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Synthesis and Evaluation of 1-Methylimidazole Modified-Functional Polymer Stationary Phase

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In order to improve the selectivity of the stationary phase of high-performance liquid chromatography (HPLC) columns, functional polymers as new separation media have seen an increasing attention, but it limited by the low column efficiency. Chemical modified polymer has shown tremendous success to increase the efficiency of the column. In this research 1-methylimidazole-modified-functional polymer was synthesized and the characteristic was investigated by FT-IR. Two xanthine derivatives (caffeine and theophylline) were used to evaluate the separation characteristics of the modified polymer stationary phase. Under the optimum mobile phase: acetonitrile/water (50:50, v/v), the two compounds can obtain better resolution (R = 3.01) on modified polymer column than resolution (R < 1.0) on blank column. The modified polymer as the stationary phase exhibited higher separation efficiency and it was a potential tool for future HPLC separation in environmental and biochemical fields.

Key Words: Synthesis, Functional polymer, Methylimidazole, HPLC separation.

INTRODUCTION

With the development of biology, medicinal, environmental science and other related scientific fields, high efficiency and high selectivity of liquid chromatographic separation technique became more and more important[1-3]. To fulfill the increasing requirement in such different fields is a main concerned work for people worked in the chromatographic fields. Separation media, as the center of chromatography separation, has been investigated and developed as the hotspot and emphases of chromatography separation during the recent few years[4,5]. Generally, normal-phase high-performance liquid chromatography (HPLC) columns with silica sorbent and reverse-phase HPLC with C_{18} as the stationary phase are the most widely used, respectively[6].

With the purpose of developing new stationary phase of HPLC, many studies put the emphasis on improvement of different stationary phases with various structures in order to improve the selectivity[7]. Recently, synthesis of functional polymers as separation media has seen an increasing attention[8-13]. Functional polymer separation media provides an accurate control over the functionality and porous structure of the support and consequently enhances its separation properties.

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Functional porous polymer as stationary phase in HPLC separation has been directed toward improving the selectivity[11], but the main limitation is the low column efficiency. In order to increasing the efficiency of the column, tailor-made polymer coatings or polymer modifications have shown tremendous success and can be a better alternative to the conventional stationary phases. Kanazawa *et al.*[14] modified the silica as the stationary phase with a thermoresponsive polymer for separation of biomolecules. Stalcup *et al.*[15] synthesized a pyridinium bromide on the surface of silica. Otherwise, function polymer as the stationary phase was modified by organic acid, alkali or ammonium has also been investigated[16]. However, there are few reports about the surface chemical modifications of the functional polymers using imidazole to improve its separation properties. So in this study, 1-methylimidazole-modified functional polymer was synthesized and the characteristic of the obtained polymer as stationary phase for HPLC separation was evaluated by using two xanthine derivatives *e.g.*, caffeine and theophylline.

EXPERIMENTAL

Methanol, acetonitrile, *n*-hexane (HPLC grade), acetic acid and diethylamine (extra pure) were purchased from Duksan Pure Chemical. Co., Ltd. (Ansan, Korea). Glycidyl methacrylete (GMA), ethylene glycol dimethacrylate (EDMA), dodecanol, cyclohexanol and azobisisobutyronitrile (AIBN) as the reactants were obtained from Junsei Chemical Co. (Tokyo, Japan). 1-Methylimidazole was purchased from Fluka (Steinkeim, Germany). Water was double distilled and filtered (FH-0.45 μm, Advantec MFS, Inc., Japan) using a decompressing pump (Division of Millipore, Waters, USA). Caffeine and theophylline (Sigma-Aldrich Co., Steinkeim, Germany) were freshly dissolved in methanol to yield a final concentration of 1.0 mg/mL.

HPLC analysis: HPLC system is comprised of a M930 solvent delivery pump (Young Lin Co. Korea), an UV detector (M720 Absorbance Detector, Young-In Scientific Co., Korea) and an integrated data system (Autochrowin. Ver. 1.42, Young Lin Co., Korea). The injection valves with 25 and 20 μL sample loops were used. The flow rate was 0.5 mL/min and UV wavelength was set at 272 nm. All the solutions must be filtered by a disposable syringe filter unit (0.2 μm) for further HPLC analysis.

Preparation of the polymer: 3.0 mL of glycidyl methacrylate, 3.0 mL of ethylene glycol dimethacrylate, 1.5 mL of dodecanol and 10.5 mL of cyclohexanol were mixed uniformly in a vial. After adding 80.0 mg azobisisobutyronitrile to the mixture, the vial was kept in water bath at 58 ºC for 24 h, followed by filling with helium gas. Afterward, the obtained polymer was oven-dried under 40 ºC and then grinding and sieving to get particles less than 32 μm. Finally, the fine particles were removed by repeated sedimentation using acetone.

Synthesis of 1-methylimidazole-modified stationary phase: The obtained particles were stirred with 3.0 mL 1-methylimidazole in dark at 75 ºC for 8 h. The

colour of the polymer powders was changed from white to pale yellow gradually. After the modification was completed and the temperature was dropped to 25 ºC in 0.5 h, all the residual reactants were removed by *n*-hexane.

FT-IR Measurement: FT-IR spectroscopy has been proven to be a powerful tool for providing conformational and structural information[17]. The RFS 100/S spectrometer (Bruker, Karlsruhe, Germany) was used in this experiment to evaluate the modified polymer. 2.0 mg powder of blank polymer and 1-methylimidazole-modified polymer were mixed with 100.0 mg KBr in an agate mortar for 1 h, respectively. Then the mixtures were molded into a disc under the pressure of 0.8-1.0 GPa.

Preparation of HPLC columns: Two HPLC columns were packed by using different kinds of polymer particles as stationary phase. The polymer particles were suspended in methanol and degassed by helium. The slurries were pressed into the hollow HPLC columns (150 mm × 4.6 mm) using a pump, respectively. After then, the packed columns were washed with by methanol until a stabile baseline was observed.

Separation condition validation: The retention factor (k) and resolution of two compounds was calculated by the following two equations:

$$k = \frac{t_R - t_0}{t_0} \quad (1)$$

$$R = \frac{2(t_{R_2} - t_{R_1})}{W_1 + W_2} \quad (2)$$

where t_R and t_0 are the retention times of analyte and unretained solutes, respectively, w_1 and w_2 are the baseline widths of the two peaks.

Assays of repeatability calculated as relative standard deviations (RSDs) were performed by injecting 0.25 mg/mL standard solutions of the two compounds 5 times in a 5-day period. The RSDs of the two compounds were also detected under different pH and temperature of mobile phase.

RESULTS AND DISCUSSION

Synthesis of 1-methylimidazole-modified-modified polymer: In the FT-IR spectra, the changes in conformation of the blank polymer and 1-methylimidazole-modified polymer are observed and two different vibration peaks between the two kinds of polymers are exhibited. The spectra of 1-methylimidazole-modified polymer have an inconspicuous peak at the wavelength of 1575 cm^{-1} in Fig. 1 and the finger print region of the amide bands[18,19] was observed from 1600-1500 cm^{-1}. This means the C-N groups interacted with blank polymer. The other obvious differences are the wavelength from 3700-3000 cm^{-1} which the intensity of nitrogen fraction (N-H) was indicated in this region[20,21]. The results validated that the 1-methylimidazole was bonded with blank polymer successfully.

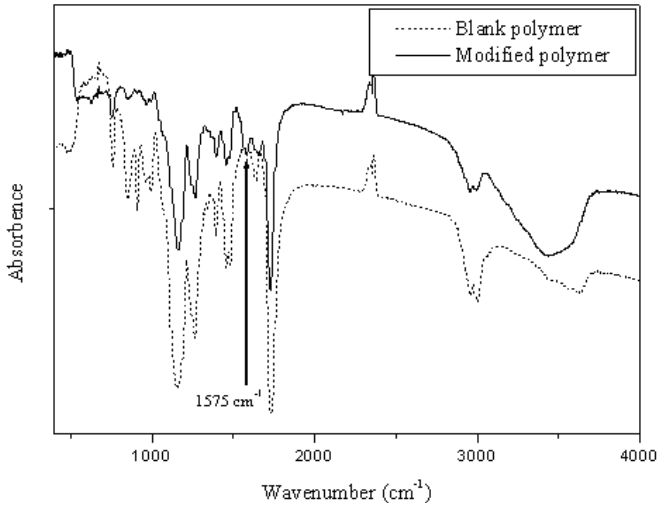

Fig. 1. Infrared spectra of blank polymer and 1-methylimidazole-modified polymer

Performance evaluation: 1-Methylimidazole-modified polymer has two major characteristics due to the porous structure of functional polymer and the performance of imidazole. The purpose of using 1-methylimidazole-modified polymer as stationary phase is to increase the molecular interactions with analytes to improve column efficiency and enlarge its application fields.

Influence of different components of mobile phases: Different components of methanol/water (v/v) and acetonitrile/water (v/v) were used as mobile phases to separate caffeine and theophylline on the obtained HPLC columns. In Table-1, the retention times (t_C, t_T) of the two compounds decreased with the components of methanol and acetonitrile increasing and theophylline have much more influence than caffeine. At the same time, the two compounds can not be separated on blank polymer column.

As the molecular structures shows in Fig. 2, there is one more methyl connect with the nitrogen atom in caffeine than in theophylline. This methyl reduced the interaction of the nitrogen atom between caffeine and 1-methylimidazole-modified polymer. Further more, acetonitrile as the aprotic solvent had low influence of the interaction than methanol which was a protic solvent. So when the acetonitrile/water was used as the mobile phase, the 1-methylimidazole-modified polymer shows the superior separation ability.

When low concentration of acetonitrile, such as 25 % was used as mobile phase, theophylline was eluted out in 10 min with a broaden peak. But with the concentration of acetonitrile increasing, the peak width of theophylline decreased and good resolution (3.15) was obtained when using 50:50 acetonitrile/water (v/v) as mobile phase. From the results shown in Table-1, acetonitrile/water (50:50, v/v) is the optimum mobile phase to separate caffeine and theophylline on the 1-methylimidazole-modified-polymer column (Fig. 3).

TABLE-1
RETENTION PARAMETERS OF CAFFEINE AND
THEOPHYLLINE BY DIFFERENT MOBILE PHASES

	Blank polymer			Modified polymer		
	k_C	k_T	R	k_C	k_T	R
Methanol/water						
100:0	0.66	1.02	< 0.1	0.18	1.05	0
75:25	0.68	1.53	< 0.2	0.24	1.44	0.84
50:50	0.96	2.81	0.64	0.38	6.36	> 1
25:75	2.48	> 7.0	< 1	1.69	> 11	> 1
0:100	4.93	> 15.	< 1	4.39	> 24	> 1
Acetonitrile/water						
100:0	0.13	0.16	0	0.12	0.45	0.13
75:25	0.20	0.27	0	0.16	0.46	0.54
50:50	0.26	0.43	0	0.18	1.31	3.15
25:75	0.34	1.16	< 1	0.21	> 7	> 3
0:100	4.93	5.72	< 1	4.39	> 24	> 3

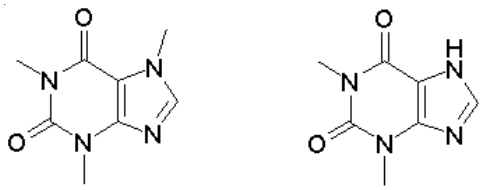

Fig. 2. Molecular structures of (a) caffeine and (b) theophylline

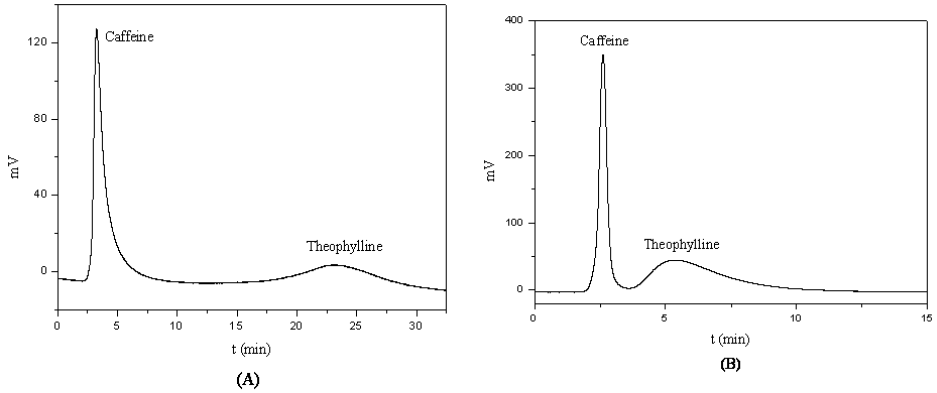

Fig. 3. Chromatograms of caffeine and theophylline. (Column: 1-methylimidazole-modified polymer, injection volume: 2 mL, 1.0 mg/mL; Mobile phase: (A) acetonitrile/water (25:75, v/v); (B) Acetonitrile/water (50:50, v/v))

Effect of mobile phase additives: In order to evaluate the effect of mobile phase additives, different proportions of acetic acid and diethylamine were investigated and the results show that the retention of both caffeine and theophylline decreased with the concentration of two additives increasing. That means both the additives can reduce the interaction between compounds and stationary phase. When the

proportions of the two modifiers in mobile phase larger than 0.1 %, the retention time were too shorten to get baseline separation. As the results shown in Table-2, acetic acid and diethylamine as mobile phase additives were not appropriate for this approach. So acetonitrile:water (50:50, v/v) without additive was used for further experiment.

TABLE-2
RETENTION PARAMETERS OF CAFFEINE AND THEOPHYLLINE BY DIFFERENT CONCENTRATION OF MOBILE PHASE ADDITIVES

	Blank Polymer			Modified Polymer		
Vol. (%)	k_C	k_T	R	k_C	k_T	R
			Actic Acid			
0.1	0.24	1.47		0.20	0.52	
0.5	0.23	1.06		0.19	0.28	
1.0	0.22	0.53	< 0.2	0.18	0.23	< 0.2
1.5	0.22	0.46		0.17	0.18	
2.0	0.21	0.30		0.16	0.17	
			Diethylamine			
0.1	0.19	0.21		0.19	0.48	
0.5	0.15	0.18		0.17	0.42	
1.0	0.09	0.14	< 0.1	0.16	0.29	< 0.1
1.5	0	0.11		0.16	0.17	
2.0	0	0.09		0.15	0.16	

Influence of ambient temperature of the column: Under the optimum separation condition, the effects of different temperature of 1-methylimidazole-modified polymer column on separation were investigated in a range of 30-50 ºC and as the results shown in Table-3. With the increasing of temperature, the retention and resolution abilities of caffeine and theophylline on the modified polymer column decreased and the two compounds can not obtain the baseline separation under high temperature. The results indicated low room temperature was suitable for the separation of the two compounds on the 1-methylimidazole-modified polymer column.

TABLE-3
RETENTION PARAMETERS OF CAFFEINE AND THEOPHYLLINE UNDER DIFFERENT TEMPERATURE ON 1-METHYLIMIDAZOLE MODIFIED POLYMER COLUMN

Temperature (ºC)	k_C	k_T	R
30	0.19	1.31	3.01
35	0.16	1.13	1.28
40	0.15	0.82	< 1
45	0.14	0.29	< 1
50	0	0.22	< 0.5

Repeatability validation: The RSD of precision tests shows that the value of caffeine was less than 6 % and of theophylline was less than 8 %. It verified that the 1-methylimidazole-modified polymer as the stationary phase for HPLC separation was stable under different pH of mobile phase and temperature.

Conclusion

In this study, a new functional polymer was successfully synthesized using chemical modification of poly(glycidyl methacrylate-co-ethylene glycol dimethacrylate) by 1-methylimidazole and applied as a new stationary phase for HPLC separation. Caffeine and theophylline were used to evaluate the characteristics of the new stationary phase and their optimum separation condition on 1-methylimidazole-modified polymer column was acetonitrile:water (50:50, v/v) under room temperature. The presented results indicated that 1-methylimidazole-modified polymer as the stationary phase was a potential tool for future HPLC separation.

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