resurgence, particularly for small polar or charged analytes [30]. Encouraging results have also been obtained with HPLC employing either polar or mixed-mode stationary phases for the separation of

ILs [28-30]. Among the more intriguing approaches ~~utilize~~is hydrophilic interaction liquid chromatography (HILIC) [29, 30], ~~the~~a ~~The~~ term HILIC was coined by Alpert in 1990 [2431] to differentiate the partitioning mechanism involved in the separation of relatively polar or charged analytes (*e.g.*, carbohydrates, amino acids, oligonucleotides) on a polar stationary phases (*e.g.*, silica, amino, amide, cyano, diol, sulfobetaine, zwitterionic) to which is adsorbed a boundary layer of water under conditions of high organic solvent content (usually 70-95% acetonitrile). Although relative contributions from dominant mechanisms in a HILIC separation may vary widely with column and mobile phase composition, , and the nature of the solutes, they include solute partitioning *via* the adsorbed water layer, anion or cation exchange with surface or boundary layer solvent ions, adsorption to polar stationary phase, and/or ion-pairing [32-36]. In all cases, this technique utilizes buffers, salts and/or acids in the eluent that tend to mask ionic interactions with surface charged groups (*e.g.* silanols). Nonetheless, because residual silanols cannot be completely suppressed, particularly with non-bonded silica phases, a HILIC separation can exhibit ionic interactions as a significant component, depending on the nature of the analytes [28]. Note that the buffers employed often comprise volatile salts (*e.g.*, ammonium acetate or trifluoroacetate), primarily because of their solubility in highly polar aprotic organic mobile phases. An added benefit of such salts is that they are often compatible with mass spectrometry [37], a particular advantage over reverse-phase techniques, which tend to have much lower sensitivity due to the higher mobile phase water content. Furthermore, the low viscosity of high acetonitrile mobile phases and increased solute diffusivities in HILIC systems has shown promise for the fast separation of ionized bases [33].

With these advantages of HILIC in mind, we have examined a separation of *N,N'*-dialkylimidazolium-based ILs (herein denoted as “C_nC_mimX”, where n and m are the carbon number of the alkyl chains and X is the counteranion, which should not affect the separation) was explored using a bare silica column. These particular ionic liquids have been the subject of intense interest (in our case, as replacements for the conventional organic solvents employed in the liquid-liquid extraction of metal ions [38]), and therefore make an excellent starting point for these investigations. To the best of our knowledge, the analytical method described here is the most efficient separation yet

129 reported for a mixture of homologous ILs, in terms of number of resolved, structurally-
130 related species in a given retention window. In addition to demonstrating the efficiency of
131 the method, we have evaluated its octanol matrix compatibility (in the interest of direct
132 IL cation hydrophobicity measurements in a water-immiscible solvent), to illustrate the
133 method's versatility, linearity, precision, sensitivity, and other figures of merit to
134 compare with other reported HPLC methods for IL quantification. Furthermore, to gain a
135 better understanding of the mechanisms of retention of ILs and robustness of this method,
136 and at the same time evaluate the robustness of this method, experiments at a variety of
137 mobile phase compositions (*i.e.*, water content and buffer concentration) and column
138 temperature were performed.

139

140 2. Experimental

141 2.1 Materials

142 All chemicals were reagent grade and used without additional purification, unless
143 noted otherwise. Aqueous solutions were prepared using deionized water with a specific
144 resistance of at least 18 MΩ·cm⁻¹. The HPLC mobile phase buffer was prepared by
145 combining equal parts of 500 mM formic acid (puriss. Grade, Sigma-Aldrich, St. Louis,
146 MO) and 500 mM ammonium formate (J.T. Baker, supplied by VWR Chicago, IL),
147 which resulted in a pH of 3.7. The aqueous buffer solution pH was measured by a pH
148 combination electrode—prior to mixing with the organic solvent. The HPLC mobile
149 phases were prepared by combining an appropriate volume of the ammonium formate
150 buffer with water and acetonitrile (Honeywell Burdick and Jackson, HPLC grade,
151 supplied by VWR Chicago, IL). The analytical mobile phase was prepared by combining
152 10.0 mL of the ammonium formate buffer with 40 mL of water and 450 mL of
153 acetonitrile to produce a 10 mM buffer concentration in 90:10 acetonitrile:water for
154 analysis. Thus, all mobile phase buffer concentrations are given as values in total
155 solution.

156 Imidazolium-based ionic liquids incorporating the *N*-methyl, *N*-ethyl, *N*-butyl, and *N*-
157 hexyl substituents were synthesized and purified by methods previously reported [36,
158 37]. In the first step, a halogenated *n*-alkane (in 3% molar excess) and an *N*-
159 alkylimidazole were combined neat, and the mixture subjected to microwave irradiation

(using a CEM Discover BenchMate Model 908005), yielding an *N,N'*-dialkylimidazolium halide. The product was washed with ethyl acetate (unless noted otherwise) four times to remove unreacted starting materials. Halide salts that were solid were either heated slightly until melted or dissolved in a small amount of deionized water to facilitate contact with the ethyl acetate. Excess solvent was removed under reduced pressure by rotary evaporation in a water bath held at 60°C. In the second step, the water-miscible salts were dissolved in deionized water and combined with an aqueous solution containing a 3 mol% excess of lithium *bis*[(trifluoromethyl)sulfonyl]imide (LiTf₂N). Although in each case, the IL phase readily formed following the addition of the aqueous LiTf₂N solution, samples were allowed to stir overnight to ensure completion of the reaction. The IL (lower) layer was then allowed to settle, separated from the aqueous phase, and washed with deionized water until a test of the water washes for the presence of halide ion (by precipitation with silver nitrate) was negative. The final products, which were nearly colorless to yellow free-flowing liquids, were dried under vacuum at 80°C for at least 24 hours.

Standards for the HPLC studies were prepared by first dissolving a known weight of the dry ILs in methanol (HPLC grade, Burdick & Jackson) and then diluting serially in acetonitrile:water 90:10 (v:v), such that the residual methanol was less than 1%.

2.2 Chromatographic Conditions

Chromatographic separations were performed under isocratic conditions on an Agilent 1200 series HPLC equipped with analytical binary pumps, an online degasser, a fixed loop (20 µL) manual injection port, and a variable wavelength UV-VIS detector, all operated using ChemStation software. The column was a 3.0 mm x 75 mm Agilent Zorbax HILIC Plus silica column with 3.5 µm average particle size and average pore diameter of 95 Å. The final conditions for HPLC analysis were an isocratic flow rate of 0.800 mL min⁻¹ with 10 mmol L⁻¹~~mM~~ ammonium formate (pH 3.7) in 90:10 (v:v) acetonitrile:water. The column temperature was maintained at 30°C for solution concentration measurements. The detection of eluting species was carried out at 210 nm, near the absorbance maximum for the imidazolium cations.

191 2.3 Methods

192 The dead time, t_0 , was measured using toluene as an unretained solute. The mobile
 193 phase composition was modified for the determination of the effect of water content by
 194 online mixing of the 10 ~~mmol L⁻¹mM~~ ammonium formate buffer (pH 3.7) in 90:10
 195 acetonitrile:water mobile phase with the appropriate amount of 10 ~~mmol L⁻¹mM~~
 196 ammonium formate buffer (pH 3.7) in water, and by preparing a solution in a 95:5
 197 acetonitrile:water mobile phase. Lower limits of detection and quantification were
 198 calculated by multiplying the ratio of the error in the standard calibration intercept to the
 199 slope by factors of 3.33 and 10, respectively.

200 The temperature range and step for the van't Hoff correlation with retention was 25-
 201 60°C in 5°C increments. Before one can calculate the entropic contribution to retention
 202 from the intercept, the phase ratio of the column must be known. Because it is impossible
 203 to know exactly the boundary between the space occupied by the stationary phase and the
 204 mobile phase in the column, however, the ratio must be calculated by $V_S / V_M = 1 - \varepsilon_T / \varepsilon_T$
 205 using the total column porosity (ε_T) provided by $\varepsilon_T = V_0 / V_C$, where V_0 is the hold-up time
 206 determined as the elution volume of a small unretained solute (toluene), and V_C is the
 207 volume of the empty column. The retention time for toluene was 0.539 ± 0.001 min,
 208 resulting in a V_0 of 0.431 mL and a phase ratio (V_S/V_M) of 0.230.

209 Investigating the effect of buffer concentration was accomplished with individual
 210 solutions of 2 to 50 ~~mmol L⁻¹mM~~ buffer in 90:10 acetonitrile:water. Under condition in
 211 which there was excessive co-elution of solute bands, individual solutions of the analytes
 212 were injected to obtain accurate retention times. Before each new set of chromatographic
 213 conditions ~~were~~-was examined, the column was equilibrated with at least 20 column
 214 volumes of mobile phase. A steady baseline UV signal and stable column temperature
 215 were taken as indicators that the system was, in fact, equilibrated.

216 Column efficiency (N) was determined by peak widths at half height and normalized
 217 to a column length of one meter by $N = 5.54 (t_R)/(w_{0.5})^2 / (0.075 \text{ m})$. Resolution was
 218 calculated using the peak width at half-height method by $R_s = 2 (t_{R,2} - t_{R,1}) / 1.7 (w_{0.5,2} +$
 219 $w_{0.5,1})$. Peak asymmetry (A_{sym}) was measured at 10% of peak height from the ratio of the

widths of the rear and front sides of the peak (B/A), as calculated using ChemStation software.

Significant carryover is a typical issue associated with the use of manual injection systems, leading to poor peak area reproducibility and low sensitivity. Acetonitrile was chosen as the needle rinse solvent because it resulted in carryover of only 0.05% of prior injection peak area with only three replicate rinses.

3. Results and Discussion

3.1 Development of an HPLC-UV method for IL analysis

3.1.1 Method optimization

In considering the array of possible chromatographic approaches to separate a mixture of charged solutes, aqueous normal phase mode (of which HILIC is a subcategory) appears to offer the most promise, in that it has been found to be much less prone to severe peak tailing and splitting than are reversed-phase separations, resulting in superior resolution [27]. Unfortunately, ~~the HILIC methods published with~~ the best retention factor windows for the HILIC methods published to date [28] ~~were are~~ very wide ($\Delta k' \geq 30$ separating $C_2C_{1im}Tf_2N$ and $C_8C_{1im}Tf_2N$), too wide for the rate of throughput sought for this method. Our approach is to use a high-purity, underivatized silica phase in an aqueous normal phase mode that will exploit the differences in IL cation charge density.. A previous study of the separation of eight neutral and ionizable solutes (not ILs) showed that good peak shape and narrower retention factor windows ($\Delta k' \sim 5$) can be obtained using underivatized silica columns and ammonium formate buffer [32]. As shown in Figure 1, which depicts the chromatograms obtained upon injection of increasing quantities of a mixture comprising a series of nine homologous $C_nC_{mim}Tf_2N$ ILs under the same conditions, good resolution of all of the analytes can be achieved in approximately six minutes.

3.1.2 Analytical performance parameters

Table 1 lists some of the pertinent peak and separations data from these experiments. Using the calibration curve method described in section 2.3 ($5\text{-}500 \mu\text{mol L}^{-1}$), the lower limits of detection for all nine IL cations were between 0.5 to $0.9 \mu\text{mol L}^{-1}$ and the lower limits of quantification were between 2 to $3 \mu\text{mol L}^{-1}$.

251

252 *3.1.2 Evaluation of the method merits*

253 Table 1 lists some of the pertinent peak and separations data from these experiments.
254 This sensitivity represents a 30-fold improvement over previously described HPLC-UV
255 methods for IL determination [24, 27] and compares favorably to that of recently reported
256 methods employing ion-pair reversed-phase chromatography [21], capillary
257 electrophoresis [24], and IC with conductivity detection [24]. Such gains in sensitivity
258 may be attributable to better efficiencies (N), although these parameters were not
259 reported in those studies indicating the lowest detection limits [29], permitting only
260 estimates from the reported chromatograms.

261 An important aim of this work was to demonstrate the method's versatility by
262 applying it to samples that may be encountered in the direct measurement of the octanol-
263 water partition coefficients of ILs. Over the course of a three-day evaluation period,
264 calibration standards in a 90:10 ACN:water diluent were used to ~~measure-quantify~~ the
265 nine IL cations. Quality control samples were prepared in the calibrator diluent and from
266 spiked 1-octanol and solutions (neat and diluted 10-fold with acetonitrile) with similar
267 results. The recoveries were between 97.5% and 103%, the reproducibility of peak
268 retention times ranged from 0.16% to 0.90% RSD, and the precision of peak areas ranged
269 from 1.26% RSD to 5.80% RSD (n = 6 replicates in three separate batches) for 500 μmol
270 L^{-1} and 10 $\mu\text{mol L}^{-1}$ solutions, corresponding to 10 nmol and 0.2 nmol on column per IL
271 in the cocktail, respectively. Good linearity ($r^2 > 0.999$) was observed for 0.1 nmol to 20
272 nmol on column per IL in the cocktail. However, at 40 nmol on column, peaks exhibiting
273 significant fronting ($A_{\text{sym}} \approx 0.5$) were observed due to overloading of the column.
274 Average column efficiencies ranged from 6500 plates m^{-1} to 46000 plates m^{-1} across the
275 nine-solute retention window. Thus, samples from aqueous phases, as well as those from
276 some water immiscible solvents, may be analyzed using a single set of standards in a
277 diluent with solvent strength similar to that of the mobile phase. Carryover was found to
278 be approximately 0.05% by injecting a blank diluent sample following the injection of a
279 500 μM standard cocktail and the needle and injection port rinse procedure (described in
280 section 2.3).

281 *3.2 Characterization of the Retention Mechanisms of the HPLC Method*

Normal-phase elution order with a significant partitioning component is a hallmark of HILIC mode separations [31]. For ionized solutes on a silica phase, ~~ion-exchange electrostatic interactions are~~ often ~~constitutes-athe~~ primary contributors to the overall retention [32-35]. While the polar, positively-charged head group of *N,N'*-dialkylimidazolium ions is capable of electrostatic ~~interactions~~attraction, hydrogen bonding, and other dipole-dipole interactions, the alkyl groups exhibit only van der Waals forces. In a separation system with a polar mobile phase and stationary phase, therefore, systematically increasing the length of the alkyl groups will result in lower retention [20], because reduction in the overall molecular polarity and charge-to-size ratio results in diminished hydrophilicity and electrostatic attraction [20].

3.2.1 Relationship between retention and IL cation carbon number

In an effort to assess the relative contributions of the electrostatic/dipolar and partition components of the retention of the IL cation, the effect of a systematic adjustment of the alkyl chain lengths of the cation was examined. Figure 2 illustrates the relationship between the logarithm of the retention factor and the carbon number of the two homologous series of ILs ($C_{10}C_n\text{im}$ and $C_nC_1\text{im}$) shown in Figure 1. Consistent with typical normal-phase separations, the trend in these plots is that of decreased retention (and therefore selectivity) with increases in the hydrophobic portions of the solutes. Nonetheless, the deviation from linearity suggests that more than just the carbon number drives that selectivity, whether the long alkyl chain or the side chain is modified. Stated another way, the slight curvature of the plots suggests that more than one mechanism influences the retention of these solutes, which may be consistent with changes in both solute hydrophilicity and electrostatic attraction.

3.2.2 Effect of solvent strength on retention

The relationship between the retention factor and the carbon number raises the question of whether the most important factor governing IL retention is partitioning or ~~adsorption~~electrostatic attraction in a system where a significant amount of water could be adsorbed to the stationary phase. Obviously, electrostatic interactions often play an important role in the behavior of ILs [45], and these interactions are thus likely to be a

significant factor in their chromatographic retention. The basic relationship governing solute retention derives from reversed-phase chromatography, for which retention is determined (ideally) by [partitioningsolvophobicity](#) [46]. Equation 1 describes the relationship between the retention factor and solvent strength for such a separation system [47].

318

$$\log k' = \log k'_A - SV_B \quad (1)$$

320

where k'_A is the theoretical value of retention factor with only the weak solvent as the mobile phase, V_B is the volume fraction of the strong solvent in the mobile phase, and S is a constant for a given solute (all other conditions constant). In conventional normal-phase systems, where retention is based on surface adsorption and subsequent displacement of the analyte by the eluent, the relationship between retention and the volume fraction of the strong solvent (*i.e.*, water), will conform to the following expression:

327

$$\log k' = \log k'_A - \frac{A_s}{n_b} \log V_B \quad (2)$$

329

where A_s and n_b are the cross-sectional areas occupied by the solute and the eluent molecules, respectively, on the surface [45]. Plots of logarithmic retention factor vs. the linear and logarithmic functions of the water contents in the eluents should therefore produce one straight line and one curve, depending on whether the dominant retention mechanism is partitioning or [adsorptionion-exchange](#). While there are many examples of such plots in the literature [30, 34, 36, 4648], this is the first with such a broad range of IL cation polarities on an [standard HPLC](#) bare silica [phasecolumn](#).

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The effect of varying the volume fraction of water in the mobile phase on $\log k'$ is presented in Figure 3. Clear evidence that partitioning cannot be the only mechanism contributing to the retention of the ILs is found in the nonlinearity. This result is in agreement with previous studies [27, 4648], which proposed that for ionic solutes, retention is also controlled by [adsorptionelectrostatic interactions](#). The demarcation lines on Figure 3 show that the plot can be divided into three distinct regions, where one mode

may play a dominant role in retention. In the first, which corresponds to the volume fraction of water from 0.05 to 0.6, IL retention decreases with increasing water content, thus defining a region ~~of-dominated by~~ normal-phase partitioning behavior that is consistent with HILIC mode. In the second region, which encompasses water volume fractions from 0.6 to 0.8, the retention factor remains essentially constant~~and~~. While all nine IL peaks co-elute at 70% water, a slight trend favoring greater retention for shorter alkyl chain lengths remains, which could reflect differences in charge-to-size ratio. However, these differences are subtle, suggesting that the difference in polarity of the solutes, and thus, hydrophilic interaction is the dominant factor in their retention- under these conditions. Lastly, in the third region (water fraction of 0.8 and above), a subtle shift to reversed-phase behavior (increasing k' with increased water in the mobile phase) is observed, albeit very weakly. These shifts in retention mode lead to the “U” shape of the curves [47].

When the logarithmic retention factors of several representative ILs are plotted versus the logarithmic volume fraction of water for region 1 (Figure 4), an excellent linear fit is obtained (as per Equation 2), which is consistent with an adsorption or ion-exchange process [2735]. ~~Thus, either of these general mechanisms may play a dominant role in the retention and selectivity under the operating conditions of this method.~~ Note, that to attain these fits, the range of the water contents was truncated from 60% for C₂C₁im to 50% for C₄C₁im, and 40% for C₇C₁im and C₁₀C₂im, because of a shift toward more reversed-phase behavior with increasing solute hydrophobicity. On the other hand, studies of hydrophobic solutes on silica phases have estimated that, from 70% to 95% ACN, the water-rich layer decreases from 13% to 4% of the pore volume, respectively [4849]. Thus, a significant portion of the pore volume may be occupied by a water-rich layer that increases in thickness with mobile phase water content. In this context, the 2-fold decreases in retention with a change in 90% ACN to 80% ACN suggests that the increasing-increased IL hydrophobic surface area (*i.e.*, IL alkyl chain length) ~~leads to additional exclusion of the solutes from the pores by hydrophobic effect~~ favors partitioning to the hydrophobic mobile phase [4950], ~~and can be taken as a leading contributor to the trends observed.~~

3.2.3 Influence of temperature on retention

Temperature often has a significant effect on retention, especially for charged or ionizable solutes [5051]. Several studies have explored the effect of temperature on retention for a variety of solutes and stationary phases [5051, -5455], and the results have provided the means for ~~fundamental~~ thermodynamic interpretations of the retention mechanisms involved. The relationship between the retention factor and ~~thermodynamic~~ temperature is described by the ~~Van't-van't~~ Hoff equation [5152, -5354] (Equation 3, using notation from reference 49):

$$\ln k' = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} + \ln \frac{V_s}{V_M} = A_i + \frac{B_i}{T} \quad (3)$$

where the parameter B_i is directly proportional to the partial standard molar enthalpy of transfer of a solute i from the mobile phase to the stationary phase ($-\Delta H^\circ$), A_i is related to the standard partial molar entropy of transfer of the solute from the mobile phase to the stationary phase (ΔS°), and the last term is the phase ratio (ratio of the volume of the stationary phase, V_s , to that of the mobile phase, V_M). Accordingly, the dependence of $\ln k'$ on inverse temperature is expected to be linear. Thus, by plotting $\ln k'$ versus $1/T$ over a sufficiently wide temperature range, the enthalpic and entropic contributions to the retention and selectivity can be estimated, $-\Delta H^\circ$ from the slope and ΔS° from the intercept. A ~~Van't-van't~~ Hoff plot exhibiting nonlinearity indicates that a change in retention mechanism has occurred over the range of temperatures studied [5253].

Figure 5 is a plot of $\ln k'$ versus $1/T$ for the various N,N' -dialkylimidazolium ILs as well as the diluent impurity. At first glance, the curves appear to be quite linear. However, the results in Table S1 indicate that the correlation for C_4C_1im ($R^2 = 0.712$) is non-linear, while the remaining IL cations exhibit much ~~more-greater~~ linearity ($R^2 > 0.95$). Given that temperature has a slight, but significant effect on the retention of C_4C_1im and the apparent curvature in the thermodynamic trend, its retention must involve a very small positive enthalpy change lying in the region of transition from a predominantly endothermic process (observed when one more CH_2 group is present) to one having a larger exothermic component (when two fewer CH_2 groups are present). Evidently, the overall retention process for C_2C_1im is exothermic, but driven by the increase in entropy

(TΔS), which may be attributable to the shift toward a larger contribution from partitioning ~~and specific adsorption~~ with the increasing polarity and greater charge density of this IL cation. The shift in enthalpy from negative to positive (*i.e.*, exothermic to endothermic) coincides with significant changes in the polarity of the ILs, affected by systematic changes in the hydrophobic chains. The observed rise in entropy with increasing IL alkyl chain length is consistent with the greater ease of dehydration of these cations and indicates the driving force underlying the change in dominant retention process to ion exchange is governed by the hydrophobic effect [4950].

3.2.4 Effect of changing buffer concentration on retention

In section 3.2.2, the changes in retention with increasing eluent water content indicated that a hydrophilic partitioning mode plays an important role in the retention of the imidazolium ions. On the other hand, Tthe extent to which ionic interactions play a role in the retention of charged solutes can be assessed by observing the effect of changing mobile phase salt concentration on retention. In fact, quaternary amines have been used to measure the activity of surface silanols in reversed-phase systems [56]. In traditional ion-exchange chromatography, the relationship between the retention factor (k') of a solute and the buffer or salt concentration $[C]$ is described by Equation 4 [5557], where s is a constant dependent on the charge of the solute and counter-ion.

$$\log k' = -s \log [C] + \text{constant} \quad (4)$$

We can define the retention of a solute (A^+) by silanol-mediated ion-exchange as the equilibrium constant of the process in Equation 5

$$K_A = \frac{[SiO^-:A^+]_s [C^+]_m}{[SiO^-:C^+]_s [A^+]_m} \quad (5)$$

where SiO^- and C^+ represent the ionized silanol group and its counter ion (*i.e.*, NH_4^+), respectively, and the subscripts s and m correspondingly denote the stationary phase and mobile phase. Expressed for a singly charged solute and a monovalent counterion, the slope of the relationship in Equation 4 would be -1. Although the relationship for our compounds is linear from 8 to 20 mmol L⁻¹ buffer, as shown in Figure 6, the deviation from unit slope observed for all of the curves (-0.37 to -0.13), provided in Table 3S2,

may be indicative of the existence of retention mechanisms other than ion exchange [5657, 5758]. Additionally, the activity of the ammonium ion may be significantly less in the bulk mobile phase due to diminished dissociation of the buffer salt in a largely aprotic solvent [5859], which could also account for some deviation of the slope from unity.

To better define the contribution of ion-exchange to the overall retention, a “two-site model” described by Yang *et al.* [5657] for the determination of the contribution from ion-exchange behavior of hydrophobic bases on reversed-phase columns was employed. The key assumption of the two-site model is that the solute molecules interact independently with two theoretical types of sites. Therefore, this approach may provide a means to estimate the extent to which normal-phase partitioning or ion-exchange contribute to retention. Equation 6 is a linear combination of the two contributions, where ϕ_{NP} , D_{NP} and ϕ_{IX} and D_{IX} are the phase ratio and distribution ratio for a pure normal-phase partitioning process and ion-exchange process, respectively, and k'_{NP} and k'_{IX} are the corresponding retention factors for these two mechanisms, the sum of which is the observed retention factor, k' . The notation used here is derived from ~~the~~that reported by Yang *et al.* [5657].

$$k' = \phi_{NP}K_{NP} + \phi_{IX}K_{IX} = k'_{NP} + k'_{IX} \quad (6)$$

No assumption is made as to the details of the normal-phase interaction, because contributions thereto may comprise more than just a partitioning process, as described by HILIC theory. As long as these two components are independent of one another, and the normal-phase equilibria are only weakly dependent on the buffer concentration, then Equation 7-6 can be rewritten as:

$$k' = \phi_{NP}K_{NP} + \frac{B_{IX}}{[C^+]_m} \quad (7)$$

This expression is the basis for the interpretation of a plot of retention factor versus the reciprocal of the mobile phase buffer concentration, where the slope of the plot (B_{IX}) is proportional to the total number of free silanol sites per unit area ($[SiO^-: C^+]$) and the

equilibrium constant for the process (K_A from Equation 5), and the y-intercept is the contribution to retention by mechanisms other than ion-exchange. Note that, for a 1:1 displacement reaction, Equation 7 leads to the conclusion that there will be no retention upon extrapolation to $1/[C^+] = 0$. It is not unprecedented to apply this approach to a normal-phase separation [5948, 60, 60-63]. In fact, the first notable use of a plot of k' versus the inverse counter-ion concentration was performed in pursuit of a second non-ion-exchange process in a mixed-mode system utilizing bare silica phases [60].

Figure 7A is a plot of the IL cation retention factor versus the reciprocal ammonium concentration of the mobile phase buffer. By employing the linear combination in Equation 6, the regression statistics and relative contributions to retention from ion-exchange (% k'_{IX}/k') with varying buffer strength (8 to 20 mmol L⁻¹) are listed in Table S3 and Table 2. All of the intercepts observed are non-zero and positive, consistent with the existence of an additional retention mechanism at “infinite” buffer concentration [61]. Assuming that two independent modes of phase transfer are present, the estimated contributions from normal phase partitioning are the difference of % k'_{IX}/k' from 100%. Although the full range of buffer concentrations assessed in these experiments was 2 to 50 mmol L⁻¹, the range of buffer concentrations (8 to 20 mmol L⁻¹)—used in these calculations is—was 8 to 20 mmol L⁻¹, that which provided measurable retention and reasonable peak shape for all solutes, maintained the relationship in Equation 7, and encompassed the analytical conditions. In accordance with Equation 7, the plots in this range of buffer concentrations are rather linear ($R^2 \geq 0.98$).

For two notable reasons, however, the changes in retention outside the 8 to 20 mmol L⁻¹ range of buffer concentrations do not follow the same trend. Firstly, the plot of retention versus buffer concentration in Figure 7B more clearly shows that the titration of the free silanols by ammonium ions in the mobile phase appears to reach an end-point near 30 mmol L⁻¹. At that point, a HILIC trend remains for the homologous series at the end of the titration. Such a trend is consistent with a study by Alpert [63] that indicated increased cation retention on a weak anion exchange phase with silanol shielding by the buffer. Secondly, the convex curves suggest that an electrostatic attraction mode is superimposed on a normal phase partitioning mechanism, which appears to some extent dependent on the buffer concentration. Under these conditions, where the organic solvent

content is high, the mobile phase will exhibit a low dielectric constant, stronger ion-pairing interactions between the IL cations and formate ions are expected. Therefore, as the buffer concentration increases both electrostatic attraction and partitioning will decrease and the contributions from these modes do not necessarily change at the same rate. More evidence for an ion-pairing effect appeared at 50 mmol L⁻¹ buffer, where C₂C₁im displayed a 90% increase in retention. Whether this trend would continue at higher buffer concentrations was not determined because the column equilibration at 50 mmol L⁻¹ buffer was as slow as two hours, in contrast to the twenty to thirty minutes necessary for equilibration between 2 and 30 mmol L⁻¹. Further increases in the buffer might have made it difficult to reach a steady state and also indicate that significant changes in the stationary phase may be responsible for the aberrant behavior of C₂C₁im at the highest buffer concentration. In this case, the increased retention may be attributable to solvation of the more charge-dense C₂C₁im by an ion-pairing mechanism. Existence of such an ion-pair mechanism in HILIC mode is supported by the identical retention for C₁₀C₁imBr and C₁₀C₁imTf₂N in Figure S1 and by similar reports in the literature [21, 25].

Such mixed-mode retention makes it difficult to quantify the individual contributions to retention across the entire range, and we can only estimate that the %contributions in Table 2 reflect those near the analytical conditions. In this region, the estimated contribution from ionic interactions increases from 20% to 80% for C₂C₁im to C₁₀C₄im, respectively. If these trends are an indication of the relative contributions, then the dominant retention mechanism for IL cations more polar than C₅C₁im is partitioning. Indeed, a shift toward a greater contribution from ionic interaction between C₂C₁im and C₅C₁im is consistent with the trends observed in the van't Hoff plots in Section 3.2.3, where the energetics of phase transfer shift from exothermic to endothermic. While the retention of the less polar IL cations may be dominated by electrostatic attraction, increasing alkyl chain length also reduces access to the charged exchange sites on the polar stationary phase. Consequently, ionic attraction may be less than that for IL cations with shorter alkyl chain lengths (*i.e.*, higher charge-to-size ratios). Considering the normal-phase behavior of the solute bands, and the likelihood that the high organic content of the mobile phase has led to a water-rich boundary on the silica phase, it is noted that as the alkyl chain length of an IL increases the polar contribution to the overall

retention is less pronounced, and it will partition more into the hydrophobic mobile phase.

~~The impurity peak, is conspicuously absent because it was not observed for the highest buffer concentration.~~

~~For an estimation of relative contributions to retention under conditions similar to those chosen for the analytical method, the prediction trends in Figure 7 is are insightful. By employing the linear combination in Equation 6, the regression statistics and relative contributions to retention from ion exchange ($\% k'_{ix}/k'$) with varying buffer strength are listed in Table S3 and Table 2. All of the intercepts observed are non-zero and positive, consistent with the existence of an additional retention mechanism at “infinite” buffer concentration [5961]. Interestingly, the estimated contribution from ion exchange (Table 2) for C_4C_{1im} and C_5C_{1im} were at or near 50%. These intermediate values of ion-exchange and partitioning contributions are in accord with the transitional break in the thermodynamic trends described in section 3.2.3. Increased relative contributions from ion exchange with respect to increasing impact from the larger hydrophobic alkyl moieties of the solutes reveal that the contribution from a partitioning mode is, to a large extent, dependent on the hydrophilicity of the solute. That is, this property may be directly associated with the solubility of the IL cations in the enriched water boundary, establishing the degree to which the solute may interact with the eluent ions. Considering the normal phase behavior of the solute bands, and the likelihood that the high organic content of the mobile phase has led to a water-rich boundary on the silica phase, it is noted that the more hydrophobic ILs may be significantly excluded from pores that are enriched in water, and will, therefore, exhibit lower retentionas the alkyl chain length of an IL increases (*i.e.*, decreased), the contribution to the overall retention is pronounced, and it tends to partition more into the hydrophobic mobile phase.~~

4. Conclusions

This paper reports an improved, routine chromatographic method to determine room temperature ionic liquids in a variety of sample types. The analytical method represents a significant improvement over previously reported ~~chromatographic-HPLC~~ methods for the determination of ILs, as nine imidazolium cations can be separated in the span of only

seven minutes with exceptionally good peak shape, excellent sensitivity and precision. In the course of investigating the retention modes, various aspects of the- robustness of the method were also ~~demonstrated~~assessed. Because the acetonitrile mobile phase employed is miscible with both organic solvents and water, as long as the water content of an injected sample is 10% or less (*i.e.*, a 10-fold dilution of an aqueous sample), the method provides a facile approach for the determination of ILs in a wide variety of samples (*e.g.*, liquid-liquid distribution measurements, environmental transport/fate, ~~—~~or radiolysis experiments). Replacement of the UV detector with a mass spectrometer could provide a means to test the entire range of IL structures and degradation products thereof. These and other similar studies are ongoing in this laboratory.

The combination of normal phase partitioning with ~~adsorption, ion-exchange~~ionic interactions, ~~or~~ and ion-pairing modes at high organic phase contents are often considered quintessential components of HILIC separations. Evaluation of the overall retention mechanisms for the nine homologous IL cations ~~demonstrated that ion-exchange suggests that is the predominant mode of retention on underivatized silica for of~~ the ionic liquid cations with this high purity, underivatized silica is primarily a superposition of partitioning and electrostatic attraction. more hydrophobic than C₅C₄im. A s~~Shifts~~ toward the predominance of a partitioning mode with higher charge-to-size ratio is consistent with the trends in retention from ~~change-ing~~ temperature, mobile phase water content, and buffer strength trends ~~observed-with-C₄C₄im~~. These studies have ~~demonstrated indicated~~ how systematic changes in the *N,N'*-imidazolium ion alkyl substituents affect the relative contributions from these modes and underline the trends imposed by their hydrophobic moieties on a silica phase designed for HILIC separations, where the overall selectivity appears to be controlled by the variation in solute polarity. Such trends could assist in developing a better understanding of the behavior of ILs, not just as solutes, but also provide insight into their behavior as bulk fluids.

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584 5. Acknowledgements

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589 **6. References**

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754

Figure Captions

Figure 1. Overlay of Chromatograms from the injection of a cocktail composed of nine homologous 1,3-dialkylimidazolium bis[(trifluoromethyl)sulfonyl]imide ionic liquids at 10, 50, and 100 $\mu\text{mol L}^{-1}$, where 1 = $\text{C}_{10}\text{C}_4\text{im}$, 2 = $\text{C}_{10}\text{C}_2\text{im}$, 3 = $\text{C}_{10}\text{C}_1\text{im}$, 4 = $\text{C}_8\text{C}_1\text{im}$, 5 = $\text{C}_7\text{C}_1\text{im}$, 6 = $\text{C}_6\text{C}_1\text{im}$, 7 = $\text{C}_5\text{C}_1\text{im}$, 8 = $\text{C}_4\text{C}_1\text{im}$, 9 = $\text{C}_2\text{C}_1\text{im}$, at 10, 50, and 100 $\mu\text{mol L}^{-1}$ in 90:10 ACN:water. 20 μL injection volume, mobile phase: 10 mM ammonium formate buffer pH 3.7 in 90:10 ACN:water, flow rate: 0.800 mL min^{-1} , Zorbax HILIC Plus 3.0 mm x 75 mm, 3.5 $\mu\text{m d}_p$, column temp. 30°C, UV detection 210 nm.

Figure 2. Variation of logarithmic retention factor with carbon number of homologous series of 1,3-dialkylimidazolium-based ILs. Fitted lines are quadratic least-squares regressions and are intended only as guides to the eye. Error bars are standard deviations ($n=3$) at the 95% confidence interval. Conditions are the same as in Figure 1.

Figure 3. The effect of varying the volume fraction of water in the eluent (V_B from Equation 1) on the logarithmic retention factor.

Figure 4. Bilogarithmic relationship of retention factor on the varying volume fraction of water in the eluent (V_B). Lines are linear least-square fits.

Figure 5. Temperature dependence of retention factors for ILs and the diluent impurity. Temperature range was 25°C to 60°C.

Figure 6. The effect of varying the ammonium formate buffer concentration (2, 5, 8, 9, and 10 mmol L^{-1}) on the retention factor.

Figure 7. Plots of (A) retention factor versus the reciprocal buffer concentration (~~8, 9, 10, and 20 mmol L^{-1}~~) and (B) retention factor versus the buffer concentration (2, 5, 8, 9, 10, 20, 30 and 50 mmol L^{-1}).

Figure 1 (color for online only)

Figure 2 (color for online only)

Figure 3 (color for online only)

Figure 4 (color for online only)

Figure 5 (color for online only)

Figure 6 (color for online only)

Figure 7 (color for online only)

Figure 1 (black and white for print only)

Figure 2 (black and white for print only)

Figure 3 (black and white for print only)

Figure 4 (black and white for print only)

Figure 5 (black and white for print only)

Figure 6 (black and white for print only)

Figure 7 (black and white for print only)

For submission to Journal of Chromatography A

Rapid quantification of imidazolium-based ionic liquids by hydrophilic interaction liquid chromatography: Methodology and an investigation of the retention mechanisms

Cory A. Hawkins*, Anna Rud, Margaret L. Guthrie, and Mark L. Dietz

Table 1. Average peak retention, average peak width, calculated peak resolution, column efficiency (N), and lower limits of detection (LLOD) and quantification (LLOQ) for ten solutes

Peak Name	Retention Time (min)	Peak Width (min)	Resolution	N (plates m ⁻¹)	LLOD (mol L ⁻¹)	LLOQ (mol L ⁻¹)
C ₁₀ C ₄ im ⁺	1.525	0.0495	----	45974	0.7	2
C ₁₀ C ₂ im ⁺	1.871	0.0609	3.69	37264	0.8	3
C ₁₀ C ₁ im ⁺	2.155	0.0687	2.58	33727	0.5	2
C ₈ C ₁ im ⁺	2.368	0.0725	1.77	33278	0.6	2
C ₇ C ₁ im ⁺	2.541	0.0755	1.38	32928	0.9	3
C ₆ C ₁ im ⁺	2.772	0.0819	1.73	30526	0.6	2
C ₅ C ₁ im ⁺	3.12	0.0876	2.42	30033	0.5	2
C ₄ C ₁ im ⁺	3.665	0.0945	3.52	30315	0.7	3
diluent impurity	5.055	0.1114	7.94	30088	ND	ND
C ₂ C ₁ im ⁺	5.952	0.2594	2.85	6534	0.8	3

ND = Not Determined because the acetonitrile impurity is not an analyte of interest

Table 2. Estimation of the ion-exchange contribution to the retention factor with 8 mmol L⁻¹ to 20 mmol L⁻¹ ammonium formate buffer

IL Cation Solute	% k' _{IX} / k' at [NH ₄ ⁺] (mmol L ⁻¹) ^a			
	8	9	10	20
C ₂ C ₁ im ⁺	22 (0.1)	20 (0.1)	19 (0.1)	10 (0.06)
C ₄ C ₁ im ⁺	45 (2)	43 (1)	41 (1)	24 (1)
C ₅ C ₁ im ⁺	54 (3)	52 (3)	50 (3)	32 (2)
C ₆ C ₁ im ⁺	60 (6)	58 (6)	57 (6)	37 (4)
C ₇ C ₁ im ⁺	64 (9)	63 (9)	61 (9)	42 (6)
C ₈ C ₁ im ⁺	67 (12)	66 (11)	64 (11)	45 (8)
C ₁₀ C ₁ im ⁺	71 (15)	70 (15)	68 (14)	50 (10)
C ₁₀ C ₂ im ⁺	75 (18)	74 (17)	72 (17)	54 (13)
C ₁₀ C ₄ im ⁺	82 (28)	81 (28)	80 (27)	65 (22)

^a Uncertainties in parentheses were calculated based on the standard deviation of retention factors (n=3) and standard errors of the linear regression parameters at the 95% confidence interval