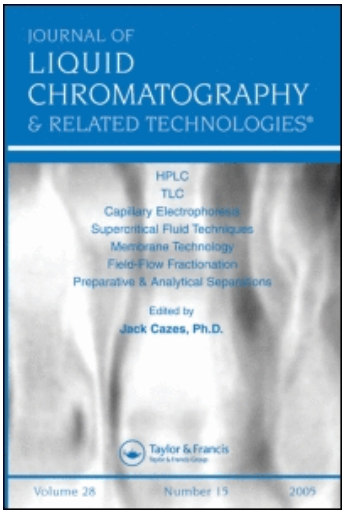

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NOVEL BI-FUNCTIONAL AMINO-IMIDAZOLIUM SILICA CONFINED STATIONARY PHASE FOR LIQUID CHROMATOGRAPHY

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NOVEL BI-FUNCTIONAL AMINO-IMIDAZOLIUM SILICA CONFINED STATIONARY PHASE FOR LIQUID CHROMATOGRAPHY

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□ *A novel amino-imidazolium stationary phase based on silica was synthesized and characterized in this paper. The material with ionic and NH_2 exhibited an exciting capacity of separation for liquid chromatography. A number of aromatic compounds were successfully separated by the material as the stationary phase. Resolutions and adsorption isotherms of these organic compounds were examined. Furthermore, the novel stationary phase showed a potential ability to separate biocompounds, such as alkaloids and tanshinones. The protonation of amino group was also investigated.*

Keywords amino-imidazolium stationary phase, aromatic compounds, biocompounds, ionic liquid, liquid chromatography, separation

INTRODUCTION

The applications of ionic liquids in liquid chromatography have started to emerge recently.[1–4] However, the application of ionic liquids in the stationary phase of liquid chromatography is relatively fewer in comparison with the use of ionic liquids as mobile-phase additives. Stalcup et al. prepared a butylimidazolium bromide stationary phase via hydrosilylation of alkenyl bromide followed by immobilization of the silane on stationary phase. The obtained column was used to test 28 small aromatic solutes to make linear solvation free energy relationship studies.[5] They found that the 1-butyl-3-heptylimidazolium bromide stationary phase was similar to conventional phenyl-based stationary phases under reversed-phase conditions for the separation of neutral aromatic solutes. Later, Colón and coworkers prepared alkyl imidazolium ionic liquids modified silica for HPLC.[6] They concluded that the separation mechanisms involved

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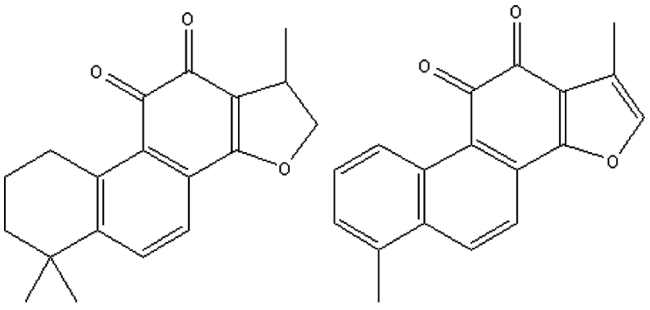

(a) (b)

FIGURE 1 Structures of bio-compounds (a) theobromine, (b) theophylline, (c) caffeine, (d) cytosine, (e) thymine, (f) adenine, (g) cryptotanshinone, and (h) tanshinone I.

multiple interactions, such as ion exchange, hydrophobic interaction, electrostatic interactions, and so on. As mentioned in the paper, it can be argued that once the ionic liquid was immobilized on a surface, it lost some properties of ionic liquid.[1,7] However, the ionic liquids stationary phases offer an approach for looking at intermolecular interactions in retention processes while offering the potential for solving some of the challenging problems confronting separations. Liu et al. confined a vinylhexylimidazolium tetrafluorobarate ionic liquid on the surface of porous silica as stationary phase to separate an ephedrine mixture using methanol/water as mobile phases.[8] The results were not as good as those obtained by using ionic liquids as additives in mobile phase with a commercial C_{18} column.[9]

In this study, the bi-functional stationary phase was synthesized. Due to the fact that the obtained materials have the same properties as both reverse phase and NH_2 column, the stationary phase was used to separate some compounds which cannot be easily separated by HPLC. In order to investigate separation behavior of this new stationary phase, the adsorption isotherms were involved. Besides these, the separation of biocompounds, such as alkaloids and tanshinones (Figure 1), was performed.

EXPERIMENTAL

Chemicals

Caffeine (99%), theophylline (99%), theobromine (99%), chlorotrimethylsilane (99%), and (3-chloropropyl)triethoxysilane (95%) were purchased from Sigma (St. Louis, MO, USA) and imidazole (99%) and 3-bromopropylamine hydrobromide (98%) were obtained from Aldrich

(Milwaukee, WI, USA). Adenine, thymine, and cytosine (TCI-GR grade) were purchased from Tokyo Kasei Kogyo Co., Ltd., (Tokyo, Japan). Cryptotanshinone and tanshinone I were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol, ethanol, and aromatic compounds were obtained from DUKSAN 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, USA) before use. The LiChrospher Si 60 silica particles and LiChrospher 100 RP-18 were purchased from Merck Chemicals Ltd. (German). All the samples were filtered by using a filter (MFS-25, 0.2 μm TF, WHATMAN, USA) before injection into the HPLC system.

Synthesis of Amino-Imidazolium Stationary Phase

Silica was first immersed in hydrochloric acid for 24 hr and then washed with deionized water until pH was 7.0. The activated silica was dried at 120°C for 12 hr. 5.0 g of silica was in 100.0 mL of dry toluene and the suspension was stirred and refluxed with an excess of 3-chloropropyltrimethoxysilane (5.0 mL). When the reaction was stopped after 24 hr and this chloropropyl silica (SilprCl) was cooled to room temperature, it was washed by toluene, ethanol, and methanol in turn. SilprCl was dry under vacuum at 80°C for 4 hr. Then, the remaining silanol groups on SilprCl were end capped by excess chlorotrimethylsilane under reflux in toluene.

Second, the chemically bonded chloropropyl group on silica surface reacted with imidazole (triethylamine as a catalyst). According to the reference,[10] 5.0 g of dry chloropropyl silica was placed in a reaction flask containing 100.0 mL of toluene and 5.93 g of triethylamine. The suspension was stirred for 30 min and imidazole (4.0 g) was then added over a period of 10 min with stirring. After a 10 hr reflux, the reaction was stopped, modified silica was cooled to room temperature, and washed with toluene, ethanol, and methanol in turn. The silica bonded with imidazole (SilprIm) was dried under vacuum at 50°C for 4 hr.

Third, same as described in E.D. Betas's report,[11] 5.0 g SilprIm was placed in a 250.0 mL flask with 100 mL ethanol and 4.0 g 3-bromopropylamine hydrobromide. The suspension was then refluxed with stirring for 24 hr. After washing with ethanol and drying under vacuum at 50°C, the amino-imidazolium silica (named as SilprImN) was obtained.

Characteristic Analysis

The carbon, hydrogen, and nitrogen contents were determined by elemental analysis that performed on an EA1112 (Italy). Thermogravimetric

measurements were obtained on a TGA unit (SCINCO thermal gravimeter S-1000) with a heating rate of 10°C/min under nitrogen. FT-IR data was obtained by a Vertex 80 V (Bruker, USA) in the range of 4000–400 cm^{-1} with a scan rate of 20 scans min^{-1}. KBr pellet was used for FT-IR analysis.

HPLC Analysis

The HPLC system is comprised of a M930 solvent delivery pump (Young Lin Co., Korea), an UV detector (M 720 Absorbance Detector, Young-In Scientific Co., Korea) and an integrated data system (Autochro-win. Ver. 1.42, Young Lin Co., Korea). The ionic liquid-modified silica particles and C_{18} particles were packed into a stainless steel column (150×4.6 mm) by the conventional slurry packing method using hexane as the solvent. The commercial C_{18} columns (5 μm particles, 10 nm pore size, 4.6×150 mm) are from RS Tech. Corporation (Daejeon, Korea). The injection volume was at 5 μL.

The retention factor (k) was calculated using the equation: $k = (t_R - t_0)/t_0$, where t_R and t_0 are the retention times of analyte and unretained solutes, respectively. Resolution was calculated using the equation $R = 2(t_2 - t_1)/(w_2 + w_1)$, where t_1 and t_2 are the retention times of two compounds, and w_1 and w_2 are the baseline peak widths of the two compounds.

Determination of Adsorbed Amount

The static method was performed on the SilprImN particles to determine the adsorbed amount. 1.0 mL of aniline, phenol, toluene and chlorobenzene mixture with seven concentrations from 0.02 g/L to 1.0 g/L was mixed with 20.0 mg particles into several flasks, respectively. The mixtures were stored at room temperature for 72 hr and then the supernatant was collected. The concentrations of unadsorbed aniline, phenol, toluene, and chlorobenzene in the solution were determined on commercial C_{18} column at room temperature. The concentrations of adsorbed compounds on the particles were calculated by subtracting the concentrations of unadsorbed compounds from the initial concentrations of these compounds.

RESULTS AND DISCUSSION

Synthesis of the Stationary Phase

The synthesis of the ionic liquids-modified stationary phases was base on a step-by-step graft on silica. The synthesis scheme was shown in

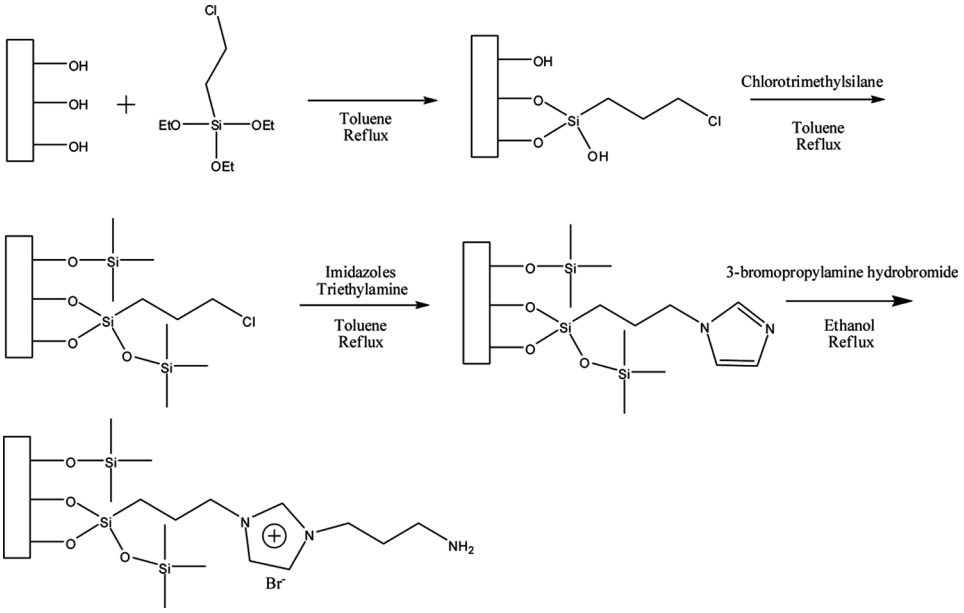

FIGURE 2 Synthesis steps used in the preparation of stationary phases.

Figure 2, which involved the silanization of unmodified silica, the reactions with imidazoles to form 1-imidazole groups and the formation of amino-imidazolium silica.

Elemental Analysis

The element contents and surface coverage of SilprIm and SilprImN silica are listed on Table 1. Based on the percentage amounts of carbon, the bonding density was determined at 3.72 μmol/m^2 for SilprCl. The bonding densities were based on the nitrogen percentages and were 1.50 μmol/m^2 and 1.13 μmol/m^2 for SilprIm and SilprImN silica, respectively. The calculation equations of the surface coverage are as follow:

$$\text{SilprCl}(\mu\text{mol/m}^2) = \frac{\%\text{C}}{36 \times (1 - \%\text{C} - \%\text{H}) \times \text{S}} \tag{1}$$

$$\text{SilprIm}(\mu\text{mol/m}^2) = \frac{\%\text{N}}{28 \times (1 - \%\text{C} - \%\text{H} - \%\text{N}) \times \text{S}} \tag{2}$$

$$\text{SilprImN}(\mu\text{mol/m}^2) = \frac{\%\text{N}}{42 \times (1 - \%\text{C} - \%\text{H} - \%\text{N}) \times \text{S}} \tag{3}$$

TABLE 1 Elemental Analysis and Surface Coverage of Bonded Silica Gels

Materials	C%	H%	N%	Coverage (μmol/m^2)
SilprCl	9.26	1.95	–	3.72
SilprIm	8.87	1.66	1.98	1.50
SilprImN	9.35	1.75	2.02	1.13

where %C, %H, and %N represent the percent of carbon, hydrogen, and nitrogen, respectively, as determined by elemental analysis shown in Table 1, S is the specific surface area of the silica support (540 m^2/g) (BET). The amount of chloropropyl groups are more than that of linked imidazole groups. That is to say, some of the chloropropyl groups were removed by the base and the chloropropyl groups in the pores are hard to react with imidazole. Although the conversion is not so good, the elemental analysis data shows a proof of immobilization on the surface of silica.

Characterization of Structure

FT-IR spectroscopy is a powerful tool for providing conformational and structural information. In Figure 3, a broad band at 3442 cm^{-1} can be

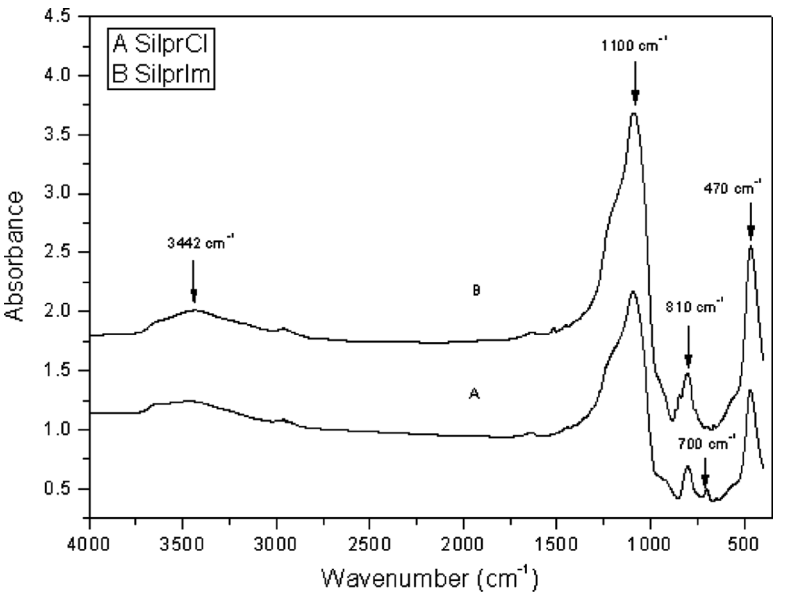

FIGURE 3 FT-IR spectra of (A) SilprCl and (B) SilprIm.

attributable to O-H stretching vibration modes of hydrogen bonded to water. The symmetric stretching Si-O vibration of silica can be observed at around 1100 cm^{-1}. At lower frequencies, the bands at around 810 and 470 cm^{-1} corresponds to asymmetric Si-O stretching and Si-O bending modes of silica.[12] The FT-IR spectra of chloropropyl silica exhibited a conspicuous peak at the wavelength of 700 cm^{-1} and the finger print region of the C-Cl group was from 704–690 cm^{-1}.[13] In the spectra of SilprIm, the finger print peak decreased. This revealed when only the imidazole was reacted with chloropropyl silica and the chlorine was replaced by imidazole. In comparison of the spectra of SilprCl and SilprIm, the finger print peak of N-H group was not observed from 3500–3300 cm^{-1}. The result proved that the SilprIm was synthesized successfully.

Thermogravimetry can be employed to determine the thermo stability of the chemically modified silica since the weight loss observed between 200°C and 800°C was found to be associated with the loss of the organic groups attached to the surface.[14] Figure 4 shows the thermogravimetric curves for SilprIm and SilprImN. From 200°C to 750°C, it presents about 13% and 20% mass loss for SilprIm and SilprImN, respectively. The SilprImN shows a higher mass loss due to the extra combination of amino group and bromine. This result proved the immobilization of amino group on imidazole was successful.

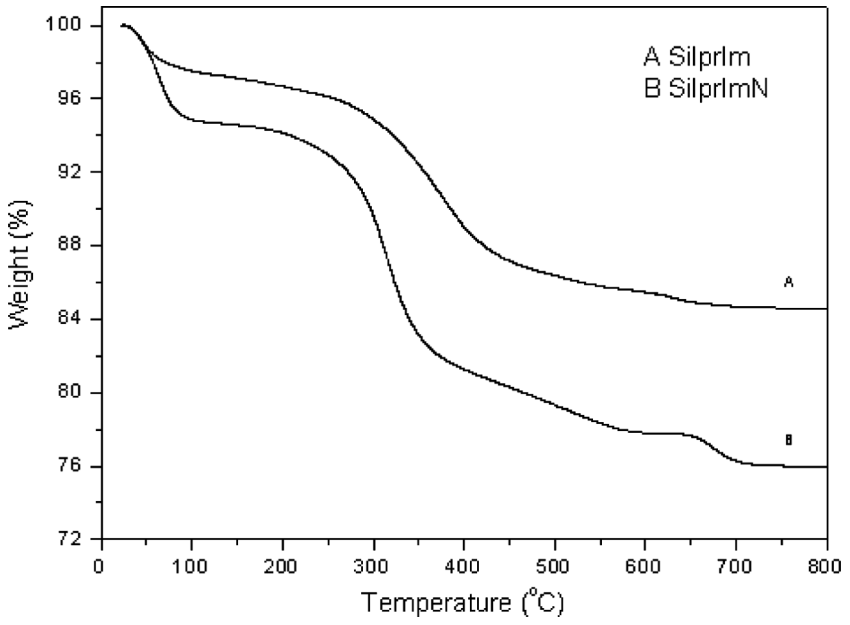

FIGURE 4 TGA curves obtain for (A) SilprIm and (B) SilprImN.

Separation of Aromatic Compounds

The separation of organic compounds was investigated first. The mixture of aniline, phenol, toluene, and chlorobenzene was injected into a SilprImN column using methanol/water (20/80, *v*/*v*) as the mobile phase (Figure 5). In comparison with preparative reverse phase column (Figure 6), the chromatogram exhibits an exciting result of separation at the same condition. The separation of mixed samples was due to the hydrophobic and amino group interactions. Especially, the aniline and phenol were separated by interacting with amino groups. The relationships between the *k* of samples and the components of methanol in mobile phase were investigated. The *k* of these compounds decreased with increasing the methanol concentrations from 10–50% on SilprImN. However, the best resolutions (*R*) (Table 2) of these samples were obtained around 20% methanol. This may be due to the band broadening at lower concentration of methanol. In order to provide this trend, several more aromatic compounds were also examined on SilprImN. Table 3 exhibits the retention factor (*k*) of these samples with different methanol concentrations. Comparing to the retention factor (*k*) of aniline, phenol, toluene, and chlorobenzene, all the samples show the consistent trend.

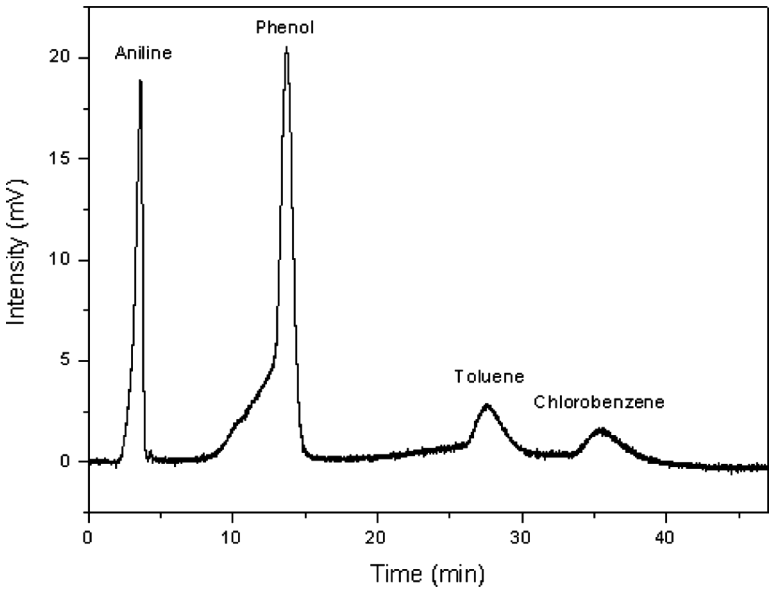

FIGURE 5 Chromatogram of aniline, phenol, toluene, and chlorobenzene on SilprImN stationary phase. (Mobile phase, Methanol/water (20/80, *v*/*v*); flow rate, 0.5 mL/min; column, 150 × 4.6 mm; detection, UV absorption at 254 nm).

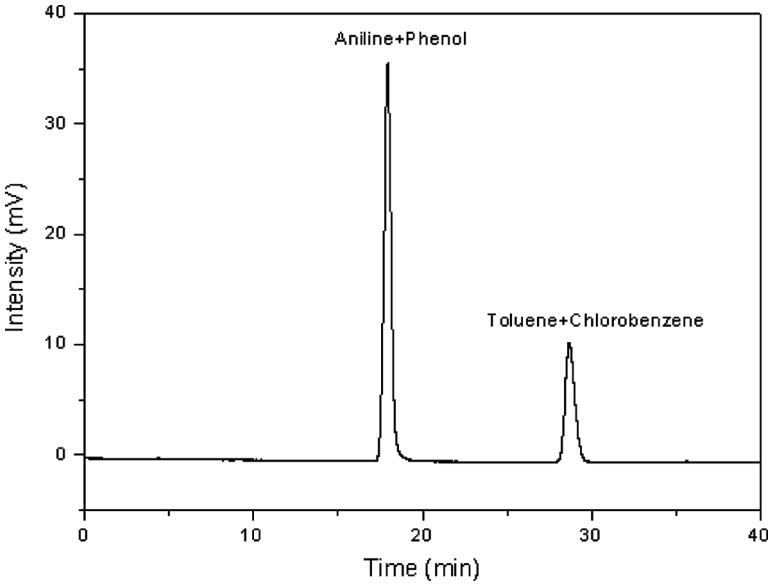

FIGURE 6 Chromatogram of aniline, phenol, toluene, and chlorobenzene on C_{18}. (Mobile phase, Methanol/water (20/80, *v*/*v*); flow rate, 0.5 mL/min; column, 150 × 4.6 mm; detection, UV absorption at 254 nm).

By previous results, the retention mechanism was presumed. In comparison with Figures 5 and 6, the separation of samples on SilprImN was due to the different interactions between solutes and stationary phase. According to the literature,[6] hydrophobic interaction is the main interaction on SilprImN to decide the elution order. This interaction causes the separation of aniline and phenol from toluene and chlorobenzene (as shown in Figure 6). By the assistance of hydrogen bonding between NH_2 group on SilprImN and the functional groups on phenyl, aniline, and phenol were separated. The separation of toluene and

TABLE 2 Resolution (*R*) of Aniline, Phenol, Toluene, and Chlorobenzene on Silprimn Stationary Phase

	Concentration of Methanol (%)				
Resolution (R)	10	20	30	40	50
R_1[a]	1.90	2.29	1.91	1.66	1.16
R_2[b]	2.82	2.49	1.90	1.43	0.88
R_3[c]	1.59	1.67	1.48	1.05	0.95

[a] R_1 is the resolution (*R*) of aniline and phenol.
[b] R_2 is the resolution (*R*) of phenol and toluene.
[c] R_3 is the resolution (*R*) of toluene and chlorobenzene.

TABLE 3 Retention Factors (k) of Organic Analytes on the Silprimn Stationary Phases

Compounds	Retention factors (k)				
	k_1 (50% MeOH)	k_2 (40% MeOH)	k_3 (35% MeOH)	k_4 (30% MeOH)	k_5 (25% MeOH)
Pyridine	0.25	0.54	0.67	0.97	1.57
Benzene	2.77	3.75	4.51	5.44	6.085
Ethylbenzene	3.67	6.21	8.04	10.92	12.735
m-Xylene	3.60	6.32	8.64	11.01	11.65
o-Xylene	3.57	6.30	8.24	10.45	11.50
p-Xylene	3.59	6.31	9.13	11.41	11.90
1,2-Chlorobenzene	4.31	8.14	12.49	16.14	17.90
o-Cresol	2.45	3.48	5.28	5.65	6.135

chlorobenzene was due to the $\pi-\pi$ interaction between the imidazole groups and samples. Attributed to the multi-interactions, the samples were successfully separated by using SilprImN as the stationary phase.

Adsorption Isotherms of Four Aromatic Compounds

The concentration was determined on the following constructed calibration curves: $Y = 10279.9X + 39.7$, $r^2 = 0.9976$ (regression coefficient) for aniline; $Y = 3088.9X + 16.7$, $r^2 = 0.9995$ for phenol; $Y = 622.9X + 5.04$, $r^2 = 0.9851$ for toluene; and $Y = 462.9X + 10.47$, $r^2 = 0.9923$ for chlorobenzene. Y is the peak area ($mV \cdot s$), and X is the concentration of samples in the injected solution (g/L). The calibration curves were determined from concentrations of 0.02 g/L to 1.0 g/L in the HPLC system at 254 nm.

The adsorption isotherms were involved to investigate the effects among samples, mobile phase, and stationary phase.[15–18] The adsorbed concentrations of aniline, phenol, toluene, and chlorobenzene adsorbed on the SilprImN particles in the flasks were measured after different concentrations of the equilibrium adsorptions were obtained. The unadsorbed concentrations of the compounds in the liquid phase were determined by HPLC quantitatively, and all analyses were carried out five times.

Figures 7 and 8 show the experimental results of the adsorption concentrations of mixture compounds on the SilprImN particles. The adsorption concentrations of aniline, phenol, toluene, and chlorobenzene on the particles increased with the concentrations of the solutes increasing. Due to the interactions between solutes and stationary phase, the adsorbed concentrations of different samples increased with increasing the k of compounds, which cause the separation of these compounds. Comparing the adsorbed concentrations of compounds in 20% and 40% methanol, a larger adsorption in 20% methanol was shown. Base on the different adsorbed

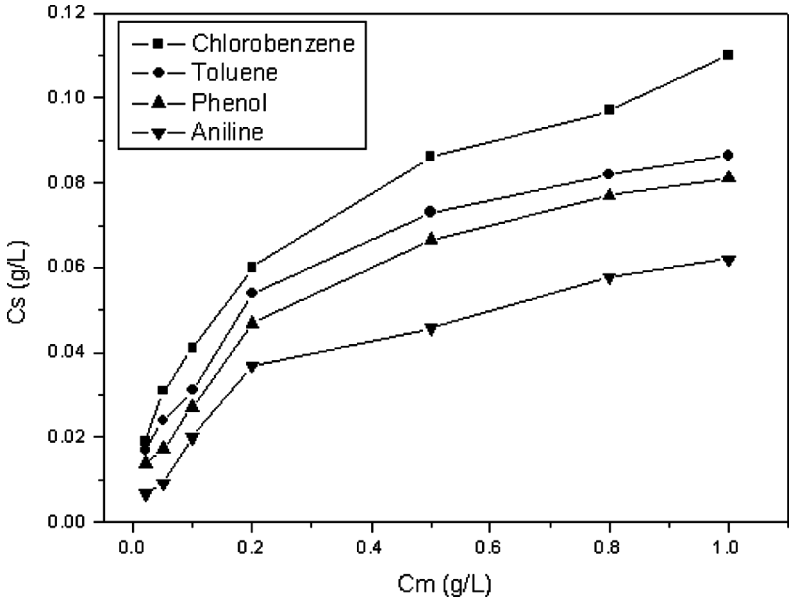

FIGURE 7 Adsorption concentrations of aniline, phenol, toluene, and chlorobenzene in the mixture compounds solutions on SilprImN. (Mobile phase, Methanol/water $(v/v) = 40/60$).

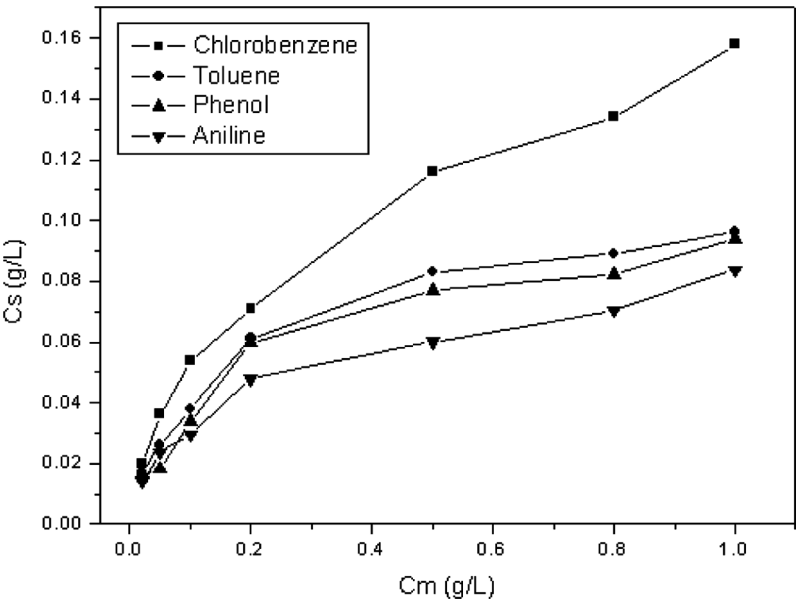

FIGURE 8 Adsorption concentrations of aniline, phenol, toluene, and chlorobenzene in the mixture compounds solutions on SilprImN. (Mobile phase, Methanol/water $(v/v) = 20/80$).

concentrations in different component of methanol, the samples show a higher retention factor in 20% methanol than those in 40% methanol.

In order to calculate the binding property, the linear, Langmuir and Freundlich models were chosen. This experiment data was fitted to the following adsorption isotherm models:

$$C_s = aC_m + b \tag{4}$$

$$C_s = \frac{aC_m}{1 + bC_m} \tag{5}$$

$$C_s = aC_m^{1/c} \tag{6}$$

where C_m (g/L) is the equilibrium concentration of the solute in the liquid phase, and C_s (g/L) is the equilibrium concentration of the solute in the solid-phase. a is the maximum adsorption capacity; b is the apparent dissociation constant, which represents the affinity between the solute

TABLE 4 Parameters in Adsorption Isotherm of Compounds on the Silprimn Particles under Different Mobile Phase Conditions

Mobile phase composition: methanol/water	Compounds	Adsorption isotherm Equation No.	Parameters			
			a	b	C	r^2
40/60	Aniline	4	0.0541	0.0134	–	0.8925
		5	0.2845	3.6987	–	0.9842
		6	0.0640	–	2.0431	0.9664
	Phenol	4	0.0686	0.0209	–	0.9021
		5	0.4250	4.2807	–	0.9905
		6	0.0848	–	2.2246	0.9795
	Toluene	4	0.0689	0.0261	–	0.8840
		5	0.5621	5.5984	–	0.9843
		6	0.0901	–	2.4822	0.9762
	Chlorobenzene	4	0.0857	0.0307	–	0.9254
		5	0.6511	5.2711	–	0.9792
		6	0.1100	–	2.4122	0.9938
20/80	Aniline	4	0.0632	0.0229	–	0.9227
		5	0.5009	5.5603	–	0.9617
		6	0.0813	–	2.4358	0.9832
	Phenol	4	0.0748	0.0259	–	0.8603
		5	0.5635	5.2449	–	0.9785
		6	0.0956	–	2.3779	0.9553
	Toluene	4	0.0757	0.0297	–	0.8626
		5	0.6648	6.0504	–	0.9922
		6	0.1000	–	2.5333	0.9708
	Chlorobenzene	4	0.1296	0.0349	–	0.9529
		5	0.6691	3.5261	–	0.9766
		6	0.1556	–	2.0862	0.9954

and adsorbent; and c is parameter. Equations (4)–(6) correspond to the linear, Langmuir and Freundlich models, respectively. Table 4 shows the parameters fitted by the three adsorption isotherm models.

Comparing the r^2 of adsorption isotherms in Table 4, Langmuir equation is more suitable for most of the samples. It also can be found that the parameter a of aniline, phenol, toluene, and chlorobenzene increased with the retention factor (k) increasing. Additionally, the parameters a in 20% methanol are larger than those in 40% methanol for the same compounds. The trends of parameters confirmed the above results of chromatographic separation. And, this study provides a possibility to optimize the conditions of the separation by adsorption theory.

Separation of Biocompounds

Because of the hydrophobic, hydrogen bonding, and π-π interactions, the stationary phase can be used to separate alkaloids with hydroxyl, ketone, and double bonds.[19] Figure 9 shows the chromatogram of the separation of caffeine, theophylline, and theobromine by pure water. The retention factors (k) were also examined with different components of methanol/water (Figure 10). Generally, the retention factor (k) decreased with the

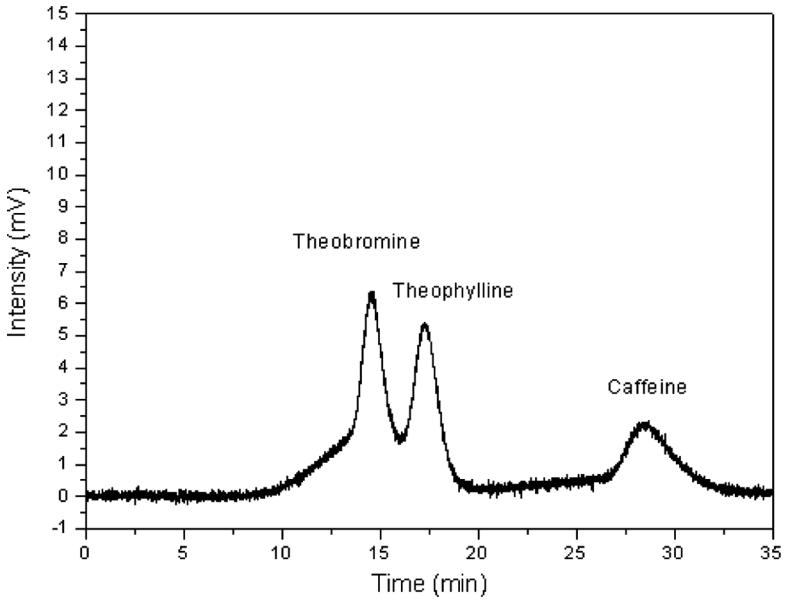

FIGURE 9 Chromatogram of caffeine, theobromine, and theophylline on SilprImN stationary phase. (Mobile phase, pure water; flow rate, 0.5 mL/min; column, 150 × 4.6 mm; detection, UV adsorption at 254 nm).

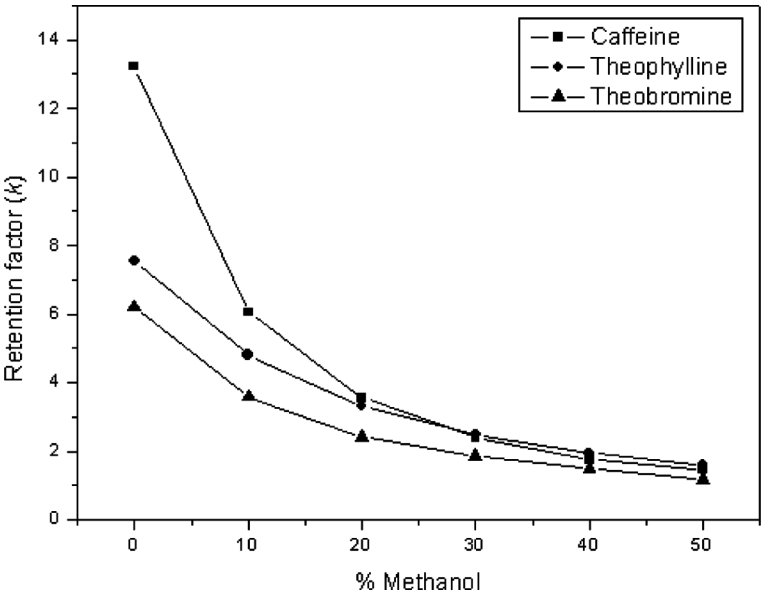

FIGURE 10 Effect of different methanol on the retention factor (k) on SilprImN stationary phase. (Mobile phase, Methanol/water; flow rate, 0.5 mL/min; column, 150 × 4.6 mm; detection, UV adsorption at 254 nm).

concentration of methanol increasing. When the concentration of methanol increased to a certain component, caffeine was eluted earlier than theophylline. This may be caused by the change of the dominative interaction between hydrophobic and amino interactions with different concentrations of organic additives. Another three alkaloids of cytosine, thymine, and adenine which had similar structures was separated successfully on SilprImN by using the same mobile phase (Figure 11).

Furthermore, according to the interaction between NH_2 and ketone group, cryptotanshinone and tanshinone I were selected and separated by using this SilprImN (Figure 12). The novel materials show exciting potential to separate biocompounds.

Protonation of Amino Group

Because the protonation is a common issue in NH_2 column, the SilprImN with amino groups showed the same behavior. In order to investigate protonation of the amino-imidazolium stationary phase, the SilprImN column was stored with methanol/water (10/90, v/v) as mobile phase, and it was used to examine the samples of pyridine and benzene within 20 d. Figure 13 illustrates the trend that the retention factor (k) of benzene decreased with that of pyridine increasing. This phenomenon was due to the protonation of amino

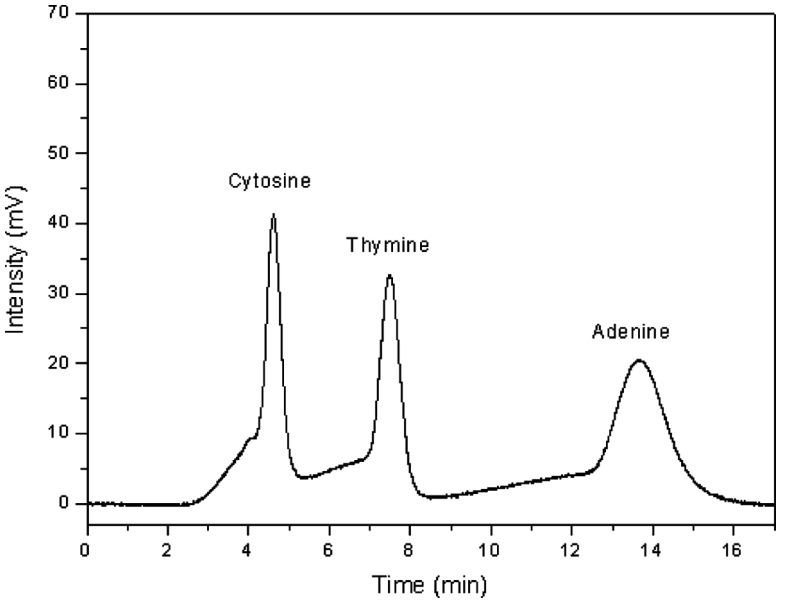

FIGURE 11 Chromatogram of cytosine, thymine, and adenine on SilprImN stationary phase. (Mobile phase, pure water; flow rate, 0.5 mL/min; column, 150 × 4.6 mm; detection, UV adsorption at 254 nm).

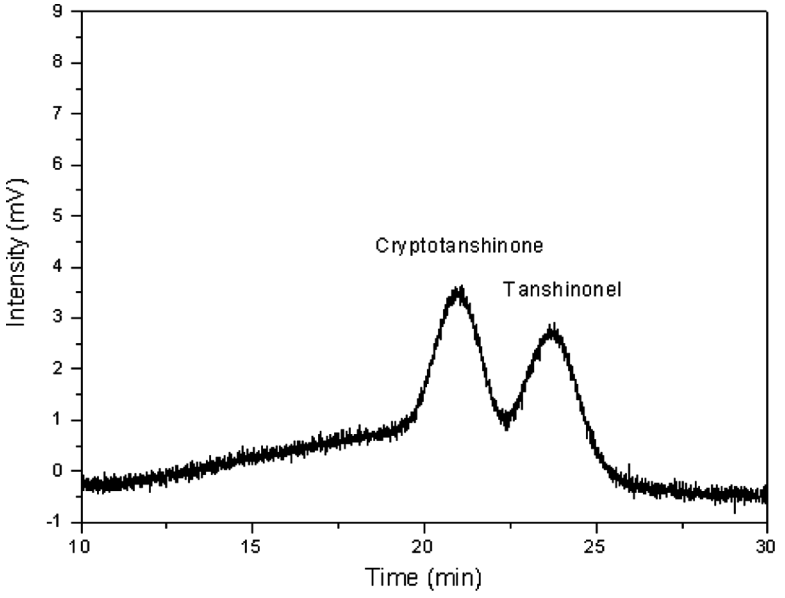

FIGURE 12 Chromatogram of cryptotanshinone, and tanshinone I on SilprImN stationary phase. (Mobile phase, Acetonitrile/water (25/75, *v*/*v*); flow rate, 0.5 mL/min; column, 150 × 4.6 mm; detection, UV absorption at 254 nm).

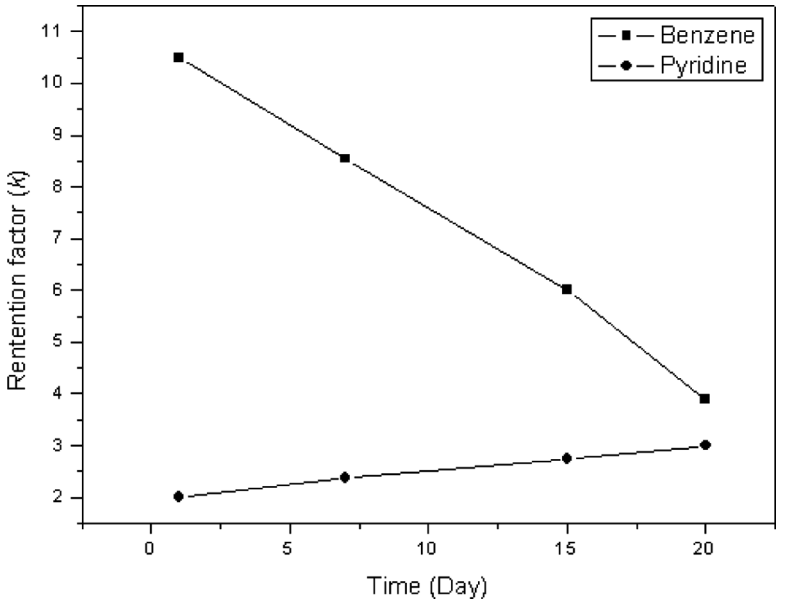

FIGURE 13 Retention factors (k) of pyridine and benzene with the protonation of stationary phase. (Mobile phase, Methanol/water (10/90, *v*/*v*); flow rate, 0.5 mL/min; column, 150 × 4.6 mm; detection, UV absorption at 254 nm).

group and damage of stationary phase which cause by the base of amino group. In this case, the column should be stored with high concentration of organic solvents to reduce the protonation.

CONCLUSION

A novel bi-functional amino-imidazolium stationary phase for high-performance liquid chromatography was described in this paper. The new stationary phase showed promising results for separation of some aromatic compounds, as well as some biocompounds. The studies of the adsorption isotherms provide a method to predict and optimize the condition for column separation by this new material. Although the protonation of amino groups still exists, the charming potential of this stationary phase is emerging. In this situation, further work is underway in our laboratory to exploit the application of this material.

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