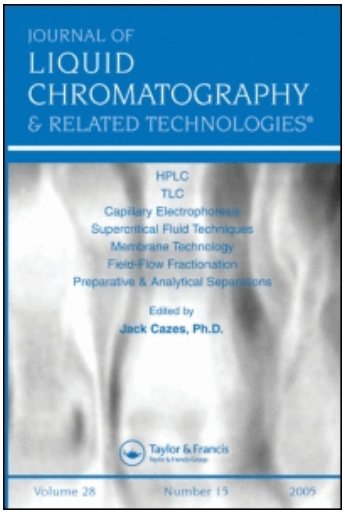

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Retention-Eluent Composition Relationships of Some Polar Compounds with Imidazolium Ionic Liquid Modifiers in RP-HPLC

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Retention-Eluent Composition Relationships of Some Polar Compounds with Imidazolium Ionic Liquid Modifiers in RP-HPLC

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Abstract: Four commonly known equations, two linear (Soczewinski and Langmuir-type) and two quadratic, have been applied for investigation of the influence of imidazolium ionic liquids modifiers on the retention eluent composition relationships in reversed-phase high performance liquid chromatography. In this study, three ionic liquids (1-butyl-3-methylimidazolium tetrafluoroborate, 1-ethyl-3-methylimidazolium methylsulfate, and 1-octyl-3-methylimidazolium methylsulfate) were evaluated as mobile phase modifiers. In the experiment, nine solutes belonging to three chemical classes, and namely: nucleic compounds (uridine 5′-monophosphate, inosine 5′-monophosphate, guanosine 5′-monophosphate, and hymidine 5′-monophosphate disodium salts), nitrogen containing heterocycles (guanine and hypoxanthine), and amino benzoic acids (orthro-, meta-, and para-isomers of amino benzoic acid) were tested. Evaluation of models of chromatographic retention was performed and discussed. The best correlations were achieved employing Langmuir-type and Soczewinski equations.

Keywords: RP-HPLC, Retention model, Mobile phase composition, Modifier, Ionic liquid

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INTRODUCTION

The reversed-phase high performance liquid chromatography (RP-HPLC) of polar and ionizable compounds is of overwhelmingly importance in the chemical, biochemical, biomedical, pharmaceutical, and environmental fields. In almost every RP-HPLC experimental case, the mobile phase is made of an organic solvent (usually methanol or acetonitrile) dissolved in water. However, such simple systems (water organic eluents) do not always satisfy analysts. In RP-HPLC separations, the minor components are present at $<5\%$ and are commonly referred to as mobile phase modifiers. The majorities of separations are impossible without various mobile phase modifiers, such as acids, salts, and organic matters. It requires the selection of a modifier, in order to fix the elution force of the mobile phase. The variations of the nature and the concentration of the modifier essentially affect the interaction energy of the analyte in the bulk mobile phase. In recent years, the interest of new modifiers is growing rapidly.

Ionic liquids are a type of salt that are liquid at low temperature (373 K).[1] It is well known that ionic liquids are excellent solvents for both inorganic and organic matters; they are nonvolatile, nonflammable, thermally stable, and recyclable solvents. Taking advantage of the special properties of ionic liquids, it is expected that the number of mobile phase modifiers in RP-HPLC can be expanded. It is no surprise that numerous works describing successful applications of ionic liquids in this field are reported annually.[2–11] The chemical nature of the ionic liquids leads to the conclusion that when they are used as the mobile phase modifiers in RP-HPLC, they exist in the mobile phase solution and they are also coated on the reversed-phase silica based column. According to authors,[3] the ionic liquids form a bonded layer (pseudo stationary phase) on the surface of the modified silica gel. Previous studies[3,12–14] have shown that ionic liquid cations can interact and compete with silanols groups on an alkyl silica base surface.

The retention of analytes in RP-HPLC is fundamentally determined by their distribution between a polar mobile phase and a stationary phase consisting of an organic layer, most often made of alkyl chains that are bonded to the silica surface. In many instances, the thickness and mobility of this layer is governed by mobile phase composition. It is related to the partition coefficient. Thus, the solute retention is affected by the thermodynamics of distribution between the stationary and mobile phases. For RP-HPLC column, the major constituent is a solvent (e.g., water), and the modifiers (e.g., methanol, acetonitrile, salts, etc.) are added to control the hydrophobic nature between solute and stationary phase. Usually, the simple mathematical models were used for finding relationships between retention and mobile phase composition. In general the modeling problem can be defined as estimating the relation between a set of predictor variables F and one or more response variables P:

$$P = f(F) + \varepsilon \tag{1}$$

in which ε contains all types of errors, including sampling and measurement errors. In our approach, P is the chromatographic retention factor, k, and F is the concentration of modifier. The Snyder equation is the most commonly encountered, and described the logarithmic relationship between k and the fraction of modifier in mobile phase.[15,16] However, in restrained terms, more elaborate equations based on the adsorption of Langmuir adsorption or other uncomplicated approaches, shows a better prediction of retention with different compositions of mobile phase.[17] In the literature, one can find other more complex models aimed at the prediction of changes in retention as a function of F.[18] Best known are: the solvatochromic model,[19] the stoichiometric displacement model,[20] and the solvophobic model.[21]

In this study, three ionic liquids were evaluated as mobile phase modifiers to test nine solutes. These substances were chromatographed by using imidazolium ionic liquids as eluent modifiers on reversed-phase columns without gradient elution. The purpose of this work is to check the commonly known, simplest models to predict the retention factors.

EXPERIMENTAL

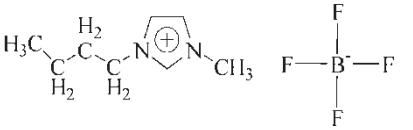
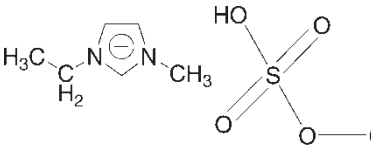
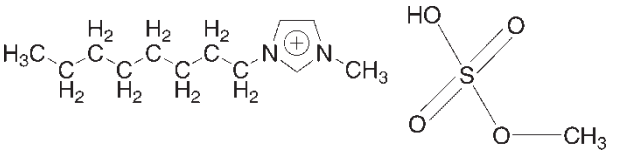
Apparatus

An analytical system with a 600 HPLC pump (Waters, U.S.A.), 486 detector (M 7200 Absorbance Detector, Young-In Scientific Co., South Korea), and Reodyne injector (Cotati, CA, USA) valve with a 20 μL sample loop were used in this study. Chromate software (Ver. 3.0 Interface Eng., South Korea) on a PC was used as a data acquisition system. Experiments were performed with the commercially available (Optimapak, South Korea) C_{18} (alkyl-) bonded phase column (4.6 × 150 mm i.d. and particle size 5 μm).

Reagents

The ionic liquids selected for these studies were purchased from C-tri Co. (Namyang, South Korea); they included 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIm][BF_4]), 1-ethyl-3-methylimidazolium methylsulfate ([EMIm][MS]), and 1-octyl-3-methylimidazolium methylsulfate ([OMIm][MS]). All ionic liquids were used as obtained, without additional pretreatment. Table 1 depicts these ionic liquids. Uridine 5′-monophosphate disodium salt (5′UMP), inosine 5′-monophosphate disodium salt (5′IMP), guanosine 5′-monophosphate disodium salt (5′GMP), thymidine 5′-monophosphate disodium salt (5′TMP), and para-amino benzoic acid (4-ABA) were purchased from Fluka (St. Louis, MO, U.S.A.); guanine (Gua), hypoxanthine (Hypo), and. orthro- and meta-amino benzoic acids (2-ABA and 3-ABA) were

Table 1. The names, abbreviations, and structures of ionic liquids

Name	Abbreviation	Structure
1-Butyl-3-methylimidazolium tetrafluoroborate	[BMIm][BF_4]	
1-Ethyl-3-methylimidazolium methylsulfate	[EMIm][MS]	
1-Octyl-3-methylimidazolium methylsulfate	[OMIm][MS]	

from Sigma-Aldrich Co. (St. Louis, MO, U.S.A.). All substances were analytical grade. Potassium nitrate (KNO_3) was obtained from Kanto Chemical Co. (Japan) and HPLC gradient grade methanol was purchased from Duksan Pure Chemical Co. (Ansan, South Korea). The distilled water was filtered with a vacuum pump (Division of Millipore, Waters, U.S.A.) and a filter (HA-0.45, Division of Millipore, Waters, U.S.A.) prior to use.

Sample Preparation

Each analyte was dissolved in water with a concentration of 1000 mg mL^{-1}. These solutions were then sonicated for 30 min and stored at 277 K. It should be emphasized that the working solutions were reprepared every 3 days so as to avoid potential errors arising from decomposition.

Chromatographic Conditions

Mobile phases were composed of methanol in water (pure reversed-phase systems). The ionic liquids from Table 1 were used as mobile phase modifiers. The information about solutes and conditions of their chromatographing (mobile phase composition) is shown in Table 2. After each experiment with the certain concentration of the ionic liquid, and before the experiment with the subsequent concentration of the ionic liquid, the column was flushed for at least 3 hours to remove the ionic liquid used at previous concentration and/or to fully equilibrate the column. Analyses were performed throughout at ambient temperature (297 K) at a flow rate of 1.0 mL min^{-1}, and the elution profiles were monitored at λ of 254 nm. The retention factor (k) was calculated according to Eq. (1).

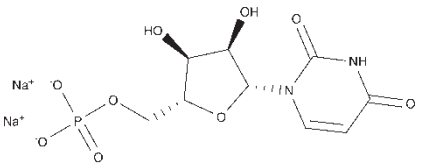
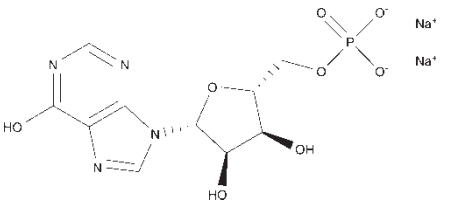
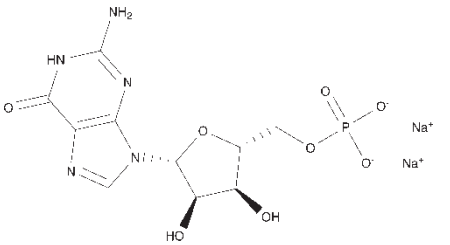
$$k = (t_R - t_M)/t_M, \tag{2}$$

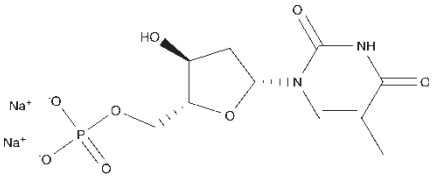
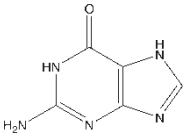
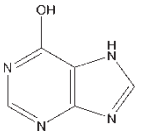
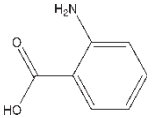
where t_M is the time of unretained compound (taken as the first deviation of the baseline following a 5 μL injection of 1% potassium nitrate solution) and t_R is the retention time. The retention factors reported in this study are the averages of at least three determinations. Evaluation of the results of the chromatographic experiments was carried out using mathematical statistic techniques. The relative error of a single measurement did not exceed 5%.

Theoretical Background

In this work, the retention factor, k, is correlated by linear and quadratic relationships involving the vol.% of ionic liquids modifier, F.

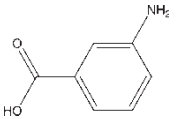
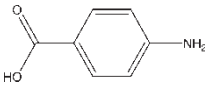
Table 2. The solutes and condition of their chromatographing (where *M* is the vol.% of methanol in water and *F* is the vol.% of ionic liquid)

Name (abbreviation)	Structure	Mobile phase composition		
			Ionic liquid	
		M	Type	Range of *F*
Uridine 5′-monophosphate disodium salt (5′UMP)			[BMIm][BF_4]	0.0097–0.2520
Inosine 5′-monophosphate disodium salt (5′IMP)		10		
Guanosine 5′-monophosphate disodium salt (5′GMP)			[EMIm][MS]	0.0093–0.1860

Hymidine 5′-monophosphate disodium salt (5′TMP)		40	[BMIm][BF_4]	0.0097–0.0776
Guanine (Gua)			[EMIm][MS]	0.0093–0.0744
Hypoxanthine (Hypo)			[OMIm][MS]	0.0133–0.1056
Ortho-amino benzoic acid (2-ABA)			[BMIm][BF_4]	0.0097–1.2416

(*continued*)

Table 2. Continued

			Mobile phase composition	
			Ionic liquid	
Name (abbreviation)	Structure	*M*	Type	Range of *F*
Meta-amino benzoic acid (3-ABA)		25	[EMIm][MS]	0.0093–1.1904
Para-amino benzoic acid (4-ABA)			[OMIm][MS]	0.0133–1.6906

Soczewinski Equation

The most successful and simplest models of retention in liquid solid chromatography are those of Snyder[15,16] and Soczewinski.[22–25] In actuality, these models are quite similar. Both of them assume that retention is the product of competitive absorption between the solute and mobile phase molecules for the active sites on the stationary-phase surface. The Soczewinski equation is:

$$k = a + b\log F, \tag{3}$$

where F is the volume fraction of an apolar component of a binary eluent and a and b are constants. Constant b is widely observed. Snyder et al. predicts that the constant b, i.e., the slope of this line, should be the ratio of the molecular areas of the solute and mobile phase, whereas the Soczewinski model predicts that this slope is the number of strongly adsorbing substituent groups on the solute.

Lagmuir Type Equation

The Langmuir type relationship between retention factor and modifier content in a mobile phase was first proposed by Row and coworkers.[17] This approach assumed that the adsorption of organic modifier is described by Langmuir isotherm. The final equation can be expressed as follows:

$$k = a + bF^{-1}, \tag{4}$$

where a and b are coefficients. The intercept, a, characterizes the adsorption interaction between the organic modifier molecules and adsorbent surface while the slope, b, relates to the solute molecules and adsorbent surface interaction.

Quadratic Equations

Finally, the retention factor is correlated by a quadratic relationship involving the volume percent of modifier (F). The simple polynomial of quadratic form is adopted and the two types of k, normal (Eq. 5) and Napierian logarithmic (Eq. 6) scale are as follows,

$$k = a + bF + cF^2, \tag{5}$$

$$\ln k = a + bF + cF^2, \tag{6}$$

where a, b, and c are constants for a given analyte and a given chromatographic system. The Eq. (6) is popularly known as a model proposed by Schoenmakers et al.[26]

Models Evaluation

All equations were performed by Origin (Microcal Software Inc., MA, USA.).[27]

RESULTS AND DISCUSSION

Ionic liquids possess exceptional properties like a coulomb field around them, which promotes strong orientation and induction interactions. Accordingly, as mobile phase modifiers, they can play variety roles, including coating residual silanols, modification of the stationary phase, and acting as ion-pairing agents. It is common knowledge that in RP-HPLC, the nonpolar alkyl groups of the stationary phase can interact with different alkyl groups of the ionic liquids cations. The addition of ionic liquids to a mobile phase leads to competition between ionic liquids cations and polar groups of solutes for the polar silanols group on an alkyl silica surface. Thus, the modifier also disables the alkyl groups of the stationary phase. If the concentration of the ionic liquid is slightly increased, their cation interactions with the silanols groups on the alkyl silica surface due to specific interactions or with the alkyl groups due to hydrophobic and non-specific interactions gradually strengthen, resulting in an increase in the carbon content of the stationary phase. With a further increase in the concentration of a modifier, cations interacts with the silanols group through electrostatic interactions, producing a weak layer electronic structure, and interact with the alkyl group through hydrophobic and non-specific interactions. Thus, it is possible to tell about the realization of the version of dynamic modification of sorbent by modifier and formation of the pseudo stationary phase. Simultaneously, the modification by ionic liquids also creates also electrostatic potential on the surface, thus providing an additional retentive force for ionized analytes. It is possible that the surface acquires some ion exchange properties, and retention is subordinated to laws governing ion exchange chromatography. Besides, ionic ionic interactions, second interactions of nonionic nature appear on the surface of sorbent due to the adsorption, and hydrogenous bonds of sorbate with the nonionic part of the sorbent due to the limited solubility of sample in the mobile phase. Thus, a sorbate, which exists in the solution as anion, is capable of making specific ionic interactions with sorbed cations of ionic liquids. In this case, the retention is caused by complicated equilibrium processes, which compete. From all that has been said, it follows that the effect of ionic liquid modifiers on separation can be very complicated. It is not inconceivable that new separation mechanisms can be made available with ionic liquids. These aspects can enable the development of more efficient separation processes.

In this work, the compounds given in Table 2 were systematically examined using mobile phases compositions expressed in the same table. In our experiment, we employed nine test solutes belonging to three chemical

classes, and namely: nucleic compounds (uridine 5′-monophosphate, inosine 5′-monophosphate, guanosine 5′-monophosphate, and hymidine 5′-monophosphate disodium salts), nitrogen-containing heterocycles (guanine and hypoxanthine), and amino benzoic acids (orthro-, meta, and para-isomers). All these organic substances have different affinity to silanols groups. Thus, all the employed solutes were apt to migrate along the chromatographic column due to the mixed retention mechanism.

Figure 1 shows chromatograms of nucleotides without (a) and with 0.0093 (b), 0.0744 (c), 0.1116 (c) and 0.1860 (d) vol.% of [EMIm][MS] in 10% methanol solution. It is clear that three nucleotides (5′-UMP, 5′-IMP, and 5′-GMP) are co-eluted with the unmodified mobile phase (Fig. 1a). It is also noted that the retention times of the solutes were very close to the dead time. Modification of the eluent by [EMIm][MS] exercises a significant influence on the retention and isolation of these nucleotides. The eluent with 0.1860 vol.% of this modifier provides better separation in an acceptable time. It is necessary to note that similar results were obtained with isolations of nitrogen containing heterocycles and amino benzoic isomers.

The performance of models was evaluated and compared with use of the squared correlation coefficient (r_{sq}) value. All regressions were carried out according to Eqs. (3–6) for each solute. The slope, intercept, and r_{sq} calculated are listed in Tables 3–5 for the modifiers [BMIm][BF_4], [EMIm][MS], and

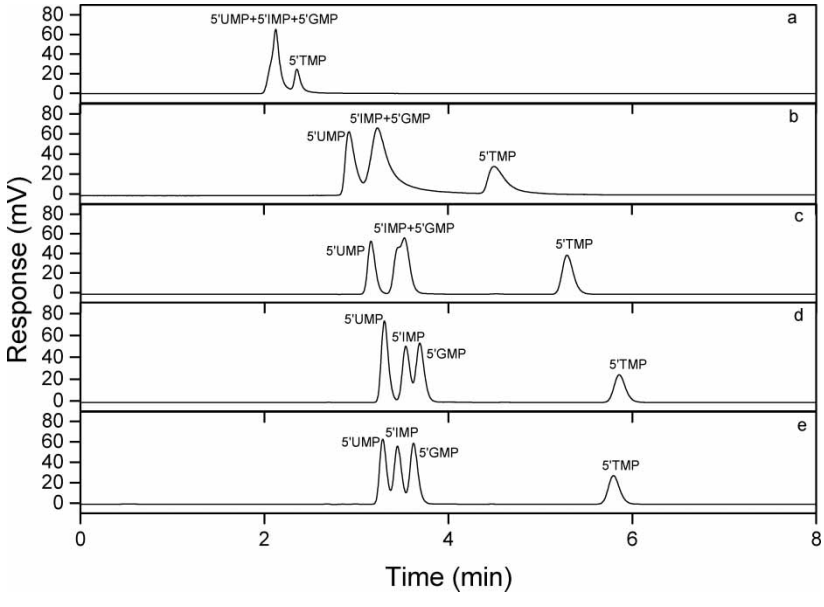


Figure 1. Chromatograms of nucleotides with mobile phase without (a) and with 0.0093 (b), 0.0744 (c), 0.1116 (c) and 0.1860 (d) vol.% of [EMIm][MS] in mobile phase.

Table 3. Calculated results of the parameters used in Eqs. (3–6) in modifier of [BMIm][BF_4]

	Eq. (3)			Eq. (4)			Eq. (5)				Eq. (6)			
Solute	*a*	*b*	r_{sq}	*A*	*b*	r_{sq}	*a*	*b*	*c*	r_{sq}	*a*	*b*	*c*	r_{sq}
5′UMP	5.45	2.58	0.8073	3.02	−0.03	0.3223	0.61	14.55	11.67	0.8745	−0.66	9.37	−7.36	0.3882
5′IMP	7.49	3.74	0.7882	3.96	−0.04	0.2945	0.67	16.22	36.22	0.8702	−0.48	9.62	−7.49	0.4116
5′GMP	8.09	4.13	0.7865	4.18	−0.04	0.2878	0.67	14.77	52.47	0.8831	−0.47	9.78	−7.65	0.4203
5′TMP	15.96	8.49	0.7701	7.88	−0.08	0.2647	0.93	26.26	122.31	0.8625	0.09	9.69	−7.66	0.4451
Gua	0.35	0.12	0.6387	0.25	$-0.1\ 10^{-2}$	0.8559	0.06	6.70	−61.41	0.7213	−2.52	44.87	−413.84	0.7203
Hypo	0.45	0.13	0.5977	0.34	$-0.1\ 10^{-2}$	0.8259	0.14	7.41	−68.82	0.6966	−1.88	31.56	−293.53	0.7029
2-ABA	1.66	0.52	0.9992	1.39	$-0.9\ 10^{-2}$	0.7218	0.65	2.66	−1.47	0.8089	0.24	2.04	−1.15	0.8283
3-ABA	0.49	0.13	0.9872	0.43	$-0.2\ 10^{-2}$	0.6364	0.21	0.83	−0.48	0.6210	−1.25	1.52	−0.85	0.8895
4-ABA	0.27	0.11	0.8482	0.22	$-0.2\ 10^{-2}$	0.8716	0.06	0.58	−0.34	0.7095	2.58	4.37	−2.75	0.4257

Table 4. Calculated results of the parameters used in Eqs. (3–6) in modifier of [EMIm][MS]

	Eq. (3)			Eq. (4)			Eq. (5)				Eq. (6)			
Solute	*a*	*b*	r_{sq}	*a*	*b*	r_{sq}	*a*	*b*	*c*	r_{sq}	*a*	*b*	*c*	r_{sq}
5′UMP	0.77	0.12	0.9812	0.65	−0.01	0.8938	0.29	6.17	−23.74	0.5036	−1.60	27.82	−119.75	0.4266
5′IMP	0.97	0.14	0.9624	0.85	−0.01	0.9225	0.39	8.19	−32.56	0.4964	−1.29	26.86	−116.66	0.4191
5′GMP	0.87	0.09	0.8429	0.79	−0.01	0.9294	0.38	7.67	−32.07	0.4579	−1.28	25.66	−113.29	0.4740
5′TMP	2.27	0.46	0.9894	1.84	−0.01	0.8709	0.82	16.03	−58.30	0.5235	−0.49	25.03	−105.87	0.4737
Gua	0.89	0.37	0.9341	0.48	$-0.3\ 10^{-2}$	0.9988	−0.01	16.53	−139.96	0.9496	−2.80	81.20	−735.31	0.8853
Hypo	0.07	0.37	0.9736	0.64	$-0.4\ 10^{-2}$	0.8895	0.05	22.26	−211.75	0.7670	−2.08	68.94	−662.01	0.7508
2-ABA	2.21	−2.33	0.9754	3.50	0.03	0.61231	4.82	−4.46	1.74	0.2187	1.83	−2.24	1.11	0.9605
3-ABA	0.13	−0.34	0.9496	0.26	$0.7\ 10^{-2}$	0.7713	0.53	−0.94	0.62	0.1511	−0.43	−3.31	2.22	0.6232
4-ABA	0.28	−0.56	0.9806	0.59	$0.9\ 10^{-2}$	0.6715	0.95	−1.26	0.58	0.2684	0.22	−2.91	1.38	0.9537

Table 5. Calculated results of the parameters used in Eqs. (3–6) in modifier of [OMIm][MS]

	Eq. (3)			Eq. (4)			Eq. (5)				Eq. (6)			
Solute	*a*	*b*	r_{sq}	*a*	*b*	r_{sq}	*a*	*b*	*c*	r_{sq}	*a*	*b*	*c*	r_{sq}
Gua	0.28	0.14	0.9654	0.14	$-0.1\ 10^{-2}$	0.8487	$-0.8\ 10^{-3}$	2.19	−6.74	0.9656	−4.49	61.67	−349.63	0.8897
Hypo	0.84	0.37	0.9017	0.49	$-0.5\ 10^{-2}$	0.9722	0.01	10.42	−59.61	0.8709	−2.67	52.98	−333.84	0.8131
2-ABA	0.61	−0.79	0.8026	7.24	0.01	0.3555	6.19	5.00	−3.11	0.0974	2.07	−0.03	−0.08	0.8683
3-ABA	−0.04	−0.89	0.8827	0.22	0.02	0.8505	1.00	−2.04	0.87	0.3761	0.27	−4.80	1.20	0.9688
4-ABA	0.70	−0.84	0.8015	0.91	0.02	0.9389	1.48	−0.95	0.25	0.1475	0.61	−1.41	0.45	0.7734

[OMIm][MS], respectively. For the estimation of predictive capability of equations we were keeping to the following rules: If the absolute value of the squared regression coefficient is approximately equal to unity $0.9 \leq r_{sq} \leq 1.0$, then it is understood that the retention mechanism proposed by equation is highly probable and the corresponding equation can be used for retention prediction. If $r_{sq} < 0.9$, then it is understood that the mixed or more complex retention mechanism is involved, with the different quantitative proportions. Next, we show a few examples, which demonstrated the effect of changes in the mobile phase composition on the parameters of equations.

Soczewinski Equation

The type of eluent and the molecular structure of the compounds significantly affected the retention, the slope *b* of Eq. (3) and, consequently, the selectivity. The analysis of data from Tables 3–5 showed that the constant *b* depends on the type and the number of substituent in the molecule, as well as on the structure. They also showed that the slope increases with increasing value of the constant *a*. The type of mobile phase modifier also influenced the *b* value. For example, tested nucleotides have the highest values of *b* with [BMIm][BF_4] than [EMIm][MS]. But at that point, the correlation coefficients of Eq. (3) are relatively low in the mobile phases modified by [BMIm][BF_4]. This fact testifies that the competitive adsorption of sample and organic modifier [BMIm][BF_4] occurred on the C_{18} surface even at low concentrations. The sole exception is the result of amino benzoic acids with [BMIm][BF_4]. All this data are obtained at a quite low content of organic modifier. This means that the Soczewinski relationship is not fully adequate, and more complex equations considering interactions between sample and modifier [BMIm][BF_4] are required. However, this equation serves for predictions of retention with other tested modifiers. As may be inferred from Tables 4 and 5, the predictive capability of the Soczewinski equation is relatively high with mobile phase modified by [EMIm][MS] or [OMIm][MS]. Figure. 2 shows the excellent agreement observed between using calculated Eq. (3) and experimental *k* for nucleic compounds with [EMIm][MS]. This agreement confirms the validity of the Soczewinski model and supports the mechanism of solute retention that is proposed. The data handling k_{calc} vs k_{exp}, sees a relatively satisfactory agreement between the experimental data points and the theoretical calculated with use of Soczewinski models, and a somewhat less satisfactory agreement for the plot calculated with use of nucleotide 5′TMP.

Langmuir-Type Equation

The dependence of *k* vs F^{-1} is characterized by the different magnitudes of slopes for each solute. In the following Langmuir-type relationship, i.e.,

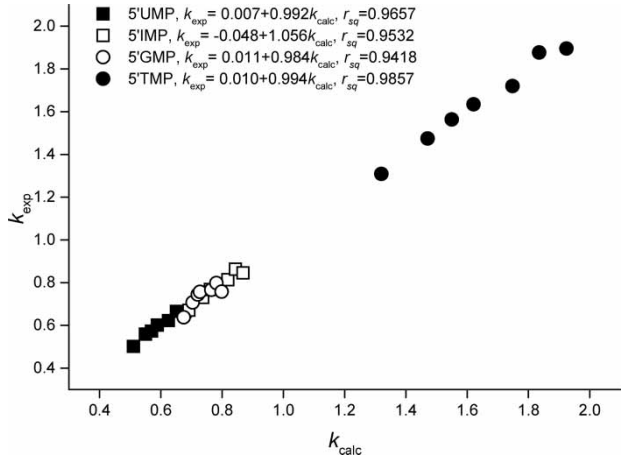


Figure 2. Comparisons of the experimental (k_{exp}) and calculated (k_{calc}) retention factors of nucleotides with [EMIm][MS] by Eq. (3).

Eq. (4), the intercept, a, and the slope, b, were obtained by the regression analysis for all compounds. Tables 3–5 indicate that the ratios of slopes are divided by the nature of samples. It is characteristic that b values were relatively close and fell into groups. For example, for nucleotides with [BMIm][BF_4] these parameters were similar and in the event of [EMIm][MS] the slope have equal values. These tables also shows that $b_{[BMIm][BF4]}$, $b_{[EMIm][MS]}$, and $b_{[OMIm][MS]}$ of amino benzoic acids are changed in a comparatively wider range from $-0.9\ 10^{-2}$ to 0.03, but the ratios of guanine and hypoxanthine are changed in a narrow range from $-0.5\ 10^{-2}$ to $-0.1\ 10^{-2}$. The Langmuir-type equation showed the different agreements with investigated modifiers. It is not too difficult to see that correlation coefficients, r_{sq}, obtained with [EMIm][MS] were almost sufficiently large (0.8709–0.9294) while with [BMIm][BF_4] r_{sq} values are unsatisfactory (0.2647–0.3223). Some conformity can be elucidated by comparison of intercepts, a, and r_{sq} values. In the first instance, intercept's values are quite close and much less than with [BMIm][BF_4]. As shown in Fig. 3, the intercepts calculated for modifiers with methylsilfate anion ionic liquids are coincident. The analogous conformity was detected in the case of other modifiers and solutes. By this means, the Langmuir-type equation describes adequately the chromatographic retention in the case of quite weak adsorption interactions between the modifier molecule and adsorbent surface.

Quadratic Equations

In the subsequent steps, the approach based on quadratic equations Eqs. (5) and (6) showed unimpressive results. The correlations coefficients (r_{sq}) are

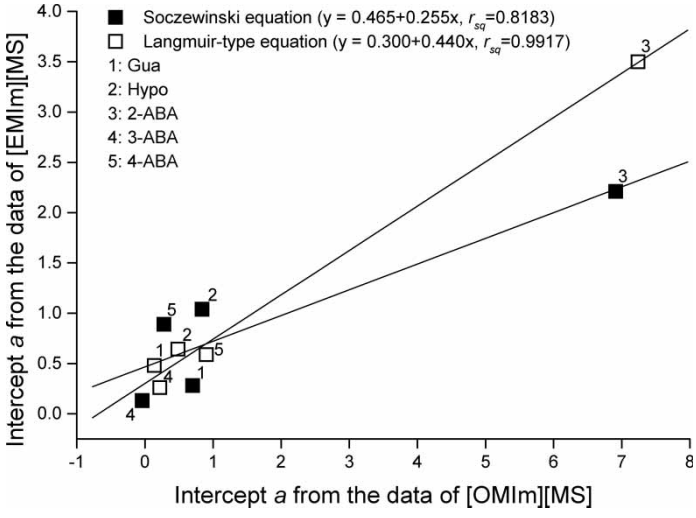


Figure 3. Comparison of intercepts, *a*, of Eqs. (3 and 4) from the data of [OMIm][MS] and [EMIm][MS].

always lower than 0.9, with the exception of the five cases, (see Tables 4 and 5). These parabolic models are adequate only for guanine with [EMIm][MS] Eq. (5) and [OMIm][MS] Eq. (6), for 2- and 4-amino benzoic acids with [EMIm][MS] Eq. (6), and for meta-amino benzoic acid with [OMIm][MS] Eq. (6). The *k* vs *F* plots shows the worst correlations. The Eq. (5) is approximates well for all experimental data of amino benzoic acids. The analogous tendency is tracked for nucleotides with for [BMIm][BF_4] Eq. (5) and for [EMIm][MS] Eqs. (5) and (6). It is noteworthy that with [BMIm][BF_4] all obtained equations displayed unacceptable correlation coefficients for predictions. It is simply evident that in the most cases these dependences have a non-parabolic character.

Performance of Models

Some overall conclusions can be drawn from a careful inspection of the numerical r_{sq} values collected in Figs 4–7. To summarize, the lowest r_{sq} values are observed with [BMIm][BF_4] and this regularity is preserved irrespective of the chemical class of the test solute. The Soczewinski model performs less accurately than the Langmuir-type model, and Schoenmakers model with [BMIm][BF_4] is the least accurate of them all. By this means, the satisfactory agreement is observed only between the experimental and theoretical data calculated for amino benzoic acids with use of [BMIm][BF_4] as modifier (Table 3 and Fig. 4). Relatively low r_{sq} values are

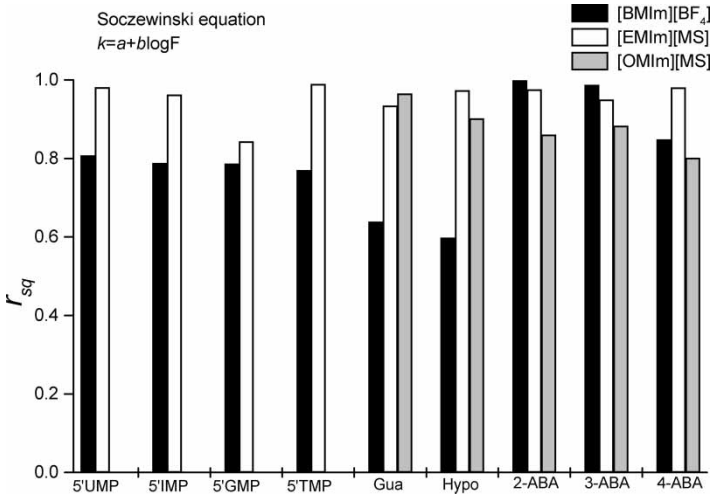


Figure 4. Effect of modifier type on the squared correlation coefficients (r_{sq}) of investigated solutes obtained with Eq. (3).

observed for nucleotides and this regularity remains valid for the quadratic models compared. In Figs 6 and 7, an additional graphical illustration of this phenomenon is offered. It is a well established fact that sufficiently low r_{sq} values of Eqs. (3) and (4) with the content of [BMIm][BF_4] in the eluent gives evidence of the predominance of the multiplex mechanism in retention, while the increase of r_{sq} values is a witness to the predominance of the adsorption partition mechanism in retention with the [EMIm][MS] and [OMIm][MS] in the employed liquid systems. However, the quality of experimental relationships between the retention parameter, k, and the mobile phase composition, which is estimated by r_{sq}, can make the distinction between the eluent modifier ranges, in which different retention mechanisms predominate. In the case of the tetrafluoroborate and methylsulfate anion containing ionic liquids, this distinction seems clear and a question can be posed as to the nature of this phenomenon. It would, however, be rather difficult to find the whys and wherefores in the nature of modifiers for which adsorption partition switches to multiplex mechanism. It is probable that there is no pure mechanism of solute retention in chromatography, and certainly, one cannot expect pure mechanisms with the modifier. A conclusion regarding Soczewinski and Langmuir-type models is quite obvious. They are the models grounded on physicochemical premises and were developed for adsorption partition liquid chromatography systems, which excludes application to the multiplex chromatography mode. As the general concept of Soczewinski and Langmuir-type models, we considered that the eluent compositions with the low numerical values of F (0.0093–0.2520 vol.%) are

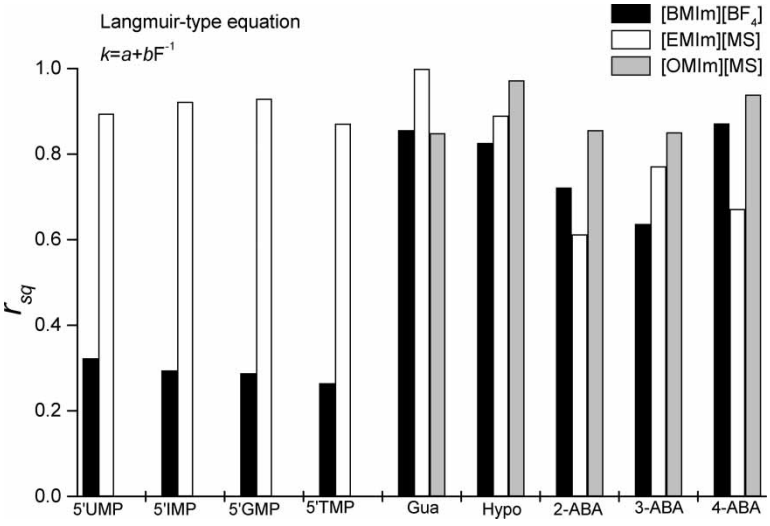


Figure 5. Effect of modifier type on the squared correlation coefficients (r_{sq}) of investigated solutes obtained with Eq. (4).

within the framework of applicability of our approach. A different conclusion can be drawn in the case of models described by Eqs. (5) and (6). These models were also founded on firm physicochemical premises and with adsorption partition liquid chromatography in mind. However, the short range of modifier contents and their weak concentrations are proving insufficient to fall outside the limits of a linear relationship. We have good reason to

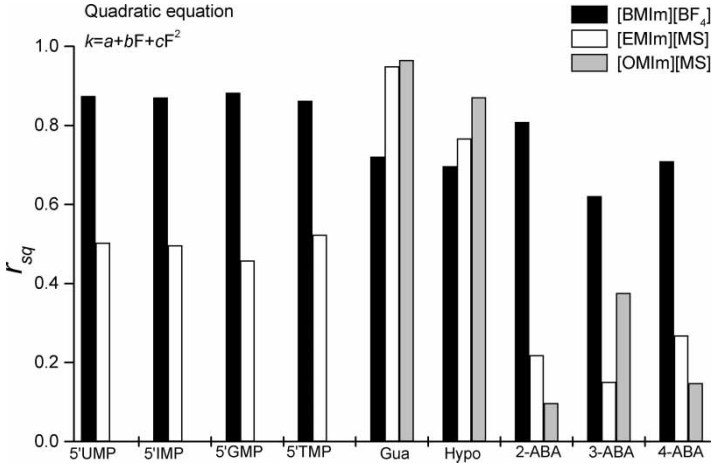


Figure 6. Effect of modifier type on the squared correlation coefficients (r_{sq}) of investigated solutes obtained with Eq. (5).

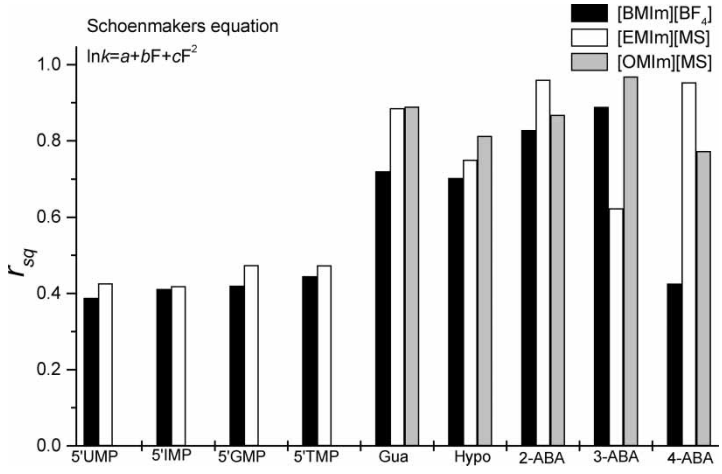


Figure 7. Effect of modifier type on the squared correlation coefficients (r_{sq}) of investigated solutes obtained with Eq. (6).

believe that some examples of satisfactory correlation coefficients have no systematic character. It is common knowledge that a parabolic relationship can easily be adapted to any observed curvilinear dependence between variables, no matter which retention mechanism actually occurs. Therefore, the performance of quadratic models in the cases studied, as proven by r_{sq} values, seems due in part to the algebraic properties of Eqs. (5) and (6) rather than to its physicochemical background, which is basically oriented on the adsorption artition liquid chromatography mode.

CONCLUSION

In this work, the influence of ionic liquids modifiers on retention eluent composition relationships in RP-HPLC was examined. Statistical evaluations of two linear and two quadratic retention models derived for nine solutes with ionic liquids as modifiers of mobile phase demonstrates that both linear models, Soczewinski and Langmuir-type, are able to describe chromatographic retention relatively precisely. Of the two, the Soczewinski equation fits better because of logarithmic scale. The best retention model was a Langmuir-type equation for nucleic compounds, guanine, and hypoxanthine, with methylsulfate-anion containing ionic liquids, and the Soczewinski equation for amino benzoic acids with [BMIm][BF_4] and [EMIm][MS]. Regression analysis demonstrates that the quadratic equations do not improve, meaningfully, the description of analytes retention. Unfortunately, one is forced to accept the fact that the influence of the ionic liquids on the separation mechanism is not yet clearly understood. More data are required,

particularly data acquired with other ionic liquids modifiers, to draw more general conclusions regarding the effect of such modifiers on the retention mechanisms in RP-HPLC.

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