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Adsorption Equilibrium of Caffeine and Theophylline on C_{18} Column Using Ionic Liquids as Additives

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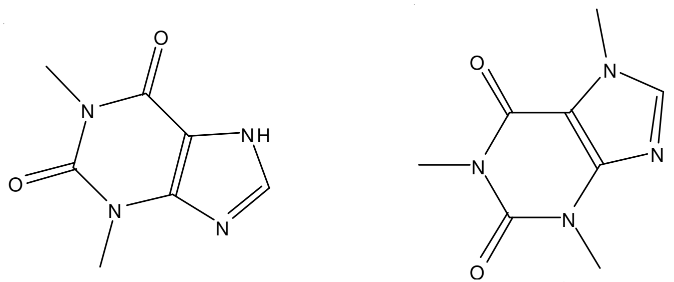
Comparison of caffeine and theophylline adsorption isotherm on C_{18} column using ionic liquids as mobile phase additives and the competitive adsorption of the two compounds were investigated. The experimental parameters of the equilibrium isotherms were determined *via* Langmuir isotherm model. The effects of the concentrations and the alkyl chain length of imidazolium ionic liquids on C_{18} particles were investigated.

Key Words: Adsorption isotherm, Ionic liquids, Caffeine, Theophylline.

INTRODUCTION

Xanthines, the derivatives of xanthine, are a group of alkaloids that are commonly used as mild stimulants and bronchodilators, particularly in treating the symptoms of asthma. Methylated xanthine derivatives from natural plant include caffeine and theophylline (Fig. 1), inhibit phosphodiesterase and antagonize adenosine. Different analytical techniques have been developed for the simultaneous determination of these three methylxanthines. However, liquid chromatography is the most commonly used method. Reversed-phase chromatography was employed because of the different characteristics of binding with C_{18}[1].



(a) (b)

Fig. 1. Structures of theophylline (a) and caffeine (b)

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The adsorption isotherm, which is a basic thermodynamic property of the separation process, shows the relationship between the solute concentration in the stationary phase and that in the mobile phase. The parameters of the adsorption isotherm can be determined by fitting the various models to the experimental data. By this method, the individual band profile of separated samples under variety of conditions was possible predicted and used to optimize the conditions[2-4]. The success of the experiments and modeling is associated with the accuracy of the adsorption isotherms and their parameters[5,6].

Ionic liquids or room-temperature ionic liquids, which are normally composed of relatively large organic cations and inorganic/organic anions, are investigated widely as green chemistry. They are good solvents, highly polar, environmentally benign, non-volatile, non-flammable and stable in air or water and so on. Because of these particular properties, in the recent years, ionic liquids played the important role of in various fields, such as catalysis in organic synthesis[7], stationary phases in gas chromatography[8], buffer electrolytes in electrochemistry[9] and mobile phase additives in reversed phase liquid chromatography[10-12].

In this study, 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF_4]), 1-ethyl-3-methylimidazolium tetrafluoroborate ([EMIM][BF_4]) and 1-hexanyl-3-methylimidazolium tetrafluoroborate ([HMIm][BF_4]) were first applied as mobile phase additives to investigate the adsorption isotherms of caffeine and theophylline. All the data were correlated to Langmuir isotherm on a C_{18} column. Furthermore, the adsorption characteristics on the C_{18} particles were obtained through the isotherms of caffeine and theophylline.

EXPERIMENTAL

Caffeine and theophylline were purchased from Sigma (St. Louis, MO, USA). Methanol was obtained from Pure Chemical Co., Ltd. (Ansan, Korea). Distilled water was filtered using a vacuum pump (Division of Millipore, Waters, USA) and a filter (HA-0.45, Division of Millipore, Waters, USA) before use. C_{18} particles were purchased from Merck KGaA Co., Ltd (RP-18, 15 μm, Darmstadt, Germany). All other solvents used in the experiment were HPLC or analytical grade. All the samples were filtered by using a filter (MFS-25, 0.2 μm TF, Whatman, U.S.A.) before injection into the HPLC system.

The chromatography system consisted of Waters 600s Multi solvent Delivery System, Waters 616 liquid chromatography (Waters Associates, Milford, MA, USA), a Rheodyne injector (20 μL sample loop) and a variable wavelength 2487 UV dual channel detector. Data processing was carried out with a Millenium 3.2 consisted of HP Vectra 500PC. Under the chromatographic condition, the flow rate was 0.5 mL/min, the injection volume was 10 μL and the UV wavelength was 274 nm. A C_{18} column (5 μm particles, 10 nm pore size, 4.6 mm × 150 mm) from RS Tech. Corporation (Daejeon, Korea), where methanol/water (30/70, v/v) were the mobile phase, were used to determine the free concentration of the compound *via* the static method.

Static methods: The static method was performed on the C_{18} particles. 20 mg C_{18} particles were placed into several flasks, respectively, to which 1.0 mL of caffeine, theophylline and mixture solution with a concentration ranging from 0.01 to 0.5 g/L was added. The procedure was repeated with different types and concentrations of ionic liquids. Then the mixture was stored at room temperature for 72 h and the supernatant was collected and filtered (0.2 μm). The concentrations of unadsorbed caffeine and theophylline in the solution were determined on a C_{18} column at room temperature. The concentrations of absorbed caffeine and theophylline on C_{18} particles were calculated according to the decreased concentrations of caffeine and theophylline in the initial solvents.

RESULTS AND DISCUSSION

Calculation of the concentrations of the two compounds: The concentrations of caffeine and theophylline adsorbed on the C_{18} particles in the flasks were measured at different concentrations after the equilibrium adsorptions of these compounds were reached. The unadsorbed concentrations of the compounds in the liquid phase were determined by HPLC quantitatively and all analyses were carried out within five times. The concentration was determined following constructed calibration curves: $Y = 64357.276X - 488.574$, $r^2 = 0.9994$ (regression coefficient) for caffeine; $Y = 69451.736X - 334.116$, $r^2 = 0.9990$ for theophylline. Y is the peak area (mAU s) and X is the concentration of target compound in the solution.

The adsorbed amount (Q) of caffeine or theophylline on C_{18} particles is determined as follows:

$$Q = \frac{(C_0 - C) \times V}{M} \tag{1}$$

where Q (mg/g) is the adsorbed amount, C_0 (g/L) is the initiator concentration, C (g/L) is the unabsorbed concentration, V (L) is the volume of the sample solvent and M (g) is the mass of the C_{18} particles.

Determination of adsorption isotherms: The different chromatographic behaviours of caffeine and theophylline were attributed to their structures (Fig. 1) and these influences of these two compounds in chromatography can affect the adsorption isotherm. Two compounds have methyl groups on different positions, which affect adsorption on the C_{18} particles.

By previous research, Langmuir adsorption isotherm was applied to the study:

$$Q = \frac{aC}{1 + bC} \tag{2}$$

where C (g/L) is the equilibrium concentrations of the solute in the liquid phase and Q (mg/g) is the equilibrium concentration of the solute in the solid-phase. a is the maximum adsorption capacity and b is the apparent dissociation constant. Tables 1 and 2 show the parameters fitted by Langmuir adsorption isotherm model. The regression coefficients (r^2) are from 0.9642 to 0.9884 for single theophylline and

TABLE-1
PARAMETERS IN ADSORPTION ISOTHERM OF CAFFEINE AND THEOPHYLLINE WITH DIFFERENT CONCENTRATIONS OF [BMIM][BF$_4$]

Concentration (mM)	Theophylline (single)			Theophylline (mixture)		
	a	b	r^2	a	b	r^2
0.00	3.3047	0.7704	0.9119	3.4312	0.9753	0.9146
0.25	1.3994	1.0054	0.9884	1.4633	1.0458	0.9768
0.50	1.3787	1.8052	0.9704	1.3771	1.0310	0.9526
1.00	1.3269	0.9732	0.9642	1.4289	1.2196	0.9774
2.00	1.3538	3.9318	0.9873	1.4564	1.1288	0.9789
3.00	1.3560	1.5491	0.9852	1.4906	1.7789	0.9154
	Caffeine (single)			Caffeine (mixture)		
0.00	3.7251	0.4182	0.9115	3.4569	0.01118	0.9747
0.25	3.2479	0.3055	0.9704	3.3012	0.3131	0.9748
0.50	2.7753	0.8974	0.9843	2.8611	1.5686	0.9683
1.00	2.9028	1.2532	0.9507	2.9804	1.3003	0.9706
2.00	3.0193	0.4439	0.9429	3.1422	0.3260	0.9573
3.00	3.2268	1.3157	0.9847	3.2154	1.7540	0.9924

TABLE-2
PARAMETERS IN ADSORPTION ISOTHERM OF CAFFEINE AND THEOPHYLLINE WITH DIFFERENT IONIC LIQUIDS AT 1 mM

Ionic liquids	Theophylline (single)			Theophylline (mixture)		
	a	b	r^2	a	b	r^2
[EMIM][BF$_4$]	1.2435	1.1802	0.9845	1.4085	1.7367	0.9944
[BMIM][BF$_4$]	1.3269	0.9732	0.9642	1.4289	1.2196	0.9774
[HMIM][BF$_4$]	1.9262	1.0053	0.9836	1.9547	0.8867	0.9476
	Caffeine (single)			Caffeine (mixture)		
[EMIM][BF$_4$]	2.4132	1.4665	0.9807	2.5013	0.7546	0.9601
[BMIM][BF$_4$]	2.9028	1.2532	0.9507	2.9804	1.3003	0.9706
[HMIM][BF$_4$]	3.2245	1.5131	0.9905	3.3155	0.9756	0.9817

from 0.9154 to 0.9789 for theophylline in mixture. It ranges from 0.9429 to 0.9843 for single caffeine and from 0.9573 to 0.9924 for mixed caffeine. All of the regression coefficients are highly proved that Langmuir equation is suitable for this adsorption isotherm. The difference of adsorption isotherms causes the separation of samples.

Effect of ionic liquid on adsorption: From Fig. 2, it can be seen that after adding a spot of [BMIM][BF$_4$], the retention factor (k) decreased obviously with adsorbed amount (Q) decreasing. It proved that [BMIM][BF$_4$] was effective to the adsorption of caffeine and theophylline. According to the mechanism of ionic liquids used as mobile phase additives[10], imidazolium cations of ionic liquids existed in both solution and coated on the stationary phase. In this study, when other change factors were abandoned, the static adsorption effects among solutes, mobile phase, C$_{18}$ particles and ionic liquid, [BMIM][BF$_4$] competed with caffeine and theophylline

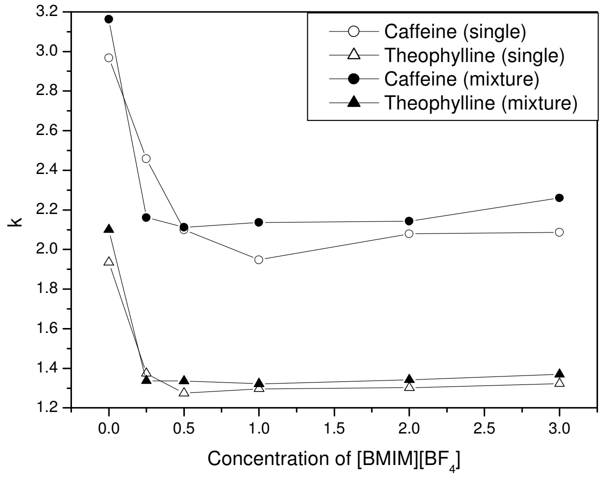

Fig. 2. Retention factors (k) of caffeine and theophylline in [BMIM][BF_4] at different concentrations

coating to C_{18} particles, which lead to the decrease of adsorbed amounts. Shorter retention time was obtained on HPLC column when comparing with the one without ionic liquids.

Effect of the concentration of ionic liquid on adsorption: The parameters of Langmuir model for single and mixed caffeine and theophylline with different concentration of [BMIM][BF_4] are shown in Table-1. The retention factors (k) of caffeine and theophylline with different concentrations of [BMIM][BF_4] were shown in Fig. 2. The chromatogram of the mixture of caffeine and theophylline at different concentrations of [BMIM][BF_4] was shown in Fig. 3.

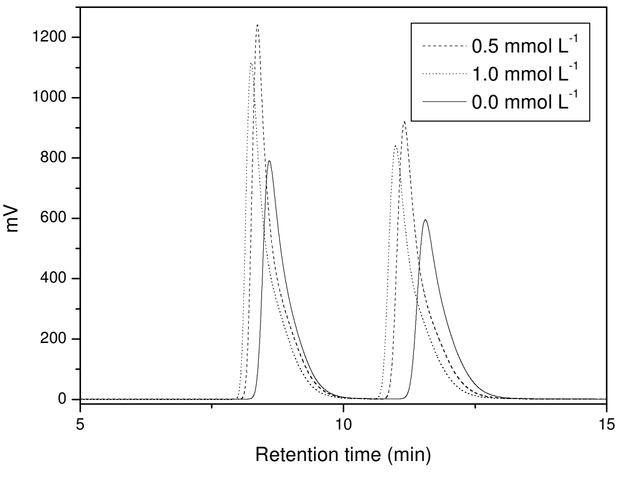

Fig. 3. Chromatogram of the mixture of caffeine and theophylline at different concentrations of [BMIM][BF_4]

In Table-1, the values of the maximum adsorption capacity (a) present a decrease at first and then increased with the concentration of [BMIM][BF_4] increasing from 0.25 mmol/L to 3.00 mmol/L and rock bottom of the concentration on the trend line is at 0.50 mmol/L. The maximum adsorption capacity of single caffeine and theophylline were smaller than the one of mixed caffeine and theophylline. The apparent dissociation constant (b) did not show the same trend but usually had small values.

In Fig. 2, the retention factor showed the same trend as the one which was obtained from adsorption isotherm. It can be seen that with the concentration of [BMIM][BF_4] increasing, the retention factor decreased at first and then increased indistinctively. In Fig. 3, the retention time decreased obviously until the concentration of [BMIM][BF_4] was 0.50 mmol/L, but increased when the concentration of [BMIM][BF_4] was more than 1.00 mmo/L. This trend revealed that low concentration of ionic liquids could compete with solutes to adsorb on C_{18} particles because of the solutes with low adsorbed amounts and short retention times. When the concentration of ionic liquids increased in the mobile phase, the dissociative ionic liquids affect solutes and increased the adsorbed amounts and retention times.

The interaction between caffeine and theophylline was also affect the adsorption. In Fig. 2, the retention factor between single and mixed caffeine was larger than theophylline which can be explained by the competitive interaction of these two compounds. In addition, the excessive methyl of caffeine would be repulsive to compete with ionic liquids.

Effects of alkyl chain length of ionic liquids on adsorption: In order to investigate the effects of alkyl chain length of ionic liquids to the adsorption of caffeine and theophylline, three kinds of ionic liquids were add to mobile phase at the same concentration of 1.0 mmol/L. The parameters in adsorption isotherm of caffeine and theophylline with different ionic liquids at 1.0 mmol/L were shown in Table-2. All the regression coefficients were higher than 0.91. It revealed that the maximum adsorption capacity (a) of caffeine and theophylline increased with the length of alkyl chain of ionic liquids increasing, which was due to the increase of the "hydrophobia" of these silanol-blocking agents.

The retention factors of single and mixture of caffeine and theophylline with [EMIM][BF_4], [BMIM][BF_4] and [HMIM][BF_4] in mobile phase were shown in Fig. 4. The retention factors show the same trend as one of the adsorption isotherm.

Conclusion

In this work, comparisons of caffeine and theophylline adsorption isotherm on C_{18} column using ionic liquids as mobile phase additives and the competitive adsorption of the two compounds were investigated. The experimental parameters of the equilibrium isotherms were estimated *via* Langmuir isotherm model. The effects of the concentrations and the alkyl chain length of imidazolium-based ionic liquids toward C_{18} particles were analysis, respectively. Ionic liquids are effective to the adsorption of caffeine and theophylline, after adding the [BMIM][BF_4], the

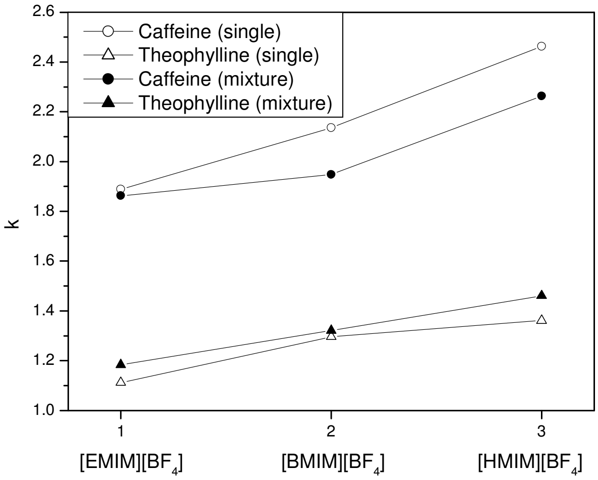

Fig. 4. Retention factors (k) of caffeine and theophylline in different types of ionic liquids at the same concentration 1 mM. (1) [EMIM][BF$_4$]; (2) [BMIM][BF$_4$]; (3) [HMIM][BF$_4$]

retention times change obviously and the chromatography peaks are more sharper than the one without ionic liquids. When increasing the concentration of [BMIM][BF$_4$], the retention time decrease at first then increase indistinctively. And the retention time increase as increasing the alkyl chain length of imidazolium based ionic liquids.

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