지적작품-내용물-변경금지 2.0 대한민국

이용자는 아래의 조건을 따르는 경우에만 사용할 수 있습니다.

- 이 저작물을 복제, 배포, 전송, 전시, 공연 및 방송할 수 있습니다.

다음과 같은 조건을 따라야 합니다:

BY: 저작자표시. 귀하는 원저작자를 표시하여야 합니다.

NO: 배포. 귀하는 이 저작물을 복사, 전송, 전시, 공연 및 방송할 수 없습니다.

EQUAL: 변경금지. 귀하는 이 저작물을 개작, 변형 또는 가공할 수 없습니다.

귀하는, 이 저작물의 저작권이나 배포의 경우, 이 저작물에 적용된 이용허락조건을 명확하게 나타내야 합니다.
귀작권자로부터 별도의 허가를 받으면 이러한 조건들은 적용되지 않습니다.

저작권법에 따른 이용자의 권리는 위의 이용에 의하여 영향을 받지 않습니다.

이것은 이용허락약관(Legal Code)을 이해하기 쉽게 요약한 것입니다.

Disclaimer
Application of Ionic Liquids as Mobile Phase Additives in
RP-SMB Chromatography to Separate Ionizable
Compounds

2007

Hoang Thai Le
Application of Ionic Liquids as Mobile Phase Additives in RP-SMB Chromatography to Separate Ionizable Compounds

2007 2
Hoang Thai Le

2007 2
Acknowledgements

To complete this work, besides my own research in this field, fortunately, I have received many helps during last two years.

First of all, I am respectively grateful to Prof. Yoon-Mo Koo for giving me the chance to study in Inha University. His support is crucial for my studies during last two years.

I respectively appreciate all professors in Department of Biological Engineering and Department of Chemical Engineering for providing interesting lectures and helpful methods, which enlarged my knowledge in Biological and Engineering major.

I also would like to thank all doctors in Bio-Nano-Process Laboratory, including Dr. Kim (SMB lab), Dr. Kim (Fermentation lab.), Dr. Chang, Dr. Ha, Dr. Lee, Dr Lim and Dr. Sohn, from whom I have learned many precious technical issues.

I also deeply thank Dr. candidate Ju Weon Lee for detail instruction and helpful advices for this work.

Another thank I would like to give to Korean seniors including Jin-Il, Jong-Gil and Hye-Jin, who have friendly helped me as well as all Vietnamese students during our Master course.

Finally, I wish to thank my parents, my sisters and brothers and all other friends for their supports and helps whenever I need.

Hoang T. Le
ABSTRACT

Separation of ionized or highly hydrophilic compounds by using Reversed-Phase Liquid Chromatography (RP-LC) where solute retention is based on the hydrophobic interaction between solute and stationary phase, is a difficult task. To overcome this problem, two methods have been used to prevent solutes from being ionized: (1)-controlling pH of mobile phase; (2)-using special salts as mobile phase additives. In the first method, mobile phase must be controlled at very low or very high pH using acidic or basic agents, respectively. This method is rarely used in the preparative or continuous chromatography. It is difficult to maintain the pH of mobile phase, in the other hand, the natural form of analytes can be destroyed by acidic or basic condition. In the second method, some special salts are added into mobile phase for neutralizing solute ion. Even though this method is used popularly nowadays, the number of additives is still limited, especially cationic additives.

Application of ionic liquids (ILs) in liquid chromatography has recently attracted the upsurge interests, especially using ILs as mobile phase additives. Therefore, the objective of this work is to study the potential
application of ionic liquids as mobile phase additives to separate ionizable compounds in RP-LC, which includes two parts:

1. Effect of ILs as mobile phase additives on the retention behaviors of ionizable compounds in RP-LC

2. Application of [hmim][BF₄] ionic liquid as mobile phase additive in Reversed-Phase Simulated Moving Bed Chromatography (RP-SMB) to separate 3-hydroxybenzoic acid (3-HBA) and 4-hydroxybenzoic acid (4-HBA)

In the first study, four kinds of ILs in the family of 1-alkyl-3-methylimidazolium tetrafluoroborate [Rmim][BF₄] were added to water/methanol mobile phase to investigate the retention behaviors of four analytes including benzoic acid, benzylamine, L-phenylalanine and L-tryptophan. The results showed that the effect of ILs on the retention behaviors of analytes can be explained by two mechanisms including the Dynamic Ion Exchange and the Ion-Pair Forming in RP-LC. The retention of benzoic acid increased significantly in the presence of ionic liquids.

The obtained results from the first study showed that highly hydrophobic ILs can be expected as good mobile phase additives to separate ionizable compounds, even some acids. Therefore, in the second study, [hmim][BF₄] was studied using as mobile phase additive in a continuous
chromatographic process (RP-SMB chromatography) to separate two ionizable isomers including 3-hydroxy-benzoic acid and 4-hydroxy-benzoic acid under the neutral pH condition of mobile phase. A good separation of these acids was obtained. In addition, comparing to the pH controlling method, new separation method of using [hmim][BF$_4$] showed the better selectivity that leaded to the higher enrichment of products and lower solvent consumption.

**Keywords:** ionic liquids, additive, retention behavior, Simulated Moving Bed, reversed phase, liquid chromatography, ionizable compound
## CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>IV</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>V</td>
</tr>
<tr>
<td>CONTENTS</td>
<td>VIII</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>X</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>XII</td>
</tr>
<tr>
<td>NOMENCLATURES</td>
<td>XIII</td>
</tr>
<tr>
<td>ABBREVIATIONS</td>
<td>XV</td>
</tr>
<tr>
<td>1. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>1-1. IONIC LIQUIDS</td>
<td>1</td>
</tr>
<tr>
<td>1-2. ROLE OF ADDITIVE IN RP-LC</td>
<td>7</td>
</tr>
<tr>
<td>1-3. BASIC CONCEPTS OF SIMULATED MOVING BED CHROMATOGRAPHY (SMB)</td>
<td>10</td>
</tr>
<tr>
<td>1-4. REVERSED PHASE SIMULATED MOVING BED CHROMATOGRAPHY (RP-SMB)</td>
<td>16</td>
</tr>
<tr>
<td>2. THEORY</td>
<td>18</td>
</tr>
<tr>
<td>2-1. MATERIAL BALANCE EQUATION IN SMB</td>
<td>18</td>
</tr>
<tr>
<td>2-2. TRIANGLE THEORY</td>
<td>18</td>
</tr>
<tr>
<td>2-3. EQUILIBRIUM ADSORPTION ISOTHERM</td>
<td>22</td>
</tr>
<tr>
<td>3. MATERIAL AND METHODS</td>
<td>23</td>
</tr>
<tr>
<td>3-1. MATERIALS</td>
<td>23</td>
</tr>
<tr>
<td>3-2. METHODS</td>
<td>28</td>
</tr>
</tbody>
</table>
4. RESULTS AND DISCUSSIONS

4-1. Effect of ILs on the retention behaviors of ionizable compounds

4-2. Application of [HMIM][BF₄] as mobile phase additive in RP-SMB chromatography to separate 3-HBA and 4-HBA

5. CONCLUSIONS

6. REFERENCES
LIST OF FIGURES

Fig. 1. Number of IL catalysis and separations publications and patents. ... 3
Fig. 2. Composition of ionic liquids............................................................. 6
Fig. 3. Illustration for the mechanism of ion-pair forming in mobile phase. 8
Fig. 4. Illustration for the mechanism of dynamic ion exchange in
stationary phase. ............................................................................... 9
Fig. 5. Illustration for the basic concepts of SMB chromatography. ........ 13
Fig. 6. Design of four-zone SMB before (a) and after (b) one switching
time. ................................................................................................ 14
Fig. 7. Illustration of column profiles at middle of switching time in cyclic
steady-state. .................................................................................... 15
Fig. 8. Triangle Theory for design of operation conditions. Regions of
separation in the (m2, m3) plane for the linear adsorption isotherm
without effect of mass transfer resistance........................................... 21
Fig. 9. Molecular structures of [Rmim][BF4] ionic liquids. .................... 25
Fig. 10. Molecular structure of chemicals in the batch experiments for
investigation of adsorption behaviors............................................... 26
Fig. 11. Molecular structure of chemicals in the SMB experiments......... 27
Fig. 12. SMB experimental procedure. ...................................................... 33
Fig. 13. Effect of ionic liquids on the retention time of benzoic acid, and benzylamine. ................................................................. 36

Fig. 14. Effect of ionic liquids on the retention time of L-phenylalanine and L-tryptophan. .............................................................. 37

Fig. 15. Surveying the mobile phase conditions for the separation of 3-HBA and 4-HBA. ................................................................. 40

Fig. 16. Isotherm curves of 3-Hydroxybenzoic acid in three mobile phase conditions........................................................................ 41

Fig. 17. Isotherm curves of 4-Hydroxybenzoic acid in three mobile phase conditions........................................................................ 42

Fig. 18. Simulation of the batch experimental results.............................. 44

Fig. 19. Operating conditions designed were based on Triangle Theory... 47

Fig. 20. The column profiles of 3-HBA and 4-HBA for three cases......... 50

Fig. 21. Operation parameters for SMB process using [hmim][BF₄] at the neutral pH................................................................. 53

Fig. 22. Comparison of experimental and simulation results for history at extract stream.............................................................. 54

Fig. 23. Comparison of experimental and simulation results for history at raffinate stream. .............................................................. 55
LIST OF TABLES

Table 1. Adsorption parameters of 3-HBA and 4-HBA............................ 45
Table 2. Operation parameters for SMB process in three cases................... 48
Table 3. Comparison of SMB simulation results of three cases.................. 51
Table 4. Purity and yield of products from the last cycle......................... 56
NOMENCLATURES

\( a, b \)  
Langmuir isotherm parameters

\( C(\text{or } c) \)  
concentration of solute in the mobile phase \([g/l]\)

\( H_A \)  
Henry constant of the strongly adsorbed component \( A \)

\( H_B \)  
Henry constant of the weakly adsorbed component \( B \)

\( E_z \)  
axial dispersion coefficient \([cm^2/min]\)

\( K_f \)  
film mass transfer coefficient \([\text{min}^{-1}]\)

\( m_j \)  
ratio of mobile phase flow rate over stationary phase flow rate in zone \( j \)

\( P_{3-HBA} \)  
purity of 3-HBA \([\%]\)

\( P_{4-HBA} \)  
purity of 4-HBA \([\%]\)

\( q_i \)  
adsorbed solid phase concentration of component \( I \) \([mg/ml]\)

\( q^* \)  
adsorbed solid phase concentration at equilibrium \([mg/ml]\)

\( Q_j \)  
volumetric flow rate in zone \( j \) \([ml/min]\)

\( t^* \)  
switching time \([\text{min}]\)

\( v_l \)  
superficial velocity of the fluid \([cm/min]\)

\( v \)  
movement velocity of solid \([cm/min]\)

\( V \)  
total column volume \([ml]\)

\( V_{F,i+1} \)  
retention volume of the inflection point of \((i+1)\) front \([ml]\)

\( V_0 \)  
column void volume \([ml]\)
\( V_D \)  
system dead volume [ml]

\( V_i \)  
retention volumes of fronts [ml]

\( V_{sp} \)  
volume of adsorbed solid phase in the column [ml]

\( u'_{i} \)  
migration linear velocities of the solutes \( i \) in the zone \( j \) [cm/min]

\( Y_{3-HBA}^{E} \)  
yield of 3-HBA at Extract stream [%]

\( Y_{3-HBA}^{R} \)  
yield of 4-HBA at Raffinate stream [%]

**Greek letters**

\( \varepsilon^* \)  
total porosity

\( \varepsilon_p \)  
intraparticle porosity

\( \varepsilon_i \)  
interparticle porosity

**Subscripts and superscripts**

\( j \)  
number of zone

\( E \)  
Extract stream

\( R \)  
Raffinate stream

\( I \)  
zone I

\( II \)  
zone II

\( III \)  
zone III

\( IV \)  
zone IV
### ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbr</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>mM</td>
<td>milimol/litter</td>
</tr>
<tr>
<td>µl</td>
<td>microlitter</td>
</tr>
<tr>
<td>nm</td>
<td>nanometter</td>
</tr>
<tr>
<td>ILs</td>
<td>ionic liquids</td>
</tr>
<tr>
<td>[Rmim][BF₄]</td>
<td>1-alkyl-3-methylimidazolium tetrafluoroborate</td>
</tr>
<tr>
<td>[emim][BF₄]</td>
<td>1-ethyl-3-methylimidazolium tetrafluoroborate</td>
</tr>
<tr>
<td>[bmim][BF₄]</td>
<td>1-butyl-3-methylimidazolium tetrafluoroborate</td>
</tr>
<tr>
<td>[hmim][BF₄]</td>
<td>1-hexyl-3-methylimidazolium tetrafluoroborate</td>
</tr>
<tr>
<td>[omim][BF₄]</td>
<td>1-octyl-3-methylimidazolium tetrafluoroborate</td>
</tr>
<tr>
<td>RP-LC</td>
<td>Reversed-phase liquid chromatography</td>
</tr>
<tr>
<td>RP-SMB</td>
<td>Reversed phase Simulated Moving Bed Chromatography</td>
</tr>
<tr>
<td>3-HBA</td>
<td>3-hydroxybenzoic acid</td>
</tr>
<tr>
<td>4-HBA</td>
<td>4-hydroxybenzoic acid</td>
</tr>
</tbody>
</table>
1. INTRODUCTION

1-1. Ionic liquids

Ionic liquids (ILs), or molten salts, are defined as materials containing only ionic species without any neutral molecules and having a low melting point (usually less than 100°C) [3]. In 1914, the first room-temperature IL, ethylammonium nitrate \([\text{EtNH}_3][\text{NO}_3]\) (melting point at 12°C) was synthesized [1]. This salt is liquid at room temperature, however, it usually contains a small amount of water (200-600ppm). In 1948, the first ILs with chloroaluminate ions were developed by Hurley and Wier at the Rice Institute in Texas as bath solutions for electroplating aluminum. However, these systems were not studied further until the late 1970s when the groups of Osteryoung and Wilkes rediscovered them. For the first time, they succeeded in preparing room-temperature liquid chloroaluminate melts. Research and development concentrated mainly on electrochemical application at this time [2]. In 1967, Swain and coworkers used tetra-n-hexylammonium benzoate as solvent for kinetic and electrochemical investigations. In the early 1970s, Wilkes and his colleagues had been trying to develop better batteries for missiles, nuclear warheads, and space probes. The team's batteries required molten salts to operate, but such
substances were hot enough to damage nearby materials. So, the chemists looked for salts that remain liquid at lower temperatures. Eventually, they identified one salt that is liquid at room temperature [2]. In 1980s the groups of Seddon and Hussey began to use chloroaluminate melts as nonaqueous, polar solvents for the investigation of transition metal complexes. In 1992 the concept of ionic liquids received a substantial boost by the work of Wilkes’s group when they described the synthesis of systems with significantly enhanced stability against hydrolysis. ILs with tetrafluoroborate ions have been successfully used, for example, in the catalyzed hydroformylation of olefins. Based on Wilkes’s work, it became clearly apparent that ILs can be formed from a whole range of cation/anion combination. Recently, the number of published article and patent relating to ILs, specifically addressing a broad range of catalysis and separation processes utilized in the chemical industry [5], has grown rapidly as shown in Fig. 1.
Fig. 1. Number of IL catalysis and separations publications and patents.

1-1-a. Composition of ionic liquids

In general, ionic liquid is a liquid that consists of only ions. IL cations are often large organic ions, while IL anions are often small inorganic ions. There are a number of different cation and anion combinations that may result in salts having low melting points; examples of some of the different cation structures and anion pairs that may result in an IL are shown in Fig. 2 [7].

ILs are "designer solvents". Chemists are free to pick and choose among dozens of small anions and hundreds of thousands of large cations to make a liquid that suit to a particular need, such as dissolving certain chemicals in a reaction or extracting specific molecules from a solution [8].

1-1-b. Physicochemical properties of ionic liquids

The physical and chemical properties of ILs can be specifically varied over a wide range by the selection of suitable cations and anions. Comparing to organic solvent, ILs have many fascinated properties such as [9]:

- Negligible vapour pressure
- Non-volatile
- Non-flammable
- High thermal, chemical and electrochemical stability
- Liquid over a wide temperature range
• Dissolution of many organic and inorganic compounds
• Miscibility with water and various organic solvents
Most commonly used cations:

N-alkyl-pyridinium  1-alkyl-3-methylimidazolium  Tetraalkyl-phosphonium  Tetraalkyl-ammonium

Some possible anions:

Water immiscible  Water miscible

[PF$_6$]$^-$  [BF$_4$]$^-$  [CH$_3$CO$_2$]$^-$
[N(SO$_2$CF$_3$)$_2$]$^-$  [CF$_3$SO$_3$]$^-$  [CF$_3$SO$_2$]$^-$
[BR$_1$R$_2$R$_3$R$_4$]$^-$  [NO$_3$]$^-$, [Cl$^-$]

Fig. 2. Composition of ionic liquids.
1-2. Role of additive in RP-LC

In the presence of mobile phase additives, the retention mechanism of solutes in RP-LC is quite complex and dependent on many factors, especially on the nature of additives. Highly hydrophobic additives participate mainly in mechanism of dynamic ion exchange in stationary phase while hydrophilic additives do in mechanism of ion-pair forming in mobile phase[10, 11, 12].

The mechanism of ion-pair forming in mobile phase

If additive agents are highly hydrophilic, they exit in mobile phase as ionic forms which interact with analyte ions to form the neutral ion pairs that sorb strongly on the stationary phase, as shown in Fig. 3. Therefore, retention of analytes increase significantly when this kind of additives are added to mobile phase.

The mechanism of dynamic ion exchange in stationary phase

If additive agents are hydrophobic, they adsorb easily onto the hydrophobic surface of stationary phase. And then, the sorbed additives on stationary phase interact with analyte ions by the mechanism of dynamic ion exchange, as shown in Fig. 4. Thereby, the retention time of analytes is
decreased or increased by the repulsive or attractive interaction between additive ions and analyte ions, respectively.
Fig. 3. Illustration for the mechanism of ion-pair forming in mobile phase.

(A⁺ and A⁻ are additive ions, C⁺ and C⁻ are solute ions)
Fig. 4. Illustration for the mechanism of dynamic ion exchange in stationary phase.

(A⁺ and A⁻ are additive ions, C⁺ and C⁻ are solute ions)
1-3. Basic concepts of Simulated Moving Bed Chromatography (SMB)

Simulated moving bed (SMB) chromatography is a continuous chromatographic process developed in the 1960’s by UOP (United Oil Products). SMB chromatography has the advantages of a high purity, high yield and small mobile phase consumption over that of batch chromatography. In addition, its large-scale applications can reduce the separation costs. Thus, it has been used in the petrochemical and sugar industries for large-scale separation, and for amino acids and chiral separation in fine chemical industries [13, 14].

Basically, illustration of the basic concept of SMB chromatography is shown in Fig. 5 which is explained in following steps [15]:

(1) A mixture of two components is injected in the centre of a stationary column. If these two components have different affinity with stationary phase, they move to the direction of mobile phase flow at different speeds and then separate.

(2) Now, if the column is moved oppositely to the mobile phase flow at a speed halfway between that of the solutes, the two solutes move in different directions relative to a stationary observer.
(3) If the column is very long, the bands will continue to separate.

(4) There are problems in a continuous system:

(a). The column needs to be of infinite length.

(b). The actual moving of solids is very difficult.

(c). Some way to introduce and remove the sample and the products are needed.

(5) To solve these problems, the column is cut into many small segments. Then, the feed and solvent inlets are now placed between the segments, thus, the moving of solid phase is simulated by moving the feed and solvent inlets each time a segment. A segment is moved from one end to the other.

(6) Products are removed by bleeding off a carefully calculated flow at suitable exit points. This changes the velocity of the bands in the column and forces the products to move toward the ports. This ensures that the column segments are clean before they are moved and that the solvent can be recycled directly back through the system.

(7) In the design of four-zone SMB (Fig. 6), each zone contains two columns connected in series. The feed, desorbent, extract, raffinate ports are placed between these columns. The port between the columns allows to open or close the inlet (feed, desorbent) and outlet (extract,
raffinate) streams at a specified switching time. The counter-current movement between the stationary and mobile phase is simulated by port movements. The feed is fed between zone II and III. The high affinity and low affinity solutes are separated and collected in the extract and raffinate port, respectively. The desorbent is fed between zone I and IV. In order to achieve successful separation, the migration linear velocities of the solutes, $I$, in the zone $j$, $u_j^I$ must satisfy the following constrains:

$$u_{3-HBA}^I - v > 0,$$
$$u_{4-HBA}^II - v > 0,$$
$$u_{3-HBA}^III - v < 0,$$
$$u_{4-HBA}^IV - v < 0$$

(1)

Where $v$ is the simulated volumetric flow rate of solid phase.

(8) After a period of time (switching time), feed, desorbent inlets and extract, and raffinate outlets moves simultaneously to the next position in the direction of the mobile phase flow (Fig. 6b). Column profiles of 4-zone SMB at the middle of switching time are illustrated in Fig. 7.
Fig. 5. Illustration for the basic concepts of SMB chromatography.
Fig. 6. Design of four-zone SMB before (a) and after (b) one switching time.
Fig. 7. Illustration of column profiles at middle of switching time in cyclic steady-state.
1-4. Reversed phase Simulated Moving Bed Chromatography (RP-SMB)

Reversed-phase simulated moving bed chromatography (RP-SMB) combined from SMB chromatography and Reversed-phase liquid chromatography (RP-LC). RP-LC has been the most widely used branch of liquid chromatography (LC) for analysis and purification of a wide variance of substances. Most common RP-LC employs microparticulate alkyl-silica-boned phases, such as octadecylated silica (C18 silica), which offers high separation efficiency combined with versatility and reproducibility [16]. In RP-LC, the mechanism of solute retention is governed by hydrophobic interactions between solute and stationary phase. Therefore, it is difficult to separate ionizable compounds in RP-LC. In order to solve this problem, conventionally, methods of control at extreme pH or using mobile phase additives can be used. However, it is rarely allowed to use the former methods in the preparative chromatography or continuous chromatography, because it is difficult to maintain the pH of mobile phase, the natural form of analytes can be destroyed by acidic or basic condition. Nowadays, the method of using mobile phase additives has been popular; however, a small number of the additive agents has been
disadvantage, especially for separation of acidic compounds. In this work, using ionic liquids as mobile phase additives to separate ionizable compounds in Reversed Phase Simulated Moving Bed Chromatography (RP-SMB) was studied.
2. THEORY

2-1. Material balance equation in SMB

The following material balances [17] are used between the stationary phase and mobile phase.

\[
\varepsilon^* \frac{\partial C_i}{\partial t} + (1 - \varepsilon^*) \frac{\partial q_i}{\partial t} + v_i \frac{\partial C_i}{\partial Z} = \varepsilon^* E_z \frac{\partial^2 C_i}{\partial Z^2}
\]

\[
\frac{\partial q_i}{\partial t} = K_f (q_i^* - q_i)
\]

\[
q_i^* = f_{eq}(C_i)
\]

where \( C_i \) is the concentration of component \( i \) in mobile phase, \( q_i \) is the equilibrium concentration of component \( i \) in the stationary phase, \( \varepsilon^* \) is the total porosity, \( v_i \) is the superficial velocity of the fluid, \( E_z \) is the dispersion coefficient, \( K_f \) is lumped mass transfer coefficient, and \( q_i^* \) is the equilibrium value of \( q_i \) for a mobile phase concentration equal to \( C_i \).

2-2. Triangle Theory

To determine the operating conditions such as the flow rate of four zones as well as switching time, M. Morbidelli et al. proposed the Triangle Theory, which is based on the equilibrium theory [18]. The equilibrium theory
neglects the axial dispersion and mass transfer resistance. The parameter $m_j$ is the flow rate ratio, and is defined as the ratio of the net fluid flow rate over the solid phase in zone $j$.

$$m_j = \frac{Q_{j}^{\text{SMB}} t^* - V \epsilon^*}{V (1 - \epsilon^*)}$$

(3)

Where $Q_{j}^{\text{SMB}}$ is the internal volumetric flow rate in zone $j$, $t^*$ is the switching time, $V$ is the volume of a single column, and $\epsilon^*$ is the overall void fraction of the bed, defined as:

$$\epsilon^* = \epsilon_i + (1 - \epsilon_i) \epsilon_p$$

(4)

where $\epsilon_b$ is bed or interparticle void fraction and $\epsilon_p$ is intraparticle void fraction.

Because of the different functions in SMB process, each zone has its own constrain for determination of zone flow rate. While the function of zone I is to desorb the more retained component and to regenerate the adsorbent solid, that of zone IV is to adsorb the less retained component and regenerate the desorbent.

$$H_A < m_1; m_4 < H_B$$

(5)

Where $H_A$ and $H_B$ are the Henry constants of the strongly adsorbed component $A$ and the weakly adsorbed component $B$, respectively. The
function of zone II and III is to desorb the weakly adsorbed component $B$ and to adsorb the strongly adsorbed component $A$, respectively.

$$H_B < m_2 < m_3 < H_A$$

(6)

Because zone II and III are separation zones, the determination of component migrations and the flow ratio in these zones are important to separate pure products at *extract* and *raffinate* ports.
Fig. 8. Triangle Theory for design of operation conditions. Regions of separation in the (m2, m3) plane for the linear adsorption isotherm without effect of mass transfer resistance.
2-3. Equilibrium adsorption isotherm

The Langmuir isotherm for a single component is:

\[ q = \frac{ac}{1 + bc} \]  \hspace{1cm} (7)

where \( q \) and \( c \) are the concentrations of the solute in stationary phase and mobile phase at equilibrium, respectively [15] and \( a \) and \( b \) are characteristic parameters of the solute in a given system. The parameters are estimated using a single component frontal analysis of each component. The amount adsorbed onto the stationary phase can be calculated by the following mass balance equation:

\[ q_{i+1} = q_i + \frac{(c_{i+1} - c_i)(V_{F,i+1} - (V_0 + V_D))}{V_{sp}} \]  \hspace{1cm} (8)

where \( V_F \) is the retention volume of the inflection point of the breakthrough curve, \( V_0 \) and \( V_D \) the column void and system dead volumes, respectively, and \( V_{sp} \) is the volume of adsorbent in the column. The subscript \( i \) relates to the number of step changes in the concentration.
3. MATERIAL AND METHODS

3-1. Materials

3-1-1. Chemicals

Ionic liquids [emim][BF₄], [bmim][BF₄], [hmim][BF₄] and [omim][BF₄], in Fig.9, were kindly supplied from C-tri company (Korea). All compounds (L-phenylalanine, L-tryptophan, Benzoic acid, Benzylamine, 3-hydroxybenzoic acid and 4-hydroxybenzoic acid), in Fig. 10 and Fig. 11, were purchased from Sigma-Alldrich Co. (USA). Mobile phases were prepared with HPLC grade methanol solvent from J. T. Baker Company (USA) and deionized water from Milli-Q purification system, Millipore (USA). Potassium phosphate monobasic (KH₂PO₄) and dibasic (K₂HPO₄) from Duksan pure chemical company (Kyungkido, Korea) were used as buffer agents in mobile phase. Hydrochloric acid from MatSunoen chemical Company (Osaka, Japan) and amonia solution 28.0-30.0% from Samchun pure chemical Company (Kyungkido, Korea) were added dropwise as needed to adjust the desired pH.
3-1-2. Apparatus

The HPLC system was composed of a pump (model LC-6AD Shimadzu, Kyoto, Japan), a UV detector (model SPD-M10A vp Shimadzu), an auto-injector (SIL-10AD vp Shimadzu), a column oven (model CTS-30 Younglin, Korea). The Licosep Micro SMB system from Novasep company (France) is used to perform SMB experiment in pilot scale. A C18 Kromasil column (1 cm x 10 cm) with 100 Å-25 µm spherical silica particles was used. The pH meter (model 420) and pH electrode were from Thermal Orion, USA.
Fig. 9. Molecular structures of [Rmim][BF$_4$] ionic liquids.

(a) 1-ethyl-3-methyl-imidazolium tetraflouroborate([emim][BF$_4$])
(b) 1-butyl-3-methyl-imidazolium tetraflouroborate([bmim][BF$_4$])
(c) 1-hexyl-3-methyl-imidazolium tetraflouroborate([hmim][BF$_4$])
(d) 1-octyl-3-methyl-imidazolium tetraflouroborate ([omim][BF$_4$])
Fig. 10. Molecular structure of chemicals in the batch experiments for investigation of adsorption behaviors.

(a) benzoic acid (M.W. 122.12)
(b) benzylamine (M.W. 137.14)
(c) L-tryptophan (M.W. 204.23)
(d) L-phenylalanine (M.W. 165.19)
Fig. 11. Molecular structure of chemicals in the SMB experiments.

(a) 3-hydroxybenzoic acid (M.W. 138.12)

(b) 4-hydroxybenzoic acid (M.W. 138.12)
3-2. Methods

3-2-1. Effect of ILs on the retention behaviors of ionizable compounds

Mobile phase was prepared by adding 25 mM KH₂PO₄ buffer agent into 20% methanol aqueous solution which was adjusted pH 4.0 by using hydrochloric acid. IL additives ([emim][BF₄], [bmim][BF₄], [hmim][BF₄] and [omim][BF₄]) were added directly into mobile phase solution with the fixed concentration of 17.5 mM. Experiment was carried out at temperature of 30°C, mobile phase flow rate of 3 ml/min. After equilibrating the column, 100 µl of sample solutions were injected to column and detected by UV detector at a few specific wavelengths of 254 nm, 300 nm and 330 nm.

In the experiments for surveying the retention behaviors of analytes, sample solutions were prepared by dissolving analytes into mobile phase solution at the concentration of 1 mM.
3-2-2. Application of [hmim][BF$_4$] as mobile phase additive to separate 3-hydroxybenzoic acid and 4-hydroxybenzoic acid in SMB process

Some initial experiments were carried out to survey the optimum conditions of mobile phase such as concentrations of buffers, IL additives and pH values.

The neutral pH was controlled by using 100 mM potassium phosphate buffer (50 mM KH$_2$PO$_4$ and 50 mM K$_2$HPO$_4$). Optimum concentration of [hmim][BF$_4$] for separation was 1.5 ml/l. New method of using [hmim][BF$_4$] at neutral pH is compared to traditional method of control at pH 3.0 and pH 3.5.

Measurement of adsorption parameters

In batch experiments, four conditions of mobile phases of 20% methanol aqueous solution including (1) at pH 3.0; (2) pH 3.5; (3) neutral pH (pH 7.1 ~ 7.3); (4) using [hmim][BF$_4$]) at neutral pH were surveyed to find the optimum conditions for mobile phase. Two pumps were used in this experiment: one for mobile phase solution, and the other for sample solution (concentration of 1 g/l).

In order to determine the isotherm for each 3-hydroxybenzoic acid and 4-hydroxybenzoic acid, single step frontal analysis experiments were carried
out at several concentrations (0; 0.2; 0.4; 0.6; 0.8; and 1 g/l). And then, concentrations of adsorbed analytes were calculated from mass balance. These adsorption data were fitted the linear and Langmuir isotherm equation.

Estimated the axial dispersion coefficient

To estimate the axial dispersion coefficient, elution profile of single frontal analysis was simulated by using VERSE tool. The estimated value of axial dispersion coefficient is obtained when simulation and experiment results have a good agreement.

Design operation condition for SMB process

Basing on Triangle Theory, (m2, m3) planes were built to design the operation conditions for SMB process. The vertex of triangle-shape operation region is the optimum operation point, from where operation point for SMB process was chosen at 20% safety margin.

Operation conditions were optimized by SMB simulation.

SMB experiment

In SMB experiment, desorbent solution was prepared by adding 1.5 ml/l [hmim][BF₄] additive into 20% methanol aqueous solution controlled at
neutral pH by 100 mM phosphate buffer. Feed solution was prepared by mixed 3-HBA (1 g/l) and 4-HBA (1 g/l) into desorbent solution.

The desorbent and feed solutions were pumped continuously into the system. Extract and raffinate were collected during every switching time. Column profiles were calibrated by sampling at the injection loop of 250 µl, at every middle switching time of the last cycle.

In analysis experiments, mobile phase of 20% methanol aqueous solution at pH 3.0 was used to analyze the collection from SMB operation by using HPLC. UV detector was used at wavelength of 236 nm for 3-HBA and 262 nm for 4-HBA.

Purities and yields of extract and raffinate streams from SMB experiment were calculated from the last cycle of SMB operation (steady state) by two following formulas:

\[
P_{3\text{-HBA}} = \frac{C_{3\text{-HBA}}^E}{C_{3\text{-HBA}}^E + C_{4\text{-HBA}}^E}
\]

\[
P_{4\text{-HBA}} = \frac{C_{4\text{-HBA}}^R}{C_{3\text{-HBA}}^R + C_{4\text{-HBA}}^R}
\]

\[
Y_{3\text{-HBA}}^E = \frac{Q_E \times C_{3\text{-HBA}}^E}{Q_E \times C_{3\text{-HBA}}^E + Q_R \times C_{3\text{-HBA}}^R}
\]

\[
Y_{4\text{-HBA}}^R = \frac{Q_R \times C_{4\text{-HBA}}^R}{Q_E \times C_{4\text{-HBA}}^E + Q_R \times C_{4\text{-HBA}}^R}
\]
3-2-3. Numerical simulation

All simulation works were performed by using VERSE tool. VERSatile Reaction SEparation (VERSE), which is a dynamic simulation package for batch and Simulated Moving Bed (SMB) Chromatography, was made by the Purdue Research foundation. VERSE simulation is based on a detailed rate model, which takes into account (1) competitive adsorption (or ion exchange) in a multicomponent mixture, (2) detailed mass transfer mechanisms (including extracolumn dispersion, intracolumn dispersion, film mass transfer, pore diffusion, and surface diffusion), (3) slow intrinsic adsorption or desorption (compared to convection or mass transfer rates), and (4) reactions among solutes in the solution phase or among adsorbed solutes.
Do batch experiments to determine the isotherm parameters

Do batch simulation to estimate the axial dispersion coefficients

Calculate operating parameters for SMB process

Do SMB simulation to optimize

Operate SMB process

Fig. 12. SMB experimental procedure.
4. RESULTS AND DISCUSSIONS

4-1. Effect of ILs on the retention behaviors of ionizable compounds

Fig. 13 shows that using [emim][BF\textsubscript{4}] additive increased retention time of both benzoic acid and benzylamine comparing to the cases of no IL additives. However, with increasing the length of carbon chain in ionic liquid cation from 4 carbons ([bmim][BF\textsubscript{4}]) to 8 carbons ([omim][BF\textsubscript{4}]), retention time of benzylamine decreased whereas that of benzoic acid increased.

It’s worthy to notice that among 4 kinds of ionic liquids, [emim][BF\textsubscript{4}] has shortest alkyl chain (only 2 carbons). This ionic liquid is hydrophilic and easily exits as ionized form in mobile phase. Therefore, it is dominant for ion-pair forming mechanism that increases the retention time of both cationic and anionic solutes. For [bmim][BF\textsubscript{4}], [hmim][BF\textsubscript{4}] and [omim][BF\textsubscript{4}], because of their long carbon chains in cation, these ionic liquids are hydrophobic and dominantly adsorb on the stationary phase. Therefore, they comply with dynamic ion exchange mechanism that
decreases retention time of cationic analyte and increases that of anionic analyte.

L-phenylalanine and L-tryptophan are zwitterions which have both amine and carboxyclic functional groups (Fig. 10). Because L-phenylalanine and L-tryptophan have pI values of 5.65 and 5.86, respectively, both of them exist in cationic forms in the mobile phase controlled at pH 4.0. Therefore, their retention behaviors were similar to benzylamine (Fig.14).
Fig. 13. Effect of ionic liquids on the retention time of benzoic acid (open square, left axis) and benzylamine (open round, right axis).
Fig. 14. Effect of ionic liquids on the retention time of L-phenylalanine (open square) and L-tryptophan (open round).
4-2. Application of [hmim][BF₄] as mobile phase additive in RP-SMB chromatography to separate 3-HBA and 4-HBA

4-2-1. Surveying the conditions of mobile phase for separation

At the neutral pH of mobile phase, both 3-HBA and 4-HBA exist as ionic forms. So, both are hydrophilic and have poor interaction with stationary phase, consequently, selectivity is poor. When mobile phase condition was controlled at low pH (e.g. pH 3.0 and pH 3.5) or at neutral pH with using [hmim][BF₄], ionic forms of 3-HBA and 4-HBA were prevented and adsorbed on the stationary phase. Therefore, these 3 cases of mobile phase condition gave good selectivity as shown in Fig. 15 (b, c and d) and were used for the next experiments.

4-2-2. Determination of adsorption parameters

Single-step frontal analysis experiments were carried out to obtain the isotherms of 3-HBA and 4-HBA. When the solute concentrations in mobile phase increased from 0 to 1 g/l, the adsorbed concentrations in stationary phase increased. Based on adsorption data, almost cases were fitted to Langmuir isotherm, only the case of using [hmim][BF₄] at neutral pH for 4-HBA was fitted to linear isotherm as shown in Fig. 16 and Fig. 17. The
isotherm slopes of pH 3.0 and pH 3.5 cases were similar together and much steeper than the case of using [hmim][BF₄] at neutral pH. In the other words, with mobile phase controlled at pH 3.0 or pH 3.5, experiments require a long running time for separation.

In addition, using [hmim][BF₄] at neutral pH gave better selectivity than the others as shown in Table 1.
Fig. 15. Surveying the mobile phase conditions for the separation of 3-HBA (solid line) and 4-HBA (dash line); (a) neutral pH; (b) using [hmim][BF₄] at neutral pH; (c) pH 3.0; (d) pH 3.5.
Fig. 16. Isotherm curves of 3-Hydroxybenzoic acid in three mobile phase conditions: pH 3, pH 3.5 and using [hmim][BF₄] at neutral pH.
Fig. 17. Isotherm curves of 4-Hydroxybenzoic acid in three mobile phase conditions: pH 3, pH 3.5 and using \([\text{hmim}][\text{BF}_4]\) at neutral pH.
4-2-3. Estimation of axial dispersion coefficients

To estimate the axial dispersion coefficients, single step frontal analysis experiments were simulated by using VERSE tool. The stacked chromatograms in the Fig. 18 shows that simulation and experimental results had good agreements. The estimation results showed in Table 1 are necessary to design operation conditions for SMB.
Fig. 18. Simulation (dash line) of the batch experimental results (solid line)

(a), (c) and (e): 3-HBA in three cases pH 3.0, pH 3.5 and using [hmim][BF₄] at neutral pH, respectively.

(b), (d) and (f): 4-HBA in three cases pH 3.0, pH 3.5 and using [hmim][BF₄] at neutral pH, respectively.
Table 1. Adsorption parameters of 3-HBA and 4-HBA

<table>
<thead>
<tr>
<th></th>
<th>$a$ [-]</th>
<th>$b$ [ml/g]</th>
<th>$D_e$ [cm$^2$/min]</th>
<th>Selectivity $\frac{a_{3-\text{HBA}}}{a_{4-\text{HBA}}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>pH 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-HBA</td>
<td>11.80</td>
<td>0.200</td>
<td>0.047</td>
<td>1.499</td>
</tr>
<tr>
<td>4-HBA</td>
<td>7.87</td>
<td>0.200</td>
<td>0.075</td>
<td></td>
</tr>
<tr>
<td><strong>pH 3.5</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-HBA</td>
<td>11.30</td>
<td>0.155</td>
<td>0.042</td>
<td>1.585</td>
</tr>
<tr>
<td>4-HBA</td>
<td>7.13</td>
<td>0.120</td>
<td>0.056</td>
<td></td>
</tr>
<tr>
<td><strong>[hmim][BF$_4$] at the neutral pH</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-HBA</td>
<td>2.82</td>
<td>0.213</td>
<td>0.027</td>
<td><strong>2.070</strong></td>
</tr>
<tr>
<td>4-HBA</td>
<td>1.36</td>
<td>0.000</td>
<td>0.017</td>
<td></td>
</tr>
</tbody>
</table>
4-2-4. Designing operation conditions for SMB process

Basing on Triangle Theory, \((m_2:m_3)\) plane was built to determinate operation conditions in three cases as shown in Fig. 19. The triangle-shape region in \((m_2:m_3)\) plane showed the operation conditions for extract and raffinate purity. The operating points were chosen at 20% safety margin from the vertex of the triangle-shape region at which the operation conditions are optimum. The operation parameters are shown in Table 2. With the same feed flow rate of 0.7 ml/min, the case of using [hmim][BF₄] gave milder values of all operation conditions than two cases of controlling at pH 3.0 and pH 3.5. Especially, low flow rate of eluent was expected to get a low desorbent consumption.
Fig. 19. Operating conditions designed were based on Triangle Theory; pH 3.0 (a), pH 3.5 (b), using [hmim][BF₄] at neutral pH (c).
Table 2. Operation parameters for SMB process in three cases: pH 3.0, pH 3.5 and [hmim][BF₄] at neutral pH

<table>
<thead>
<tr>
<th></th>
<th>Switching time (min)</th>
<th>Pump flow rate (ml/min)</th>
<th>SMB zone flow rate (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Extract</td>
<td>Raffinate</td>
</tr>
<tr>
<td>pH 3</td>
<td>8.13</td>
<td>2.41</td>
<td>1.04</td>
</tr>
<tr>
<td>pH 3.5</td>
<td>10.95</td>
<td>1.82</td>
<td>0.86</td>
</tr>
<tr>
<td>[hmim][BF₄] at neutral pH</td>
<td>4.90</td>
<td>1.34</td>
<td>0.63</td>
</tr>
</tbody>
</table>
4-2-5. SMB simulation results

Using the operation parameters in Table 2, SMB processes for three cases were simulated by VERSE tool. By the simulation results, the plots of column profile in the Fig. 20 shows that 3-HBA and 4-HBA can be well separated at neutral pH by using [hmim][BF₄] as well as at pH 3.0 or pH 3.5. In fact, the simulation results in Table 3 shows that purities and yields at extract and raffinate streams were similar in three cases. The differences among them were less than 2.5%. In addition, using [hmim][BF₄] at neutral pH gave higher concentration of products and lower desorbent consumption than two cases of controlling at low pH.

With the batch experiment and SMB simulation results, therefore, 3-HBA and 4-HBA were expected to be separated at the neutral pH in SMB process by new separation method of using [hmim][BF₄] additive.
Fig. 20. The column profiles of 3-HBA (solid line) and 4-HBA (dash line) for three cases: pH 3.0 (a), pH 3.5 (b), using [hmim][BF₄] at neutral pH (c).
Table 3. Comparison of SMB simulation results of three cases

<table>
<thead>
<tr>
<th></th>
<th>3-HBA</th>
<th>4-HBA</th>
<th>3-HBA</th>
<th>4-HBA</th>
<th>3-HBA</th>
<th>4-HBA</th>
<th>Solvent consumption [ml/cycle]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>pH 3</strong></td>
<td>100.00</td>
<td>100.00</td>
<td>99.96</td>
<td>100.00</td>
<td>0.290</td>
<td>0.670</td>
<td>2.40</td>
</tr>
<tr>
<td><strong>pH 3.5</strong></td>
<td>100.00</td>
<td>100.00</td>
<td>99.97</td>
<td>100.00</td>
<td>0.385</td>
<td>0.814</td>
<td>1.86</td>
</tr>
<tr>
<td>[hmim][BF₄] at neutral pH</td>
<td>100.00</td>
<td>100.00</td>
<td>99.94</td>
<td>97.85</td>
<td><strong>0.524</strong></td>
<td><strong>1.085</strong></td>
<td><strong>1.37</strong></td>
</tr>
</tbody>
</table>
4-2-6. SMB operation – Novasep system

Fig. 21 shows the operating illustration and parameters of four-zone SMB process. The SMB experiment with Novasep system was carried out within 8 cycles. Results from High Performance Liquid Chromatography (HPLC) analysis of extract and raffinate streams were compared to SMB simulation in Fig. 22 and Fig. 23. Accordingly, 3-HBA and 4-HBA were obtained separately at extract and raffinate streams, respectively. Concentrations of products in the experiment have reached approximately to average concentration of those in simulation. However, from the switching number 16 to 40 of SMB process, some fluctuation of pump flow rate occurred, leading the change in zone flow rate and some contamination of 3-HBA at raffinate stream. After the switching number 40 of SMB operation, the pump flow rate was stable, which gave better purities.

As shown in Table 4, purities and yields of extract stream calculated from the last cycle were 97.62 % and 100.00%, and those of raffinate stream were 100.00% and 97.38%, respectively. In simulation results, those of extract stream were 100.00% and 99.94%, and those of raffinate stream were 100.00% and 97.85%, respectively. The experimental and simulation results of SMB had good agreement.
Fig. 21. Operation parameters for SMB process using [hmim][BF₄] at the neutral pH.

Switching time: 4.9 min

**Feed continuous injection**

- Desorbent (1.16 ml/min)
  - I: (3.13 ml/min)
  - II: (1.80 ml/min)
  - III: (2.50 ml/min)
  - IV: (1.87 ml/min)

- Extract (1.23 ml/min)
- Raffinate (0.63 ml/min)

(0.7 ml/min)
Fig. 22. Comparison of experimental and simulation results for history at *extract* stream.
Fig. 23. Comparison of experimental and simulation results for history at raffinate stream.
Table 4. Purity and yield of products from the last cycle

<table>
<thead>
<tr>
<th></th>
<th>Experiment</th>
<th>Simulation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Purity (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-HBA (Extract)</td>
<td>97.62</td>
<td>100.00</td>
</tr>
<tr>
<td>4-HBA (Raffinate)</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td><strong>Yield (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-HBA (Extract)</td>
<td>100.00</td>
<td>99.94</td>
</tr>
<tr>
<td>4-HBA (Raffinate)</td>
<td>97.38</td>
<td>97.85</td>
</tr>
</tbody>
</table>
5. CONCLUSIONS

In RP-LC, [Rmim][BF₄] ionic liquids as mobile phase additives has effects on the retention behaviors of ionizable compounds in two mechanisms: Ion-pair forming in mobile phase and Dynamic ion exchange in stationary phase. For short length of alkyl chain IL ([emim][BF₄]), the mechanism of ion-pair forming dominates and increases the retention volume of both cationic and anionic analytes. For [bmim][BF₄], [hmim][BF₄] and [omim][BF₄], the mechanism of dynamic ion exchange dominates and increases retention volume of anionic analytes (Benzoic acid), and decreases that of cationic analyte (Benzyalmine) and zwitterionic analytes (L-phenylalanine and L-tryptophan).

In SMB process, [hmim][BF₄] ionic liquid can be used as mobile phase additive to separate 3-hydroxybenzoic acid and 4-hydroxybenzoic acid at neutral pH (pH 7.1 ~7.3) of mobile phase. Comparing to the conventional methods of controlling at low pH (pH 3.0 and pH 3.5), new separation method of using [hmim][BF₄] at neutral pH yielded a good selectivity, short separating time in batch experiment, and high concentration of output products, low desorbent consumption in SMB simulation. Furthermore, the experimental and simulation results of SMB had good agreement. Purity
and yield were 97.62% and 100.00% in extract streams, 100.00% and 97.38% in raffinate stream, respectively.

This study showed that ionic liquids, can be used as mobile phase additives to separate ionizable compounds even at neutral pH condition, in RP-LC and RP-SMB chromatography. Ionic liquids, which can be easily designed to have specific physicochemical properties, are believed to be useful for separation of wide range of solutes.
6. REFERENCES


[20] Lijun He, Wenzhu Zhang, Liang Zhao, Xia Liu, Shengxiang Jiang* (2003) Effect of 1-alkyl-3-methylimidazolium-based ionic liquids as
the eluent on the separation of ephedrines by liquid chromatography.  


