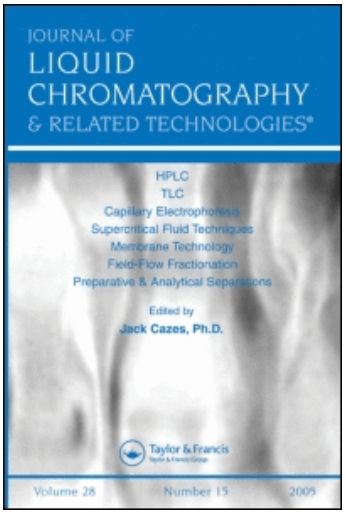



**Journal of Liquid Chromatography & Related Technologies**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

# Analysis of Linear Regressions Applied to Water-Methanol Eluents Modified with Ionic Liquid

Yulia Polyakova[a]; Kyung Ho Row[a]
[a] Center for Advanced Bioseparation Technology and Department of Chemical Engineering, Inha University, Incheon, South Korea

**To cite this Article** Polyakova, Yulia and Row, Kyung Ho(2007) 'Analysis of Linear Regressions Applied to Water-Methanol Eluents Modified with Ionic Liquid', Journal of Liquid Chromatography & Related Technologies, 30: 17, 2557 — 2573
**To link to this Article: DOI:** 10.1080/10826070701540472
**URL:** http://dx.doi.org/10.1080/10826070701540472

*Journal of Liquid Chromatography & Related Technologies*®, 30: 2557–2573, 2007

ISSN 1082-6076 print/1520-572X online
DOI: 10.1080/10826070701540472

# Analysis of Linear Regressions Applied to Water-Methanol Eluents Modified with Ionic Liquid

**Yulia Polyakova and Kyung Ho Row**
Center for Advanced Bioseparation Technology and Department of Chemical Engineering, Inha University, Incheon, South Korea



**Abstract:** Five equations, two linear (Soczewinski and Langmuir-type) and three multiple linear have been applied for investigation of the influence of 1-butyl-3-methylimidazolium tetrafluoroborate ionic liquid modifier on the retention eluent composition relationships in reversed-phase high performance liquid chromatography. In the experiment, five solutes belonging to amino acids, namely N-carbobenzyloxy-D-phenylalanine, D-tryptophan, and orthro-, meta-, and para-isomers of amino benzoic acid were tested. Statistical evaluation of models of chromatographic retention was performed and discussed. This study shows that Soczewinski and Langmuir-type equations are able to describe chromatographic retention of molecules which do not form stable ions in the aqueous, relatively precisely. At the same time, these models are not applicable for the description of N-CBZ-D-phenylalanine retention, which exists in the water solution as anion and more complex models, considering that interactions between ionizable sample and eluent are required. Multiple linear regressions with two variables (methanol and ionic liquid modifier contents) improve significantly the description of N-CBZ-D-phenylalanine retention.

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

Address correspondence to Kyung Ho Row, Center for Advanced Bioseparation Technology and Department of Chemical Engineering, Inha University, #253, Yonghyun-Dong, Nam-Ku, Building 2S 335, Incheon 402-751, South Korea. E-mail: rowkho@inha.ac.kr

## INTRODUCTION

Ionic liquids are room temperature fluids composed entirely of ions, typically large organic cations and small inorganic anions.[1] The components of ionic liquids (ions) are constrained by high coulombic forces, and thus exert practically no vapor pressure above the liquid surface. Importantly, the near zero vapor pressure (nonvolatile) property of ionic liquids means they do not emit the potentially hazardous volatile organic compounds associated with many solvents during their handling and use. The thermodynamics and mechanism of processes carried out in ionic liquids are different from those in conventional media. Nevertheless, the physicochemical nature of ionic liquids is not well understood. The reason why they are liquids is yet to be elucidated.[2–4]

This creates new opportunities for chemical synthesis, catalysis, fine chemistry, and separations. The literature and patents describe numerous applications with increased success rate and advantages.[5–11] The prospects for ionic liquid use are vast. Many ionic liquids are miscible with water and a number of common organic solvents, providing flexibility for chromatographic separation process, and they are nonvolatile even at elevated temperatures. Several ionic liquids are highly polar, ideal for modification of mobile phase in reversed-phase high performance liquid chromatography (RP-HPLC).[12–25]

The simplest biphasic systems (water-organic eluents) do not always satisfy analysts, and majorities of separations are impossible without various mobile phase additives. In RP-HPLC separations, the minor components are present at $<5\%$ and are commonly referred to as mobile phase modifiers. In recent years the interest of new modifiers is growing rapidly. One of the new types of mobile phase modifiers are the ionic liquids. Ionic liquids are clearly complex entities compared to the relatively simple modifiers used in most chromatographic processes. Several reasons are essential to attaining the goal of accelerated application of chromatographic separation processes based on ionic liquids.

The chemical nature of the ionic liquids allows concluding that they exist in the polar mobile phase as ions and they also coated on the reversed-phase silica-based column. According to authors,[15,26] the ionic liquids form a bonded layer (pseudo-stationary phase) on the surface of the modified silica gel. Previous studies[15,27–31] have shown that ionic liquid cations can interact and compete with silanol groups on an alkyl silica base surface.

The retention of analytes in RP-HPLC is fundamentally determined by their distribution between a liquid 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. Usually, simple mathematical models are used for finding relationships between retention and mobile phase composition. In the case of ionic liquid modifiers, this approach requires parallel efforts on (1) developing the fundamental understanding of ionic liquids so that they can be deliberately designed to meet HPLC needs, (2) applied research/development to prove that ionic liquids can efficiently affect a

separation processes in RP-HPLC, and (3) fundamental understanding of mobile phase composition versus chromatographic performance. This requires a robust understanding of the fundamental physicochemical principles involved in the separation mechanisms with ionic liquids. To realize the potential of ionic liquids, a theoretical approach to the development of new ionic liquid modifier and the separation processes in which they could be used must be studied. In the literature one can find complex models aimed at the prediction of changes in retention as a function of mobile phase composition.[32] Best known are: the Snyder model,[33,34] the Langmuir approach,[35] the solvatochromic model,[36] the stoichiometric displacement model,[37] the solvophobic model,[38] and the Schoenmakers model.[39] Many researchers investigated the separation of organic chemicals with different types of mobile phases and modifiers in RP-HPLC. It must be emphasized, that in all the previous experiments only common modifiers were used and ionic liquids have never been applied for this purpose. It is reasonably safe to suggest that the present study is a pioneer work and should be of immediate interest to separation science.

In this paper, linear equations, two linear (Soczewinski and Langmuir-type) and three multiple linear, have been applied for investigation of the influence of 1-butyl-3-methylimidazolium tetrafluoroborate ionic liquid modifier on the retention eluent composition relationships in reversed-phase high performance liquid chromatography.



## EXPERIMENTAL

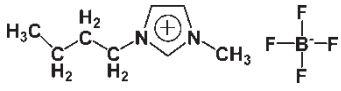
### Apparatus

An analytical system with 600 HPLC pump (Waters, U.S.A.), 486 detector (M 7200 Absorbance Detector, Young-In Scientific Co., South Korea), and Reodyne injection (Cotati, CA, USA) valve with 20 μL sample loop were used. 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 liquid, 1-butyl-3-methylimidazolium tetrafluoroborate, selected for these studies was purchased from C-tri Co. (Namyang, South Korea) (Table 1). This ionic liquid was used as obtained, without additional pretreatment. Amino acids, n-carbobenzyloxy-D-phenylalanine, D-tryptophan, and orthro- and meta-amino benzoic acids, were product of Sigma-Aldrich Co. (St. Louis, MO, U.S.A.). Para-amino benzoic acid was purchased from

*Table 1.* The name, abbreviation, and structure of ionic liquid

| Name | Abbreviation | Structure |
|---|---|---|
| 1-Butyl-3-methylimidazolium tetrafluoroborate | [BMIm][$BF_4$] |  |

Fluka (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.

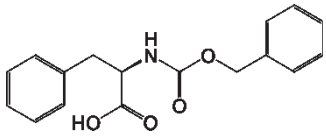
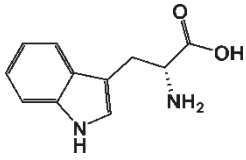
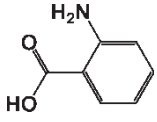
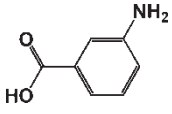
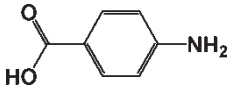
## Chromatographic Conditions

Mobile phases were composed of methanol in water (pure reversed-phase systems). The ionic liquid -butyl-3-methylimidazolium tetrafluoroborate was used as mobile phase modifiers. The modified mobile phases were prepared by dissolving known amounts of 1-butyl-3-methylimidazolium tetrafluoroborate in the water-methanol eluent. The information about solutes and conditions of their analysis (mobile phase composition) is shown in Table 2. 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 $\lambda$ of 254 nm. The eluent was flowed in isocratic mode. The retention factor ($k$) was calculated according to Eq. (1)

$$k = (t_R - t_M)/t_M, \tag{1}$$

where $t_M$ is the dead time (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

***Table 2.*** The solutes and condition of their analysis (where $F$ is the vol.% of methanol in water and $I$ is the vol.% of ionic liquid [BMIm][$BF_4$])

| Name (abbreviation) | Structure | Mobile phase composition | |
|---|---|---|---|
| | | $F$ | $I$ |
| N-CBZ-D-Phenylalanine (D-Phe) |  | | |
| D-tryptophan (D-Try) |  | 55–80 | 0.0097–0.2520 |
| Ortho-amino benzoic acid (2-ABA) |  | | |
| Meta-amino benzoic acid (3-ABA) |  | 15–50 | 0.0097–1.2416 |
| Para-amino benzoic acid (4-ABA) |  | | |

was carried out using mathematical statistic techniques. The relative error of a single measurement did not exceed 5%.

## Theoretical Background

In this work, the mobile phase composition versus chromatographic retention factor, $k$, was estimated. 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 $P$:

$$P = f(M) + \varepsilon \tag{2}$$

in which $\varepsilon$ contains all types of errors, including sampling and measurement errors. In our approach, $P$ is the chromatographic retention factor, $k$, and $M$ is the concentration of modifier.

Soczewinski Equation

The most successful and simplest linear models of retention in liquid-solid chromatography are those of Snyder[34] and Soczewinski.[40–43]

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

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

where $I$ is the volume fraction of a polar component of a binary eluent (in our case it was ionic liquid) 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.



Langmuir Type Equation

The Langmuir type relationship between retention factor and modifier content in a mobile phase was first proposed by Row and co-workers.[35] This approach assumed that the adsorption of organic modifier is described by the Langmuir isotherm. The final equation can be expressed as follows:

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

where $a$ and $b$ are experimental 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.

Multiple Linear Regressions

Multiple linear regressions are extensions of simple linear regression with more than one dependent variable.

In general then, the multiple regression procedures will estimate a linear equation of the form:

$$P = a_0 + a_1X_1 + a_2X_2 + \cdots + a_kX_k \tag{5}$$

where $k$ is the number of predictors. Note, that in this equation the regression coefficients (or $a_1 \ldots a_k$ coefficients) represent the independent contributions of each independent variable to the prediction of the dependent variable.

In our case, the regression coefficients for each mobile phase composition were obtained by multiple linear regression analysis for the measured

retention factors using the following equations:

$$k = a_0 + a_1 F + a_2 I \tag{6}$$

$$k = a_0 + a_1 \log F + a_2 \log I \tag{7}$$

$$k = a_0 + a_1 F^{-1} + a_2 I^{-1} \tag{8}$$

where $F$ is the vol.% of methanol in water and $I$ is the vol.% of ionic liquid [BMIm][$BF_4$].

## Data Analysis

Linear and multiple linear regression analysis and statistical tests were performed using the program Origin (Microcal Software Inc., MA, USA.).[44] The quality of the fits was estimated using the squared correlation coefficient ($r^2$).



## RESULTS AND DISCUSSION

In this work, five solutes given in Table 2 were examined using water-methanol mobile phases with and without ionic liquid modifier [BMIm][$BF_4$]. The ionic liquids have been chosen because they are water-soluble (hydrophilic) and also combine with the most common cations and anions.

The modification of the eluents by [BMIm][$BF_4$] is really affected the solute retention. Figure 1 shows chromatograms of amino benzoic acids without (a) and with 0.1552 (b) vol.% of [BMIm][$BF_4$] in 25% methanol solution. It is clear that two amino benzoic acids (3-ABA and 4-ABA) are co-eluted with the unmodified mobile phase (Figure 1a). It is also noted that the retention times of the solutes were very close to the dead time. Solutes with two strongly polar groups behave differently; in some cases, ortho-isomers are adsorbed more strongly than meta- and para-isomers on polar bonded stationary phases in some eluents containing a high concentration of polar modifier in an aqueous mobile phase. Chromatograms illustrating isolation of N-CBZ-D-phenylalanine and D-tryptophan with and without [BMIm][$BF_4$] in the mobile phase (65% methanol in water) are presented in Figure 2. It is readily apparent that the increase of the modifier concentration causes increased isolation of the sorbats. Identical tendencies are obtained for all methanol concentrations. Furthermore, this modifier has a major impact on the analyte retention. It is axiomatic that the separation should be designed to minimize the required volume of ionic liquid modifier. It is easily seen that adjusting the eluent by a mere 0.0388 vol.% of [BMIm][$BF_4$] provides baseline separation in an acceptable

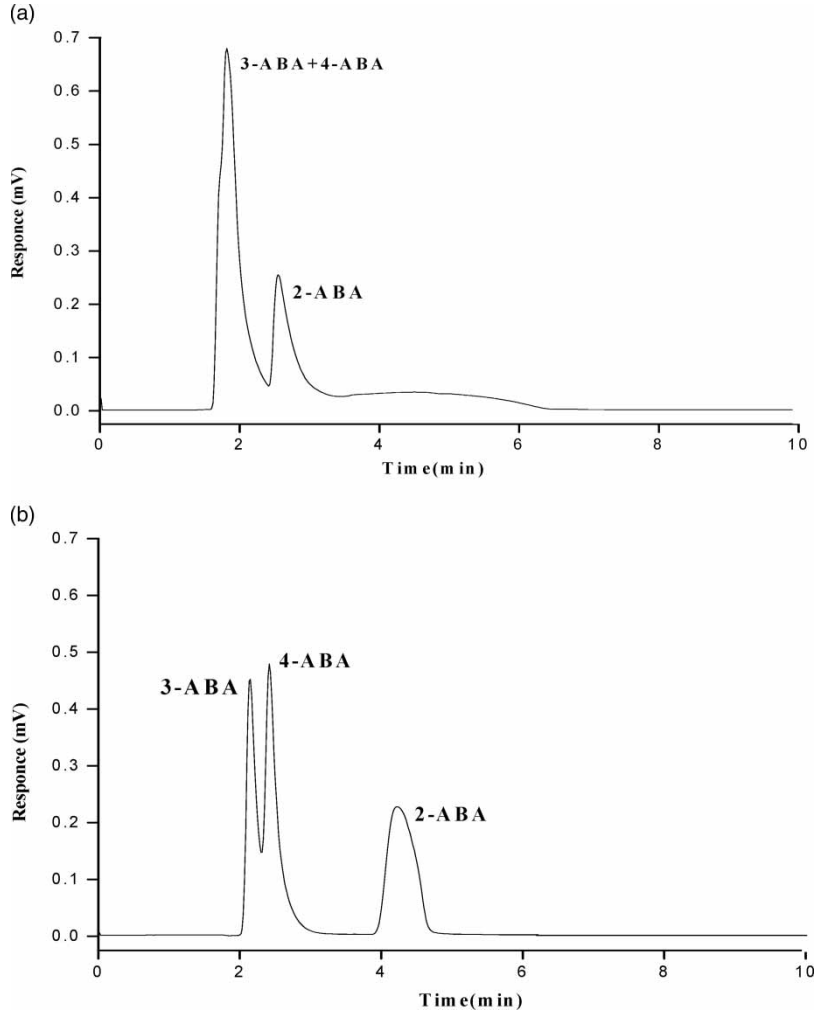



*Figure 1.* Chromatograms of amino benzoic acids without (a) and with 0.1552 (b) vol.% of [BMIm][$BF_4$] in mobile phase (25% methanol in water).

time (Figure 2a). As a result, excellent separations of amino benzoic isomers were achieved using 0.3104 vol.% of [BMIm][$BF_4$] in 25% methanol solution. The full isolation of N-CBZ-D-phenylalanine and D-tryptophan is possible, with even lower concentrations ranging from 0.0388 (b) vol.% of [BMIm][$BF_4$] in mobile phase (65% methanol in water). Also important to note is that the experimental technique should minimize the loss of the ionic liquids and be capable of removing used ionic liquid from the column. After each experiment with the certain concentration of the ionic liquid, and before

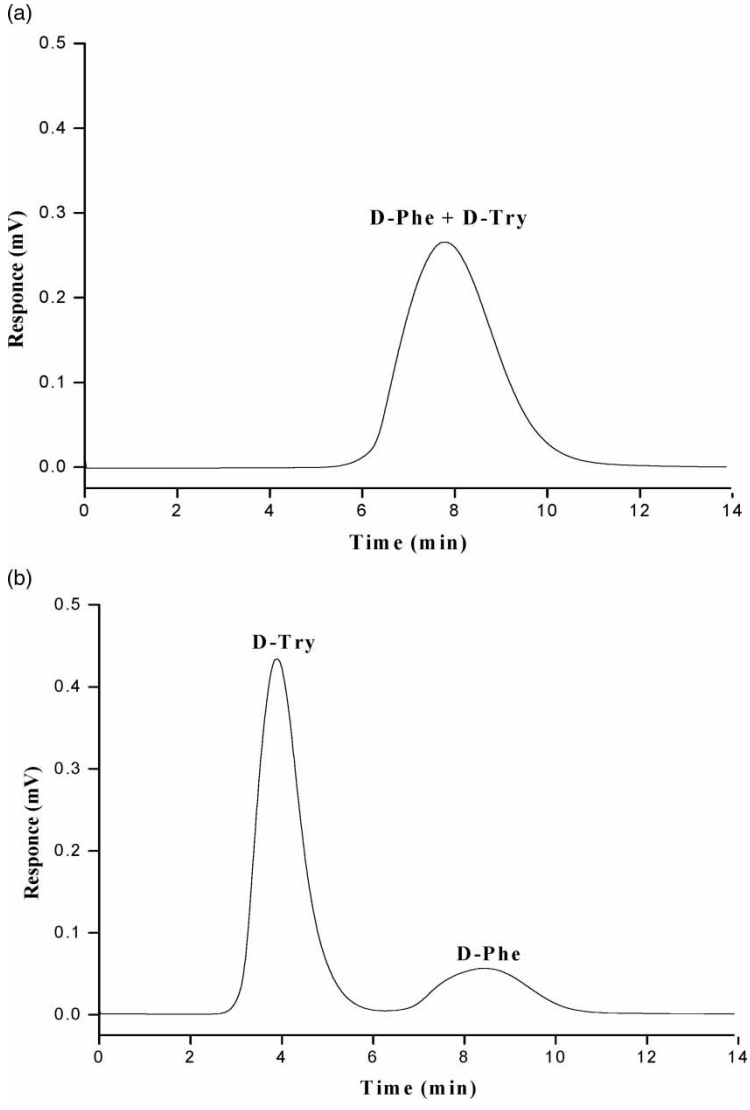



***Figure 2.*** Chromatograms of N-CBZ-D-phenylalanine and D-tryptophan without (a) and with 0.0388 (b) vol.% of [BMIm][$BF_4$] in mobile phase (65% methanol in water).

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. The constancy in efficiency and peak tail factor showed that the use of ionic liquid is not harmful to the column.

Usually in RP-HPLC the retention time will increase as the lipophilicity of the substance is increased and as the polarity of the mobile phase is decreased. The nature and concentration of any competing modifier added to the eluent will determine the retention times and elution order for the solute. In this experiment, the amino acids were tested as model compounds in order to study the contributions of ionic liquid adjustment. All used solutes are polar solutes having a different affinity to silanols and to the polar groups. Thus, all the employed test solutes were apt to migrate along the chromatographic column due to the mixed retention mechanism, although quantitative proportions between adsorption and partition depended on quantitative proportions of modifier to water and on the chemical nature of the solute molecules.

In this work, the retention factor, $k$, is correlated by simple linear relationships involving the vol.% of ionic liquids modifier. In addition, the multiple linear regressions were based on the variables of two terms: vol.% of methanol in water and the vol.% of ionic liquid. The performance of models was evaluated and compared with use of the squared correlation coefficient ($r^2$) value. All regressions were carried out according to Eqs. (3–8) for each amino acid. The slope, intercept, and $r^2$ calculated are listed in Tables 3 and 4. For the estimation of predictive capability of equations we were keeping to the following rules. If the value of the squared regression coefficient is approximately equal to unity $0.9 < r^2 < 1.0$, then it is understood that the retention mechanism proposed by the corresponding equation is highly probable and this equation can be assessable. If $r^2 < 0.9$, we assume that the mixed or more complex retention mechanism is involved with the different quantitative proportions. In the following we show a few examples, which demonstrated the effect of changes in the mobile phase composition on the parameters of linear equations.

The dependence of $k$ vs $F$ is characterized by the different magnitudes of slopes for each solute. In the following simple linear relationships, (Eqs. (3) and (4)), the intercept, $a$, and the slope, $b$, were obtained by the regression analysis for all compounds. The chemical structure is influenced also on the $a$ value. For example, the tested N-CBZ-D-phenylalanine has higher values of $a$ of Eqs. (3 and 4) than D-tryptophan. The intercept of Eq. (4), $a$, characterizes the adsorption interaction between the organic modifier molecules. Some conformity can be elucidated by comparison of intercepts and $r^2$ values. In all cases, if the correlation coefficients were satisfactory, $a$ values were low. 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.

The analysis of data from Table 3 showed that the constant $b$ depends on the type and the number of substituent in the molecule, as well as on the structure. Note that, in this case, the coefficients b obtained with the Eq. (3) are substantially smaller than similar coefficients obtained by the equation Eq. (4) for N-CBZ-D-phenylalanine and D-tryptophan. Table 3 also shows

***Table 3.*** Calculated results of the parameters used in Eqs. (3) and (4) in modifier of [BMIm][$BF_4$] (where $F$ is the vol.% of methanol in water)

| | Eq. (3) | | | Eq. (4) | | |
|---|---|---|---|---|---|---|
| $F$ | $a$ | $b$ | $r^2$ | $a$ | $b$ | $r^2$ |
| D-Phe | | | | | | |
| 55 | 12.31 | −0.05 | 0.0021 | 12.23 | $0.6\ 10^{-2}$ | 0.0855 |
| 60 | 5.52 | −0.19 | 0.1916 | 5.68 | $0.2\ 10^{-2}$ | 0.0809 |
| 65 | 4.84 | 0.79 | 0.1701 | 4.18 | $0.9\ 10^{-2}$ | 0.1006 |
| 80 | 4.02 | 1.52 | 0.5523 | 3.094 | −0.04 | 0.3071 |
| D-Try | | | | | | |
| 55 | 1.04 | −0.63 | 0.8921 | 1.49 | 0.01 | 0.9429 |
| 60 | 0.99 | −0.48 | 0.8949 | 1.33 | $0.8\ 10^{-2}$ | 0.9742 |
| 65 | 0.86 | −0.52 | 0.9156 | 1.23 | $0.8\ 10^{-2}$ | 0.9732 |
| 80 | 2.09 | −0.11 | 0.5953 | 2.15 | $0.5\ 10^{-2}$ | 0.8323 |
| 2-ABA | | | | | | |
| 15 | 3.36 | 1.34 | 0.9302 | 2.42 | −0.02 | 0.5536 |
| 20 | 2.35 | 0.81 | 0.9547 | 1.79 | −0.01 | 0.5815 |
| 25 | 1.66 | 0.53 | 0.9991 | 1.39 | $-0.9\ 10^{-2}$ | 0.7218 |
| 50 | 0.24 | 0.16 | 0.6119 | 0.13 | $-0.2\ 10^{-2}$ | 0.2880 |
| 3-ABA | | | | | | |
| 15 | 0.97 | 0.42 | 0.9780 | 0.70 | $-0.6\ 10^{-2}$ | 0.7063 |
| 20 | 0.59 | 0.20 | 0.9745 | 0.46 | $-0.3\ 10^{-2}$ | 0.6245 |
| 25 | 0.49 | 0.13 | 0.9872 | 0.43 | $-0.2\ 10^{-2}$ | 0.6363 |
| 50 | 0.02 | 0.09 | 0.8337 | −0.04 | $-0.1\ 10^{-2}$ | 0.6123 |
| 4-ABA | | | | | | |
| 15 | 0.40 | 0.19 | 0.9768 | 0.28 | $-0.3\ 10^{-2}$ | 0.6931 |
| 20 | 0.26 | 0.12 | 0.9971 | 0.18 | $-0.2\ 10^{-2}$ | 0.7413 |
| 25 | 0.27 | 0.11 | 0.8483 | 0.22 | $-0.2\ 10^{-2}$ | 0.8716 |
| 50 | −0.05 | 0.05 | 0.9293 | −0.09 | $-0.1\ 10^{-2}$ | 0.8874 |



that $b$ (Eq. (3)) of amino benzoic acids are changed in a comparatively wider range from 0.09 to 1.34.

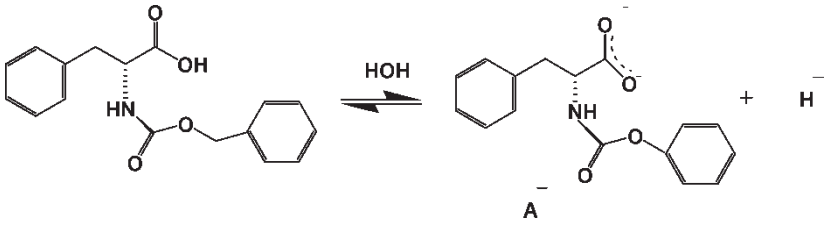
It is not too difficult to see that correlation coefficients of Eqs. (3) and (4), $r^2$, obtained with D-tryptophan were almost sufficiently large, while with N-CBZ-D-phenylalanine $r^2$ values are unsatisfactory (0.0021–0.5523). In case of amino benzoic acid isomers, the correlation coefficients obtained with Eq. (3) were suitable, while with Eq. (4) $r^2$ are unacceptable.

These phenomena can be explained from the point of view of intermolecular interactions between the sorbate and the solid and mobile phases. The special features of the chemical structure of the investigated amino acids determine possible interaction modes. The phenylalanine has an N-carbobenzyloxyl group which is blocking amine nitrogen. Because of this, the molecule

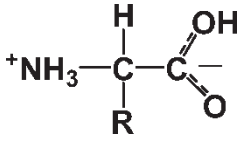
***Table 4.*** Calculated results of the parameters used in Eqs. (6–8) in modifier of [BMIm][$BF_4$]

| | Eq. (6) | | | | Eq. (7) | | | | Eq. (8) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Solute | $a_0$ | $a_1$ | $a_2$ | $r^2$ | $a_0$ | $a_1$ | $a_2$ | $r^2$ | $a_0$ | $a_1$ | $a_2$ | $r^2$ |
| D-Phe | 57.88 | −0.84 | 1.10 | 0.8751 | 214.22 | −116.53 | 0.18 | 0.8222 | −43.78 | 3054.71 | $-7.5 \times 10^{-4}$ | 0.8991 |
| D-Try | 3.69 | −0.03 | −2.25 | 0.6523 | 8.54 | −4.27 | −0.54 | 0.8875 | −0.51 | 111.09 | $-0.8 \times 10^{-2}$ | 0.9498 |
| 2-ABA | 1.91 | −0.04 | 1.44 | 0.7251 | 6.54 | −3.35 | 0.68 | 0.8683 | −0.20 | 37.39 | −0.01 | 0.7348 |
| 3-ABA | 0.56 | −0.01 | 0.45 | 0.7259 | 2.06 | −1.10 | 0.21 | 0.8762 | −0.12 | 11.87 | $-0.3 \times 10^{-2}$ | 0.7305 |
| 4-ABA | 0.23 | $-0.7 \times 10^{-2}$ | 0.28 | 0.7179 | 1.05 | −0.58 | 0.13 | 0.8634 | −0.11 | 6.11 | $-0.2 \times 10^{-2}$ | 0.7076 |

acquires expressed acidic properties and in the aqueous solutions dissociates according to the scheme:




Thus, the N-CBZ-D-phenylalanine in water media is an organic acid and generally exists in the anionic form ($A^-$). The molecule of D-tryptophan has weak nucleophilicity due to aromatic indolyl, however, as a whole it is neutral, and in the aqueous medium forms the zwitterion. In this structure there has been an intramolecular proton transfer from the -OH of the carboxylic acid group to the amine group:




In this case, this molecule does not form the stable ions in the aqueous solution and it remains neutral as a whole. In the case of amino benzoic acid isomers the comparative weakness of the parent substances (aniline and benzoic acid) again renders the zwitterion structure more likely. There is a good chance that the amino benzoic acid exists as a zwitterion ($COO^-$ and $NH_4^+$).

Thus, the N-CBZ-D-phenylalanine, which exists in the solution as an anion, is capable of making specific ionic interactions with the sorbed cations of [BMIm][$BF_4$] (pseudo-stationary phase). Such chromatographic behavior can be explained by the realization of the ion-exchange mechanism of retention. This means that the Soczewinski and Langmuir-type relationships are not adequate, and more complex models considering interactions between ionizable sample and eluent are required. At the same time, the chromatographic behavior of D-tryptophan and amino benzoic acid isomers are satisfactorily described by the Eq. (3) and (4). As may be inferred from Table 3, the predictive capabilities of these models are relatively high with mobile phase modified by [BMIm][$BF_4$].

We are interested in estimating a multiple linear regression equation based on the information about methanol and modifier contents according to Eqs. (6–8). Different combinations of the variables representing each of two terms described above were tried to obtain the equation of best fit, which would relate to the retention of the reference compounds to their mobile phase composition. The values of the calculated results of

the parameters used in Eqs. (6–8) of different amino acids, using the water-methanol mobile phases at different contents of [BMIm][$BF_4$], are given in Table 4. It is not too difficult to see that multiple linear regression Eq. (6) with two variables produced correlation coefficients with low values (the best was $r^2 = 0.8751$ with N-CBZ-D-phenylalanine). The most acceptable results were obtained using Eq. (7). Retention of all investigated substances predict well with the aid of this equation. Equation (8) is applicable for the prediction of the N-CBZ-D-phenylalanine and D-tryptophan retention. It is interesting to note that all obtained multiple linear regressions in the case of N-CBZ-D-phenylalanine give better correlation coefficients than with simple linear equations. The introduction of parameters describing methanol content that is less significant resulted in an increase of the multiple correlation coefficients, $r^2$ in the case of N-CBZ-D-phenylalanine.

## CONCLUSION

In this paper, the influence of mobile phase composition on retention of some amino acids in RP-HPLC was examined. Statistical evaluations of two simple linear and three multiple linear equations derived for solutes with ionic liquid [BMIm][$BF_4$] as modifier of mobile phase, demonstrates that both simple linear models, Soczewinski and Langmuir-type, are able to describe chromatographic retention of molecules, not forming stable ions in the aqueous, relatively precisely. The N-CBZ-D-phenylalanine, which exists in the solution as anion, is capable of making specific ionic interactions with the sorbed cations of [BMIm][$BF_4$] (pseudo-stationary phase). Such chromatographic behavior can be explained by the realization of the ion-exchange mechanism of retention. Soczewinski and Langmuir-type models are not applicable for the description of N-CBZ-D-phenylalanine retention, which exists in the water solution as anion and more complex models considering interactions between ionizable sample and eluent are required. Multiple linear regressions with two variables (methanol and [BMIm][$BF_4$] modifier contents) demonstrate that used equations do meaningfully improve the description of N-CBZ-D-phenylalanine retention.



## ACKNOWLEDGMENT

The authors are grateful for financial support from the Center for Advanced Bioseparation Technology, Inha University.

## REFERENCES

1. Welton, T. Room-temperature ionic liquids. Solvents for synthesis and catalysis. Chem. Rev. **1999**, *99* (8), 2071–2083.

2. Anthony, J.L.; Maginn, E.J.; Brennecke, J.F. Solution thermodynamics of imidazolium-based ionic liquids and water. J. Phys. Chem. B **2001**, *105* (44), 10942–10949.
3. Aki, S.; Brennecke, J.F.; Samanta, A. How polar are room-temperature ionic liquids? Chem. Commun. **2001**, *5*, 413–414.
4. Tokuda, H.; Tsuzuki, S.; Mabh, S. How ionic are room-temperature ionic liquids? An indicator of the physicochemical properties. J. Phys. Chem. B **2006**, *110* (39), 19593–19600.
5. Koel, M. Ionic liquids in chemical analysis. Crit. Rev. Anal. Chem. **2005**, *35* (3), 177–192.
6. Liu, L.F.; Jonsson, L.A.; Jiang, G.B. Application of ionic liquids in analytical chemistry. TrAC. Trends Anal. Chem. **2005**, *24* (1), 20–27.
7. Sheldon, R.A.; Lau, R.M.; Sorgedrager, M.J.; Van Rantwijk, F.; Seddon, K.R. Biocatalysis in ionic liquids. Green Chem. **2002**, *4* (2), 147–151.
8. Koel, M.; Kaljurand, M. Application of the principles of green chemistry in analytical chemistry. Pure Appl. Chem. **2006**, *78* (11), 1993–2002.
9. Pacholec, F.; Butler, H.T.; Poole, C.F. Molten organic salt phase gas-liquid-chromatography. Anal. Chem. **1982**, *54* (12), 1938–1941.
10. Ding, J.; Welton, T.; Armstrong, D.W. Chiral ionic liquids as stationary phases in gas chromatography. Anal. Chem. **2004**, *76* (22), 6819–6822.
11. Anderson, J.L.; Armstrong, D.W. High-stability ionic liquids. A new class of stationary phases for gas chromatography. Anal. Chem. **2003**, *75* (18), 4851–4858.
12. Shetty, P.H.; Younberg, P.J.; Kersten, B.R.; Poole, C.F. Solvent properties of liquid organic salts used as mobile phases in microcolumn reversed-phase liquid chromatography. J. Chromatogr. **1978**, *411*, 61–79.
13. Poole, C.F.; Kersten, B.R.; Ho, S.; Coddens, M.E.; Furton, K.G. Organic salts, liquid at room temperature, as mobile phase in liquid chromatography. J. Chromatogr. **1986**, *352*, 407–425.
14. Wang, Q.; Baker, G.; Baker, S.; Colon, L. Surface confined ionic liquid as a stationary phase for HPLC. Analyst **2006**, *131* (9), 1000–1005.
15. He, L.; Zhang, W.; Zhao, L.; Liu, X.; Jiang, S. Effect of 1-alkyl-3-methylimidazolium-based ionic liquids as the eluent on the separation of ephedrines by liquid chromatography. J. Chromatogr. A **2003**, *1007*, 39–45.
16. Zhang, W.; He, L.; Gu, Y.; Liu, X.; Jiang, S. Effect of ionic liquids as mobile phase additives on retention of catecholamines in reversed-phase high-performance liquid chromatography. Anal. Lett. **2003**, *36*, 827–838.
17. Xiaohua, X.; Liang, Z.; Liu, X.; Shengxiang, J. Ionic liquids as additive in HPLC-analysis of amines and the interaction mechanism of ionic liquids. Anal. Chim. Acta **2004**, *519*, 207–211.
18. Zhang, W.; He, L.; Jiang, S. Ionic liquids as mobile phase additives for separation of nucleotides in high-performance liquid chromatography. Chin. J. Chem. **2004**, *22*, 549–552.
19. Marszałł, M.P.; Bączek, T.; Kaliszan, R. Reduction of silanophilic interactions in liquid chromatography with the use of ionic liquids. Anal. Chim. Acta **2005**, *547* (2), 172–178.
20. Polyakova, Y.; Koo, Y.M.; Row, K.H. Application of ionic liquids as mobile phase modifier in HPLC. Biotechnol. Bioproc. Eng. **2006**, *11*, 1–6.
21. Polyakova, Y.; Jin, Y.; Zheng, J.; Row, K.H. Effect of concentration of ionic liquid 1-butyl-3-methylimidazolium tetrafuoroborate for retention and separation of some amino and nucleic acids. J. Liq. Chromatogr. & Rel. Technol. **2006**, *29*, 1687–1701.

22. Jin, Y.; Le, T.H.; Polyakova, Y.; Koo, Y.M.; Row, K.H. Influence of ionic liquid for separation of D-tryptophan and N-CNZ-phenylalanine. Korean Chem. Eng. Res. **2006**, *44* (5), 453–459.
23. Ruiz-Angel, M.J.; Carda-Broch, S.; Berthod, A. Ionic liquids versus triethylamine as mobile phase additives in the analysis of $\beta$-blockers. J. Chromatogr. A **2006**, *1119* (1–2), 202–208.
24. Zheng, J.Z.; Polyakova, Y.; Row, K.H. Retention factor and resolution of amino benzoic acid isomers with some ionic liquids. Biotechnol. Bioproc. Eng. **2006**, *11* (6), 477–483.
25. Waichigo, M.; Hunter, B.M.; Riechel, T.L.; Danielson, N.D. Alkylammonium formate ionic liquids as organic mobile phase replacements for reversed-phase liquid chromatography. J. Liq. Chromatogr. & Rel. Technol. **2007**, *30* (2), 165–184.
26. Liu, S.L.; Zhou, F.; Zhao, L.; Xiao, X.H.; Liu, X.; Liang, S.X. Immobilized 1,3-dialkylimidazolium salts as new interface in HPLC separation. Chem. Lett. **2004**, *33* (5), 496–497.
27. Yanes, E.G.; Gratz, S.R.; Baldwin, M.J.; Robison, S.E.; Stalcup, A.M. Capillary electrophoretic application of 1-alkyl-3-methylimidazolium-based ionic liquids. Anal. Chem. **2001**, *73*, 3838–3844.
28. Imaz, C.; Carreras, D.; Navajas, R.; Rodrıguez, C.; Rodrıguez, A.F.; Maynar, J.; Cortes, R. Determination of ephedrines in urine by high-performance liquid chromatography. J. Chromatogr. A **1993**, *631*, 201–205.
29. Imaz, C.; Navajas, R.; Carreras, D.; Rodrıguez, C. Comparison of various reversed-phase columns for the simultaneous determination of ephedrines in urine by high-performance liquid chromatography. J. Chromatogr. A **2000**, *870*, 23–28.
30. Marszall, M.P.; Baczek, T.; Kaliszan, R. Evaluation of the silanol-suppressing potency of ionic liquids. J. Sep. Sci. **2006**, *29* (8), 1138–1145.
31. Marszall, M.P.; Baczek, T.; Kaliszan, R. Reduction of silanophilic interactions in liquid chromatography with the use of ionic liquids. Anal. Chim. Acta **2005**, *547* (2), 172–178.
32. Kaliszan, R. *Structure and Retention in Chromatography. A Chemometric Approach*; Harwood Academic Publishers: Amsterdam, 1997.
33. Snyder, L.R.; Quarry, M.A. Computer simulation in HPLC method development. Reducing the error of predicted retention times. J. Liq. Chromatogr. **1978**, *10*, 1789.
34. Snyder, L.R. *Principles of Adsorbtion Chromatography*; Marcel Dekker, Inc.: New York, USA, 1968; Ch. 8.
35. Kim, J.D.; Row, K.H.; So, M.S.; Polunina, I.A.; Larin, A.V. Chromatographic behavior of deoxyribonucleosides with respect to organic modifier content in the mobile-phase. J. Liq. Chromatogr. & Rel. Technol. **1995**, *18*, 3091–3104.
36. Sadek, P.C.; Carr, P.W.; Doherty, R.M.; Kamlet, M.J.; Taft, R.W.; Abraham, M.H. Study of retention processes in reversed-phase high-performance liquid chromatography by the use of the solvatochromic comparison method. Anal. Chem. **1985**, *57*, 2971–2978.
37. Valko, K.; Snyder, L.R.; Glajch, J.L. Retention in reversed-phase liquid chromatography as a function of mobile-phase composition. J. Chromatogr. A **1993**, *656*, 501–520.
38. Melander, W.R.; Horvath, Cs. In *High-Performance Liquid Chromatography. Advances and Perspectives*; Horvath, Cs., Eds.; Academic Press: NewYork, 1980, Vol. 2.

39. Schoenmakers, P.J.; Billiet, H.A.H.; Tijssen, R.; Galan, L. Gradient selection in reversed-phase liquid chromatography. J. Chromatogr. **1978**, *149*, 519–537.
40. Soczewinski, E. Solvent composition effects in thin-layer chromatography systems of the type silica gel-electron donor solvent. Anal. Chem. **1969**, *41*, 179–182.
41. Soczewinski, E. Solvent composition effects in liquid-solid systems. J. Chromatogr. **1977**, *130*, 23–28.
42. Soczewinski, E.; Golkievicz, W. A simple molecular model for adsorption chromatograpgy. VII. Relationship between the $R_M$ value and the composition for phenol in system of the type (cyclohexane + polar solvent)/silica. Chromatographia **1973**, *6*, 269–273.
43. Soczewinski, E. Mechanistic molecular model of liquid solid chromatography. Retention eluent composition relationship. J. Chromatogr. A **2002**, *965*, 109–116.
44. Smith, T.J.; Stevenson, K.J. Origin 7.0 OriginLab Corporation, One Roundhouse Plaza, Northampton, MA 01060. 1-800-969-7720. www.OriginLab.com. Suggested Price $699.00 (Retail, Single User), $529.00 (Educational, Single User). Contact Company for Other Pricing Options. J. Am. Chem. Soc. **2003**, *125*, 3669–3669.

Received March 20, 2007
Accepted May 15, 2007
Manuscript 6081