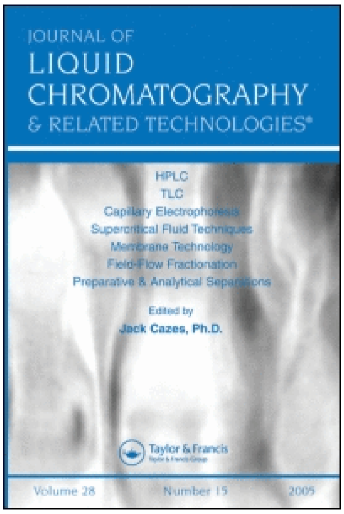

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Effect of Ionic Liquids as Additives on the Separation of Bases and Amino Acids in HPLC

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Abstract: In this paper, four bases, including cytosine, thymine, adenine, 6-chlorouracil and four amino acids, including L-histidine, L-tyrosine, L-phenylalanine, and DL-tryptophane, were separated with different types of ionic liquids as additives for the mobile phase in high-performance liquid chromatography. Four different ILs, including 1-ethyl-3-methylimidazolium tetrafluoroborate, 1-butyl-3-methylimidazolium tetrafluoroborate, 1-hexyl-3-methylimidazolium tetrafluoroborate, and 1-butyl-3-methylimidazolium chloride were used. The effects of the length of the alkyl group on the imidazolium ring and its counterion, the concentrations of the ionic liquids on the retention factor of the bases and amino acids are investigated. The results showed that the retention and resolution of the bases and amino acids were improved when ionic liquids were used as additives. The mechanism at the separation with ionic liquids as additive to mobile phase is discussed.

Keywords: Ionic liquid, Additive, Base, Amino acid, High-performance liquid chromatography

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INTRODUCTION

Ionic liquids (ILs),[1] also called room temperature ionic liquid or room temperature molten salts, are investigated widely as "green" organic solvents. They are usually composed of organic cations and inorganic or organic anions; most of them have good solubility in water and are stable in air. Ionic liquids have many availability characters in chemical reactions; they are non-volatile, nonflammable, thermally stable, and recyclable solvents, and they have some particular properties.

Over the past years, ILs played the important role of in various fields, such as organic synthesis, biology, catalysis, and electrochemistry. Similarly, ILs also have a wide range of applications in separation and analysis.[2] Armstrong et al.[3,4] used high-stability ionic liquids as stationary phases for gas chromatography. Ionic liquids were also used to modify silica as stationary phases for liquid chromatography.[5,6] Ionic liquids have been used as the novel separation media (additives) in capillary electrophoresis;[7–9] they display unique separation and analysis character. In the application of mobile phases for HPLC, Jiang et al.[10–13] used ionic liquids as additives in eluents to separate ephedrines, amines, catecholamines, and nucleotides. The results demonstrated that the addition of ionic liquid has great effects on the separation of these basic compounds, such as the decrease of band tailing, reduction of band broadening, and improvement of resolution. Kaliszan et al.[14,15] concluded that the ionic liquids can suppress the deleterious effects of silanols after ionic liquids have been used as eluents. Berthod et al.[16,17] also pointed out that the silanol screening effect is due to the cations of ionic liquids, when it is possible to increase or to decrease the solute retention factors acting upon the hydrophobic nature or chaotropic character of its anion. Until now, more and more researches that ionic liquids are used as additives to improve the separation with HPLC.[18–23]

Bases are the components of DNA and RNA; they play an important role in cell metabolism, and they have an indelible effect in the genome. Analysis of bases in body tissues and cells is widely used in biochemical, medical, and pharmacological studies. In addition, there are many products which have the additive components of DNA and RNA; also, for quality control in the food industry, concentrations of biologic molecules also need to be determined.

Amino acids are an indispensable species of nutriments in cellular and biological systems. Amino acids are the basic units which comprise proteins. They participate in the body's metabolism and physiological processes. Separation of amino acids is also widely used in the food industry, feedstuffs, and in pharmaceutical and clinical investigations. Many amino acids have been separated by HPLC, but the method usually needs a long analysis time. So, separation of amino acids by HPLC still needs some research.

Ionic liquids as mobile phase additives were not used to separate bases and small molecular amino acids. The purpose of this paper is to broaden the potential applications of ILs as mobile phase additives in liquid chromatography. In this study, four imidazolum based ionic liquids were used as additives to separate bases and amino acids on a common reversed-phase C_{18} column without any other modifiers. The possible separation mechanisms have been explored and are discussed.

EXPERIMENTAL

Apparatus

All chromatographic tests were performed with a Agilent 1100 Series modular HPLC system with a binary pump, a 20 μL sample loop and a UV–Vis detector. Separations were carried out using a column (150 mm × 4.6 mm I.D.) packed with C_{18} silica particles.

Reagents

Four ILs, 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIm][BF_4]), 1-butyl-3-methylimidazolium chloride ([BMIm][Cl]), 1-ethyl-3-methylimidazolium tetrafluoroborate ([EMIm][BF_4]), and 1-hexyl-3-methylimidazolium tetrafluoroborate ([HMIm][BF_4]), were supplied from the Center Green Chemistry and Catalysis, Lanzhou Institute of Chemical Physics (Lanzhou, China). Cytosine, thymine, adenine, and 6-chlorouracil were all domestic analytical reagents (AR). L-his, L-tyr, L-phe, and DL-trp were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The column used in the experiments was slurry packed with 5 μm C_{18} silica gel which was purchased from Fuji Silysia Chemical Ltd. (Japan). Deionized water was filtered through a 0.45 μm nylon membrane filter and was degassed ultrasonically prior to use. All the other compounds used in the experiments were of analytical grade and were used without further purification.

Chromatographic Conditions

The mixtures were stored at 277 K avoid potential errors arising from decomposition. The mobile phases were pure water or water with different concentrations of ILs in the water. The column was flushed for at least 15 minutes to remove the ILs used at the previous concentration and to fully equilibrate the column. The injection volume of the mixture was 20 μL. Analyses

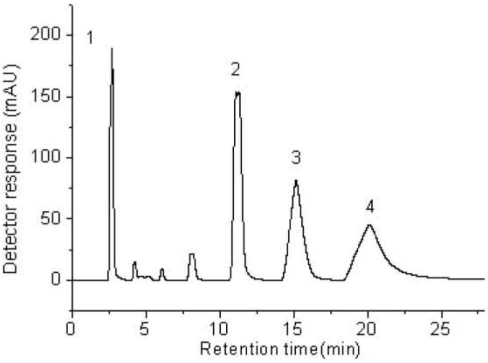

Figure 1. Separation of four bases including cytosine (1), thymine (2), adenine (3) and 6-chlorouracil (4) with pure water as the mobile phase. Chromatographic conditions: C18 (150 mm × 4.6 mm I.D.), flow-rate: 1 ml min^{-1}, injection volume: 20 μl and detection: UV at 254 nm.

were performed throughout at ambient temperature at a flow rate of 1.0 mL/min in isocratic mode, and the UV detector was set at λ of 254 nm.

RESULTS AND DISCUSSION

Effect of Concentrations of [BMIm][BF_4] on Separation of Bases and Amnio Acids

First, separation of bases with pure water as mobile phase was examined as shown in Figure 1. The retention time is long and the peak is broad.

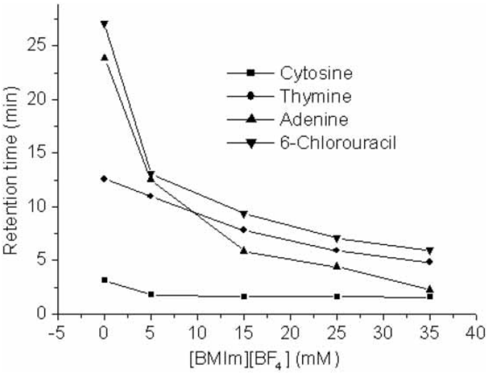

Figure 2. The retention times of bases with different concentrations of [BMIm][BF_4] as the mobile phases. Other chromatographic conditions are the same as in Figure 1.

Figure 2 shows the retention times of the bases when different concentrations of [BMIm][BF_4] (0–35 mM) were added to water as additives to the mobile phase. With the increase of the concentration of [BMIm][BF_4], the retention times of the bases were decreased, apparently. And, the retention of adenine and 6-chlorouracil were influenced much more than cytosine and thymine. For example, when the mobile phase was changed from pure water to 5 mM [BMIm][BF_4], the retention times of adenine and 6-chlorouracil were shortened nearly twice as much as before. Changes of retention times of cytosine and thymine with different concentrations of [BMIm][BF_4] are relatively small.

Separation of amino acids was performed with the same chromatographic conditions of bases. Separation of four amino acids, including L-his, L-tyr, L-phe, and L-trp with pure water as the mobile phase was examined first, as shown in Figure 3. The dependencies of retention times on different concentrations of [BMIm][BF_4] (0–35 mM) in the eluent were also investigated, as shown in Figure 4, and similar dependencies are obtained for all analytes. The increase of the [BMIm][BF_4] concentration in the eluents causes a decrease in retention of all analytes.

Effect of Alkyl Groups of the Imidazolium Cation of Ionic Liquids

The effect of different alkyl groups on imidazolium cations was investigated. Addition of the same concentration of three ionic liquids with different alkyl groups ([EMIm][BF_4], [BMIm][BF_4], and [HMIm]BF_4) was used to study this effect on the separation of bases (Figure 5). Compared with the separation of bases with pure water as mobile phase

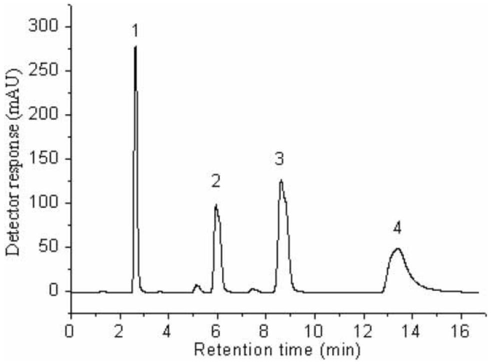

Figure 3. Separation of four amino acids including L-his (1), L-tyr (2), L-phe (3), and DL-trp (4) with pure water as the mobile phase. Chromatographic conditions: C18 (150 mm × 4.6 mm I.D.), flow-rate: 1 ml min^{-1}, injection volume: 20 μl and detection: UV at 254 nm.

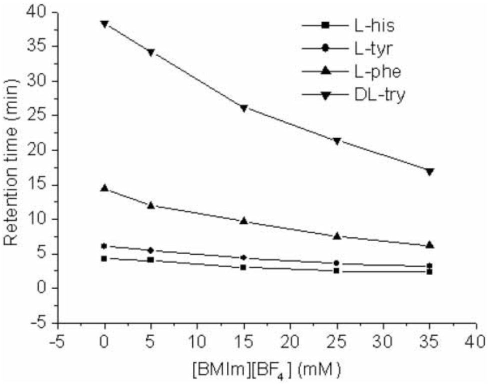

Figure 4. The retention times of amino acids with different concentrations of [BMIm][BF$_4$] as the mobile phases. Other chromatographic conditions are the same as in Figure 3.

(Figure 1), the separation of bases using ILs as mobile phase modifiers were time saving and efficient. With the increase of alkyl chain length on the imidazolium cations, the retentions of bases are decreased and the peak shapes are improved.

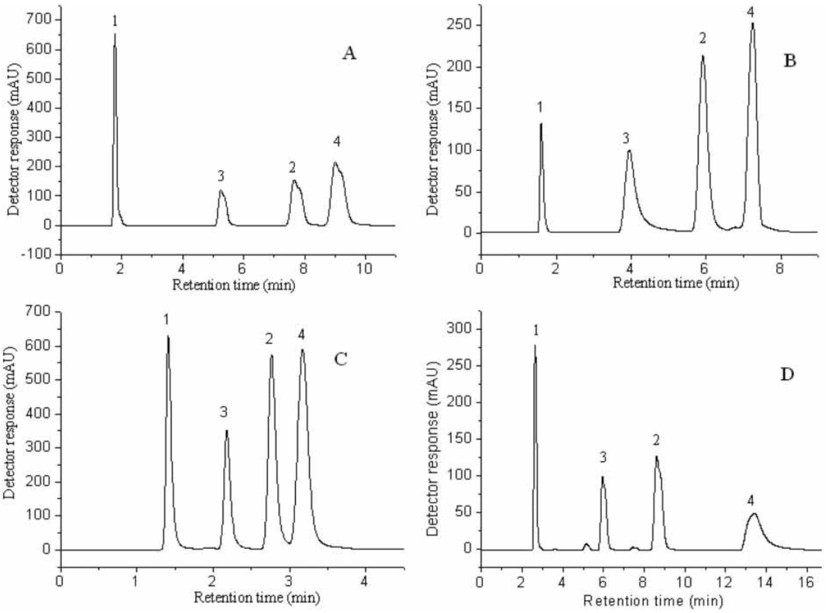

Figure 5. Separation of four bases including cytosine (1), adenine (3), thymine (2) and 6-chlorouracil (4) with (A) 25 mM [EMIm][BF$_4$], (B) 25 mM [BMIm][BF$_4$], (C) 25 mM [HMIm][BF$_4$], and (D) 25 mM [BMIm][Cl] as the mobile phase. Other chromatographic conditions are the same as in Figure 1.

These effects[10] may be attributed to the competition between imidazolium cations and the polar groups of the analytes for the silanol group on the alkylsilica surface, and also to the formation of weak bilayer electronic structures on the C_{18} column. In our experiments, the results demonstrate ILs could become adsorbed onto the C_{18} column and formed a dynamic pseudostationary phase; the phenomenon illustrates that the longer alkyl chain of the imidazolium cations may become adsorbed easier for the stronger hydrophobic interaction between ILs and C_{18} stationary phase. Bases have experienced more electrostatic repulsion from pseudostationary phase when [HMIm][BF_4] is used as mobile phase additive. This phenomenon shows that the character of the cation and the length of the hydrocarbon chain were so important that it has a strong influence on the processes of sorbate distribution between stationary and mobile phases. The phenomenon is apparent retention time in the shortened times for exponential times. With the existance of [HMIm] cationic modifiers, the interactions of the silanol and alkyl groups become dominant, resulting in low numerical values of k.

The separation of amino acids was also performed with three different alkyl groups in ionic liquids ([EMIm][BF_4], [BMIm][BF_4], and [HMIm]BF_4) as mobile phase additive, as shown in Figure 6. Compared with pure water as

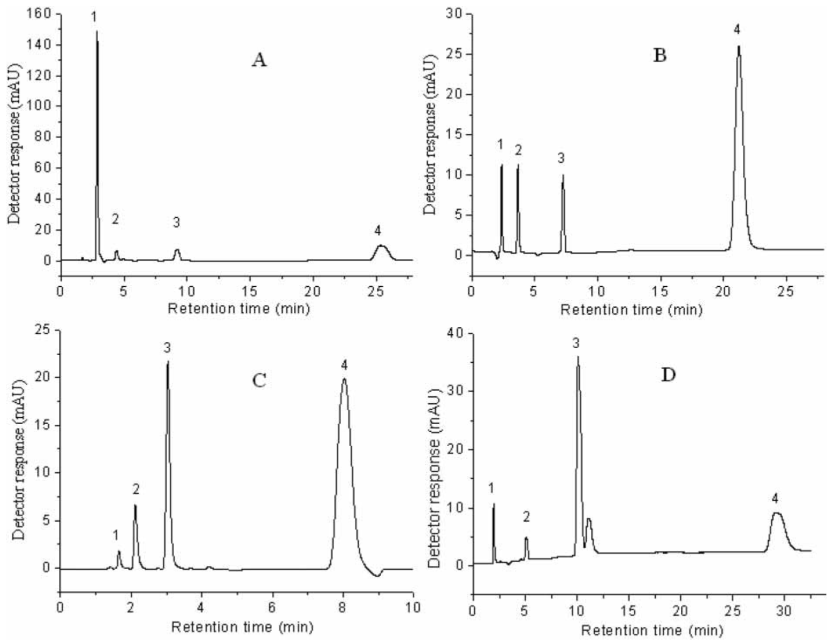

Figure 6. Separation of four amino acids including L-his (1), L-tyr (2), L-phe (3), DL-trp (4) with (A) 25 mM [EMIm][BF_4], (B) 25 mM [BMIm][BF_4], (C) 25 mM [HMIm][BF_4], and (D) 25 mM [BMIm][Cl] as the mobile phase. Other chromatographic conditions are the same as in Figure 3.

mobile phase, when [EMIm][BF_4] (Figure 6A) and [BMIm][BF_4] (Figure 6B) were used as modifiers, the retention times for the separation of amino acids is not shortened. It is possible that amino acids are zwitterionic analytes, especially acidic amino acids such as L-tyr, L-phe, and DL-trp. Acidic analytes will have some electrostatic attraction with the pseudostationary phase, so the retention time may be increased. But, with [HMIm][BF_4] as the mobile phase additive, the retention times of amino acids were shortened a small amount, as shown in Figure 6C. These phenomena explain that there exist multiple interactions with the pseudostationary phase and amino acids, such as electrostatic interaction, hydrogen bonding, ion-pairing effects, etc.

Effect of Different Counterions of Ionic Liquids

The counterions of the imidazolium ionic liquids also have significant effects on separation of bases and amino acids. In this work, two different ionic liquids, [BMIm][BF_4] and [BMIm][Cl], were used to separate bases (Figures 5B and 5D) and amino acids (Figures 6B and 6D). The modifier with BF_4^- as a counterion provides much better separation for bases and amino acids, and the retention times are shorter. The better separation was obtained with [BMIm][BF_4] as mobile phase modifier, compared with [BMIm][Cl^-] as modifier, as shown in the figures. It seems that $[BMIm]^+$ with BF_4^- is superior to that with Cl^- in the separation of bases and amino acids.

Mechanism of Ionic Liquids as Additives on the Separation of Bases and Amino Acids

We note that the positively permeated imidazolium groups not only existed in the bulk solution, but also was coated onto the RP C_{18} column wall when ionic liquids were used in HPLC. The coated ionic liquids could form a dynamic pseudostationary phase with imidazolium cations. Imidazolium cations can interact with silanol groups and compete for the silanol groups on the alkylsilica surface with the polar group of the analytes. Therefore, it can effectively shield the residual silanols[14] and improve the peak shapes, while also decreasing the retention time of the analytes such as bases. But, if the analytes are acidic or zwitterionic, the retention times may not be shortened. The dynamic stationary phase may bring multiple interactions between the specimens and dynamically coated ionic liquids.

CONCLUSIONS

In this paper, the successful separation of bases and amino acids has been achieved using [BMIm][BF_4], [BMIm][Cl], [EMIm][BF_4], and

[HMIm][BF_4] as mobile phase modifiers. The concentrations of ionic liquids, the lengths of alkyl groups on the imidazolium ring, and the different counterions of imidazolium ionic liquids can also affect the separation. The separation of specimens such as bases and amino acids can be improved with ionic liquids as additives to water as mobile phase; the retention time was shortened and the column efficiency was increased. Ionic liquids could become coated dynamically onto the C_{18} silica for the hydrophobic interaction, and the coated ionic liquid would form a dynamic pseudostationary phase to interact with specimens like bases and amino acids.

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