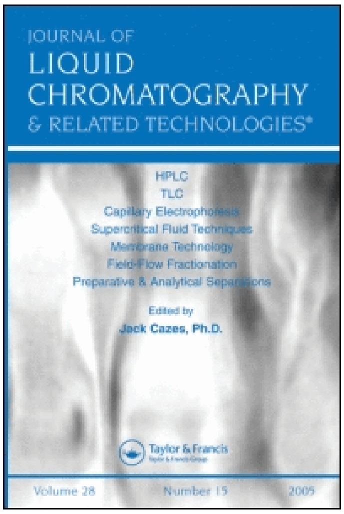

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RETENTION MECHANISM OF NINE SOLUTES BY THE USE OF LINEAR SOLVATION ENERGY RELATIONSHIPS ON A C_{18} COLUMN

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RETENTION MECHANISM OF NINE SOLUTES BY THE USE OF LINEAR SOLVATION ENERGY RELATIONSHIPS ON A C_{18} COLUMN

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□ *Linear solvation energy relationships are used to investigate the fundamental chemical interactions governing the retention of 9 aromatic solutes in 36 mobile phases in reversed phase liquid chromatography. The systems studied involve sodium dodecyl sulfate (0.03–0.09 M) and two kinds of ionic liquids (0.003–0.009 M), with 5 to 20% acetonitrile as mobile phase modifiers in water. The mechanism of retention in two kinds of additive were analyzed and compared. The results suggest that LSER formalism is able to reproduce adequately the experimental retention factors of the solutes studied in the different experimental conditions investigated.*

Keywords chromatographic retention, ionic liquid, linear solvation energy relationships, modifier, surfactant

INTRODUCTION

Retention prediction and selectivity optimization are very important in rapid method development in reversed phase liquid chromatography (RPLC).[1] These years many practical retention models[2] used to study the complicated process in RP-HPLC, such as linear solvation energy relationships (LSERs), have been developed and widely used.

In order to increase the chromatography effect, additives were added into the mobile phase. There are many additives that can be used in an LSER model to investigate the retention in the RP-HPLC, such as surfactant and ionic liquid (IL).

The technique of the system which uses surfactant is called Micellar Liquid Chromatography (MLC). Since its introduction by Armstrong and Henry in 1980,[3] MLC has seen solid growth in its use. The major advantages of MLC over most separation techniques as well as its unique capabilities have been widely investigated, with more than one hundred papers on this

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subject as well as reviews[4–6] and several books having been published.[7,8] According to reported studies, intermolecular solute-solvent interactions play a major role not only in separation science but also in many other areas of chemistry, as well as synthesis, spectroscopy, and pharmaceuticals. Since retention prediction and selectivity optimization have been very important in the rapid method development of MLC, it is imperative to achieve a better understanding of the factors that control separation efficiency.

Ionic liquids are widely recognized as one of the key components of "green" chemistry. The solvent properties make them possible candidates in chromatography. And most applications of ILs in RP-HPLC have been mainly as mobile phase additives.[9] The chemical nature of the ILs makes it possible to conclude that when they are used as the mobile phase additives in HPLC, they exist in the mobile phase solution and they are also coated on the reversed phase silica based column. Unfortunately, the influence of ILs modifiers on chromatographic retention is still currently unclear.

In this study, 9 solutes (acetophenone, aniline, caffeine, methylparaben, o-cresol, m-cresol, p-cresol, phenol, and pyridine) have been in terms of LSER. Several systems using anionic surfactant sodium dodecyl sulfate (SDS) and two kinds of IL: 1-Hexyl-3-methylimidazolium tetrafluoroborate ([Hmim][BF_4]) and 1-Methyl-3-octylimidazolium tetrafluoroborate ([Omim][BF_4]) as additives in acetonitrile/water mobile phases were characterized using the previously mentioned solvation parameter LSER model.

EXPERIMENTAL

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 µL 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 C_{18} column (Optimapak, Korea, 150 mm × 4.6 mm, 5 µm). An injection volume of 2 µL was applied throughout the experiments. All procedures were carried out at 30°C.

Materials

All of the LSER test solutes, the surfactant SDS were purchased from Daejung (Korea), and the two ILs were from C-TRI (Korea). The mobile phase modifier acetonitrile was purchased from Duksan (Korea).

Deionized water was obtained via a water purification system from Millipore Corp. (Milford, MA).

Preparation of Mobile Phases and Standard Solutions

The solutions of SDS were prepared by first dissolving 0.1 gram of surfactant in 5.0 mL of deionized water. The final volume was adjusted to 100.0 mL with deionized water. The same sequence was followed for the preparation of mixed mobile phases, but the ILs were added into the acetonitrile/water solution directly. The corresponding molar concentrations of SDS were 0.03 M, 0.06 M, and 0.09 M, and the molar concentrations of ILs were 0.003 M, 0.006 M, and 0.009 M. The mixed mobile phase contained 5, 10, 15, and 20% (v/v) acetonitrile modifiers for the surfactant mixture. After thorough mixing in an ultrasonic 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 more minutes prior to the experiments. A mobile phase was refrigerated after each use. All stock solute solutions were prepared at concentrations of 1.0 mg/mL each. All of the nine solute samples were dissolved in methanol. It should be emphasized that the working solutions were reprepared every 3 days so as to avoid potential errors arising from decomposition.

THEORETICAL BACKGROUND AND CALCULATION

The general LSER equation used in this work is:[10]

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

Here k is the experimental retention factor. The $V_x\pi^{\mathrm{H}}$, $\Sigma\alpha_2^{\mathrm{H}}$, $\Sigma\beta_2^{H}$ and R_2 terms are solute descriptors, where V_x represents the solute's size/polarizability, π_2^{H} is the dipolarity/polarizability, $\Sigma\alpha_2^{\mathrm{H}}$ is the hydrogen bond (HB) acidity, $\Sigma\beta_2^{\mathrm{H}}$ 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[11] 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.[12–15]

Retention Factor Estimation

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

$$k = (t_R - t_M)/t_M \tag{2}$$

where 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 μL injection of 1% sodium nitrite solution. The retention factors reported in this study are the averages of at least three determinations. Evaluation of the results of the chromatographic experiments was carried out using mathematical statistic techniques. The relative error of a single measurement did not exceed 5%.

Linear Solvation Energy Relationship Estimations

Retention factors were determined for the 9 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. Solute interactions with the micellar systems occur through a variety of mechanisms such as surface adsorption, coaggregation, or partitioning into the hydrophobic core of the micelles. Due to these different mechanisms, the LSER constants for different kinds of solutes are not identical.

RESULTS AND DISCUSSION

The retention behaviors of the 9 test solutes (acetophenone, aniline, caffeine, methylparaben, o-cresol, m-cresol, p-cresol, phenol, and pyridine) in each system were examined and compared using the solvation parameter LSER model, i.e., model described in Equation (2). The test solutes and their descriptors used in this study are given in Table 1.

TABLE 1 Test Solutes and Their Descriptors for the Solvation Parameter Model

	Descriptors				
Solute	V_x (cm^{3}mol)	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
p-Cresol	0.82	0.87	0.57	0.31	0.916
Methylparaben	0.9	1.37	0.69	0.45	1.131
Acetopenone	0.818	1.01	0	0.48	1.0139
Aniline	0.955	0.96	0.26	0.5	0.8162
o-Cresol	0.84	0.86	0.52	0.3	0.916
Pyridine	0.631	0.84	0	0.52	0.6753
m-Cresol	0.822	0.88	0.57	0.34	0.916

LSER Coefficients in SDS and ILs Systems

The coefficients of the LSER equations obtained from SDS and IL mobile phases were grouped in Table 2, in which correlation coefficients ranged from −1.76 to 1.83 SDS mobile phase, from −1.73 to 2.95 for [Hmim][BF$_4$] mobile phase, and from −2.39 to 3.39 for [Omim][BF$_4$] mobile phase; and standard errors ranging from 0.08 to 0.30 for SDS, from 0.09 to 0.1.02 for [Hmim][BF$_4$] mobile phase, and from 0.06 to 0.26 [Omim][BF$_4$] mobile phase.

LSER coefficients as a function of SDS, [Hmim][BF$_4$] and [Omim][BF$_4$] concentrations were showed in Figures 1–3, respectively. As shown in the figures, the value of all of the 5 coefficients (*m, s, a, b* and *r*) change regularity as the concentrations of additives or acetonitrile change, and the situation is different between [Hmim][BF$_4$] and [Omim][BF$_4$] systems.

In all of the SDS mobile phase, most of the values of coefficients *s, a,* and *b* were negative, it mean that an increase in the solute dipolarity/polarizability, HB acidity, and HB basicity decreases the overall retention of the molecule. Furthermore, most of the values of *m* and *r* were positive in all studies, indicating that increases in the solute volume and excess molar acidity will make increases in the solute volume and excess molar. In view of the value range, the coefficients of HB basicity (*b*) and the excess molar refractivity (*r*) generally play the largest roles in determining the retention of solutes in all studies. The solute dipolarity/polarizability (*s*) is also an important factor in mobile phase to the retention.

While in the IL mobile phases, most of the values of *b* and *s* were negative, most of the values of *m* and *r* were positive. This means that an increase in the HB basicity and the solute dipolarity/polarizability decreases the overall retention of the molecule, the increase of the solute volume and the excess molar refractivity will cause an increase in the solute volume

TABLE 2 Constants for the Chromatographic Mobile Phases Using Solvation Parameter Model

Additive		[Hmim][BF$_4$]			[Omim][BF$_4$]			SDS		
Concentration of Additive, M		0.003	0.006	0.009	0.003	0.006	0.009	0.03	0.06	0.09
Concentration of modifier						5% ACN				
Constants	log k$_0$	0.77(0.43)	0.65(0.14)	0.64(0.32)	0.66(0.08)	0.75(0.08)	0.79(0.11)	0.82(0.08)	0.78(0.09)	0.77(0.08)
	m	0.46(0.99)	0.47(0.33)	0.56(0.75)	0.17(0.18)	0.29(0.18)	0.46(0.26)	−0.06(0.18)	0.03(0.22)	0.15(0.18)
	s	−1.73(0.79)	−1.45(0.27)	−1.02(0.59)	−2.39(0.14)	−2.13(0.14)	−2.01(0.21)	−1.12(0.14)	−0.90(0.17)	−0.81(0.14)
	a	0.10(0.50)	−0.05(0.17)	−0.11(0.37)	0.25(0.08)	0.22(0.09)	0.19(0.13)	−0.17(0.09)	−0.18(0.11)	−0.23(0.09)
	b	−1.42(1.02)	−1.60(0.34)	−1.64(0.76)	−0.89(0.18)	−1.03(0.18)	−1.06(0.26)	−0.71(0.18)	−0.77(0.22)	−0.77(0.18)
	r	2.95(0.88)	2.76(0.29)	2.17(0.66)	3.39(0.16)	3.31(0.16)	3.29(0.23)	2.02(0.16)	1.57(0.19)	1.26(0.16)
Concentration of modifier						10% ACN				
Constants	log k$_0$	0.80(0.11)	0.77(0.13)	0.48(0.11)	0.42(0.07)	0.40(0.07)	0.40(0.08)	0.80(0.11)	0.77(0.13)	0.55(0.12)
	m	0.07(0.25)	0.27(0.31)	0.65(0.25)	0.05(0.17)	0.07(0.17)	0.07(0.20)	0.07(0.25)	0.27(0.30)	0.65(0.28)
	s	−1.18(0.19)	−0.76(0.25)	−0.61(0.20)	−1.14(0.14)	−1.07(0.14)	−1.07(0.16)	−1.18(0.19)	−0.76(0.25)	−0.52(0.22)
	a	0.04(0.12)	−0.20(0.15)	−0.33(0.13)	0.18(0.08)	0.18(0.09)	0.12(0.10)	0.04(0.12)	−0.20(0.15)	−0.38(0.14)
	b	−0.86(0.25)	−1.25(0.31)	−1.58(0.26)	−1.40(0.17)	−1.54(0.18)	−1.56(0.19)	−0.86(0.25)	−1.25(0.31)	−1.76(0.28)
	r	1.75(0.22)	1.37(0.27)	1.31(0.22)	2.82(0.15)	2.80(0.15)	2.76(0.17)	1.75(0.22)	1.37(0.27)	1.24(0.25)
Concentration of modifier						15% ACN				
Constants	log k$_0$	0.62(0.09)	0.62(0.09)	0.70(0.11)	0.39(0.08)	0.37(0.09)	0.22(0.06)	0.62(0.10)	0.61(0.10)	0.64(0.10)
	m	0.23(0.22)	0.25(0.22)	0.30(0.26)	−0.64(0.18)	0.17(0.21)	0.18(0.14)	0.22(0.22)	0.25(0.22)	0.30(0.22)
	s	−1.01(0.18)	−0.86(0.18)	−0.67(0.21)	−2.36(0.15)	−1.21(0.17)	−1.12(0.11)	−1.01(0.18)	−0.86(0.18)	−0.76(0.17)
	a	−0.27(0.11)	−0.28(0.11)	−0.30(0.13)	0.055(0.09)	0.052(0.10)	0.033(0.07)	−0.26(0.11)	−0.27(0.11)	−0.28(0.11)
	b	−1.17(0.22)	−1.23(0.23)	−1.31(0.27)	−0.71(0.19)	−1.04(0.21)	−1.27(0.14)	−1.05(0.22)	−1.17(0.23)	−1.31(0.23)
	r	1.83(0.19)	1.54(0.19)	1.26(0.23)	3.21(0.16)	2.69(0.18)	2.60(0.13)	1.83(0.19)	1.55(0.19)	1.31(0.20)
Concentration of modifier						20% ACN				
Constants	log k$_0$	0.64(0.09)	0.54(0.11)	0.51(0.11)	0.39(0.07)	0.39(0.07)	0.43(0.06)	0.66(0.08)	0.52(0.11)	0.51(0.11)
	m	0.27(0.21)	0.48(0.25)	0.58(0.25)	0.26(0.17)	0.29(0.16)	0.38(0.15)	0.23(0.19)	0.46(0.25)	0.61(0.25)
	s	−1.09(0.17)	−0.84(0.19)	−0.67(0.19)	−1.26(0.14)	−1.16(0.13)	−1.10(0.12)	−1.09(0.15)	−0.84(0.20)	−0.68(0.20)
	a	−0.24(0.10)	−0.31(0.12)	−0.36(0.12)	0.03(0.09)	0.01(0.08)	−0.01(0.07)	−0.25(0.10)	−0.31(0.12)	−0.34(0.12)
	b	−1.13(0.21)	−1.24(0.25)	−1.31(0.25)	−1.34(0.18)	−1.39(0.16)	−1.41(0.15)	−1.13(0.19)	−1.25(0.25)	−1.27(0.25)
	r	1.70(0.18)	1.26(0.22)	1.18(0.22)	2.26(0.15)	2.22(0.14)	2.18(0.13)	1.71(0.17)	1.25(0.22)	1.18(0.22)

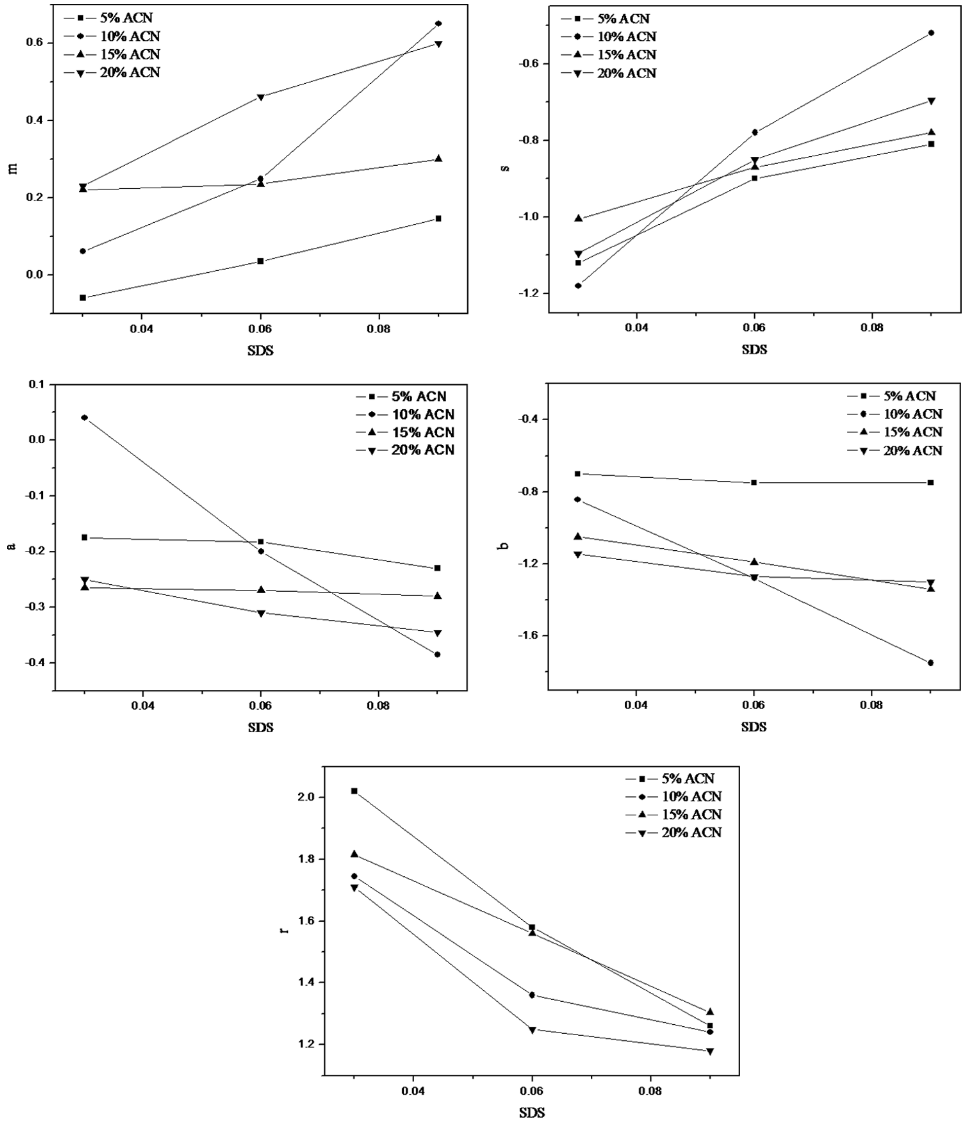


FIGURE 1 LSER coefficients as a function of SDS concentrations. Modifiers are: ■, 5% ACN; ●, 10% ACN; ▲, 15% ACN; and ▼, 20% ACN. Error bars have been omitted for clarity.

and excess molar. And, the coefficient s and r, also b, play the largest role in determining the retention of solutes in all studies. However, the coefficient of $\Sigma\alpha_2^H$ is negative in [Hmim][BF$_4$] systems but positive in [Omim][BF$_4$], for that the length of the alkyl groups of the two ILs interact with the hydrogen bond acidity effectively.

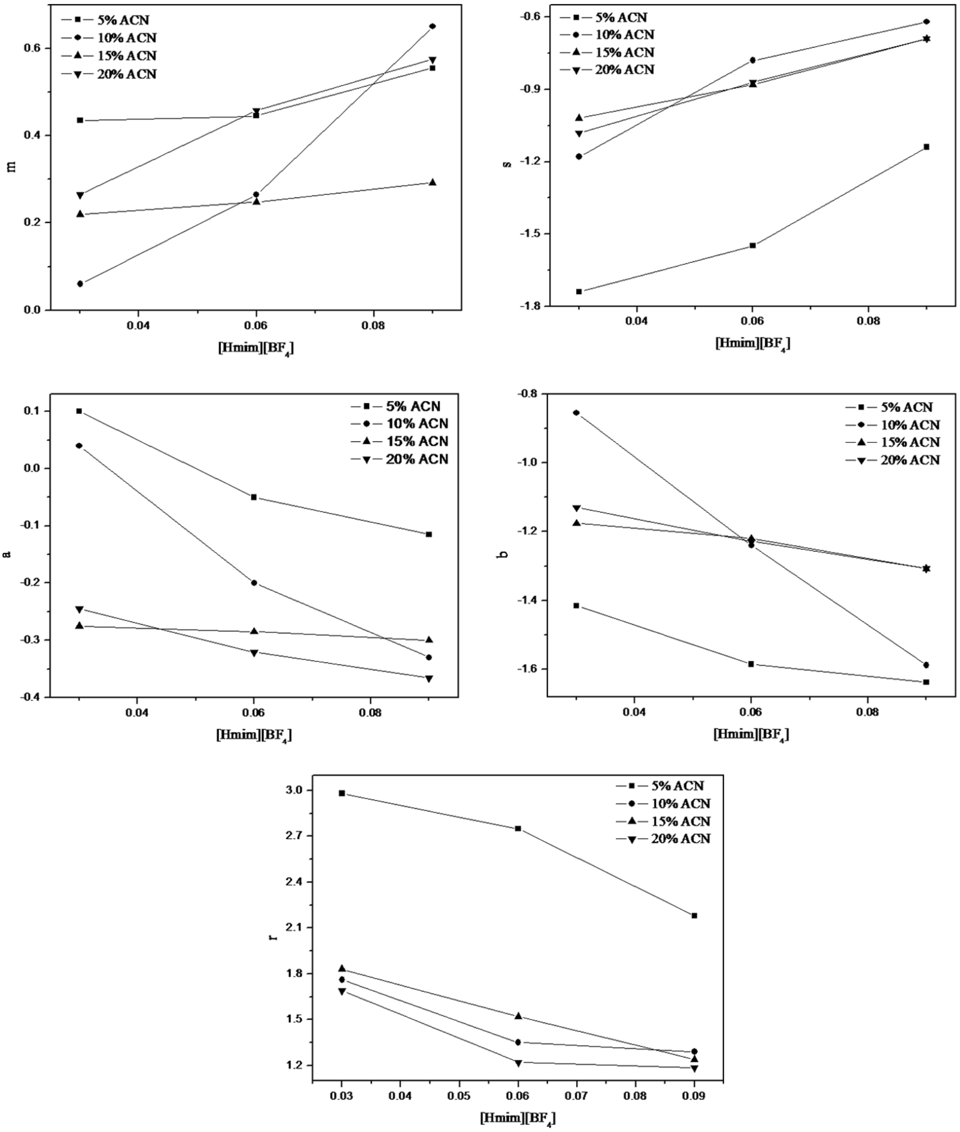


FIGURE 2 LSER coefficients as a function of [Hmim] [BF_4] concentrations. Modifiers are: ■ 5% ACN; ● 10% ACN; ▲ 15% ACN; and ▼ 20% ACN. Error bars have been omitted for clarity.

Effect of the Addition of Acetonitrile on the Retention in SDS and ILs Systems

As shown in the Figures 1–3, according to comparison of the general trend of the coefficients at different concentrations of acetonitrile in mobile phase, the coefficient *m* and *s* increases, but the coefficient *a, b,*

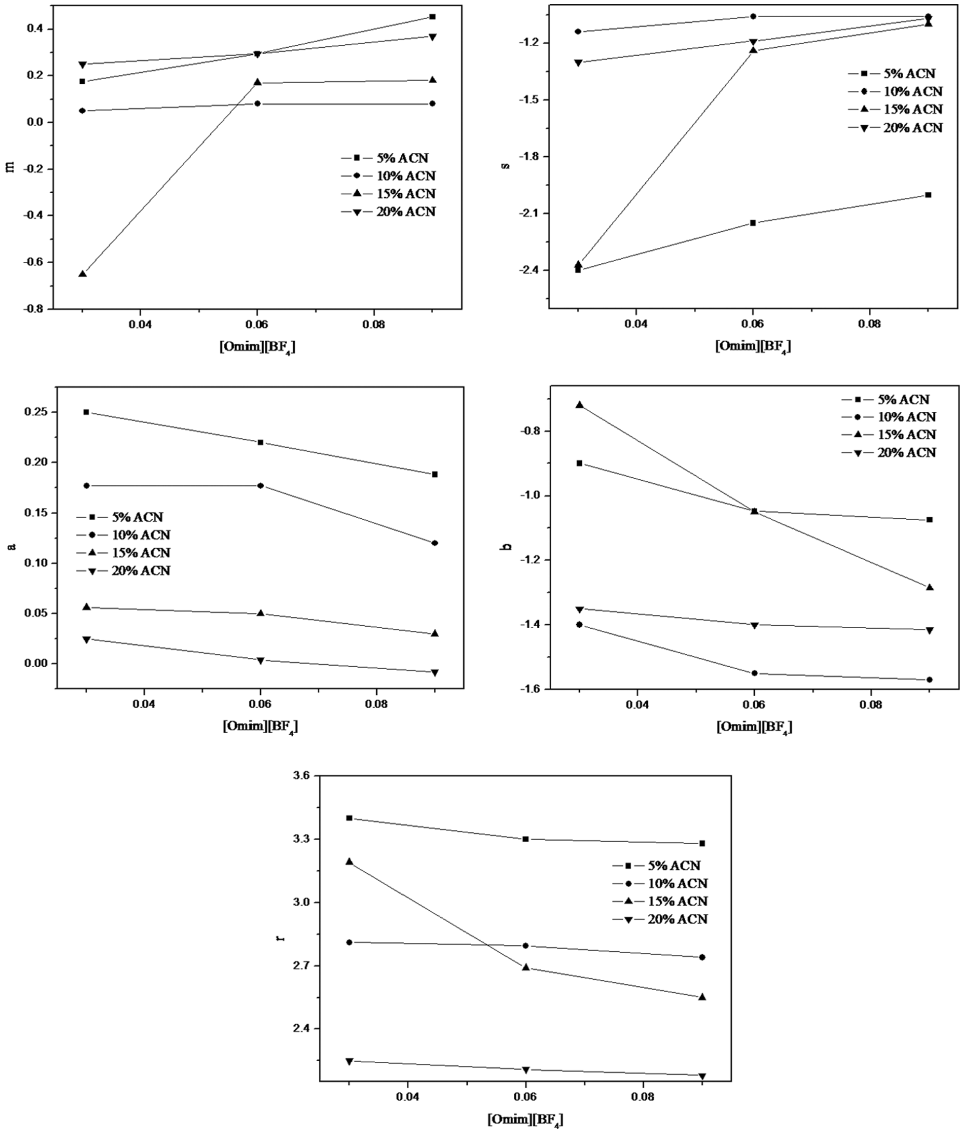
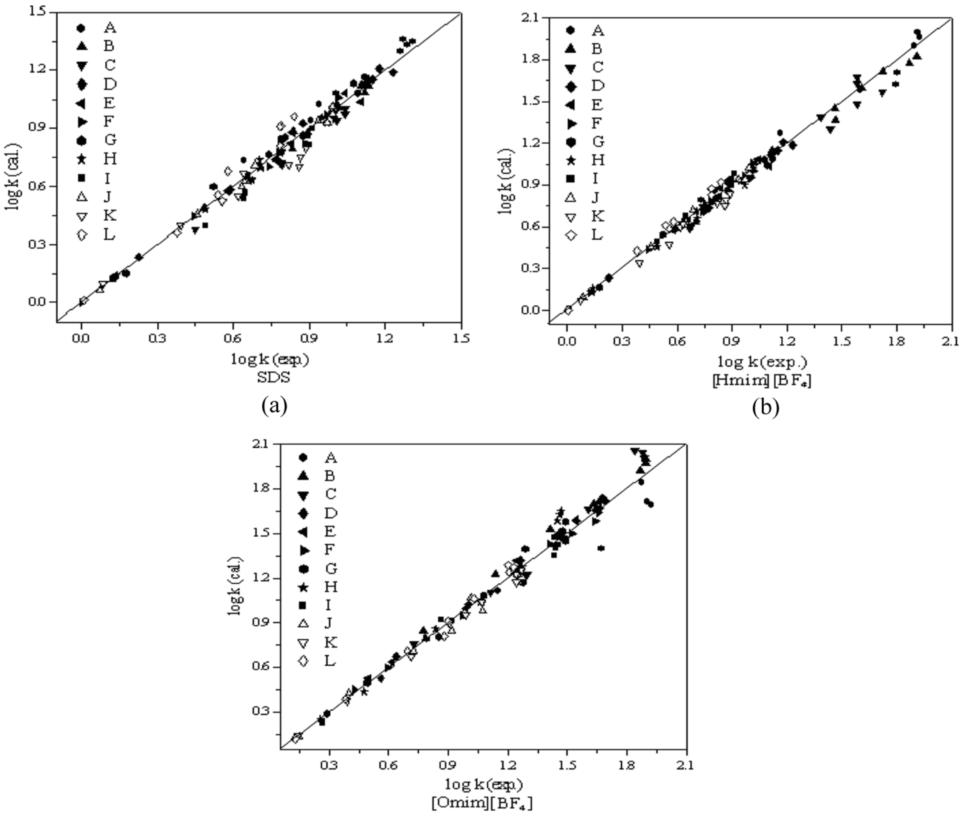


FIGURE 3 LSER coefficients as a function of [Omim][BF$_4$] concentrations. Modifiers are: ■ 5% ACN; ● 10% ACN; ▲ 15% ACN; and ▼ 20% ACN. Error bars have been omitted for clarity.

and r decreases as the concentration of acetonitrile decreasing, both in SDS systems and IL systems. In addition, sometimes the comparison is not so obvious; several trends are not so regular. This is because the modifier, acetonitrile, cannot form the equilibrium between the HB acidity and HB basicity, which was the special characteristics of water. So, as the concentration of acetonitrile in the mobile phase increases, the polarity of the mobile



(a) (b) (c)

FIGURE 4 The correlation between experimental (exp) and calculated (cal) log *k*. (For SDS, *A, B* and *C* represent 0.03 M, 0.06 M and 0.09 M additive in 5% ACN; *D, E* and *F* represent 0.03 M, 0.06 M and 0.09 M additive in 10% ACN; *G, H* and *I* represent 0.03 M, 0.06 M and 0.09 M additive in 15% ACN; *J, K* and *L* represent 0.03 M, 0.06 M and 0.09 M additive in 20% ACN. For IL, *A, B* and *C* represent 0.003 M, 0.006 M and 0.009 M additive in 5% ACN; *D, E* and *F* represent 0.003 M, 0.006 M and 0.009 M additive in 10% ACN; *G, H* and *I* represent 0.003 M, 0.006 M and 0.009 M additive in 15% ACN; *J, K* and *L* represent 0.003 M, 0.006 M and 0.009 M additive in 20% ACN.).

phase changed in spite of any other effect with the stationary phase and solutes.

EFFECT OF THE ADDITIVES ON THE RETENTION IN SDS AND ILS MOBILE PHASES

Comparing the effects of SDS and ILs in terms of retention, the longest retention time of the solutes was less than 1 hr in the SDS system, but the longest one was more than 3 hr in the ILs system. Therefore, this infers that the effect of the SDS concentration upon retention was larger than that of the ILs. But, the concentration of SDS was 10 times of the concentration of

ILs; if the retention times of the solutes are proportional to the concentration of the modifiers, the longest retention time in the ILs mobile phase will be less than the one in the SDS mobile phase at the same concentration. According to this premise, the concentration of ILs is more effective than SDS in terms of retention. In addition, the mechanism of the SDS system is different from that of the ILs'. In the SDS system, the surfactant forms micelles and acts upon the stationary phase; the primary effect among these is a hydrophobic effect. In the ILs system, as mobile phase additives, the ILs could play multiple roles, such as blocking the residual silanols groups and modifying the stationary phase or acting as ion pairing agents.

A problem worthy to be pointed out is that the retention times of solutes will generally decrease when the concentration of SDS or ILs increases. And, in all additive systems, the general trends of the coefficients are: when the concentrations of additives increase, the coefficients *m* and *s* increase, but *a, b,* and *r* decrease. Chemically, the *m* coefficient decreases because increasing the additive concentration increases the concentration of interacting cells among additive, solutes, and mobile phase. Also, when the concentration of the additive increases, the dipolarity increases, but the interacted cells are hard to polarize, which makes the coefficient *b* increase. But, the hydrogen bond donating and accepting ability will decrease as the polar additives areadded to the mobile phase.

CONCLUSION

Surfactant SDS and Two Kinds of IL

[Hmim][BF_4] and [Omim][BF_4] were applied as additives to the mobile phases, which contained acetonitrile as a modifier. The LSER model was successfully applied to investigate the effect of the additive concentrations on retentions of nine aromatic compounds in RP-HPLC. The results obtained from the solvation parameter model provide comparable information, such as the mechanism of the SDS and the IL in the RP-HPLC as additives. The correlation between experimental (exp) and calculated (cal) log *k* proved the LSER models; it also proved that the effect of [Omim][BF_4] is better than [Hmim][BF_4] as additive for the mobile phase in RP-HPLC (Figure 4). This model is a helpful tool to understand the solute-additive interactions and to evaluate the retention characteristics of liquid chromatography.

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