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N-Methylimidazolium-functionalized monolithic silica column for mixed-mode chromatography

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ABSTRACT

The development of mixed-mode stationary phase to achieve multiple separation capabilities in one column is very important for high performance liquid chromatography. In this paper, a new specific stationary phase based on grafting *N*-methylimidazolium to a monolithic silica column was successfully prepared for performing capillary liquid chromatography. The characteristics of the column were evaluated by the separation of different types of compounds including inorganic anions, aromatic acids, nucleotides, polycyclic aromatic hydrocarbons, alkylbenzenes, and phenols. The mechanisms for the separation of these compounds were investigated and appeared to involve the mixed interactions including anion-exchange, hydrophilic, π–π, dipole–dipole, and hydrophobic interactions.

1. Introduction

The preparation of monolithic silica columns has attracted significant attention in chromatographic area and a great number of related researches have been reported [1–3]. Attributed to the high porosity and large through-pore size with small-sized skeletons, much higher separation efficiency can be achieved with monolithic columns than that of particle-packed columns at a similar pressure drop [4]. In comparison with traditional packed columns, monolithic silica columns offer many improvements, including fast mass transfer, absence of end frits, and elimination or significant reduction of certain operation problems inherent in packed columns due to the presence of end frits. Therefore, monolithic silica columns are good alternatives to particle-packed columns for their unique advantages [3,5].

Up to date, most of publications dealing with monolithic silica columns focused on reversed-phase separations represented by octadecyl (C18) columns [3,6], which are useful for the separation of nonpolar analytes. With different modifications of columns, various kinds of chromatographic modes could be realized such as ion-exchange chromatography and hydrophilic interaction chromatography, etc. [7–12]. In order to combine the advantages of different kinds of separation modes together, some approaches have been made to obtain monolithic columns with mixed-mode stationary phases for CEC and HPLC [13–15]. However, studies in this area are still rather weak, and more studies are needed to develop mixed-mode stationary phase so as to achieve multiple separation capabilities in one column.

Ionic liquids (ILs) containing relatively large asymmetric organic cations and inorganic or organic anions possess many fascinating properties (including low volatility, high stability, good solubility for organics and inorganics, and so on), which make them being successfully applied in most subdisciplines of analytical chemistry [16]. Immobilizing ILs on silica particles as stationary phases of packed columns in HPLC has aroused much interest recently. *N*-Methylimidazolium- and imidazolium-based ILs have been bonded on silica particles as HPLC stationary phases for the separation of inorganic anions, organic acids, and some organic amines based on anion-exchange and hydrophobic interactions [17,18]. Wang et al. synthesized two alkylimidazolium-modified silica stationary phases for the separation of aromatic carboxylic acids [19]. Since *N*-methylimidazolium as one of the cations to compose ILs possesses a π conjugated system and a cation of imidazole ring, it is a potential material to be applied in the preparation of mixed-mode stationary phases including π–π and anion-exchange interactions.

In order to combine the characteristics of *N*-methylimidazolium-based ionic liquids and monolithic silica columns, a novel *N*-methylimidazolium-functionalized monolithic silica column was prepared. Its performance was investigated through the separation of some compounds including inorganic anions, aromatic acids, nucleotides, phenols, alkylbenzenes and PAHs. And the retention mechanism for the separation of these compounds was also discussed.

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2. Experimental

2.1. Materials and reagents

Tetramethoxysilane (TMOS) and methyltrimethoxysilane (MTMS) were obtained from Sigma (St Louis, MO, USA). Polyethylene glycol (PEG, MW 10,000) was purchased from Sinopharm Chemical Reagent Company (Shanghai, China). 3-Chloropropyltrimethoxysilance was obtained from Acros (New Jersey, USA). *N*-Methylimidazole was obtained from J&K chemicals (Beijing, China). Triethylamine was purchased from Guangzhou Chemical Regent Factory (Guangzhou, China). Polystyrene standards with molecular masses ranging from 1300 to 2,000,000 were purchased from Alfa (Tianjin, China). Tetrahydrofuran (THF), acetonitrile (ACN) and methanol (HPLC grade) were purchased from SK Chemicals (Ulsan, South Korea). Purification water was obtained from an Elga water purification system (ELGA, London, UK).

Sodium nitrite, sodium bromide, potassium nitrate, sodium iodide, sodium bromate, potassium iodate, *p*-hydroxybenzoic acid, benzoic acid, salicylic acid, and 3,5-dinitrosalicylic acid were purchased from Guangzhou Jinhuada Chemical Regent Factory (Guangzhou, China). Adenosine-5′-monophosphate (5′-AMP), guanosine-5′-monophosphate (5′-GMP), cytidine-5′-monophosphate (5′-CMP), and uridine-5′-monophosphate (5′-UMP) were purchased from Genbase Bio-Technology Company (Guangzhou, China). Benzene, naphthalene, fluorene, anthracene, methylbenzene, ethylbenzene, propylbenzene, butylbenzene, 4-hydroxyphenol, 2-chlorophenol, 4-nitrophenol, and 2,4-dinitrophenol were obtained from Alfa Aesar (Heysham, UK). The chemicals used in experiments were of analytical grade and used without further purification. The phosphate buffer solution (PBS) was prepared by dissolving the desired amount of Na_2HPO_4 in water and regulated to the required pH with 1 M H_3PO_4 or NaOH. Mobile phase was prepared by mixing PBS or water with organic solvents in appropriate volume ratio. The running mobile phase was filtered through a 0.45 μm filter and degassed ultrasonically prior to use.

2.2. Instrumentation

A capillary liquid chromatography system was used for the characterization of the monolithic silica capillary column. The system consisted of a microflow pump (MP711, GL Sciences, Tokyo, Japan), an UV detector with an optical fiber flow cell (MU701, GL Sciences, Tokyo, Japan), and a manual injection valve (Valco VICI, Schenkon, Switzerland) with an internal sample loop of 50 nL. The chromatographic data were processed with HW-2000 workstation (Qianpu Software Company, Shanghai, China). A fused-silica capillary (GL Sciences, Japan), 8 cm × 50 μm i.d., was used to connect the outlet end of analytical column with the optical fiber flow cell (light path 3 mm). For column fabrication, temperature-controlling process was carried out using a gas chromatography (GC) system 7890II (Techcompany, Shanghai, China). All separations were carried out at room temperature, which was kept at 25 °C using air conditioning system. Fused-silica capillaries (200 μm i.d. × 365 μm o.d.) were purchased from Hebei Yongnian Ruipu Chromatogram Equipment Company (Handan, China). Scanning electron microscopy (SEM) of the monolithic column was carried out on a XL-30 environmental scanning electron microscope (Philips, Eindhoven, Netherlands).

2.3. Column preparation

2.3.1. Preparation of monolithic silica column

A fused-silica capillary was first treated with 1 M NaOH at 40 °C overnight, followed by a flush of water for 30 min. Subsequently, it was flushed with 1 M HCl for 1 h, then water and acetone for 30 min, respectively. Finally, the capillary was purged with nitrogen at 180 °C for 3 h prior to use.

The preparation condition of the monolithic silica column was similar to that reported by Miyamoto et al. [20]. Briefly, a 0.45 mL TMOS/MTMS mixture (v/v 3:1) was added to a solution containing PEG (0.0513 g) and urea (0.1013 g) in 0.01 M acetic acid (1 mL) at 0 °C and stirred for 30 min. The homogeneous solution was then stirred for 10 min at 40 °C, charged into the pretreated capillary, and allowed to react at 40 °C overnight. Then, the temperature was raised slowly and the column was treated for 4 h at 120 °C to form mesopores with the ammonia generated by the hydrolysis of urea, then cooled and washed with water and methanol in turns. After drying in a GC oven, heat treatment was carried out at 330 °C for 25 h to decompose the organic moieties in the column.

2.3.2. Chemical modification of monolithic silica column with *N*-methylimidazolium

Surface modification of the monolithic silica column was carried out by two steps, as shown in Fig. 1. First, 3-chloropropyltrimethoxysilane (50 μL) and triethylamine (5 μL) (as a catalyst [18,21]) were added to 600 μL of toluene. The mixture was stirred and then charged into the column, which was rinsed with toluene in advance, and then reacted at 90 °C for 15 h. Afterwards, the column was washed with toluene to remove the unreacted reagents. Subsequently, *N*-methylimidazole (50 μL) was added to 600 μL of toluene, stirred and then charged into the column and reacted with chloropropyl group which was bonded on the silica surface during the first step at 80 °C for 10 h. Finally, the column was rinsed with toluene followed by methanol.

3. Results and discussion

3.1. Column characterization

3.1.1. SEM observation and column permeability

Fig. 2 shows the SEM micrographs of the monolithic silica column before and after modification by *N*-methylimidazolium. As can be seen from the micrographs, the general morphology of the column did not have obvious change after the surface modification procedure. The fractured surface of the monolithic column was a typical continuous bed structure which was composed of skeleton and large through-pores. Moreover, no shrinkage or cracking of the column was found. So sufficient attachment of silica monolithic skeletons to the capillary wall was achieved. These results were mainly attributed to the addition of MTMS as an organic modifier to TMOS changing the chemical or physical characteristics of sol–gel process [22]. During aging procedure, appropriate mesopores were formed with ammonia generated by the hydrolysis of urea [23]. As a consequence, large surface area was obtained, which would be helpful for the modification to the column.

Column permeability (K) reflects through-pore size and external porosity, or a domain size (a combined size of a through-pore and a skeleton) at a constant through-pore size/skeleton size ratio [24]. The permeability of the column was calculated according to Eq. (1)

$$K = \frac{F \times \eta \times L}{\Delta P \times \pi \times r^2} \quad (1)$$

where F, η, L, ΔP, and r stand for volume flow rate of the mobile phase, dynamic viscosity of the mobile phase, the column length, the column back pressure, and the inner radius of the column, respectively [25]. The permeability of the column before and after modification was calculated to be 6.6×10^{-14} m^2 and 6.1×10^{-14} m^2, respectively, which indicated that *N*-methylimidazolium has been successfully bonded to the monolithic silica skeleton. In comparison with the particle-packed

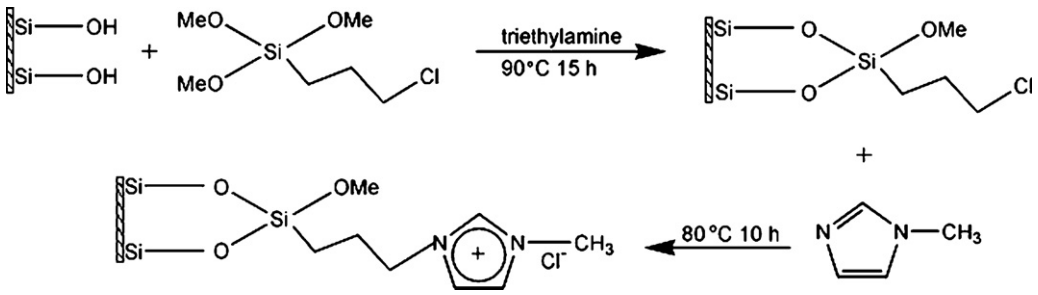

Fig. 1. Schematic diagram for the preparation of *N*-methylimidazolium functionalized monolithic silica column.

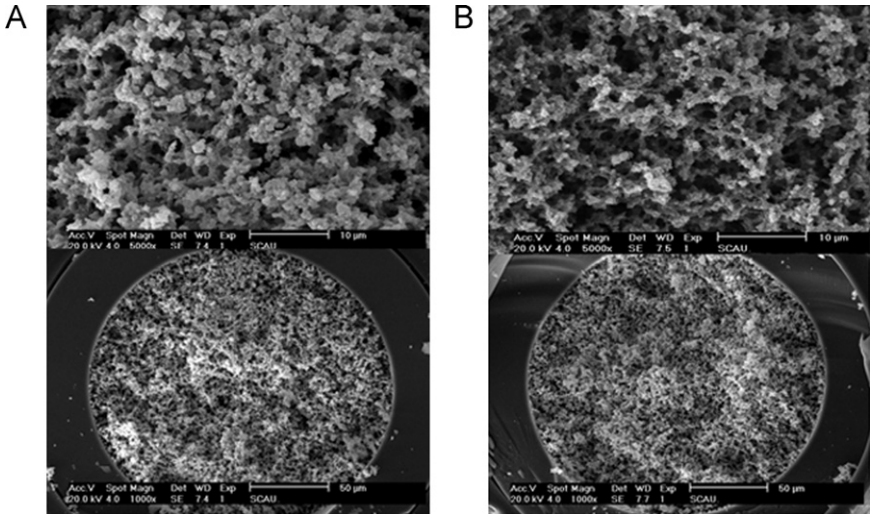

Fig. 2. Scanning electron micrographs of the monolithic silica capillary column before (A) and after (B) modification with *N*-methylimidazolium.

column (silica base, 5 μm particle modified with diethylamino group) having a permeability of 2.0×10^{-14} m^2 [26], the prepared *N*-methylimidazolium-functionalized monolithic silica column had more than 3 times the permeability of the particle-packed column.

3.1.2. Pore characterization of column by size exclusion chromatography

The pore characterization of the column before and after modification was measured by size exclusion chromatography using polystyrene standards as solutes and THF as mobile phase [27]. Fig. 3 shows the relationship between the molecular weight of polystyrene standards and the elution volume of THF for the column before and after modification. Methylbenzene and a polystyrene standard with a molecular weight of 2×10^6 were used for determining the total porosity and external porosity, respectively. The internal porosity is derived from the difference between the total and external porosities. Table 1 summarizes the pore characterization of the column before and after modification. As shown in Table 1, the internal porosity (mesopore volume) of the column decreased obviously after modification. The difference in the internal porosity before and after modification could be regarded as the volume occupied by the *N*-methylimidazolium moieties.

3.1.3. Column efficiency

The column efficiency was evaluated on the basis of van Deemter equation.

$$H = A + \frac{B}{u} + Cu \quad (2)$$

where H, u represent the plate height and the linear velocity of the mobile phase, A, B and C are three coefficients and respect eddy dispersion, longitudinal diffusion, and mass transfer resistance, respectively [28]. Fig. 4 presents the van Deemter curves for the *N*-methylimidazolium-functionalized column with 5′-UMP (an anionic solute) and ethylbenzene (a neutral solute) as the test solutes. At an optimal velocity, the minimum plate height of the

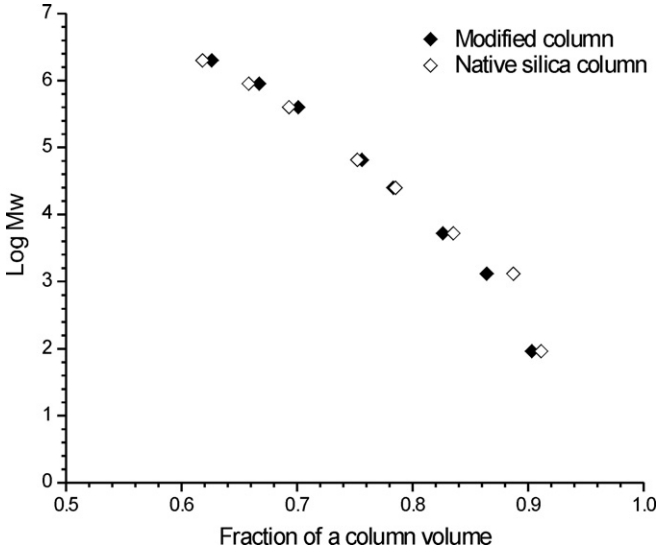

Fig. 3. The relationship between the molecular weight of polystyrene standards and the elution volume of THF for the column before and after modification with *N*-methylimidazolium.

Table 1
Porosities of the monolithic silica column before and after modification by *N*-methylimidazolium.

Column	Total porosity (ε_T)	External porosity (ε_e)	Internal porosity (ε_i)	Bond phase (V_s)	Phase ratio (V_s/ε_T)
Native	0.911	0.618	0.293		
Modified	0.903	0.626	0.277	0.016	0.018

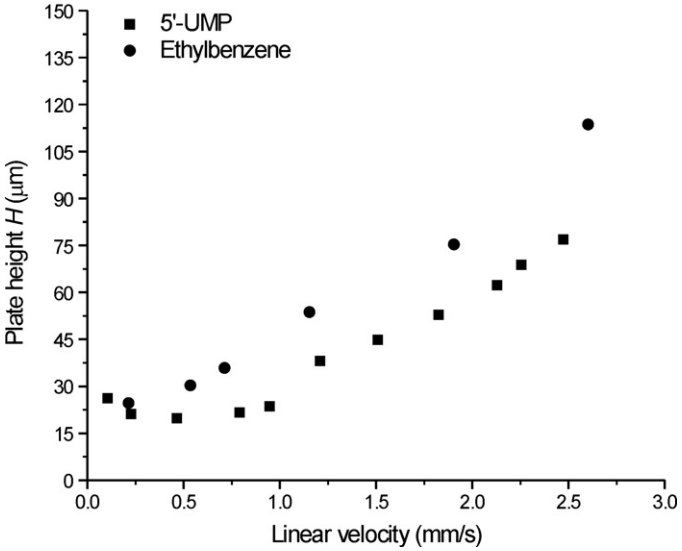

Fig. 4. van Deemter plot of *N*-methylimidazolium modified column. Experimental conditions for 5′-UMP: column, 39 cm × 200 μm i.d.; mobile phase, 20 mM Na_2HPO_4 (pH 3.0); detection, UV at 254 nm. For ethylbenzene: column, 40 cm × 200 μm i.d.; mobile phase, methanol–water (60:40, v/v); detection, UV at 214 nm.

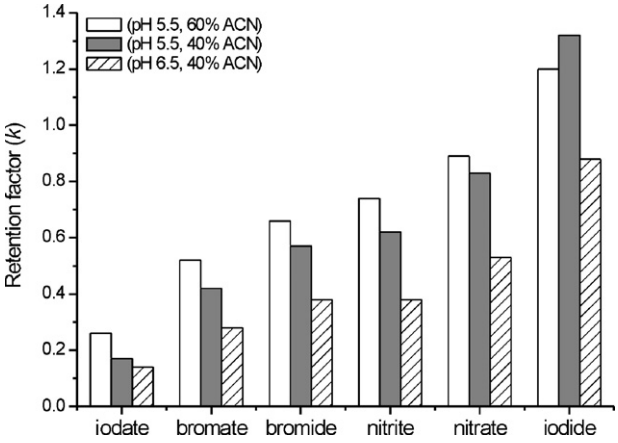

Fig. 6. *k* values of inorganic anions under different mobile phase conditions. Conditions: phosphate concentration, 20 mM; column, 32.5 cm × 200 μm i.d.; linear velocity, 0.69 mm/s; detection, UV at 200 nm.

column was about 20 μm for 5′-UMP (0.46 mm/s) and 25 μm for ethylbenzene (0.21 mm/s), respectively. In comparison with on-column detection, it is obvious that the dead volumes derived from the post-column detection used in the study have greatly reduced the separation efficiency. Consequently, an on-column detection mode should be applied in our future work to improve the column performance.

The performance of the *N*-methylimidazolium modified monolithic column and the unmodified native silica column was compared using six inorganic anions and four alkylbenzenes as the test samples. As shown in Fig. 5, the inorganic anions and alkylbenzenes obtained good resolution using the *N*-methylimidazolium modified column, whereas they were not separated on the native silica column. The results demonstrated that the *N*-methylimidazolium modified column possessed better separation abilities than the native silica column for these compounds. Meanwhile, the results also proved that *N*-methylimidazolium was successfully attached to the monolithic silica skeleton.

3.2. Applications

3.2.1. Separation of inorganic anions

A mixture containing six inorganic anions was separated to investigate the retention mechanism of the column. As shown in Fig. 6 the retention factors (*k*) of various anions decreased with the

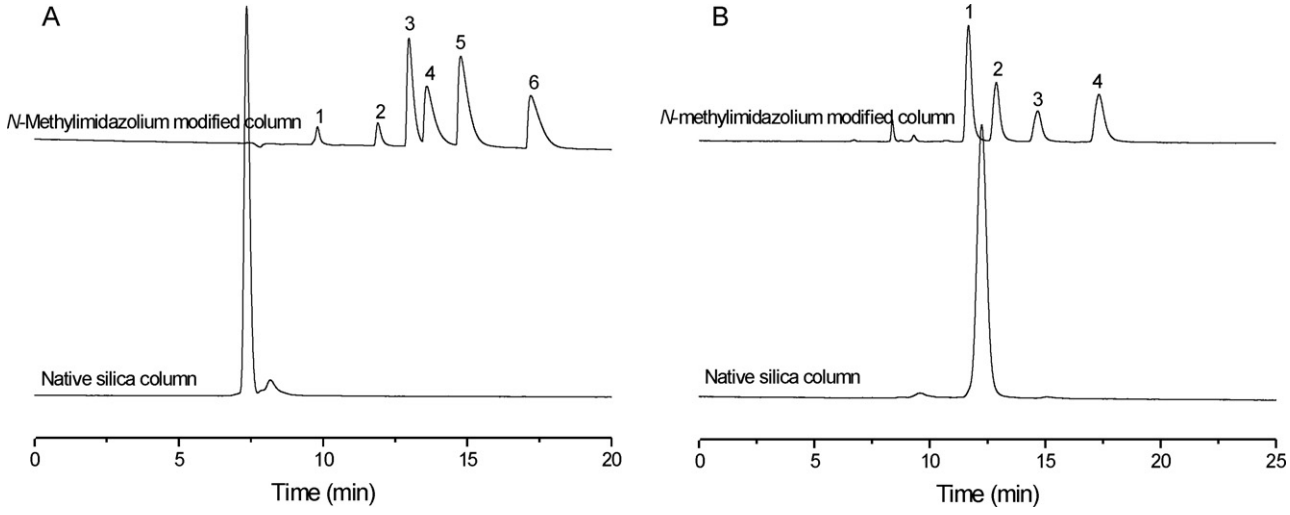

Fig. 5. Chromatograms of inorganic anions and alkylbenzenes separated on *N*-methylimidazolium modified column and native silica column. Experimental conditions: (A): solute, inorganic anions; column, 32.5 cm × 200 μm i.d.; mobile phase, 20 mM Na_2HPO_4 (pH 5.5)-ACN (40:60, v/v); linear velocity, 0.69 mm/s; detection, UV at 200 nm. Peak identification: 1, iodate; 2, bromate; 3, bromide; 4, nitrite; 5, nitrate; 6, iodide. (B): solute, alkylbenzenes; column, 39 cm × 200 μm i.d.; mobile phase, methanol-water (70:30, v/v); linear velocity, 0.97 mm/s; detection, UV at 214 nm. Peak identification: 1, methylbenzene; 2, ethylbenzene; 3, propylbenzene; 4, butylbenzene.

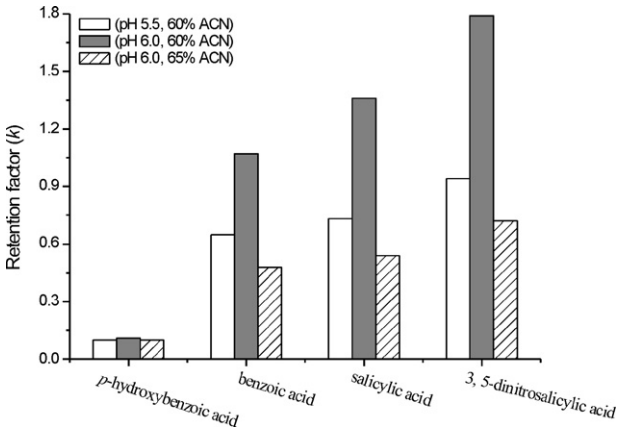

Fig. 7. *k* values of aromatic acids under different mobile phase conditions. Conditions: phosphate concentration, 20 mM; column, 25 cm × 200 μm i.d.; linear velocity, 0.74 mm/s; detection, UV at 230 nm.

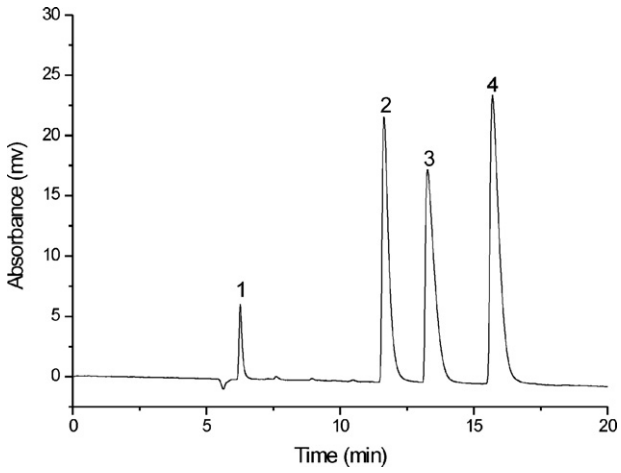

Fig. 8. Separation of aromatic acids using *N*-methylimidazolium modified column. Experimental conditions: column, 25 cm × 200 μm i.d.; mobile phase, 20 mM Na_2HPO_4 (pH 6.0)-ACN (40:60, v/v); linear velocity, 0.74 mm/s; detection: UV at 230 nm. Peak identification: 1, *p*-hydroxybenzoic acid; 2, benzoic acid; 3, salicylic acid; 4, 3,5-dinitrosalicylic acid.

increase of buffer pH from 5.5 to 6.5. The results demonstrated that the increase of the pH of the mobile phase reduced the electrostatic interactions between the anions and the stationary phase owing to the increase of HPO_4^{2-} in the mobile phase, which possessed a stronger electrostatic interaction with the stationary phase than $H_2PO_4^-$ (pKa_1, pKa_2, and pKa_3 of phosphoric acid are 1.97, 6.82, and 12.5, respectively [29]). With the increase of the ACN content, the *k* values of most anions increased except iodide. This result showed that the hydrophilic interactions of the anions and the stationary phase also played a role for the separation of the anions. It should be noted that *k* value of iodide showed the opposite tendency to that of other anions. The reason is unclear. The investigations of the effects of the mobile phase pH and ACN content on the *k* values of anions exhibited that anion-exchange and hydrophilic interactions played main roles for the separation of inorganic anions on the *N*-methylimidazolium modified monolithic column. Fig. 5A shows the chromatogram of the six inorganic anions.

The elution order of the anions on the column is iodate, bromate, bromide, nitrite, nitrate, and iodide. In ion-exchange chromatography, ion valence and radius are two major determinants for the retention and selectivity of the ions. In our experiment, since each of the anions has one valence, the elute order is mainly decided by their ionic radius. A later-eluting ion possessed a larger ionic radius than an earlier-eluting one, leading to a lower charge density, and thus causing weak solvation. As a result, the solvated ionic radius of the later-eluting ion becomes smaller, which makes it easier to interact with the stationary phase.

3.2.2. Separation of aromatic acids

A mixture of aromatic acids including *p*-hydroxybenzoic acid (p*K*a 4.58), benzoic acid (p*K*a 4.20), salicylic acid (p*K*a 2.97), and 3,5-dinitrosalicylic acid (p*K*a 2.82) was used for further studying the separation mechanism [30]. As shown in Fig. 7, the *k* values of aromatic acids increased with the increase of the buffer pH from 5.5 to 6.0. It may be a higher buffer pH leads to a higher degree of ionization of these aromatic acids, thus causing a stronger electrostatic interaction between the aromatic acids and the stationary phase. *N*-Methylimidazolium possessing a π conjugated system may interact with some molecules having a similar construction through π–π interaction. Since aromatic acids contain π conjugated systems, they have π–π interactions with the stationary phase. As shown in Fig. 7, the *k* values of aromatic acids decreased with the increase of ACN content from 60% to 65%. The result may be due to the decrease of the π–π interaction between the analytes and the stationary phase with an increased ACN content. The elution order of these aromatic acids has relationship with their p*K*a values. The smaller the p*K*a value of the aromatic acid was, the later it eluted. At pH 6.0, each of the aromatic acids took one negative charge according to their p*K*a values. The difference in their ionic radius was probably responsible for the difference in their retention on the column. In terms of their structures of the solutes, the difference in dipole–dipole interaction was also probably responsible for the difference in retention of these solutes. The above investigations exhibited that anion-exchange, dipole–dipole and π–π interactions played main roles for the separation of aromatic acids on the *N*-methylimidazolium modified monolithic column. Fig. 8 presents the chromatogram for the separation of the four aromatic acids on the column.

3.2.3. Separation of nucleotides

The chromatographic characteristic of the column was also examined using four nucleotides (5′-UMP, 5′-AMP, 5′-CMP, and 5′-GMP). Fig. 9 shows the chromatogram of the four nucleotides. The elution order of the nucleotides was 5′-AMP, 5′-CMP, 5′-UMP, and 5′-GMP. The p*K*a values of the primary phosphate groups were 1.02 (5′-UMP), 0.89 (5′-AMP), 0.80 (5′-CMP), and 0.70 (5′-GMP), respectively [31]. Under the experimental conditions (buffer pH

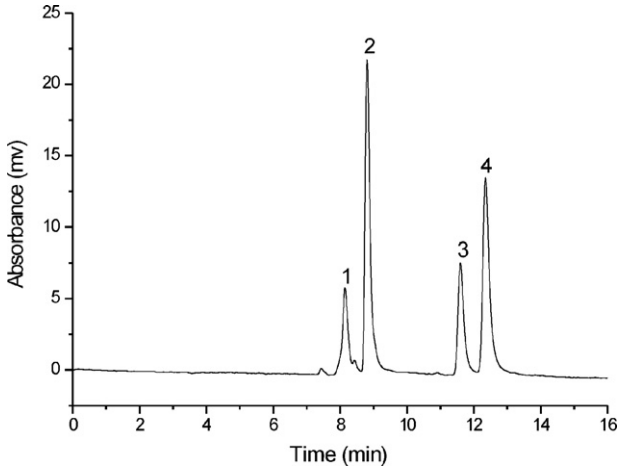

Fig. 9. Separation of nucleotides using *N*-methylimidazolium modified column. Experimental conditions: column, 32.5 cm × 200 μm i.d.; mobile phase, 20 mM Na_2HPO_4 (pH 3.0); linear velocity, 0.73 mm/s; detection, UV at 254 nm. Peak identification: 1,5′-AMP; 2,5′-CMP; 3,5′-UMP; 4,5′-GMP.

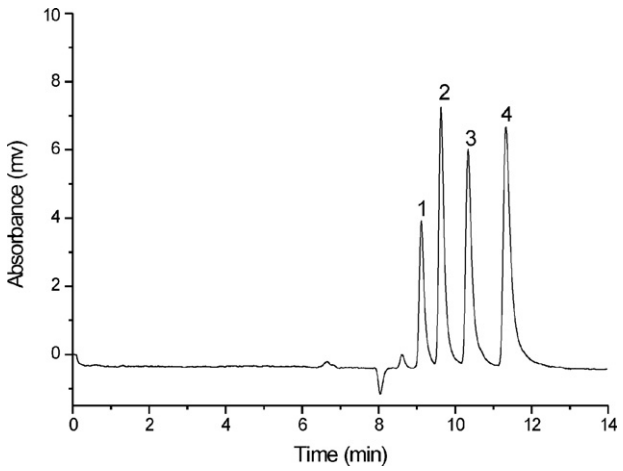

Fig. 10. Separation of PAHs using *N*-methylimidazolium modified column. Experimental conditions: column, 32.5 cm × 200 μm i.d.; mobile phase, methanol–water (70:30, v/v); linear velocity, 0.67 mm/s; detection, UV at 254 nm. Peak identification: 1, benzene; 2, naphthalene; 3, fluorene; 4, anthracene.

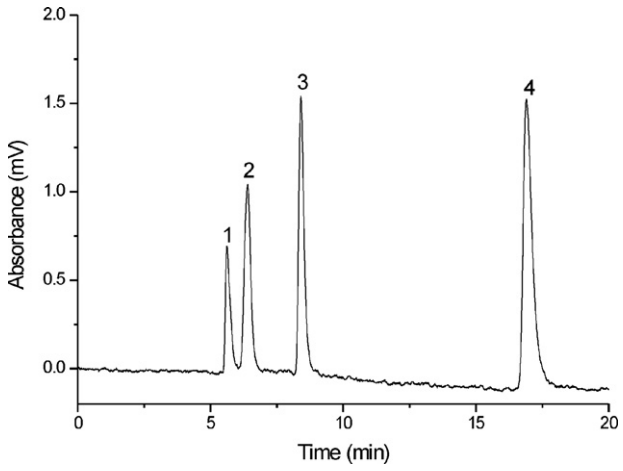

Fig. 11. Separation of phenols using *N*-methylimidazolium modified column. Experimental conditions: column, 25 cm × 200 μm i.d.; mobile phase, methanol–water (60:40, v/v); linear velocity, 0.82 mm/s; detection, UV at 254 nm. Peak identification: 1,4-hydroxyphenol; 2,2-chlorophenol; 3,4-nitrophenol; 4,2,4-dinitrophenol.

3.0), each of the nucleotides has one negative charge. Therefore, the anion-exchange interaction between the analytes and the stationary phase plays a role for the separation of the nucleotides. 5′-AMP and 5′-GMP have purine bases, and 5′-CMP and 5-UMP have pyrimidine bases. Purine and pyrimidine have π conjugated systems. Therefore, π–π interaction between the analytes and the stationary phase also influenced the separation of the nucleotides on the column. Based on the above discussion, the separation of the nucleotides on the *N*-methylimidazolium modified column is mainly decided by the anion-exchange and π–π interactions between the analytes and the stationary phase.

3.2.4. Separation of PAHs

To demonstrate that π–π interaction between the analytes containing π conjugated systems and the stationary phase really affects the separation of these compounds. A PAHs mixture including benzene, naphthalene, fluorene, and anthracene was utilized as the test sample since they are neutral compounds containing π conjugated systems. The polarizabilities of these compounds are 0.52 (benzene), 0.92 (naphthalene), 1.03 (fluorene), and 1.34 (anthracene), respectively [32]. If the π–π interaction between the analytes and the stationary phase mainly played a role for the separation of them on the *N*-methylimidazolium modified column, the elution order of these compounds would have relationship with the degrees of their π conjugation. Fig. 10 shows the chromatogram of the four PAHs. In this case, the *k* values of the analytes were 0.13 (benzene), 0.20 (naphthalene), 0.28 (fluorene), and 0.41 (anthracene), respectively. These compounds eluted in the order of benzene, naphthalene, fluorene, and anthracene, which is in agreement with the order of the increase of their π conjugation degree. The higher the degree of the π conjugation of the compound was, the stronger the π–π interaction of the compound with the stationary phase was, and thus the later the compound eluted. The retention time decreased with the increase of methanol content in the mobile phase since the π–π interaction between the analytes and the stationary phase decreased with the increase of methanol content. The results proved that π–π interaction between the analytes and the stationary phase mainly played a role for the separation of PAHs.

3.2.5. Separation of alkylbenzenes

The stationary phase of the column is composed of two parts, one is an *N*-methylimidazolium group which is responsible for π–π and anion-exchange interactions and the other is the $-(CH_2)_3-$ spacer which provides sufficient hydrophobic properties. In order to test the hydrophobic characteristic of the column, four alkylbenzenes (methylbenzene, ethylbenzene, propylbenzene, butylbenzene) were selected as the specimens. Fig. 5B shows the chromatogram of the alkylbenzenes. The polarizabilities of these compounds are 0.52 (methylbenzene), 0.51 (ethylbenzene), 0.50 (propylbenzene), and 0.51 (butylbenzene), respectively [32,33], which demonstrated that the compounds have similar π conjugation level. Log *P* (octanol–water partition coefficient) value reflects hydrophobicity of methylbenzene (2.73), ethylbenzene (3.15), propylbenzene (3.72), and butylbenzene (4.38), respectively [34]. The experimental results showed that the alkylbenzenes eluted in the order of methylbenzene, ethylbenzene, propylbenzene, and butylbenzene, which was in accordance with the increase of their log *P* values. It was found that the *k* values of the four alkylbenzenes were 0.76 (methylbenzene), 0.96 (ethylbenzene), 1.23 (propylbenzene) and 1.62 (butylbenzene) with a mobile phase containing 70% methanol, and then decreased to 0.42 (methylbenzene), 0.46 (ethylbenzene), 0.51 (propylbenzene), and 0.58 (butylbenzene), respectively, with the methanol content increased to 80%. These results demonstrated that the π–π and hydrophobic interactions of the analytes with the stationary phase mainly played the roles for the separation of alkylbenzenes.

3.2.6. Separation of phenols

In order to make a further examination of the separation capability of the column, a mixture of phenols including 4-hydroxyphenol, 2-chlorophenol, 4-nitrophenol, 2,4-dinitrophenol was separated using the column. Through regulating the composition of mobile phase, a good resolution of the four phenols was obtained with methanol–water (60:40, v/v) as the mobile phase, as shown in Fig. 11.

3.3. Reproducibility and column stability

A *N*-methylimidazolium functionalized column showed stable chromatographic performance for more than three months. The reproducibility of the column was evaluated in terms of the retention factor of salicylic acid. The intraday relative standard deviation (RSD) was 2.4% ($n = 6$) and the interday RSD was 4.9% ($n = 6$).

4. Conclusions

In this study, a silica-based monolithic column modified with *N*-methylimidazolium has been successfully prepared. Its char-

acteristics for the separation of different types of compounds were evaluated. The *N*-methylimidazolium group together with the $-(CH_2)_3-$ spacer as a linker exhibited different kinds of interactions for the separation of various types of compounds. Based on anion-exchange and hydrophilic interactions, six inorganic anions were well separated under the optimized conditions. Efficient separations of nucleotides, aromatic acids, and phenols were achieved based on the anion-exchange, dipole–dipole, and $\pi-\pi$ interactions. Four PAHs and four alkylbenzenes were also well separated based on $\pi-\pi$ and/or hydrophobic interactions. The results demonstrated that the *N*-methylimidazolium functionalized column had great potentiality in the separation of many different types of compounds based on anion-exchange and/or hydrophilic and/or $\pi-\pi$ and/or hydrophobic interactions.

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