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Application of [hmim][BF_4] ionic liquid in reversed-phase simulated moving bed for the separation of 3- and 4-hydroxybenzoic acid

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Abstract−In reversed-phase liquid chromatography (RP-LC), the separation of ionized compounds at neutral pH of the mobile phase is ineffective because these compounds easily become ionized and too hydrophilic to adsorb on the hydrophobic C_{18} stationary phase. The common method to separate ionized compounds in RP-LC is the usage of the ion pairing agent and the control of pH in the mobile phase. In this work, [hmim][BF_4] ionic liquid was adopted as a mobile phase additive in reversed-phase simulated moving bed chromatography (RP-SMB) to overcome the problem mentioned above. The results showed that when 1.5 ml// [hmim][BF_4] was added to mobile phase, 3-hydroxy benzoic acid (3-HBA) and 4-hydroxy benzoic acid (4-HBA) were separated at neutral pH in the RP-SMB. In particular, purities of 3-HBA and 4-HBA products were 97.62% and 100%, respectively, and had a good agreement with simulation results.

Key words: Ionic Liquid, Ionized Compounds, Mobile Phase Additive, Reversed-phase, Simulated Moving Bed, Continuous Chromatography

INTRODUCTION

In reversed-phase liquid chromatography (RP-LC), it is difficult to separate ionized compounds, because these compounds tend to easily turn into ionic forms when they are dissolved in an aqueous mobile phase solution. In other words, these ionic forms are highly hydrophilic in mobile phase, and thus have very weak interaction to the hydrophobic adsorbent such as C_{18} stationary phase. To separate such ionized compounds, it is necessary to prevent them from being ionized. To constrain the analytes to be ionized, the pH control of the mobile phase has been commonly used to enhance the adsorption capacity and selectivity. When pH (pOH) condition of mobile phase is lower than the ionization constant, pK_a (pK_b), of analyte, a large portion of analyte exists as non-ionic form; therefore, it can interact with the hydrophobic stationary phase [1,2]. However, this method could be applied only for a small number of compounds which natural structures are stable in acidic or basic aqueous solution. In addition, it is hard to constantly maintain the pH of the mobile phase in some separation processes that require a long operating time, such as preparative chromatography and the continuous chromatographic process. Therefore, if it is possible to make the ionized compounds be non-ionized at the neutral pH condition, the above two problems can be solved.

Ionic liquids (ILs), salts existing as liquid phase at room temperature, are composed of ionic species, particularly large organic cations, and inorganic or organic anions. Recently, ILs have been studied to be used as a mobile phase additives in RP-LC, because of their special physicochemical properties, such as very low vapor pressure, non-flammability, high ionic mobility, and excellent chemical stability. Especially, the physicochemical properties of ionic liquids can be easily designed by varying the lengths and branches of the alkyl chains in the anionic core and cationic precursor [3-8]. In 2003, He et al. studied to use 1-butyl-3-methylimidazolium tetrafluoroborate ([bmim][BF_4]) ionic liquid as the mobile phase additive to separate ephedrines [9], and to investigate the adsorption mechanism of 1-alkyl-3-methylimidazolium tetrafluoroborate ([Rmim]BF_4) ionic liquids as mobile phase additives in HPLC for the separation of some amines [10]. Recently, several imidazolium tetrafuoroborate ionic liquids have been studied for the possibility of suppression of deleterious effects of free silanols in liquid chromatography [11-13]. In these studies, the silanol suppressing potency of imidazolium tetrafluoroborates ionic liquids was demonstrated to markedly exceed that of the conventional mobile phase additives, like triethylamine, dimethyloctylamine and ammonia. It is also possible to design the hydrophobic properties of ionic liquids. In other words, ionic liquids can contribute to increase the hydrophobic interaction between the analytes, especially ionized solutes and reversed-phase stationary phase.

Compared to the common inorganic salts used as the mobile phase additives, ionic liquids have hydrophobic organic cations that strongly interact with the reversed-phase stationary phase. Therefore, when they are used as the mobile phase additives in RPLC, ionic liquids not only prevent the analytes from being ionized and the silanol groups on the C_{18} stationary phase surface from interacting with ionic forms, but also enhance the adsorption capacity of the ionized analytes on the C_{18} stationary phase [9].

In this study, to separate two ionized anionic compounds in reversed-phase simulated moving bed chromatography (RP-SMB) under the neutral pH condition of desorbent, 1-hexyl-3-methylimidazolium tetrafluoroborate ionic liquid ([hmim][BF_4]) was used as the desorbent additive. This ionic liquid has hexyl branch in cation, and thus is a hydrophobic cationic additive agent. According to Dai and Carr [14,15], a hydrophobic additive agent is dominant in the

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mechanism of dynamic ion exchange in the stationary phase, which enhances the ability of anionic compounds to adsorb on the C_{18} stationary phase. Simulated moving bed (SMB) chromatography is a continuous chromatographic process developed in the 1960's by UOP (United Oil Products) [16]. SMB chromatography has the advantages of 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 [17-19].

THEORY

To simulate the SMB process, the equilibrium-dispersion model and the linear lumped kinetic model [20] were used.

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

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

$$q_i^* = f_{eq}(C_i) \tag{1}$$

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

To determine the operating conditions such as the flow rate of four zones as well as switching time, Morbidelli et al. [21] proposed the Triangle theory, which is based on the equilibrium theory. 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.

$$m_j = \frac{Q_j^{SMB} t^* - V\varepsilon^*}{V(1-\varepsilon^*)} \tag{3}$$

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

$$\varepsilon^* = \varepsilon_i + (1-\varepsilon_i)\varepsilon_p \tag{4}$$

where ε_i is bed or inter-particle void fraction and ε_p is intra-particle void fraction. Because of the different functions in SMB process, each zone has its own constraint for the determination of zone flow rate. While the function of zone I is to desorb the more retained component and to regenerate adsorbent, that of zone IV is to adsorb the less retained component and regenerate desorbent.

$$H_A < m_1;\ m_4 < H_B \tag{5}$$

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.

$$H_B < m_2 < m_3 < H_A \tag{6}$$

Because zone II and III are separation zones, the determination of component migrations and the flow ratio in these zones is important in order to separate pure products at *extract* and *raffinate* ports. In this study, Aspen Chromatography® was used for batch chromatography and SMB processes in order to verify the adsorption and mass transfer parameters and design methods.

The linear adsorption isotherm for a single component is:

$$q = H_c \tag{7}$$

The Langmuir adsorption isotherm for a single component is:

$$q = \frac{ac}{1+bc} \tag{8}$$

where H_i is the Henry constant of component i, q and c are the concentrations of the solute in stationary phase and mobile phase at equilibrium, respectively [20], and a and b are characteristic parameters of the solute in a given system. The parameters are estimated by using a single component frontal analysis of each component.

MATERIAL AND METHODS

[hmim][BF_4] ionic liquid was kindly supplied from C-tri Company. Analytes of 3-hydroxybenzoic acid and 4-hydroxybenzoic acid were purchased from Sigma-Alldrich, USA. Mobile phases were prepared with methanol (HPLC grade solvent) from J. T. Baker Company, USA and deionized water from Milli-Q purification system, Millipore, USA. Potassium phosphate monobasic (KH_2PO_4) and dibasic (K_2HPO_4) from Duksan Pure Chemical (Kyungkido, Korea) were used as buffer agents in mobile phase. Hydrochloric acid 35% from MatSunoen Chemical (Osaka, Japan) and ammonia solution 28.0-30.0% from Samchun Pure Chemical (Kyungkido, Korea) were added dropwise as needed to adjust the desired pH.

The HPLC system was composed of a pump (model LC-6AD, Shimadzu, Kyoto, Japan), a PDA detector (model SPD-M10Avp Shimadzu), an auto-injector (SIL-10ADvp Shimadzu), a column oven (model CTS-30 Younglin, Kyungkido, Korea). The Licosep Micro SMB system from Novasep co. (France) was used to perform an SMB experiment at laboratory scale. Eight C18 Kromasil columns (1 cm×10 cm) with 100 A°-25 μm spherical silica particles were used. The pH meter (model 420) and pH electrode were from Thermal Orion, USA.

The effect of [hmim][BF_4] ionic liquid on separation of 3-HBA and 4-HBA was examined in this experiment. The batch chromatography experiments were carried on the C18 column (1.0×10 cm) with 3 ml/min of the mobile phase flow-rate and 30 °C of column temperature. The feed solutions, 1 g/L 3-HBA and 4-HBA, were pumped into column for several minutes. Three different mobile phase conditions, pH 3.0 and neutral pH (pH 7.1-7.3) without additive, and neutral pH with [hmim][BF_4] additive, were used. Mobile phase used was 20% methanol in aqueous solution, degassed under vacuum condition during 30 minutes. To control pH 3.0, HCl solution was dropwisely added. To control neutral pH, 100 mM of phosphate buffer (50 mM KH_2PO_4 and 50 mM K_2HPO_4) was used.

"The concentration of [hmim][BF_4] ionic liquid used for all experiments in this work was 1.5 ml/l mobile phase. To obtain this parameter, we investigated the effect of ionic liquid concentration on the selectivity of 3- and 4-HBA using HPLC system with one

single column. Six mobile phases were made by adding 0, 0.5, 1.0, 1.5, 2.0, 2.5 ml [hmim][BF_4] into 1 liter of 20% methanol containing 100 mM phosphate buffer. Then, the 1.5 ml/*l* [hmim][BF_4] was chosen for the best selectivity of 3- and 4-HBA."

In this work, the SMB process was carried out with the Licosep SMB system (Novasep, France) using eight commercial columns which have the same size and packing characteristics. The column efficiency of all eight columns was measured at flow rate 3 ml/min by injecting 5 µl of 0.02 mol/L L-phenylalanine solution at 25 °C. The height equivalent to one theoretical plate (HETP) of all columns was calculated by the following formula [22]:

$$HETP = \frac{L}{N} \tag{9}$$

$$N = 5.54 \times \left(\frac{t - t_0}{w_{0.5}}\right)^2 \tag{10}$$

where N and L are the number of theoretical plates and the length of one column, respectively, $W_{0.5}$ is the peak width at 50% peak height, t and t_0 are retention time and dead time, respectively The average of HETP for eight columns was 0.288 mm with standard deviation of 0.009. Total bed void fraction (ε) and inter-particle void fraction (ε_i) were measured by injecting 20 µl of 0.1 M NaCl, and 20 µl of diluted Blue dextran solution, respectively. Total column porosity and inter-particle porosity are 0.544, and 0.336, respectively.

Multi-step frontal analysis was carried out for both 3-HBA and 4-HBA with one of 8 columns (L=100 mm, I.D.=10 mm) at 30 °C. At pH 3.0, five concentration steps (0.2, 0.4, 0.6, 0.8, and 1 g/L) were used for 3-HBA, and four concentration steps (0.1, 0.3, 0.5, and 0.7 g/L) were used for 4-HBA. At neutral pH with [hmim][BF_4], eight concentration steps (0.05, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, and 1 g/L) were used for both 3-HBA and 4-HBA. Each multi-step frontal analysis was carried out with column and without column.

The Licosep Micro SMB system (Novasep, France) was used to perform SMB experiment at laboratory scale. In the design of four-zone SMB, eight columns were divided into four zones with the configuration of 2-2-2-2. Desorbent was 20% methanol in deionized water added with 100 mM phosphate buffer (50 mM KH_2PO_4 and 50 mM K_2HPO_4) and [hmim][BF_4] at concentration 1.5 ml/*l* desorbent. During an SMB run, the system temperature was controlled at 30 °C by using a water bath (Lauda, Germany). Feed solution was prepared by dissolving 1 g/L 3-HBA and 1 g/L 4-HBA into desorbent solution. Columns were cleaned with 50% methanol in deionized water and equilibrated with desorbent for 1 hour prior to operate SMB equipment. Extract and raffinate samples were collected from effluents accumulated within each switching interval. Extract and raffinate samples from SMB operation were analyzed by HPLC system with one of the eight columns in the SMB experiment. The 3-HBA and 4-HBA products were detected by UV detector at 236 nm and 262 nm, respectively. Purities and yields of *extract* and *raffinate* streams from SMB experiment were calculated from the last cycle of SMB operation (steady state) by the following formulas:

$$P^E_{3\text{-}HBA} = \frac{C^E_{3\text{-}HBA}}{C^E_{3\text{-}HBA} + C^E_{4\text{-}HBA}} \tag{11}$$

$$P^R_{4\text{-}HBA} = \frac{C^R_{4\text{-}HBA}}{C^R_{3\text{-}HBA} + C^R_{4\text{-}HBA}} \tag{12}$$

$$Y^R_{3\text{-}HBA} = \frac{Q^E \times C^E_{3\text{-}HBA}}{Q^E \times C^E_{3\text{-}HBA} + Q^R \times C^R_{3\text{-}HBA}} \tag{13}$$

$$Y^R_{4\text{-}HBA} = \frac{Q^R \times C^R_{4\text{-}HBA}}{Q^R \times C^E_{4\text{-}HBA} + Q^R \times C^R_{4\text{-}HBA}} \tag{14}$$

where P, Y, and Q were the purity, yield, and volumetric flow rate, respectively; superscripts E and R represent the extract and raffinate ports, respectively; subscripts 3-HBA and 4-HBA represent 3-hydroxy benzoic acid and 4-hydroxy benzoic acid, respectively.

RESULTS AND DISCUSSION

A mobile phase controlled neutral pH is good for maintaining the chemical structure of a fragile solute, but it usually makes acidic or basic compounds to be ionized. Because the ionic forms are highly hydrophilic, these are hard to be adsorbed to the hydrophobic stationary phase. Consequently, chromatographic resolution of ionized compounds is very poor when mobile phase is at neutral pH. The 3-HBA and 4-HBA contain carboxylic group, [-COOH], in benzyl ring (Fig. 1). At the neutral pH (7.1-7.3), this functional group, which has a pKa value lower than neutral pH, as shown in Table 1, tends to exist as ionized form [$-COO^-$] in an aqueous solution. Thus, solutes containing [$-COO^-$] group are hydrophilic and strongly eluted by the mobile phase without any significant adsorption to the stationary phase. As shown in Fig. 2(a), selectivity of 3-HBA and 4-HBA is very poor at the neutral pH of mobile phase.

On the contrary, when mobile phase is controlled at pH 3.0, this [-COOH] group is protonated and hardly turned into [$-COO^-$]. At pH 3.0, therefore, 3-HBA and 4-HBA have strong adsorption and better selectivity as shown in Fig. 2(c). At the neutral pH of mobile

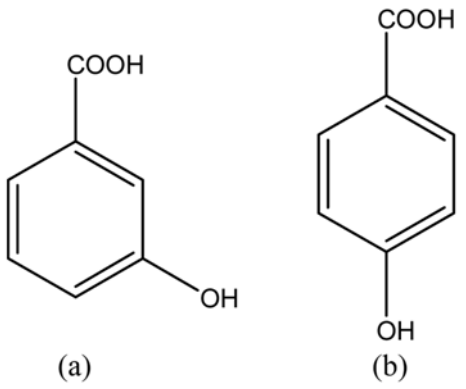

Fig. 1. Molecular structures of 3-hydroxybenzoic acid (a), and 4-hydroxy-benzoic acid (b).

Table 1. Molecular weights and ionization constants of 3- and 4-HBA

	MW [g/mol]	pKa
3-Hydroxy benzoic acid	138.1	4.06
4-Hydroxy benzoic acid	138.1	4.48

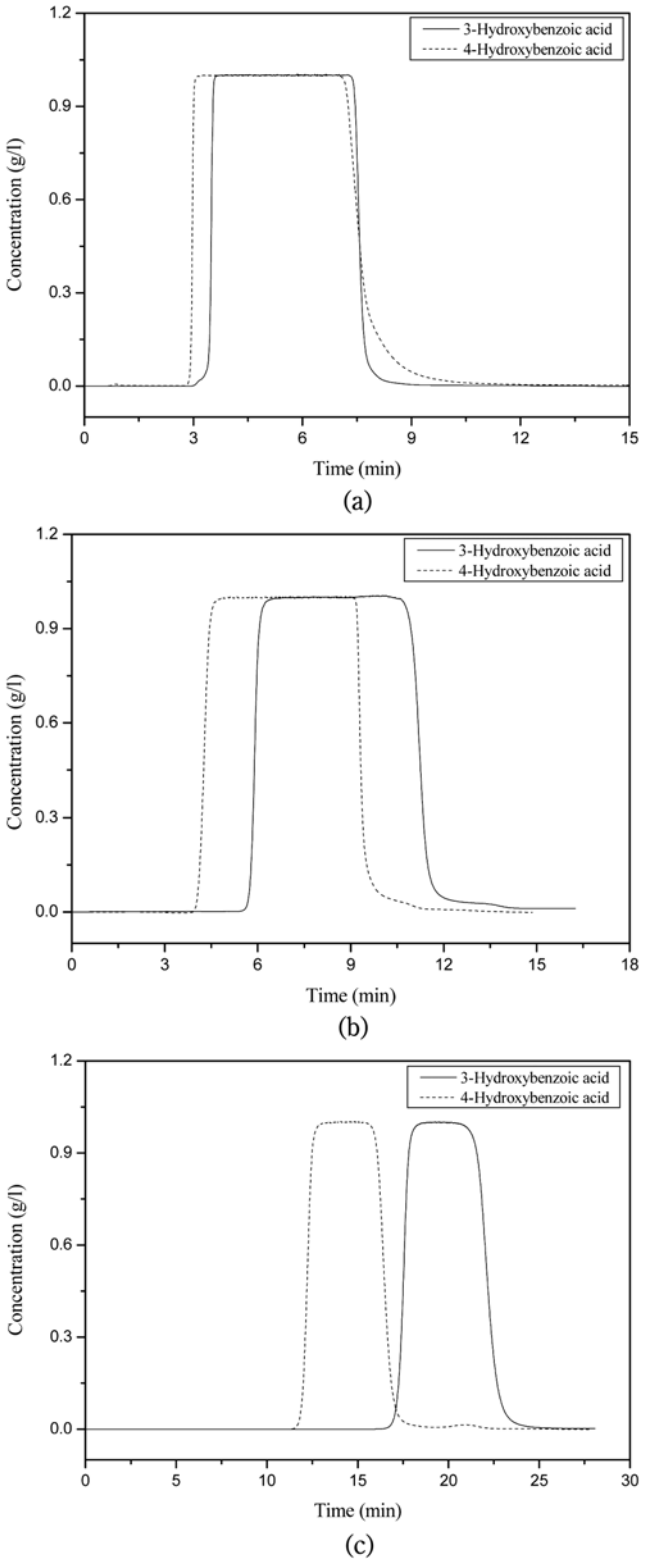

Fig. 2. Elution profiles of 3-HBA (solid line) and 4-HBA (dash line) in three different mobile phase condition. (a) neutral pH without [hmim][BF_4], (b) neutral pH with [hmim][BF_4], (c) pH 3.0.

phase, however, the selectivity of these compounds can be substantially improved when [hmim][BF_4] ionic liquid is added to the mobile phase, as shown in Fig. 2(b). Since [Hmim][BF_4], which is a special salt with a long carbon chain (hexyl) in cation, has a hydrophobic tail, this tail can strongly adsorb on the C_{18} stationary phase. It is the adsorbed $[hmim]^+$ cation on the stationary phase that interacts with the $[-COO^-]$ anion of 3-HBA and 4-HBA molecules in the mechanism of dynamic ion exchange [14,15]. Consequently, it improves the selectivity of 3-HBA and 4-HBA as shown in Table 2.

Table 2. Selectivity of 3-HBA and 4-HBA in different mobile phase conditions

Condition	Selectivity
Neutral pH w/o [hmim][BF_4]	1.33
Neutral pH w/ [hmim][BF_4]	1.57
pH 3.0	1.46

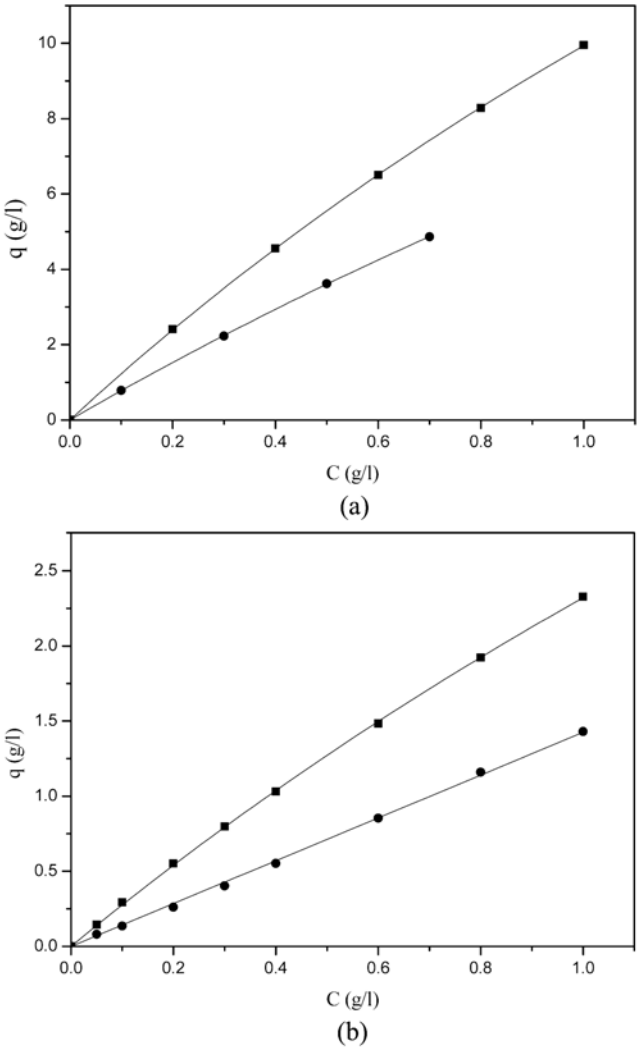

Fig. 3. Isotherm curves of 3-HBA (square) and 4-HBA (round) at pH 3.0 (a) and neutral pH with [hmim][BF_4] (b).

Retention factor and selectivity coefficient are calculated by the following formulas:

$$k' = \frac{\text{The number of moleculars in the stationary phase}}{\text{The number of moleculars in the mobile phase}} \quad (15)$$

$$k' = \frac{V_{analyte} - V_o}{V_o} \quad (16)$$

$$\alpha = \frac{k'_{3\text{-}HBA}}{k'_{4\text{-}HBA}} = \frac{V_{3\text{-}HBA} - V_o}{V_{4\text{-}HBA} - V_o} \quad (17)$$

where k', α, V_o, V are the retention factor, the selectivity coefficient, the total dead volume and the retention volume of analyte, respectively, subscripts of 3-HBA, 4-HBA are 3-hydroxy benzoic acid, 4-hydroxy benzoic acid, respectively. The total dead volume of column is defined as retention volume of NaCl when 20 µl of 0.1 M NaCl was injected to the column at 30 °C.

The adsorption isotherm was measured at 30 °C with two mobile phase conditions including pH 3.0 and neutral pH with using [hmim][BF_4]. In both conditions, 3-HBA and 4-HBA have adsorption isotherms in agreement with Langmuir model (Fig. 3). In other words, the concentration ratio (q/c) of these solutes between solid phase and liquid phase decreases as the concentration of solutes in the liquid phase increases to a nonlinear level. The adsorption parameters in Table 3 show that isotherm parameters a at pH 3.0 are much higher than those at neutral pH using [hmim][BF_4]. In other words, it takes more time to elute analytes at pH 3.0 of mobile phase, although the selectivity at pH 3.0 is lower than at neutral pH with using [hmim][BF_4], as shown in Table 2. To estimate the axial dispersive coefficients for each analyte in both conditions of mobile phase, single-step frontal analysis is carried out at each condition and simulated. The estimated parameters of the axial dispersive coefficients are also shown in Table 3.

Based on the estimated adsorption parameters in Table 3 and column parameters in Table 4, SMB operation conditions for two mobile phase conditions were designed by using the Triangle theory proposed by Morbidelli [21]. The design was based on the criteria of at least 99% purity and 99% yield of extract and raffinate products. The results in Table 5 show that operation parameters of SMB system at neutral pH with [hmim][BF_4] are milder and easier to operate than at pH 3.0. At the same feed flow rate and feed concentration, in particular, flow rates in four zones in the case of using [hmim][BF_4] are about two times smaller than those in the case of pH 3.0.

The operation parameters of SMB process were tested by using the VERSE simulator to confirm that the design should give the desired separation results for 3-HBA and 4-HBA. Column profiles of SMB simulation in Fig. 4 show that 3-HBA and 4-HBA could be well separated at pH 3.0 as well as at neutral pH by using [hmim]

Table 3. Langmuir isotherm parameters of 3- and 4-HBA

	3-HBA	4-HBA
Isotherm parameter a [-]		
pH 3.0	11.8	7.87
Neutral pH w/[hmim][BF_4]	2.82	1.36
Isotherm parameter b [ml/g]		
pH 3.0	0.20	0.20
Neutral pH w/[hmim][BF_4]	0.213	0
Apparent axial dispersion coefficient [cm^2/min]		
pH 3.0	0.047	0.075
Neutral pH w/[hmim][BF_4]	0.027	0.017

Table 4. System parameters

Configuration	2-2-2-2
Column length [cm]	10
Column diameter [cm]	1
Particle diameter [ìm]	25
Inter-particle void fraction [-]	0.336
Intra-particle void fraction [-]	0.314
Total column void fraction [-]	0.544
Dead volume between columns [ml]	0.6

Table 5. Operating conditions of SMB process designed for two mobile phase conditions

	Neutral pH w/[hmim][BF_4]	pH 3.0
Feed flow rate (ml/min)	0.7	0.7
Feed concentration (g/*l*)	1	1
Switching time (min)	4.9	8.13
Zone 1 flow rate (ml/min)	3.13	6.24
Zone 2 flow rate (ml/min)	1.8	3.83
Zone 3 flow rate (ml/min)	2.5	4.53
Zone 4 flow rate (ml/min)	1.87	3.49

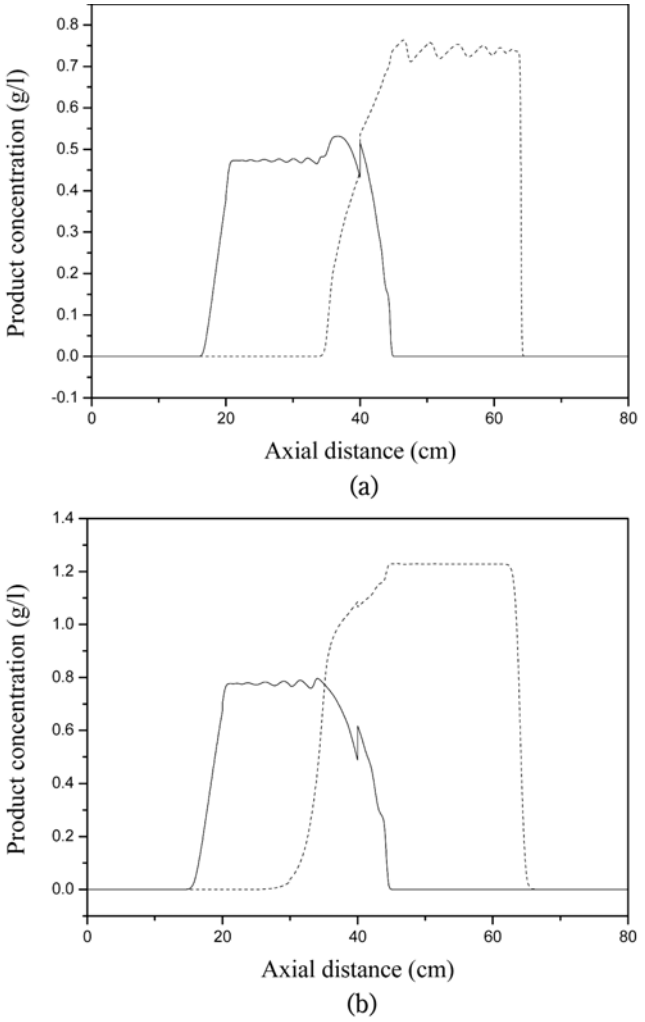

Fig. 4. Internal profiles of 3-HBA (solid line) and 4-HBA (dash line) at pH 3.0 (a) and neutral pH with [hmim][BF_4] (b).

Table 6. SMB simulation results

	Extract (3-HBA)	Raffinate (4-HBA)
Product purity [%]		
pH 3.0	100.00	100.00
Neutral pH using [hmim][BF_4]	100.00	100.00
Product yield [%]		
pH 3.0	99.96	100.00
Neutral pH using [hmim][BF_4]	99.84	97.85
Product concentration [g/L]		
pH 3.0	0.291	0.670
Neutral pH using [hmim][BF_4]	0.524	1.085
Desorbent consumption [ml/cycle]		
pH 3.0	2.40	
Neutral pH using [hmim][BF_4]	1.37	

Table 7. Experimental and simulation results of SMB at neutral pH with [hmim][BF_4]

	Extract (3-HBA)	Raffinate (4-HBA)
Product purity [%]		
Experimental	97.62	100.00
Simulation	100.00	100.00
Recovery yield [%]		
Experimental	100.00	97.38
Simulation	99.84	97.85

[BF_4] as desorbent additive. According to the simulation results of SMB process in Table 6, purities and yields at extract and raffinate ports were similar for both cases; however, in the case of neutral pH with using [hmim][BF_4], the concentrations of extract and raffinate products are about twice as much as at pH 3.0. In addition, the SMB process under neutral pH using [hmim][BF_4] consumed only 1.37 ml/cycle compared to 2.40 ml/cycle under pH 3.0.

The SMB process for the separation of 3-HBA and 4-HBA at neutral pH was operated by using the Novasep system with operation parameters in Table 5 during eight cycles. The extract and raffinate stream from SMB process are fractionated at every switching time and analyzed by high performance liquid chromatography (HPLC). Histories at extract and raffinate ports as in Fig. 5 and Fig. 6 showed that 3-HBA and 4-HBA were collected separately at extract and raffinate streams, respectively. Product concentrations in the experiment reached approximately to average concentrations in simulation that are about 0.5 g/*l* at extract port and 1.0 g/*l* at raffinate port.

From switching number 16 to 40 of the SMB process, some amount of extract product was eluted at the raffinate port, which makes the purity of the raffinate stream decreasing. It can be explained that there was some competition between 3-HBA and 4-HBA for stationary phase when the binary mixture was fed into the column. This phenomenon must be described by a competitive isotherm model, different from the noncompetitive Langmuir isotherm measured by using single-component frontal analysis. However, we can assume that 3-HBA and 4-HBA are noncompetitive and follow the noncompetitive Langmuir isotherm because a competitive isotherm model in the presence of additive agents is complex and difficult to describe accurately; in addition, this contamination is small and occurred before the SMB process reached steady-state.

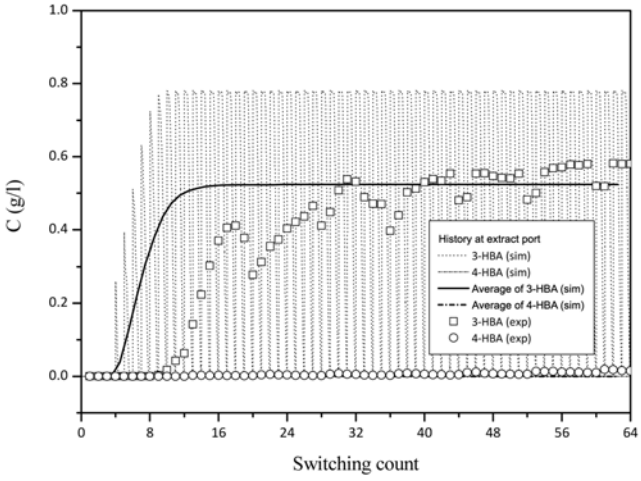

Fig. 5. Comparisons of experimental and simulation results for history at *extract* stream.

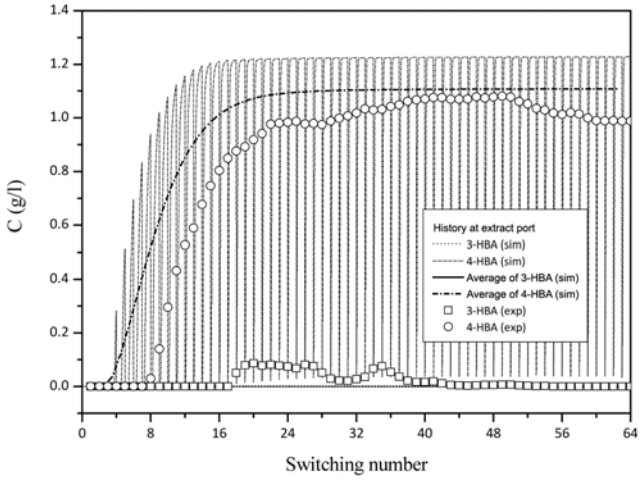

Fig. 6. Comparisons of experimental and simulation results for history at *raffinate* stream.

In fact, purities and yields of products calculated from the last cycle of SMB process showed good agreement between simulation and experimental results. In particular, 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.

CONCLUSION

Ionic liquid [hmim][BF_4], which belongs to the family of 1-alkyl-3-imidazolium tetrafluoroborate ([Rmim][BF_4]), has a long carbon chain on the cationic core that interacts strongly with C18 surface in the mechanism of dynamic ion exchange [14]. When [hmim][BF_4] exists in a desorbent solution, $[hmim]^+$ acts as a connector between solute ion and C18 surface. Therefore, the retention time of acidic compounds such 3-HBA and 4-HBA increases. Consequently, 3-HBA and 4-HBA could be separated under the neutral pH (7.1-7.3)

of mobile phase by using reversed-phase columns when [hmim][BF_4] was added into mobile phase. At the steady state of SMB process, purity and yield of 3-HBA were 97.62% and 100%, and those of 4-HBA were 100% and 97.38%, respectively.

Compared to the method controlling mobile phase at pH 3.0, the new separation method using [hmim][BF_4] as mobile phase additive showed some advantages in separation of ionized compounds in RP-LC such as mild operation conditions, high enrichment of products, and reduced solvent consumption.

The results obtained in this work show the potential application of ionic liquids in the reversed phase chromatographic separation process for ionized mixture. Especially, if the easy tunability of physicochemical properties of ionic liquids is considered, ionic liquids will be widely used for the chromatographic separation process in the near future.

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