

Effects of Ionic Liquid on the Separation of 2-Chlorophenol and 2,4,6-Trichlorophenol in RP-HPLC

J. Zheng and K.H. Row*

Center for Advanced Bioseparation Technology, Department of Chemical Engineering, Inha University, Incheon 402-751, South Korea

Abstract

In this study, the use of ionic liquids as additives in the separation of 2-chlorophenol and 2,4,6-trichlorophenol in reversed-phase high-performance liquid chromatography is reported. The ionic liquids used were 1-butyl-3-methylimidazolium tetrafluoroborate, 1-ethyl-3-methylimidazolium tetrafluoroborate, 1-ethyl-3-methylimidazolium methylsulfate ([EMIm][MS]), and 1-octyl-3-methylimidazolium methylsulfate ([OMIm][MS]), because of their high hydrophobicity and good solvating properties. The effects of the ionic liquid on retention factor and resolution, and the change of detector response using the different ionic liquids were studied. Separation with the [MS] anion ionic liquid in the eluent resulted in better resolution than with the [BF₄] anion ionic liquid. The best result was achieved using 3.0 mM/L of [OMIm][MS] and 1.0 mM/L [EMIm][MS] as mobile phase additives.

Introduction

Unlike traditional salts, ionic liquids are liquids composed of relatively large organic cations and inorganic or organic anions (1). Ionic liquids have some unique properties, such as negligible vapor pressure, good thermal stability, tunable viscosity, strong polarity, and miscibility with water and organic solvents, as well as good extractability for various organic compounds and metal ions (2). Ionic liquids have been applied to analytical chemistry (2), catalysis (3,4) and biocatalysis (5), synthesis (6), and separation science (7–16). When ionic liquids are diluted or immobilized on a stationary support, they may not possess all the properties of the ordinary ionic liquids; in some cases, they may keep several kinds of intermolecular interactions. For this reason, ionic liquids can be useful for chromatographic separations (1,15,17).

The interest in ionic liquids for their potential application in separation science is increasing because ionic liquids present a variety of desirable properties. In past decades, the dialkylimidazolium-based ionic liquids have generated enormous attention.

The purpose of the present study is to investigate the potential application of different ionic liquids as additives for the separation of 2-chlorophenol and 2,4,6-chlorophenol. Four types of ionic liquids were used as mobile phase modifiers in reversed

phase-high performance liquid chromatography (RP-HPLC) to isolate the samples. The retention factors of these chloro-substituted solutes were determined with mobile phases containing four ionic liquids in water–methanol. Four ionic liquids, 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIm][BF₄]), 1-ethyl-3-methylimidazolium tetrafluoroborate ([EMIm][BF₄]), 1-ethyl-3-methylimidazolium methylsulfate ([EMIm][MS]), and 1-octyl-3-methylimidazolium methylsulfate ([OMIm][MS]) were used. The effects of the concentration and chemical nature of the ionic liquids on the chromatographic retention and separation of 2-chlorophenol and 2,4,6-chlorophenol were studied; the influence of the ionic liquid on the detector response in the UV was also studied.

Experimental

Reagents

Four ionic liquids (99.99%), [BMIm][BF₄], [EMIm][BF₄], [EMIm][MS], and [OMIm][MS] were purchased from C-tri Co. (Namyang, South Korea). 2-Chlorophenol and 2,4,6-trichlorophenol were purchased from Sigma-Aldrich Co. (St. Louis, MO), and all reagents were of analytical grade. Potassium nitrate (KNO₃) was purchased from Kanto Chemical Co. (Tokyo, Japan) to measure the dead volume. HPLC-grade methanol (CH₃OH) was purchased from Duksan Pure Chemical Co. (Ansan, South Korea); distilled water was filtered with a vacuum pump (Millipore, Waters, Milford, MA) and filter (HA-0.45, Millipore) before use.

Apparatus

The instrument used in this study consisted of a 600 HPLC pump (Waters), 486 detector (M 7200 Absorbance Detector, Young-In Scientific Co., Seoul, South Korea), and Rheodyne injection valve with 20- μ L sample loop. Chromate software (Ver. 3.0 Interface Eng., South Korea) connected to a PC was used as the data acquisition system. The experiments were carried out using a Chromolith RP 18-end-capped column (4.6 \times 100 mm i.d.).

Chromatographic conditions

Stock solutions of the standards of 0.5 mg/mL each were prepared by dissolving the individual sample standards in pure methanol. A mixture of chlorophenols was prepared as aliquots of the individual solutions of 2-chlorophenol and 2,4,6-

* Author to whom correspondence should be addressed: Kyung Ho Row, Center for Advanced Bioseparation Technology and Department of Chemical Engineering, Building 25 335, Inha University, #253, Yonghyun-Dong, Nam-Ku, Incheon 402-751, South Korea; email rowkho@inha.ac.kr.

trichlorophenol in the ratio of 1:10. Mobile phases were 80.0 vol.% of methanol in water (pure reversed-phase systems). The flow rate was fixed at 1.0 mL/min and used in the isocratic mode. The constant injection volume of the mixture was 15 μ L. Detection was at a wavelength of 254 nm. The solutions were stored at 4°C and the working standards were re-prepared every 2 days to avoid potential errors from decomposition of the targets. Retention factor (k) values were calculated using the formula:

$$k = (t_R - t_0) / t_0 \quad \text{Eq. 1}$$

where t_R is the retention time of the analyte and t_0 is the retention time of the non-retained peak (taken as the first deviation of the baseline following the injection of 5 μ L KNO₃). Three replicated injections were made to determine the retention time, and the average values were used to calculate the retention factors.

Evaluation of the results of the chromatographic experiments was carried out by mathematical statistical techniques. The relative error of a single measurement did not exceed 5%. All experimental procedures were performed at an ambient temperature.

Results and Discussion

Chemically modified silica with aqueous–methanol eluents has been widely used in RP-HPLC. The parameters which affect the sorption of the substances onto the stationary phase and hence the retention of solutes include many effects, such as the nature of the stationary phase, the lipophilicity of the substance, the concentration of the solute in the mobile phase, the ionic strength of the mobile phase, and the nature and concentration of any competing modifier added to the eluent. The strength of a mobile phase is defined by its polarity, the ability to dissolve more polar compounds. Solvent selectivity, on the other hand, is the ability to dissolve compounds that have the same polarity. Each solvent will show a favorable kind of interaction with sample solutes having the same polarity. The optimization of solvent strength and/or selectivity is a major goal in chromatographic method development.

To investigate the chromatographic behavior of chlorophenols with a pure reversed-phase system, binary mobile phases of different methanol content (0–100 vol %) in water were tested. These pilot experiments showed that for a wide range of methanol concentrations, if higher than 80 vol.%, samples could

not be analysed due to the fast elution of the analytes. The retention times of investigated phenols decreased with increasing methanol concentrations in the mobile phase. For these reasons, the eluent with 80 vol % of methanol was chosen and used in future experiments. It should be noted that in all cases with water–methanol eluent, the retention factors of the two samples were very low and did not exceed 0.9. These experiments indicated that in a pure reversed-phase system, no satisfactory separation could be achieved using methanol as the only mobile phase modifier.

As reported by other authors (1,17), ionic liquid cations in the eluent will adsorb onto the C18 silica-based surface and cause changes in their properties. Imidazolium cations can interact with silanol groups and compete with the polar group of the analytes for the silanol groups on the alkylsilica surface in a column. Therefore, it can effectively shield residual silanols and improve peak shape, while also decreasing the retention time of the analytes. Another application of ionic liquid in chromatography is suppression of the deleterious effects of free silanols by using imidazolium tetrafluoroborate ionic liquid (18). The addition of imidazolium tetrafluoroborate ionic liquid to the mobile phases at concentrations of 0.5–1.5 vol % as silanol-blocking additives was markedly more efficient than the addition of the standard mobile phase additives, such as triethylamine and dimethyloctylamine.

Ionic liquids containing imidazolium cation or an inorganic anion absorb UV light, making detection problematic. Alkyl-imidazolium cations have a strong absorbance from 200–280 nm. So, [BMIm][BF₄] in water at the concentration 5×10^{-5} mol/L has an absorbance maximum at approximately 215 nm (15). A 10% (v/v) solution of 1-butyl-3-methyl-imidazolium chloride in acetonitrile has an absorbance of 0.5–2.5 AU in the UV region using a 1-cm cuvette (19). Accordingly, a signal strength weakening at the UV detection wavelength was observed. The influence of the ionic liquid on the detection of the analytes was also evaluated by measuring the peak heights of the individual standards in different concentrations of ionic liquids. The experiments showed that the decreasing trend of the peak heights with increasing ionic liquid concentration in range of 1.0–5.0 mM/L is negligible. However, this reduction was negligible at 254 nm and had no effect on the results of the experiments.

In the next step, the influence of the ionic liquid concentration in the range of 1.0–5.0 mM/L was tested. It is important to note here that after each experiment with a certain concentration of the ionic liquid and before the experiment with the subsequent

Table I. Retention Factors of 2-Chlorophenol and 2,4,6-Trichlorophenol with Different Ionic Liquids and Concentrations

Ionic liquid conc. (mM/L)	[BMIm][BF ₄]		[EMIm][BF ₄]		[EMIm][MS]		[OMIm][MS]	
	2-chlorophenol	2,4,6-trichlorophenol	2-chlorophenol	2,4,6-trichlorophenol	2-chlorophenol	2,4,6-trichlorophenol	2-chlorophenol	2,4,6-trichlorophenol
0.0	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22
1.0	0.21	0.24	0.17	0.23	0.23	0.87	0.22	0.84
2.0	0.16	0.22	0.16	0.26	0.23	0.87	0.22	0.83
3.0	0.39	0.31	0.22	0.22	0.23	0.86	0.22	0.83
5.0	0.23	0.23	0.22	0.22	0.23	0.89	0.22	0.81

concentration of the ionic liquid, the column was flushed for at least 3 h to remove the ionic liquid used at the previous concentration and to fully equilibrate the column. The constancy of efficiency and peak tailing showed that the use of ionic liquids is not harmful to the column. In order to test the possible effects of the ionic liquid on the C18 (alkyl-) bonded phase, the column was evaluated before and after exposure to the ionic liquid, using aqueous-methanol media as the mobile phase with the solutes and benzene as the testing marker.

Table I shows the retention factors of solutes with different ionic liquids and concentrations. The retention times of the solutes are very close to the dead time. The retention factors are less than 1.0 in all cases. Modification of the eluent by ionic liquid exercises a significant influence on the retention and isolation of these solutes. The presence of the [MS] anion in the ionic liquid caused an increase in the retention of the 2,4,6-trichlorophenol, so good resolution was obtained. It is clear that [MS] anion decreases the elution force of the mobile phase, which leads to an increase in the retention time. The retention times of the two solutes are independent of the concentration of the two ionic liquids after the addition of small amounts of ionic liquids.

The different separations resulting from two ionic liquids with different counterions as the eluent modifiers may be due to the association with solutes at the water-methanol media, and it seems that the cation on ionic liquid with [MS] is superior to that with [BF₄] in the separation of 2-chlorophenol and 2,4,6-trichlorophenol. However, further investigations on the mechanism of such interaction are needed before clearer explanations of the phenomenon can be provided.

The resolution of the samples with concentration of the ionic liquid is shown in Figure 1. The authors believe that some observed maximums and minimums for the trends do not have systematic character. Therefore, the trends appear to be due in part to measurement inaccuracy rather than to physicochemical factors. In chromatographic experiments, the optimum separation condition is determined by factors such as baseline resolution, reduction of concentration of the additive, and minimum time of analysis. Complete separation of the solutes was achieved with [EMIm][MS] and [OMIm][MS]. With [BMIm][BF₄] and [EMIm][BF₄], too little resolution was seen. Excellent separation of the solutes was achieved with an eluent of 3.0 mM/L [OMIm][MS] and 1.0 mM/L [EMIm][MS].

The previously mentioned data already showed that the nature of the ionic liquid distinctly affects the chromatographic behavior of the solutes. Mobile phases containing ionic liquids gave better results in the separation of 2-chlorophenol and 2,4,6-trichlorophenol than those without any ionic liquids. Figure 2 shows the separation of the analytes without and with 1 mM/L of ionic liquids. It is interesting to note that without ionic liquids, the peaks of 2-chlorophenol and 2,4,6-trichlorophenol are com-

pletely overlapped (Figure 2A); whereas after the addition of ionic liquids to the mobile phase, these compounds are partially (Figures 2B and C) or completely (Figures 2D and E) resolved. Also, in comparison with a pure reversed-phase system (in our case, water-methanol systems), these ionic liquids significantly improve the peak shape, decrease the peak tailing, and increase resolution. Note from Figure 2 that the detector response of 2,4,6-trichlorophenol changed significantly when the counter

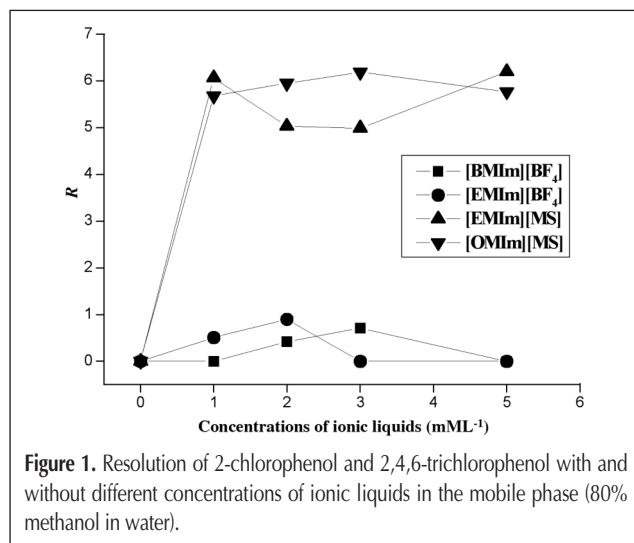


Figure 1. Resolution of 2-chlorophenol and 2,4,6-trichlorophenol with and without different concentrations of ionic liquids in the mobile phase (80% methanol in water).

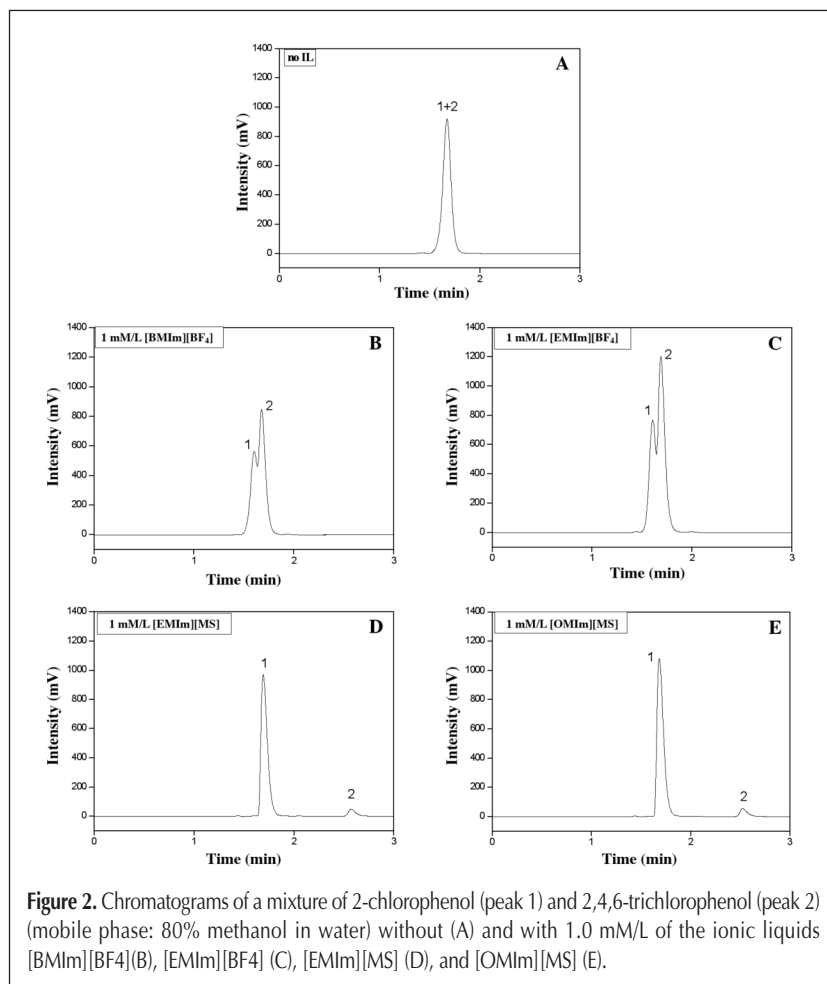


Figure 2. Chromatograms of a mixture of 2-chlorophenol (peak 1) and 2,4,6-trichlorophenol (peak 2) (mobile phase: 80% methanol in water) without (A) and with 1.0 mM/L of the ionic liquids [BMIm][BF₄](B), [EMIm][BF₄] (C), [EMIm][MS] (D), and [OMIm][MS] (E).

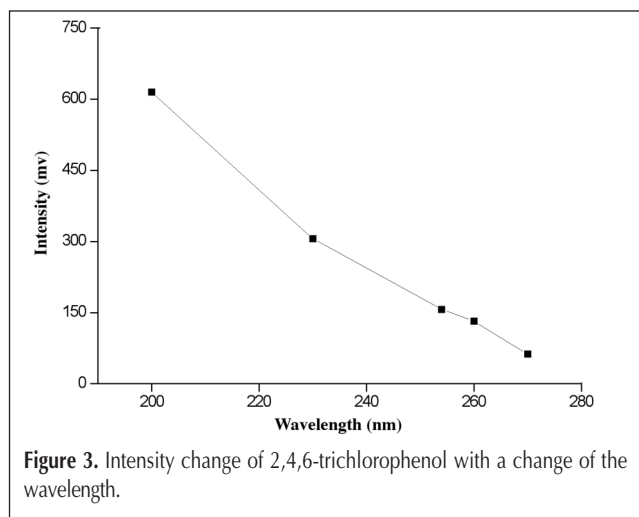


Figure 3. Intensity change of 2,4,6-trichlorophenol with a change of the wavelength.

ion used was $[\text{BF}_4]$ versus $[\text{MS}]$. Generally, the change in detector response intensity varied with the wavelength of the UV spectrum. This phenomenon was investigated and shown in Figure 3. The detector response decreases with decreasing UV wavelength. We assume that the decrease of UV absorption of 2,4,6-trichlorophenol is related to the fact that the maximum of absorption for this substance is located in the low-wave region of the UV spectrum.

Unfortunately, our investigations presented here cannot provide a satisfactory explanation for the complex effects of ionic liquids on separation. At this time, additional experiments with acetonitrile and other solvents to separate similar compounds are in progress, and a comprehensive evaluation of the interaction mechanism of ionic liquids and their application in HPLC is being developed.

Conclusions

In this paper, the effects of four ionic liquids on the retention, separation, and changes of the UV intensity of 2-chlorophenol and 2,4,6-trichlorophenol were discussed. Ionic liquids showed promising performance as additives in the separation of these solutes. The concentrations of ionic liquids and nature of ionic liquid anions can affect the separation. According to these results, it is assumed that the separation mechanism is complex. When ionic liquids are used as additives in RP-HPLC, the strong interactions between the imidazolium cation and its counterion and solutes will also play important roles. Part of the ionic liquids coat the surface of the stationary phase, on which they suppress the free silanol groups and improve the shape and resolution of the peaks. As a result, excellent separation of these solutes was achieved using 3.0mM/L $[\text{OMIm}][\text{MS}]$ and 1.0 mM/L $[\text{EMIm}][\text{MS}]$ as the modifier of eluents. The role of the ionic liquids is multiplex, and further investigations are needed in order to qualitatively and quantitatively explain the phenomena.

Acknowledgment

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

References

- X. Xiao, Z. Liang, L. Xia, and S. Jiang. Ionic liquids as additives in high performance liquid chromatography. Analysis of amines and the interaction mechanism of ionic liquids. *Anal. Chim. Acta* **519**: 207–211 (2004).
- J. Liu, J. Jonsson, and G. Jiang. Application of ionic liquids in analytical chemistry. *Trends Anal. Chem.* **24**: 20–27 (2005).
- D. Zhao, M. Wu, Y. Kou, and E. Min. Ionic liquids: Applications in catalysis. *Catal. Today* **74**: 157–189 (2002).
- C.M. Gordon. New developments in catalysis using ionic liquids. *Appl. Catal. A* **222**: 101–117 (2001).
- Z. Yang and W. Pan. Ionic liquids. Green solvents for nonaqueous biocatalysis. *Enzyme Microb. Technol.* **37**: 19–28 (2005).
- J. Sun, S. Fujita, and M. Arai. Development in the green synthesis of cyclic carbonate from carbon dioxide using ionic liquids. *J. Organomet. Chem.* **690**: 3490–3497 (2005).
- J. Liu, N. Li, G. Jiang, J. Liu, J.A. Jonsson, and M. Wen. Disposable ionic liquid coating for headspace solid-phase microextraction of benzene, toluene, ethylbenzene, and xylenes in paints followed by gas chromatography-flame ionization detection. *J. Chromatogr. A* **1066**: 27–32 (2005).
- J. Peng, J. Liu, G. Jiang, C. Tai, and M. Huang. Ionic liquid for high temperature headspace liquid-phase microextraction of chlorinated anilines in environmental water samples. *J. Chromatogr. A* **1072**: 3–6 (2005).
- K. Tian, S. Qi, Y. Cheng, X. Chen, and Z. Hu. Separation and determination of lignans from seeds of *Schisandra* species by micellar electrokinetic capillary chromatography using ionic liquid as modifier. *J. Chromatogr. A* **1072**: 181–187 (2005).
- C. He, S. Li, H. Liu, K. Li, and F. Liu. Extraction of testosterone and epitestosterone in human urine using aqueous two-phase systems of ionic liquid and salt. *J. Chromatogr. A* **1072**: 143–149 (2005).
- L. He, W. Zhang, L. Zhao, X. Liu, and S.X. Jiang. Effect of 1-alkyl-3-methylimidazolium-based ionic liquids as the eluent on the separation of ephedrine by liquid chromatography. *J. Chromatogr. A* **1007**: 39–45 (2003).
- L. He, W.Z. Zhang, B. Wen, X. Liu, and S.X. Lang. Separation of ephedrine using 1-butyl-3-methylimidazolium tetrafluoroborate ionic liquids as eluent in high-performance liquid chromatography (HPLC). *Chin. Chem. Lett.* **14**: 603–604 (2003).
- M.P. Marszał, T. Bączek, and R. Kaliszan. Reduction of silanophilic interactions in liquid chromatography with the use of ionic liquids. *Anal. Chim. Acta* **547**: 172–178 (2005).
- M.M. Waichigo, T.L. Riechel, and N.D. Danielson. Separation of ephedrine using 1-butyl-3-methylimidazolium tetrafluoroborate ionic liquids as eluent in high-performance liquid chromatography (HPLC). *Chromatographia* **61**: 17–23 (2004).
- W. Zhang, L.J. He, L. Xia, and S.X. Jiang. Ionic liquids as mobile phase additives for separation of nucleotides in HPLC. *Chin. J. Chem.* **22**: 549–552 (2004).
- C.H. Lee, J.W. Lee, and K.H. Row. Optimum solvent selectivity and gradient mode for deoxyribonucleosides in reversed-phase high-performance liquid chromatography. *J. Chromatogr. A* **1072**: 337–344 (1998).
- Y. Polyakova, Y.M. Koo, and K.H. Row. Application of ionic liquids as mobile phase modifier in HPLC. *Biotechnol. Bioprocess. Eng.* **11**: 1–6 (2006).
- W. Zhang, L. He, Y. Gu, X. Liu, and S. Jiang. Effect of ionic liquids as mobile phase additives on retention of catecholamines in reversed-phase high-performance liquid chromatography. *Anal. Lett.* **36**: 827–838 (2003).
- M. Koel. Physical and chemical properties of ionic liquids based on the dialkylimidazolium cation. *Proc. Estonian Acad. Sci. Chem.* **49**: 145–155 (2000).

Manuscript received May 31, 2007;
Revision received October 8, 2007.