

Retention mechanism of some solutes using ionic liquids as mobile phase modifier in RP-high-performance liquid chromatography (HPLC)

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Abstract—Two kinds of ionic liquid, 1-Hexyl-3-methylimidazolium tetrafluoroborate ([Hmim][BF₄]) and 1-Methyl-3-octylimidazolium tetrafluoroborate ([Omim][BF₄]), were used as additives in the linear solvation energy relationships (LSERs) model to investigate the fundamental chemical interactions governing the retention of nine aromatic compounds in acetonitrile/water mobile phases on a C₁₈ column. The effects of the [Hmim][BF₄] and [Omim][BF₄] were compared and the ability of the LSERs to account for the chemical interactions underlying solute retention was shown. A comparison of predicted and experimental retention factors suggests that LSER formalism is able to reproduce adequately the experimental retention factors of the solutes studied in the different experimental conditions investigated.

Key words: Ionic Liquid, Linear Solvation Energy Relationships (LSER), Retention, Modifier

INTRODUCTION

Ionic liquids (ILs) are widely recognized as one of the key components of “green” chemistry. A quantity of scientific papers which grows progressively from year to year implies ILs have enormous advantages in high-performance liquid chromatography (HPLC) [1-4]. It has already been proven that ILs can be used as mobile-phase additives in reversed-phase chromatography when mixed with other solvents of low viscosity. And the ILs used as additives in HPLC for separation have been studied and it was proven that ILs have several mechanisms, such as block the residual silanols groups, modify the stationary phase or to act as ion-pairing agents.

Over the years many practical retention models [5] for RP-HPLC, such as linear solvation energy relationships (LSER), have been developed and widely used. In this study, we have used LSER to explain retention in RP-HPLC using two kinds of ILs as additives.

The general LSER equation used in this work is [6]:

$$\log k = \log k_0 + m(V_x/100) + s\pi_2^H + a\Sigma\alpha_2^H + b\Sigma\beta_2^H + rR_2 \quad (1)$$

where, k is the experimental retention factor. The V_x , π_2^H , $\Sigma\alpha_2^H$, $\Sigma\beta_2^H$ and R_2 terms are solute descriptors, where V_x represents the solute's size/polarizability, π_2^H is the dipolarity/polarizability, $\Sigma\alpha_2^H$ is the hydrogen bond (HB) acidity, $\Sigma\beta_2^H$ is the HB basicity, and R_2 is the excess molar refraction. The subscript “2” simply signifies that these parameters are solute descriptors.

The coefficients of these descriptors m , s , a , b , and r reflect differences in the two bulk phases between which the solute is transferring [7] and are obtained through a multiparameter linear regression. The $\log k_0$ term is simply the intercept of the regression and is comprised of constant contributions from the solutes and the chromatographic system.

We note that since the parameters V_x and π_2^H are blends of two different interactions, the coefficients of these parameters are also

blends of the corresponding properties. Specifically, m is the difference in the cohesivity/dispersive ability of the two bulk phases, and s is the difference in the ability of the two phases to interact through dipole-dipole and dipole-induced dipole interactions. Many reviews and examples of LSERs and their interpretations are available [8-11].

In this study, nine solutes (acetophenone, aniline, caffeine, methylparaben, o-cresol, m-cresol, p-cresol, phenol and pyridine) have been in terms of LSER. Several mobile phases using two kinds of ionic liquids, [Hmim][BF₄] and [Omim][BF₄], as additives in acetonitrile/water mobile phases were characterized by using the previously mentioned solvation parameter LSER model.

EXPERIMENTAL

1. Instruments

All experiments were performed on a Younglin M930 (Korea) equipped with a spectrophotometer (M 7200 Absorbance Detector, Young-In Scientific Co., Korea), and a Rheodyne injector (Hamilton Company, USA) valve with a 20 μ L sample loop. The software Chromate (Ver. 3.0 Interface Eng., Korea) was used for system control and data handling. The detector was operated at 254 nm for LSER test solutes. Experiments were performed with a commercially available C₁₈ column (Optimapak, Korea, 4.6 \times 150 mm, 5 μ m). An injection volume of 2 μ L was applied throughout the experiments. All procedures were carried out at 303 K.

2. Materials

All of the LSER test solutes and the ionic liquids were purchased from C-TRI (Korea). The two ionic liquids, 1-hexyl-3-methylimidazolium tetrafluoroborate ([Hmim][BF₄]) and 1-octyl-3-methylimidazolium tetrafluoroborate ([Omim][BF₄]), were from C-TRI (Korea). The mobile phase modifier acetonitrile was purchased from Dusan (Korea). Deionized water was obtained via a water purification system from Millipore Corp. (Milford, MA).

3. Preparation of Mobile Phases and Standard Solutions

Ionic liquids were added into the acetonitrile/water solution directly. The molar concentrations were adjusted to 0.003 M, 0.006 M,

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and 0.009 M, respectively. The mixed mobile phase contained 5, 10, 15 and 20 v/v % acetonitrile (ACN) modifiers for the mixed solution. After thorough mixing in a sonicator for 30 minutes, the final running eluents were filtered through a syringe filter (HA-0.45, Division of Millipore, Waters, USA) and then sonicated for 20 minutes more prior to the experiments. All stock solute solutions were prepared at concentrations of 1.0 mg/mL in methanol and injected, respectively. It should be emphasized that the working solutions were re-prepared every three days so as to avoid potential errors arising from decomposition.

CALCULATIONS

1. Retention Factor Estimation

The retention factor, k , of each solute was measured according to the following formula:

$$k = (t_R - t_M) / t_M \quad (2)$$

here, t_R and t_M are the retention times of the retained analyte and the retention times of the unretained analyte (also known as dead time), respectively. Sodium nitrite was used as a t_M marker and was measured from the time of injection to the first deviation from the baseline following a 5 μ L injection of 1% sodium nitrite solution. The retention factors reported in this study are the averages of at least three determinations. The results of the chromatographic experiments were evaluated by using mathematical statistic techniques. The relative error of a single measurement did not exceed 22% for [Hmim][BF₄] and 5% for [Omim][BF₄].

2. Linear Solvation Energy Relationship Estimations

Retention factors were determined for the nine compounds used in this study, and the system constants were calculated by multiple linear regressions using Origin Pro 6.0 software (Microcal Software Inc., MA, USA). The differences in LSER coefficients indicate the variations in the types of interactions between stationary phases and solutes. In the column, modification of stationary phase is the most important mechanism of ionic liquid. During the separation, the interactions among the solute, mobile phase and stationary phase were changed according to the component of acetonitrile and ionic liquids. Due to these different interactions, the LSER constants for different kinds of solutes are not identical.

RESULTS AND DISCUSSION

The retention behaviors of the nine test solutes (acetophenone, aniline, caffeine, methylparaben, o-cresol, m-cresol, p-cresol, phenol and pyridine) in each mobile phase were examined and compared using the solvation parameter LSER model, i.e., the model described in Eq. (2). The test solutes and their descriptors used in this study are given in Table 1.

The coefficients for the LSER equations obtained for [Hmim][BF₄] and [Omim][BF₄] mobile phases were grouped in Table 2 and Table 3. In all the mobile phase investigated, most of the values of b and s were negative, which means that an increase in the HB basicity and solute dipolarity/polarizability decreases the overall retention of the molecule. Furthermore, most of the values of m and a were positive in all studies, indicating that increases in the solute volume, excess molar and HB acidity will make increases in the solute vol-

Table 1. Test solutes and their descriptors for the solvation parameter model

Solute	Descriptors				
	V_x (cm ³ /mol ⁻¹)	π_2^H	α_2^H	β_2^H	R_2 (cm ³ /10)
Caffeine	1.5	1.6	0	1.35	1.363
Phenol	0.805	0.89	0.6	0.3	0.7751
<i>p</i> -Cresol	0.82	0.87	0.57	0.31	0.916
Methylparaben	0.9	1.37	0.69	0.45	1.131
Acetophenone	0.818	1.01	0	0.48	1.0139
Aniline	0.955	0.96	0.26	0.5	0.8162
<i>o</i> -Cresol	0.84	0.86	0.52	0.3	0.916
Pyridine	0.631	0.84	0	0.52	0.6753
<i>m</i> -Cresol	0.822	0.88	0.57	0.34	0.916

ume and excess molar. In view of the value range of the coefficients, the excess molar refractivity (R_2) and solute dipolarity/polarizability (V_x) generally play the largest role in determining the retention of solutes in all studies. HB basicity ($\Sigma\beta_2^H$) is also an important factor in the each mobile phase with coefficients comparable in magnitude to those of solute volume.

1. LSER Coefficients in Two Kinds of ILs Systems

LSER coefficients as a function of [Hmim][BF₄] and [Omim][BF₄] concentrations are shown in Fig. 1 and Fig. 2, respectively. The values of all of the five coefficients (m , s , a , b and r) change very complicatedly as the concentrations of ILs or acetonitrile are changing, even the situation was different between the [Hmim][BF₄] and [Omim][BF₄] systems.

In both ionic liquids systems, the value of m was positive. Furthermore, only one negative m value shows that the endoergic cavity formation term does not have the most important effect on retention. According to Eq. (2), a positive sign of m indicates that the solute will preferentially transfer from the aqueous phase to the surfactant phase. As the concentration of the ILs changed, the value of m changed very complexly; it can reflect that the effect of the ILs was less important than ACN for the retention of nine solvents from side.

The difference in dipolarity/polarizability is represented by the coefficient s . A negative sign for this coefficient indicates that the solutes experience a microenvironment that has less dipolar/polarizable characteristics than the aqueous phase. All of the values of s were negative in [Hmim][BF₄] and [Omim][BF₄] system. As the concentration of IL increased, the holistic trend of s was 20% > 15% > 10% > 5% for ACN.

The coefficient a is an important factor in the solvatochromic model in the two systems studied here. This coefficient represents the difference in the hydrogen bond accepting basicity of the ionic liquid mobile phase and that of the aqueous phase. Most of the values of a of [Hmim][BF₄] system were negative but the same values of [Omim][BF₄] system were positive. The results means the hydrogen bond accepting ability of the mobile phase with [Omim][BF₄] is better than [Hmim][BF₄].

The coefficient b is the second most important factor in the LSER solvation parameter model used in this study. A comparison of the

Table 2. Constants for the liquid chromatography systems using solvation parameter model ([Hmim][BF₄])

Surfactant concentration, M		0.03	0.06	0.09
Modifier concentration, % v/v		5		
Constants	c	0.77 (0.43)	0.65 (0.14)	0.64 (0.32)
	m	0.46 (0.99)	0.47 (0.33)	0.56 (0.75)
	s	-1.73 (0.79)	-1.45 (0.27)	-1.02 (0.59)
	a	0.10 (0.50)	-0.05 (0.17)	-0.11 (0.37)
	b	-1.42 (1.02)	-1.60 (0.34)	-1.64 (0.76)
	r	2.95 (0.88)	2.76 (0.29)	2.17 (0.66)
Statistics	r ²	0.9490	0.9934	0.9542
	SD	0.1793	0.05961	0.1343
	F	11.1747	90.9116	12.4995
Modifier concentration, % v/v		10		
Constants	c	0.80 (0.11)	0.77 (0.13)	0.48 (0.11)
	m	0.066 (0.25)	0.27 (0.31)	0.65 (0.25)
	s	-1.18 (0.19)	-0.76 (0.25)	-0.61 (0.20)
	a	0.040 (0.12)	-0.20 (0.15)	-0.33 (0.13)
	b	-0.86 (0.25)	-1.25 (0.31)	-1.58 (0.26)
	r	1.75 (0.22)	1.37 (0.27)	1.31 (0.22)
Statistics	r ²	0.9928	0.9884	0.9916
	SD	0.04427	0.05523	0.04517
	F	83.185	50.9075	71.1838
Modifier concentration, % v/v		15		
Constants	c	0.62 (0.09)	0.62 (0.09)	0.70 (0.11)
	m	0.23 (0.22)	0.25 (0.22)	0.30 (0.26)
	s	-1.01 (0.18)	-0.86 (0.18)	-0.67 (0.21)
	a	-0.27 (0.11)	-0.28 (0.11)	-0.30 (0.13)
	b	-1.17 (0.22)	-1.23 (0.23)	-1.31 (0.27)
	r	1.83 (0.19)	1.54 (0.19)	1.26 (0.23)
Statistics	r ²	0.9943	0.9925	0.9903
	SD	0.03938	0.04016	0.04685
	F	105.4138	79.5973	61.0685
Modifier concentration, % v/v		20		
Constants	c	0.64 (0.09)	0.54 (0.11)	0.51 (0.11)
	m	0.27 (0.21)	0.48 (0.25)	0.58 (0.25)
	s	-1.09 (0.17)	-0.84 (0.19)	-0.67 (0.19)
	a	-0.24 (0.10)	-0.31 (0.12)	-0.36 (0.12)
	b	-1.13 (0.21)	-1.24 (0.25)	-1.31 (0.25)
	r	1.70 (0.18)	1.26 (0.22)	1.18 (0.22)
Statistics	r ²	0.9943	0.9899	0.9899
	SD	0.03761	0.04421	0.04398
	F	104.5399	59.0105	58.5859

coefficients for each concentration of ionic liquid reveals that b and r have the largest absolute values among all coefficients for all concentrations presented here. The b coefficient is proportional to the difference in the hydrogen bond donating ability of the mobile phase of an ionic liquid and that of the aqueous phase. A larger b coefficient reflects that more solutes partition out of the aqueous environment of the mobile phase and the average solute environment appears to be less polar and higher hydrogen bond donating ability strength of the mobile phase.

As discussed in an earlier study, the *r* coefficient represents the excess molar refraction of the solute. All mobile phases with ionic liquid have a positive coefficient *r*. With the component of methanol in mobile phases and the concentrations of ionic liquids increasing, the coefficient *r* decreased. It shows that the stationary phase is slightly better able to bond with polar molecules than in the mobile phase and it is statistically significant for all ionic liquid systems.

In view of the analysis above for five coefficients, and using previous study [4] for reference, we got the relationship between the

Table 3. Constants for the liquid chromatography systems using solvation parameter model ([Omim][BF₄])

Surfactant concentration, M		0.03	0.06	0.09
Modifier concentration, % v/v		5		
Constants	c	0.66 (0.08)	0.75 (0.08)	0.79 (0.11)
	m	0.17 (0.18)	0.29 (0.18)	0.46 (0.26)
	s	-2.39 (0.14)	-2.13 (0.14)	-2.01 (0.21)
	a	0.25 (0.08)	0.22 (0.09)	0.19 (0.13)
	b	-0.89 (0.18)	-1.03 (0.18)	-1.06 (0.26)
	r	3.39 (0.16)	3.31 (0.16)	3.29 (0.23)
Statistics	r ²	0.9979	0.9981	0.9962
	SD	0.03168	0.03244	0.04658
	F	291.0455	307.6477	156.9109
Modifier concentration, % v/v		10		
Constants	c	0.42 (0.07)	0.40 (0.07)	0.40 (0.08)
	m	0.051 (0.17)	0.067 (0.17)	0.073 (0.20)
	s	-1.14 (0.14)	-1.07 (0.14)	-1.07 (0.16)
	a	0.18 (0.08)	0.18 (0.09)	0.12 (0.10)
	b	-1.40 (0.17)	-1.54 (0.18)	-1.56 (0.19)
	r	2.82 (0.15)	2.80 (0.15)	2.76 (0.17)
Statistics	r ²	0.9982	0.9983	0.9979
	SD	0.03041	0.03102	0.03518
	F	340.3541	347.2503	285.3102
Modifier concentration, % v/v		15		
Constants	c	0.39 (0.08)	0.37 (0.09)	0.22 (0.06)
	m	-0.64 (0.18)	0.17 (0.21)	0.18 (0.14)
	s	-2.36 (0.15)	-1.21 (0.17)	-1.12 (0.11)
	a	0.055 (0.09)	0.052 (0.10)	0.033 (0.07)
	b	-0.71 (0.19)	-1.04 (0.21)	-1.27 (0.14)
	r	3.21 (0.16)	2.69 (0.18)	2.60 (0.13)
Statistics	r ²	0.9979	0.9974	0.9989
	SD	0.03291	0.03707	0.02561
	F	293.6587	230.6257	191.4947
Modifier concentration, % v/v		20		
Constants	c	0.39 (0.07)	0.39 (0.07)	0.43 (0.06)
	m	0.26 (0.17)	0.29 (0.16)	0.38 (0.15)
	s	-1.26 (0.14)	-1.16 (0.13)	-1.10 (0.12)
	a	0.028 (0.09)	0.006 (0.08)	-0.007 (0.07)
	b	-1.34 (0.18)	-1.39 (0.16)	-1.41 (0.15)
	r	2.26 (0.15)	2.22 (0.14)	2.18 (0.13)
Statistics	r ²	0.9977	0.9981	0.9982
	SD	0.03117	0.02832	0.02676
	F	260.7977	317.7132	332.7768

concentration of ILs modifier and the chromatographic retention that the significant addition of ILs modifier to a mobile phase leads to competition between IL cations and solutes absorbing to the silica. This results in a decrease in the retention times, but with a further increase in the concentration of an ILs modifier, cations interact with the silanols groups through electrostatic interactions, producing a weak bilayer electronic structure. This repulses basic sorbates and interacts with the alkyl groups through hydrophobic and non-specific interactions, so the retention of solutes decreases due to

these repulsive and hydrophobic interactions. As mobile phase additives, ILs could play multiple roles, such as blocking the residual silanols groups, modifying the stationary phase or acting as ion-pairing agents.

2. Estimation of LSER Equations

Calculated log k values of the test solutes were computed for [Hmim][BF₄] and [Omim][BF₄] mobile phases using Eq. (2). The relationship between calculated (cal) and experimental (exp) log k is shown in Fig. 3.

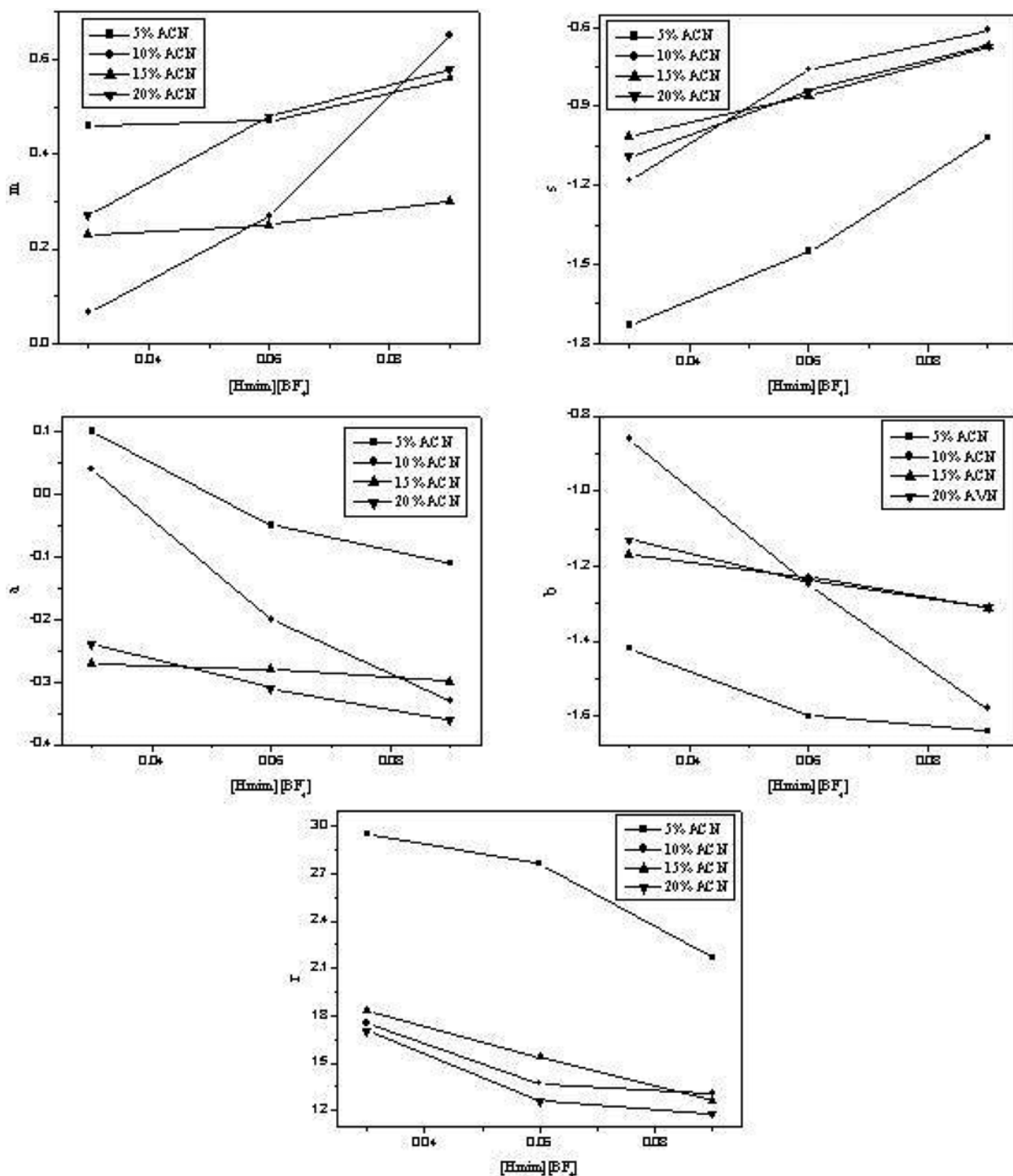


Fig. 1. LSER coefficients as a function of [HMim][BF₄] concentrations. Modifiers are: ■ 5%, ● 10%, ▲ 15% and ▼ 20% ACN. Error bars have been omitted for clarity.

The solvation parameter model is found to provide results statistically and chemically. The correlation between experimental (exp) and calculated (cal) log k (mobile phases composed from acetonitrile 5 v/v % with different concentrations of [HMim][BF₄] and [Omim][BF₄]) is demonstrated in Fig. 3. It shows that LSERs are able to approximately reproduce the experimental log k values for the solutes studied in the different mobile phases. And comparing the two series

of the data, the relative errors in [Omim][BF₄] system were obviously smaller and the coefficient of the regression curve in Fig. 3 was higher than the ones in the [HMim][BF₄] system. Because of different carbon chain of cations, the hydrophobicity [Omim][BF₄] is stronger than [HMim][BF₄]. In this case, [Omim][BF₄] had less relative error in reversed phase chromatography and the repercussion of LSERs model in [Omim][BF₄] mobile phase system was

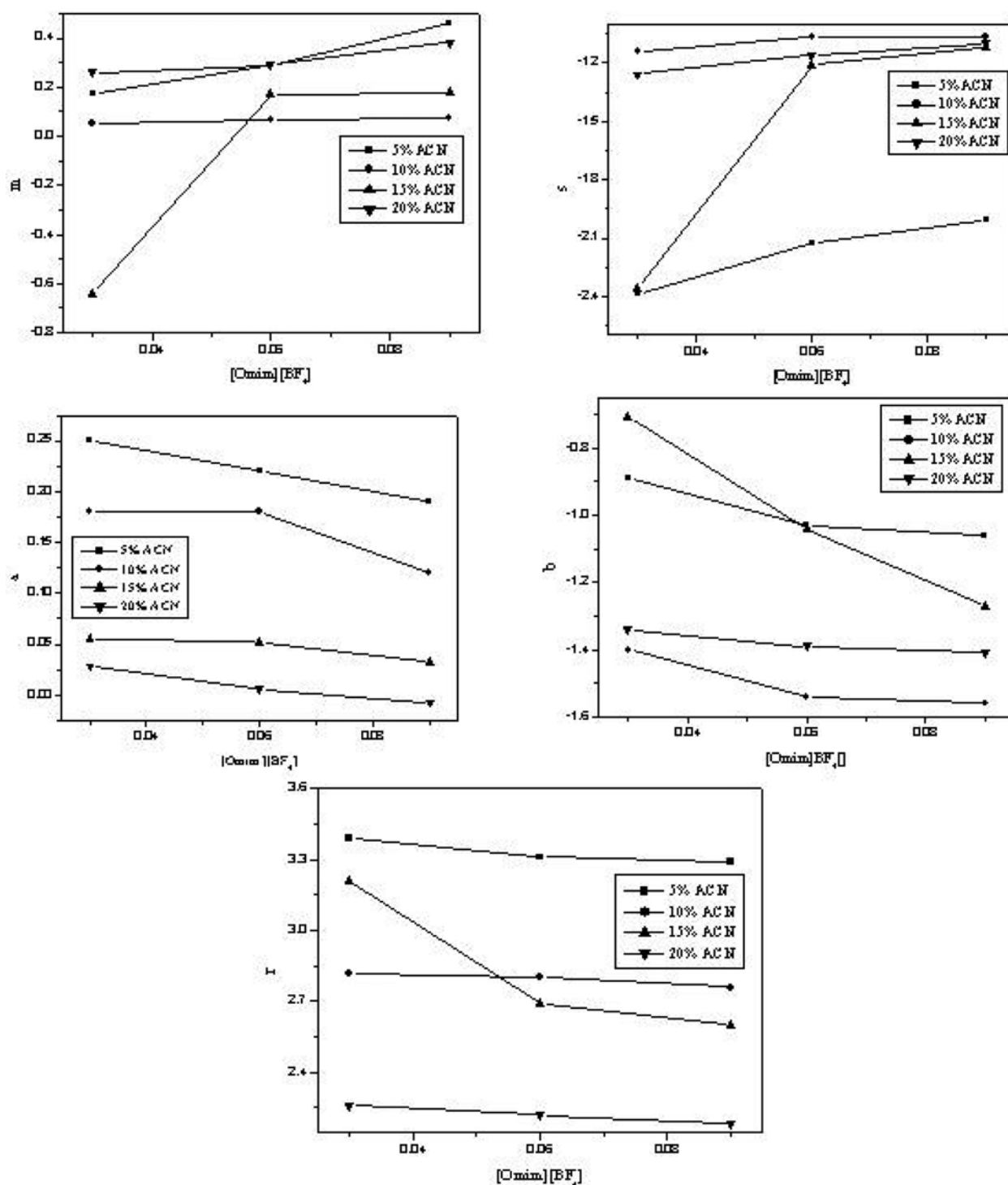


Fig. 2. LSER coefficients as a function of [Omim][BF₄] concentrations. Modifiers are: ■ 5%, ● 10%, ▲ 15% and ▼ 20% ACN. Error bars have been omitted for clarity.

better than [Hmim][BF₄] mobile phase system.

CONCLUSION

Ionic liquids [Hmim][BF₄] and [Omim][BF₄] were applied as additives in the mobile phases using acetonitrile as modifier. The LSER model, i.e., the solvation parameter model, was successfully

applied to investigate the effect of the additive concentrations on retention of nine aromatic compounds in RP-HPLC. The results obtained from the solvation parameter model provide comparable information, for example, coefficient *s* and coefficient *r* play the most important role in retention behavior in all ionic liquid systems. It is worth noting that, using the obtained LSER models, it is possible to predict retention factors with high correlation coefficients

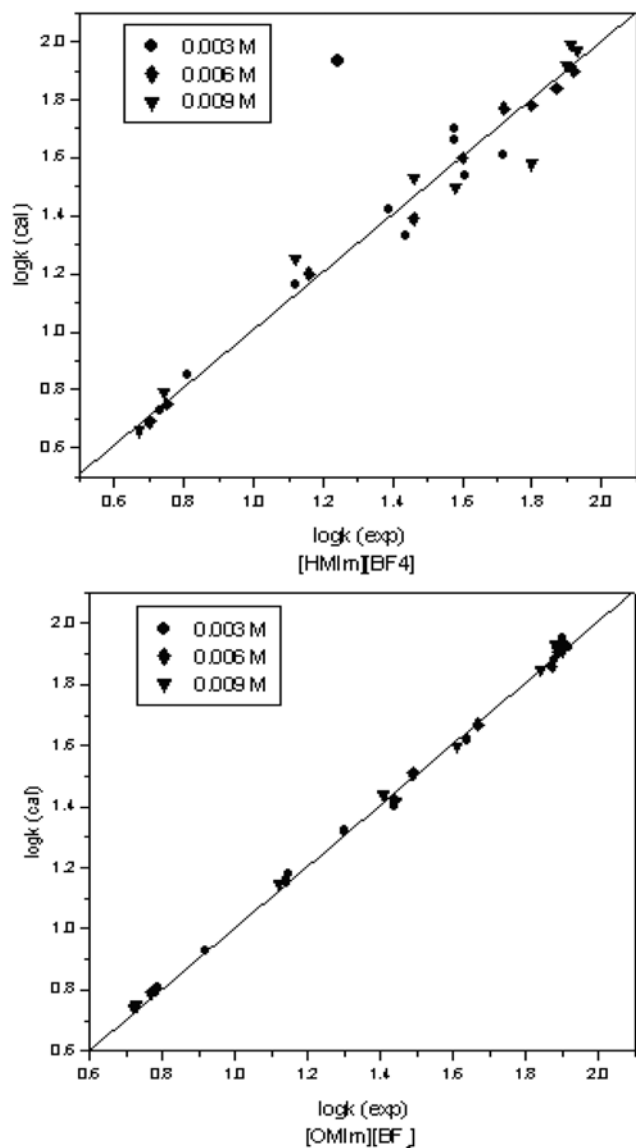


Fig. 3. The correlation between experimental (exp) and calculated (cal) log k (mobile phases composed from 5 v/v % ACN with different concentrations of [Hmim][BF₄] and [Omim][BF₄]).

($r^2 > 0.99$ in [Omim][BF₄] system and $r^2 = 0.96$ in [Hmim][BF₄] system). And it also proved that the effect of [Omim][BF₄] is better than [Hmim][BF₄] as additive for mobile phase in RP-HPLC. It is evident from the results of the LSER model that the excess molar refraction and HB basicity have a dominant effect role on the solute ionic liquid interaction. This model is a helpful tool for understanding the solute-ionic liquid interactions and evaluating the retention characteristic of liquid chromatography.

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