A New Ionic Liquids-Based Monolithic Column for Determination of Caffeine and Theophylline

Tao Zhu, Wentao Bi, Kyung Ho Row

Department of Chemical Engineering, Inha University, Nam-Ku, Incheon 402-751, Korea

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ABSTRACT: The present study has concentrated on finding a new stationary phase in liquid chromatography. To improve the selectivity of monolithic column, a new ionic liquids–based (ILs-based) monolithic column (150 × 4.6 mm i.d.) is synthesized. Characteristic and evaluation of monolithic column are investigated by field emission-scanning electron microscopy (FE-SEM) and determination of caffeine and theophylline in high performance liquid chromatography (HPLC). FE-SEM images show that this monolithic column has a porosity structure. At the condition of mobile phase was 0.06 mol L^{-1} Na_2HPO_4 (pH 9.0) and flow rate was 0.7 mL min^{-1}, a good linear relationship was demonstrated when the concentrations of caffeine and theophylline were in the range of 0.1–60.0 μg mL^{-1}. These two compounds can obtain better resolution on the ILs-based monolithic column than non-ILs monolithic column, and the recoveries ranged from 97.40% to 108.00% and the interday and intraday relative standard deviations were less than 5%. The HPLC method, developed in this study, was proved to be acceptable for drugs assay, and this ILs-based monolithic column as the stationary phase was a potential tool for future HPLC separation. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 118: 3425–3430, 2010

Key words: HPLC; ionic liquids; monolithic column; caffeine; theophylline

INTRODUCTION

Room temperature ionic liquids (RTILs) are salts that are composed of cations and anions as important components of green chemistry.[1,2] The cations and anions of RTILs are larger and more complex, and their crystalline structure breaks down easily and the salt becomes liquid.[3] RTILs have possessed high thermal stabilities and negligible vapor pressures making them attractive alternatives to environmentally unfriendly organic solvents.[4] They have obtained increasing interest in the last few years as novel solvents for synthesis, separations, electrochemistry, and process chemistry, and they have been used in a variety of different areas within modern chemistry and shown to provide unique properties.[5–11]

Monolithic column is a single rod in stainless column, which is made up of highly interconnected channel network with high effective porosity and low column backpressure. Depending on the nature of the monolithic material, it can be divided into two major types: organic polymer-based monolithic material and silica-based monolithic material.[12–14] It had attracted considerable attention in HPLC due to its large adsorption capacity, simple preparation procedure and excellent performance. Based on these advantages, ionic liquid-based polymer sorbent was successfully used as a special sorbent in a solid-phase extraction (SPE) process to isolate caffeine and theophylline from green tea.[15] It is true that reverse-phase HPLC with C_{18} column as the stationary phase is the most widely used in the world, and the present study has concentrated on finding a new stationary phase instead of C_{18} column for analysis of drugs. With this purpose, many studies put the emphasis on improvement of different stationary phases with various structures in order to improve the efficiency and selectivity.[16,17] According to the advantages of ILs and monolithic column, a new ILs-based monolithic material was synthesized, and it can be as an ideal alternative to the conventional stationary phases with faster separations and low backpressure.

Caffeine is a methylxanthine that occurs naturally in some beverages and is also used as a pharmacological agent. Its pharmacological effect is to stimulate the central nervous system, relaxes smooth muscle, and produce agitation. Theophylline, as a member of the xanthine family, bears a structural and pharmacological similarity to caffeine.[18] They are widely distributed in some plants, such as tea,

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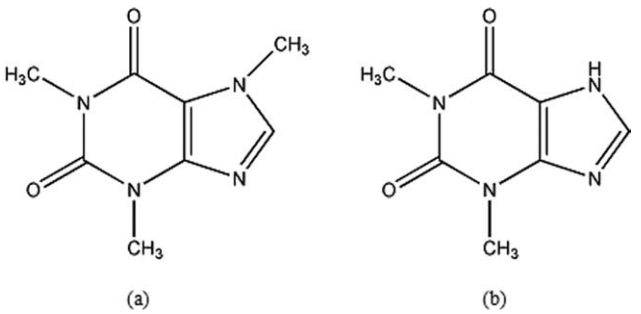

Figure 1 Chemical structures of caffeine (a) and theophylline (b).

cocoa,[19] and so on, and they are clinical drugs which used in therapy for respiratory diseases such as asthma under a variety of brand names.[20,21] Therefore, a rapid and cheap analytical method needs to be established for their simultaneous determination. The structures of caffeine and theophylline are shown in Figure 1.

The aim of this work was to synthesize a new ILs-based monolithic material as a stationary phase material in HPLC. After characteristic and testing of the obtained material, a method was developed for determination of caffeine and theophylline using the ILs-based monolithic column.

EXPERIMENTAL

Reagents and materials

Methacrylic acid (MAA) and glycidyl methacrylate (GMA) were bought from Sigma (St Louis, MO). Ethylene glycol dimethacrylate (EGDMA) was purchased from Fluka (Buchs, Switzerland). Dodecanol was obtained from Acros organics (NJ). Cyclohexanol and 2,2′-azobis (isobutyronitrile) (AIBN) were purchased from Junsei Chemical (Japan) and refined before use. Chlorobutane was bought from Tokyo Chemical (Japan) and imidazole was obtained from Aldrich (Milwaukee, WI). Methanol, toluene, and sodium phosphate was bought from Pure Chemical (Ansan, Korea). All the other solvents used in the experiment were HPLC or analytical grade.

Apparatus

The chromatography system consisted of Waters 600s Multi Solvent Delivery System, Waters 616 liquid chromatography (Waters Associates, Milford, MA), a Rheodyne injector (20 μL sample loop) and a variable wavelength 2487 UV dual channel detector. Autochro-2000 software (Younglin, Korea) was used as data acquisition system. Deionized water was filtered with a vacuum pump (Division of Millipore, Waters) and a filter (HA-0.45, Division of Millipore, Waters) before use. All the samples were filtered by using a filter (MFS-25, 0.2 μm TF, WHATMAN) before injection into the HPLC system.

Preparation of ILs and ILs-based monolithic column

Chlorobutane (5 g, 0.054 mol) and imidazole (3.68 g, 0.054 mol) was added to a clean, dry round bottom flask containing a magnetic stir bar, then toluene (50 mL) was added into the bottle as solvent. The reaction was carried out at 100°C for about 12 h. The obtained viscous product of ionic liquid was washed several times with cold hexane.

The ILs-based monolithic column was prepared by an *in situ* polymerization. First, a mixture consisting of 0.25 mL MAA, 1.0 mL GMA, 1.50 mL EGDMA, 1.50 mL dodecanol, 1.50 mL cyclohexanol, 0.6 mL ILs, and 0.06 g AIBN were purged with helium gas for 15 min. Then the stainless steel column (150 × 4.6 mm i.d.) sealed at the bottom was filled with the polymerization mixture and then sealed at the top. After the polymerization was allowed to proceed at 55°C for 24 h, the column was flushed with methanol to remove the porogen and other soluble compounds present in the polymer rod. Finally, the column was washed with 0.05 mol L^{-1} acetate buffer followed by deionized water until the eluent was neutral. The non-ILs monolithic column was prepared and treated in an identical manner. Synthesis processes of ILs and ILs-based monolithic column were depicted in Figure 2.

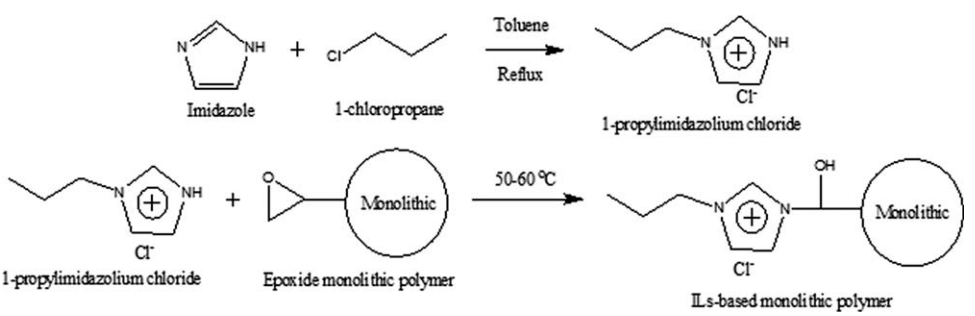

Figure 2 Synthesis processes of ILs and ILs-based monolithic column.

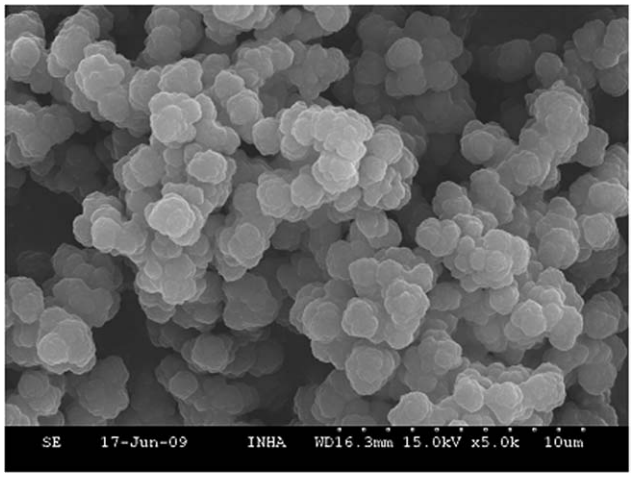

Figure 3 FE-SEM images of non-ILs blank polymers (a) and ILs polymers (b).

Characteristic

The monolithic materials in the column were pumped out and cut into small pieces followed by drying under vacuum at 50°C overnight. Microstructures of the dried monolithic samples were observed by FE-SEM (S-4300 model, Hitachi, Japan,) and it was operated at the voltage of 15 kV.

Sample preparation and chromatography

Stock solutions of caffeine and theopyhlline were prepared at a concentration of 1000 μg mL^{-1} by dissolving 10 mg of drugs in 10 mL of methanol, respectively. For method development, a series mixed standard solution containing caffeine and theopyhlline was prepared at seven concentration levels covering a range of 0.1–60.0 μg mL^{-1}. Phosphate buffers used in the mobile phase were prepared by adding phosphate to deionized water at concentrations ranging from 0.01 to 0.10 mol mL^{-1}. pH was adjusted as required by adding appropriate amounts of phosphoric acid.

The analysis was performed on an ILs-based monolithic column (150 × 4.6 mm i.d.), and the monolithic column was operated at 25°C. The optimum mobile phase was 0.06 mol mL^{-1} Na_2HPO_4 (pH 9.0), and the flow rate was 0.7 mL min^{-1}. The chromatogram was monitored at a wavelength of 274 nm.

RESULTS AND DISCUSSION

Synthesis of ILs-based monolithic column

The monomer ratio MAA/GMA was an important factor for the properties of the monolithic column. Five ILs-based monolithic columns were synthesized with different ratio of MAA/GMA in the polymerization mixture while keeping other experimental conditions constant. The porosity structure of the five monolithic columns was evaluated by FE-SEM, and the separation efficiencies of the five monolithic columns were tested by injecting 10 μL mixture of caffeine and theophylline at the concentration of 5.0 μg mL^{-1} into the ILs-based monolithic columns in HPLC when 0.06 mol L^{-1} Na_2HPO_4 (pH 9.0) was used as the mobile phase at UV 274 nm. The results of FE-SEM and HPLC showed that when the volume ratio increased (0, 1/5, 1/4, 1/3, 1/2, 1/1, respectively), the porosity structure of monolithic column became more and more poor, and the resolution of caffeine and theophylline decreased (0, 1.84, 1.62, 1.45, 1.33, 1.18, respectively). There is an epoxy group in the structure of GMA, and this group can be easily broken and can react with the ILs. Based on this reason, if the ratio of GMA is relatively small, it will have not enough epoxy group reacts

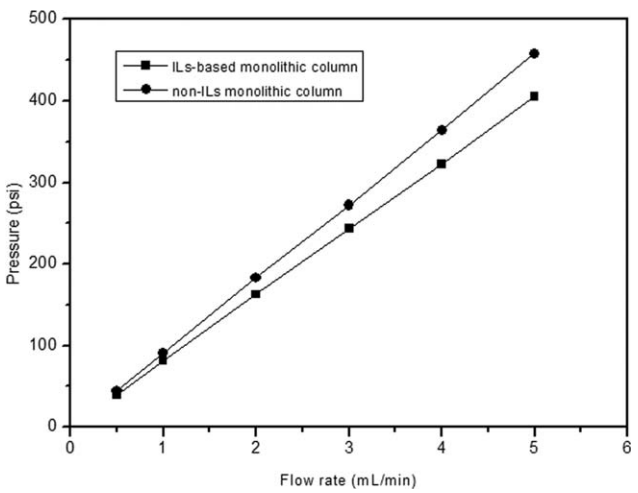

Figure 4 Change of backpressure with different flow rate for ILs-based monolithic column and non-ILs monolithic column in HPLC (Mobile phase: deionized water; column size: 150 × 4.6 mm i.d.).

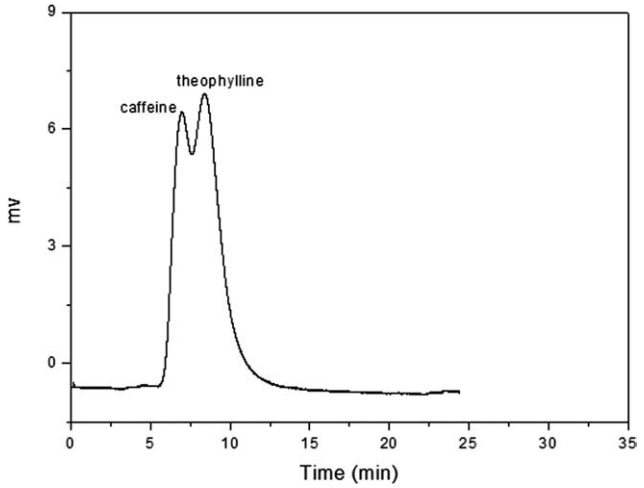

Figure 5 Chromatogram of caffeine and theophylline (5.0 μg mL^{-1}) on non-ILs monolithic column (Mobile phase composition: 0.06 mol L^{-1} Na_2HPO_4, pH 9.0).

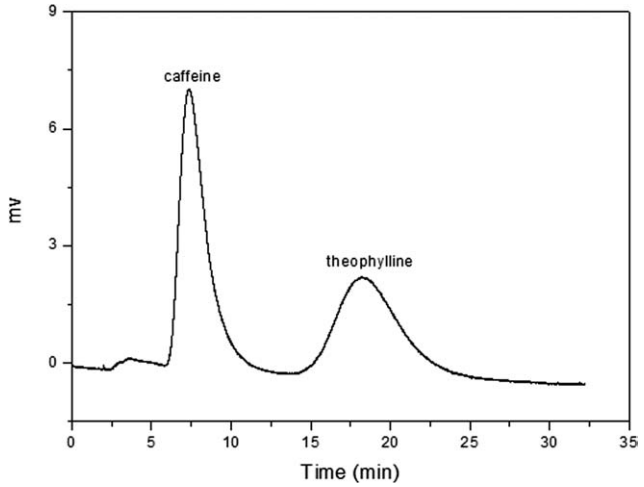

Figure 6 Chromatogram of caffeine and theophylline (5.0 μg mL^{-1}) on ILs-based monolithic column (Mobile phase composition: 0.06 mol L^{-1} Na_2HPO_4, pH 9.0).

with the ILs and the performance of the ILs-based monolithic column will be reduced, and if the ratio of GMA is so large, the reaction of epoxy group and ILs will become more difficult. Considering various factors, the optimum MAA/GMA volume ratio was 1/4.

Performance evaluation

Non-ILs monolithic column and ILs-based monolithic column were fractured into small pieces, and studied by FE-SEM (Fig. 3). There are many macropores and interconnected channel networks in these two monolithic columns. These macropores and channels allowed mobile phase to flow through the column with lower flow backpressure at higher flow rates. The relationships between backpressures and different flow rates of monolithic column in HPLC were showed in Figure 4. From Figures 3 and 4, non-ILs monolithic column, and ILs-based monolithic column both have the similar pore structures, and have low backpressures in HPLC. The properties of the monolithic column were more likely originated from the polymerization process. The polymerization temperature, type of solvent and composition of solvent were the three greatest factors on the pore properties of monolithic column. Moreover, ILs-based monolithic column using GMA and ILs were synthesized in a stainless column at 55°C for 24 h. There is a chemical reaction between epoxy group of GMA and ILs. The non-ILs monolithic column was prepared and treated in an identical manner. There is no significant difference on pore structures and properties between these two monolithic columns. The purpose of using ILs in monolithic column is to increase the molecular interactions with analytes to improve column efficiency and enlarge its application fields. In the next step, determination of caffeine and theophylline on these two monolithic columns in HPLC was performed for the detection of ILs to improve the selectivity of monolithic column.

Optimization of chromatographic conditions

The chromatographic conditions were optimized in order to determine the most favorable separation and chromatographic efficiency. To simplify the operation, 0.05 mol L^{-1} NaH_2PO_4 and 0.05 mol L^{-1} Na_2HPO_4 were tested as eluting solution at the beginning of chromatography experiments. The experimental results showed that an almost complete separation was achieved at a flow rate of 0.7 mL min^{-1} with detection at 274 nm when 0.05 mol L^{-1} Na_2HPO_4 as mobile phase. But 0.05 mol L^{-1} NaH_2PO_4 solution could not do like this. Therefore,

TABLE I
Calibration Curve (n = 7), LOD, and LOQ for the Quantification of Caffeine and Theophylline

	Regression equation	r^2	Linear range (μg mL^{-1})	LOD (μg mL^{-1})	LOQ (μg mL^{-1})
Caffeine	$A = 108.48C - 26.29$	0.9999	0.1–60.0	0.03	0.10
Theophylline	$A = 189.03C - 54.35$	0.9998	0.1–60.0	0.03	0.10

TABLE II
Intraday and Interday Precisions, Accuracies and Recoveries of Caffeine and Theophylline with Three Different Concentrations

		Intraday		Interday		
	Concentration ($\mu g\ mL^{-1}$)	Measured concentration ($\mu g\ mL^{-1}$)	Precision RSD (%)	Measured concentration ($\mu g\ mL^{-1}$)	Precision RSD (%)	Recovery (%)
Caffeine	0.5	0.53	4.35	0.49	3.89	106.00
	5.0	5.12	2.53	5.12	3.12	102.40
	30.0	31.28	2.77	30.86	3.36	102.87
Theophylline	0.5	0.54	3.18	0.48	4.14	108.00
	5.0	4.87	2.92	5.02	3.25	97.40
	30.0	30.95	1.46	30.83	2.08	103.17

a series of Na_2HPO_4 solutions over the concentration range of 0.01–0.10 mol L^{-1} were used as eluting solution to separate caffeine and theophylline. At last, the optimum condition was found to be a mobile phase consisting of 0.06 mol L^{-1} Na_2HPO_4 (pH 9.0), and chromatograms of caffeine and theophylline (5.0 $\mu g\ mL^{-1}$) on non-ILs monolithic column and ILs-based monolithic column were shown in Figures 5 and 6, respectively.

Method validation

Calibration curves, LOD, and LOQ

The calibration curves were used to calculate concentrations of caffeine and theophylline from the measured peak area. Standard calibration curves (concentration against chromatographic peak area) for caffeine and theophylline were linear over the concentration range of 0.1–60.0 $\mu g\ mL^{-1}$. Each calibration curve included seven data points and each point was run at least three times. The correlation coefficient for each regression equation was better than 0.9998. The sensitivity of a method is expressed by the limit of detection (LOD) and the limit of quantification (LOQ). The LOD and LOQ for caffeine and theophylline, established as the amounts for which the signal-to-noise- ratios were 3 : 1 and 10 : 1, respectively, were 0.03 and 0.10 $\mu g\ mL^{-1}$, respectively. The data were showed in Table I. In previous studies[22] about analysis of caffeine and theophylline on C_{18} column, the LOD and LOQ of both compounds were 0.04 and 0.08 $\mu g\ mL^{-1}$, respectively.

Precision, accuracy, and recovery

The accuracy and precision of the method were assessed by performing replicate analyses of quality control samples at three different concentrations of caffeine and theophylline in five replicates in the same day and consecutive days. The results showed that the intra-day relative standard deviations and interday relative standard deviations of the proposed method were less than 4.35 and 4.14%, respectively. Recovery was calculated at the three concentration levels (low, medium and high) presented for assessing accuracy and precision. The recoveries of these two drugs ranged from 102.40 to 106.00% and from 97.40 to 108.00 %, respectively, which confirms the reliability of this method. The results are shown in Table II and the data of previous studies[22] are shown in Table III.

CONCLUSIONS

In this study, a new ILs-based monolithic column was successfully synthesized as a new stationary

TABLE III
Intraday and Interday Precisions, Accuracies and Recoveries of Caffeine and Theophylline with Three Different Concentrations in Human Urine

		Intraday		Interday		
	Concentration ($\mu g\ mL^{-1}$)	Measured concentration ($\mu g\ mL^{-1}$)	Precision RSD (%)	Measured concentration ($\mu g\ mL^{-1}$)	Precision RSD (%)	Recovery (%)
Caffeine	0.5	0.51	3.25	0.49	3.36	102.00
	5.0	5.01	4.83	5.12	4.99	100.20
	30.0	30.54	2.37	30.86	3.12	101.80
Theophylline	0.5	0.49	4.11	0.48	4.53	98.00
	5.0	4.99	3.27	5.02	3.08	99.80
	30.0	30.75	3.65	30.83	3.86	102.50

phase for HPLC separation. Determination of caffeine and theophylline was used to evaluate the characteristics of the new material, and this analysis method showed high sensitivity as well as the appropriate precision, accuracy and recovery. Moreover, this monolithic column had lots of advantages, such as easy to make, low cost and good stability, and the cost of analysis can be reduced by reusing the monolithic column up to 100 times injections without significant changes in the analyte recovery or the column back pressure. Comparing to previous studies,[22] the linear ranges, mean recoveries and LOD of the proposed method in this study were not bad and were proved to be acceptable for drugs assay. In conclusion, this ILs-based monolithic column as stationary phase will become a potential tool for future HPLC separation.

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