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Benzimidazole modified silica as a novel reversed-phase and anion-exchange mixed-mode stationary phase for HPLC

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ABSTRACT

A novel stationary phase based on benzimidazole modified silica was prepared and evaluated as reversed-phase and anion-exchange mixed-mode stationary phase for high performance liquid chromatography (HPLC). Using the phase, polycyclic aromatic hydrocarbons, phthalates, anilines and phenols were respectively separated depending on formation of different types of chemical bonds like hydrogen bonding, π–π stacking, electrostatic forces and hydrophobic interactions by reversed-phase chromatography; inorganic and organic anions were also separated mainly through anion-exchange and π–π interactions by anion-exchange chromatography with high resolution and column efficiency. The proposed benzimidazole modified silica is a promising mixed-mode stationary phase for the separation of complex samples in HPLC.

1. Introduction

High performance liquid chromatography (HPLC) provides many separation modes such as reversed-phase, normal phase, ion-exchange chromatography, chiral chromatography, etc. [1]. The reversed-phase chromatography is the most popular for its successful separation of most organic compounds based on the hydrophobic interaction mechanism [2,3]. Ion-exchange chromatography, as an efficient supplement for reversed-phase chromatography, is the commonly used separation mode for ionic analytes via the ion-exchange mechanism [4]. The existence of variable separation modes increases the separation efficiency of HPLC and widens its application in different fields.

The chromatographic performance of the stationary phase depends on its separation mechanism [5,6]. Most stationary phases only provided single or simple separation mechanism [7,8] and were applied in single chromatographic mode. Mixed-mode stationary phase, relative to single-mode, is a promising stationary phase which can be applied in different modes on single column based on multiple interaction mechanisms. This kind of stationary phase can reduce the cost of testing and improve work efficiency to a great extent, especially for the analysis of some complex samples. It deserves wide and further investigations. Until now, several sorts of stationary phases with mixed-mode performance have been investigated in laboratory [8–16], such as phenylpropanolamine [8], phenylamine [9,10], 8-quinolinol [11], poly(1-allylimidazole) [12], imidazole [13,14], pyridine [15,16] and dipyridine [17] functional silicas. These functional silicas were tested in reversed-phase and ion-exchange mixed-mode chromatography, to separate organic compounds and ions on the same column. Lämmerhofer and his co-workers prepared the mixed mode reversed-phase/weak anion-exchange separation materials by functionalizing thiol-modified silica with N-(10-undecenoyl)-3-aminoquinuclidine and N-(10-undecenoyl)-3-α-aminotropane, respectively [18–20]. And the mixed mode phases have distinct interaction sites on a single chromatographic ligand, such as a hydrophobic alkyl chain strand (hydrophobic domain), embedded polar functional groups (hydrophilic domains), and a terminal bicyclic quinuclidine or tropane ring which accommodate the anion-exchange site (ionic domain) [19]. Retention properties of 79 fungal metabolites (including neutral, acidic, basic, and amphoteric compounds) were evaluated on it [19]. These stationary phases took advantage of the functional groups with charged or chargeable moieties. Ionic or charge–charge interaction was involved in the separation of ionic analytes with ion-exchange chromatographic conditions [10,11,13,15,19]. In addition,

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a multiple retention mechanism including hydrogen-bonding, hydrophobic, electrostatic and π–π interactions was suggested in reversed-phase mode.

Benzimidazole modified silica was applied to sorption and preconcentration of metal ions from non-aqueous solvents [21,22]. Benzimidazole contains phenyl and imidazole in its molecular structure, which has multiple properties, e.g., large π-system, weak basic group. Firstly, the large π-system can interact with compounds with π-electron group in molecular structure. Secondly, weak basic group can be protonated to form anion-exchange site. Besides, benzimidazole group may interact with some analytes via hydrophobic interaction and hydrogen bonding. So it is a promising functional group to provide mixed-mode separation properties. As far as we know, benzimidazole modified silica has not been used as a stationary phase in HPLC. A stationary phase based on benzimidazolium-modified silica was prepared and investigated in our present work. It can be applied as a mixed-mode stationary phase for HPLC. On this stationary phase, aromatic compounds (polycyclic aromatic hydrocarbons (PAHs), phthalates, anilines and phenols) and anions were successfully separated by reversed-phase and anion-exchange chromatography, respectively. The new stationary phase has potential application to the separation of complex analytes with different physicochemical properties.

2. Experimental

2.1. Chemicals and materials

Spherical and porous silica particles of 5 μm, with 8 nm average pore diameter and 390 $m^2\,g^{-1}$ specific surface area (BET), which were made in our laboratory, were used as the support. 3-Chlorpropyltrimethoxysilane (98%) was obtained from Qufu Chenguang Fine Chemical Co. (Shandong, China). Benzimidazole (98%) was purchased from Shanghai Jingchun Industry Co. (Shanghai, China). All organic and inorganic compounds used in chromatographic tests were analytical reagents. Toluene was dried by refluxing with sodium for 24 h, and then distilled before use. All other compounds were of analytical grade quality and were used without further purification. A Kromasil C_{18} column (150 × 4.6 mm i.d., 5 μm, 10 nm) was obtained from Hanbon Science & Technology Co. (Jiangsu, China).

2.2. Preparation of stationary phase

Benzimidazole modified silica stationary phase was synthesized by the two step reaction. Firstly, 20.0 g of silica was dried under vacuum at 135 °C for 20 h and placed in a 500 mL reaction flask containing 200 mL of toluene. Under stirring, 20 mL of 3-chloropropyltrimethoxysilane was added into this mixture. The mixture was refluxed in nitrogen atmosphere for 24 h. Then, the reaction mixture was cooled to room temperature, and filtered. The solid product was washed with toluene, ethanol, ethanol–water mixture (1:1, v/v) and acetone in turn. The chloropropyl silica (SilprCl) was obtained and dried under vacuum at 65 °C for 8 h.

The chloropropyl groups on SilprCl were treated with benzimidazole. Briefly, 3.0 g of SilprCl was placed in a 100 mL reaction flask containing 30 mL of N,N-dimethylformamide. Then, 3.0 g of benzimidazole was added into the mixture. The reaction mixture was stirred under nitrogen at 140 °C for 96 h. Then the heating was stopped and the modified silica was washed with ethanol, water and methanol in turn. The benzimidazole modified silica (SilprBim) was obtained and dried under vacuum at 75 °C for 10 h, before packing and characterizations.

2.3. Characterizations

FTIR spectra of the samples in the range of 4000–400 cm^{-1} were obtained on a Thermo Nicolet 5700 FTIR spectrophotometer (Madison, WI, USA). The carbon, hydrogen and nitrogen contents of SilprCl and SilprBim were determined by elemental analysis performed on an Elementar Vario EL cube (Hanau, Germany). The average concentration of benzimidazolium groups attached onto silica can be calculated through the nitrogen content of SilprBim. X-ray Photoelectron Spectroscopy (XPS) was used to characterize the modified silicas. The XPS spectra were recorded with an Escalab 210 Axis Ultra photoelectron spectrometer (VG Scientific, UK) using an Mg K alpha excitation source. The specific surface areas (BET) of silica, SilprCl and SilprBim were determined on an ASAP 2010 Accelerated Surface Area and Porosimetry System (Micromeritics, USA).

2.4. Chromatographic conditions

All chromatographic tests were performed on an Agilent 1100 series (Santa Clara, CA, USA) with a 20 μL sample loop and a UV/vis detector. The SilprBim was slurry-packed into 150 × 4.6 mm i.d. stainless steel LC column under a constant packing pressure of 50 MPa. Mobile phases were filtered through a 0.45 μm nylon membrane filter. All tests in reversed-phase chromatography used methanol–water as the mobile phase with UV detection at 254 or 230 nm. The separation of inorganic anions used KCl solution as the mobile phase with UV detection at 210 nm, and the separation of organic anions used methanol–KH_2PO_4 solution as the mobile phase with UV detection at 200 nm. pH of a 175 mmol L^{-1} KCl eluent was modulated from 2.5 to 7.0 with hydrochloric acid and potassium hydroxide using a calibrated Sartorius PB-10 pH meter (Goettingen, Germany). All tests were performed at 20 °C and 1 mL min^{-1}. The column dead time was obtained from the mobile phase signal in the UV detection.

3. Results and discussion

3.1. Preparation of stationary phase

SilprBim was prepared through the reaction between benzimidazole and the chloropropyl groups of SilprCl, which was prepared by modifying silica with 3-chloropropyltrimethoxysilane . The synthesized process is schematically described in Fig. 1.

3.2. Characterizations

The FTIR spectra of SilprCl and SilprBim are shown in Fig. 2. The bands around 1110 cm^{-1} and broad bands around 3448 cm^{-1} are attributed to the stretching vibrations of Si–O and O–H groups respectively, and sourced from matrix of silica. Bands appeared at 2920 cm^{-1} and 2930 cm^{-1} are attributed to aliphatic C–H stretching in the spectra of SilprCl and SilprBim. In the spectrum of SilprBim, two new bands appeared at 1645 cm^{-1} and 1565 cm^{-1} are attributed to the aromatic C=C or C=N stretching vibration, and the new bands around 750 cm^{-1} are attributed to the aromatic C=C or C=N bending vibration, resulting from the presence of the benzimidazolium groups on SilprBim. Possible absorption of trace water by SilprBim or KBr resulted in the broad absorption around 3500 cm^{-1}.

The XPS spectra of SilprCl and SilprBim are shown in Fig. 3. Except for the peaks at 532.7 eV (O 1s) and 103.3 eV (Si 2p) sourced from the matrix of SiO_2, the peak at 285.0 eV is from C 1s in the spectra of SilprCl and SilprBim. The peak at 200.3 eV is from Cl 2p in the spectrum of SilprCl, and it decreases significantly in

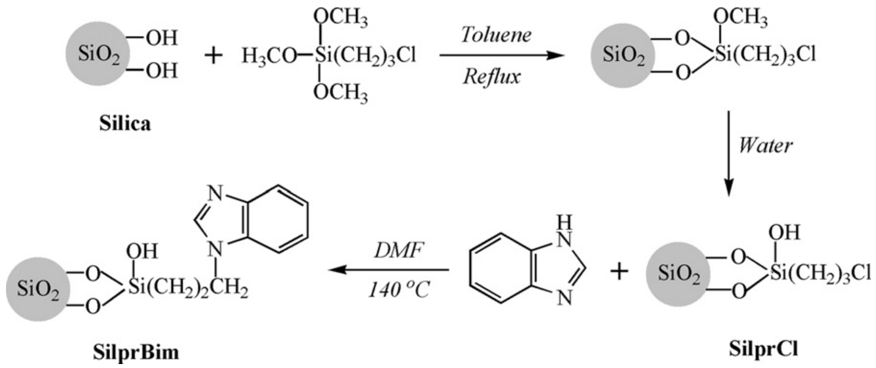

Fig. 1. Preparation process of benzimidazole modified silica stationary phase.

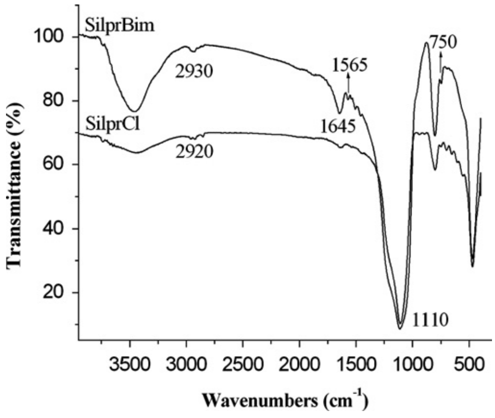

Fig. 2. FTIR spectra of SilprCl and SilprBim.

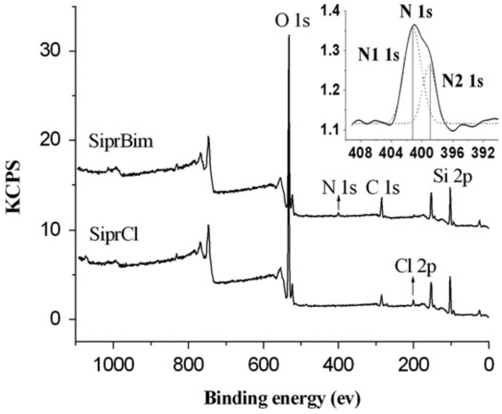

Fig. 3. XPS spectra of SilprCl and SilprBim.

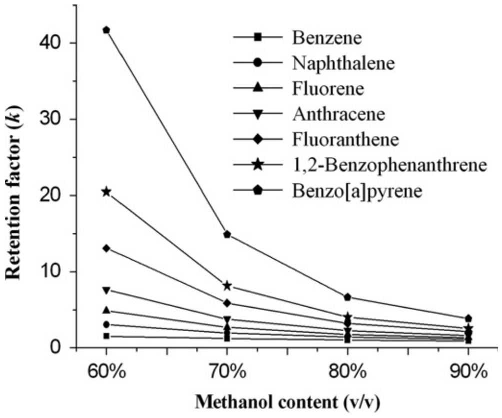

Fig. 4. Effect of methanol content on retention factors (k) of benzene (1), naphthalene (2), fluorene (3), anthracene (4), fluoranthene (5), 1,2-benzophenanthrene (6) and benzo[a]pyrene (7). Chromatographic conditions: benzimidazole modified silica column (150 × 4.6 mm i.d.), mobile phase: methanol–water (v/v), flow-rate: 1 mL min^{-1}, injection volume: 20 μL and detection: UV at 254 nm.

the spectrum of SilprBim. In the spectrum of SilprBim, the new peaks at 401.3 eV (N1 1s) and 399.2 eV (N2 1s) are from the nitrogen bonded with propyl and another nitrogen without substituent in the benzimidazolium group, respectively. The two peaks of N 1s in the SilprBim XPS spectrum evidently show that benzimidazole is attached onto the silica surface.

The elemental contents were C 4.90%, H 1.07% for SilprCl and C 7.18%, H 1.07%, N 1.18% for SilprBim. From the carbon content of SilprCl, amount of the chloropropyl groups attached onto the silica is calculated as 4.13 μmol m^{-2}. From the nitrogen content, the average concentration of benzimidazolium groups attached onto the silica surface is calculated as 1.28 μmol m^{-2} for SilprBim. The calculating formulas are as follows:

(1) Chloropropyl groups on SilprCl (μmol m^{-2})

$$=\frac{\text{C\%}\times 10^6}{36\times S_1}=4.13$$

(2) Benzimidazolium groups on SilprBim (μmol m^{-2})

$$=\frac{\text{N\%}\times 10^6}{28\times S_2}=1.28$$

where C%, H% and N% represent the percentage of carbon, hydrogen and nitrogen, respectively, as determined by elemental analysis. S_1 and S_2 are the specific surface area of SilprCl (330 m^2 g^{-1}) and SilprBim (329 m^2 g^{-1}), respectively.

3.3. Chromatographic evaluation

3.3.1. Separation of PAHs by reversed-phase chromatography

Since benzimidazolium group has the large π-system structure, SilprBim can interact with organic molecules with a π-electron by π–π interaction, similar to phenyl column. As typical molecules with the π-electron, PAHs were used to investigate the π–π interaction of SilprBim. The effect of methanol content in methanol–water eluent on the retentions of seven PAHs (benzene, naphthalene, fluorene, anthracene, fluoranthene, 1,2-benzophenanthrene and benzo[a]pyrene) was investigated. As shown in Fig. 4, under the same chromatographic conditions, the retentions increase with the increase of the π-electron system in seven PAHs, which indicates the presence of the π–π interaction between

SilprBim and PAHs. At the same time, with the increase of methanol content from 60% to 90% (v/v) in methanol–water mobile phase, the retention of PAH decreases. It is indicated that the hydrophobic interaction may exist between PAHs and SilprBim. So, the separation of PAHs on SilprBim mainly involved the comprehensive mechanism of hydrophobic and π–π interactions. As shown in Fig. 5, the baseline separation of seven PAHs is obtained on SilprBim column. A Kromasil C_{18} column was used to compare the hydrophobicity of SilprBim column. Based on retention factors (k) of benzene, naphthalene, anthracene and 1,2-benzophenanthrene in two phases, slopes of the phenyl selectivity plot were 0.20 for SilprBim and 0.39 for C_{18} using 80% (v/v) methanol–water mobile phase. In addition, we found that hydrophobicity differences became more and more obvious with the increase of water in the mobile phase. Using the same conditions, the k values of fluoranthene on Zorbax Ecplipse XDB-C_8 column and SilprBim column were 2.3 and 3.2, respectively [23]. It can be concluded that the SilprBim was less hydrophobic than C_{18} but more hydrophobic than C_8. The retention of seven PAHs on C_{18} column is much stronger than that on SilprBim column using the same conditions. Furthermore, the baseline separation of seven PAHs on C_{18} needed much more time than that on SilprBim.

3.3.2. *Separation of phthalates by reversed-phase chromatography*

A test mixture of phthalates composed of dimethyl phthalate, diallyl phthalate, di-*n*-butyl phthalate and dicyclohexyl phthalate is separated well on SilprBim column with 50% (v/v) methanol–water in Fig. 6. The octanol/water partition coefficient (logP) is often used as a measurement of the molecular hydrophobicity. log *P* values of the four phthalates are 1.64, 3.29, 4.83 and 5.64 respectively. Compounds with larger log *P* values are more hydrophobic. Comparing with the retentions of four phthalates, more hydrophobic phthalates correspond to stronger retentions, which illustrates the important role of hydrophobic interaction in the separation of phthalates on SilprBim. Besides, π–π interaction may exist between phthalates and SilprBim. Through the separation of phthalates with different hydrophobicities, the hydrophobic characteristic of SilprBim is further demonstrated. All ACD/log *P* values were from a free chemical database of RSC [24] and these data were generated using the ACD/Labs' ACD/PhysChem Suite.

3.3.3. *Separation of anilines by reversed-phase chromatography*

A test mixture of anilines containing *p*-methylaniline, *m*-nitroaniline, *o*-nitroaniline, 1-aminonaphthalene (log *P*, 2.17) and 2,4,6-tribromoaniline (log *P*, 4.43) was used in the chromatographic evaluation for SilprBim. The reason of the selection of samples was according to π-electron system in the five anilines. As shown in Fig. 7, the anilines mixture is completely separated using 55% (v/v) methanol–water as mobile phase. According to the test result and the structures of analytes, the larger π-electron system in molecule resulted in the stronger retention. The strongest retention of 2,4,6-tribromoaniline may be ascribed to the strong π–π interaction between its large π-electron system and SilprBim, as well as stronger hydrophobic interaction. Electrostatic repulsion, hydrophobic and hydrogen bond interactions may also be involved in the separation mechanism of anilines on SilprBim.

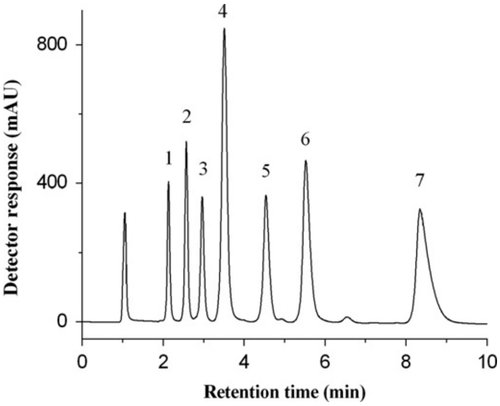

Fig. 5. Separation of test mixture of benzene (1), naphthalene (2), fluorene (3), anthracene (4), fluoranthene (5), 1,2-benzophenanthrene (6) and benzo[a]pyrene (7). Chromatographic conditions: mobile phase: methanol–water (80:20, v/v), other conditions are the same as in Fig. 4.

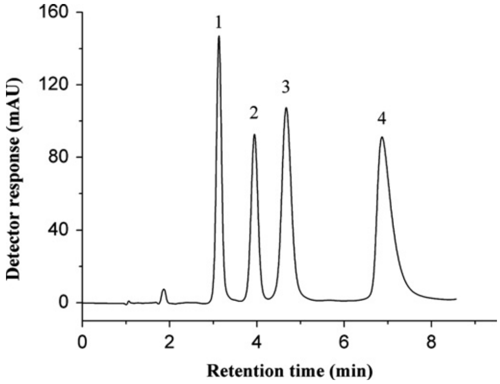

Fig. 6. Separation of test mixture of dimethyl phthalate (1), diallyl phthalate (2), di-*n*-butyl phthalate (3) and dicyclohexyl phthalate (4). Chromatographic conditions: mobile phase: methanol–water (50:50, v/v), detection: UV at 230 nm, other conditions are the same as in Fig. 4.

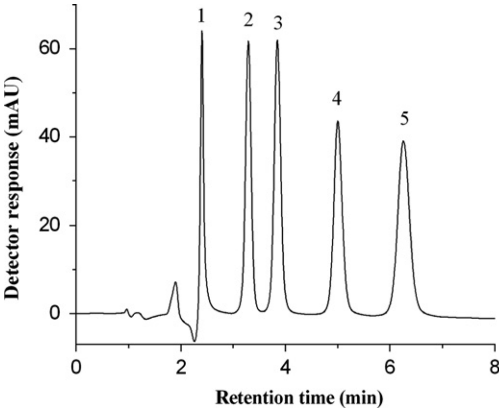

Fig. 7. Separation of test mixture of *p*-methylaniline (1), *m*-nitroaniline (2), *o*-nitroaniline (3), 1-aminonaphthalene (4) and 2,4,6-tribromoaniline (5). Chromatographic conditions: mobile phase: methanol–water (55:45, v/v), other conditions are the same as in Fig. 4.

3.3.4. *Separation of phenols by reversed-phase chromatography*

Protonated SilprBim can interact with electronegative analytes by charge–charge force. The test mixture composed of phenol (pK_a, 10.00; log *P*, 1.54), *p*-chlorophenol (pK_a, 9.38; log *P*, 2.43), methyl *p*-hydroxybenzoate (pK_a, 8.87; log *P*, 1.88), *m*-nitrophenol (pK_a, 8.39; log *P*, 1.93), 1-naphthol (pK_a, 9.45; log *P*, 2.71), *o*-nitrophenol (pK_a, 7.22; log *P*, 1.7) and *p*-nitrophenol (pK_a, 7.15; log *P*, 1.67) was used to investigate the electrostatic property of SilprBim. As shown in Fig. 8, the separation of seven phenols is obtained with 60% (v/v) methanol–water . Comparing with their log *P* values there is not so much correlation between hydrophobicity and retention of phenols. But the retentions of phenols on SilprBim were related to their pK_a except for 1-naphthol . With the decrease of their pK_a, the electronegativity of phenols increases, resulting in the increase of electrostatic interaction between phenols and SilprBim. Especially, the dependence of elution order of *m*-, *o*-, and *p*-nitrophenol on their pK_a further illustrates the electrostatic property of SilprBim. For 1-naphthol, its stronger retention than other phenols with smaller pK_a may result from stronger π–π and hydrophobic interactions between the large π-electron system with SilprBim. Besides electrostatic interaction, π–π, hydrophobic interactions and hydrogen bond may also be involved in separation of phenols on SilprBim column. All pK_a values were from references [25,26].

Under reversed-phase chromatographic conditions, PAHs, phthalates, anilines and phenols were successfully separated respectively on SilprBim. According to these chromatographic tests, it is concluded that SilprBim can be applied as the reversed-phase stationary phase with multiple interactions such as π–π, hydrophobic, electrostatic and hydrogen-bonding interactions.

3.3.5. Separation of inorganic anions by anion-exchange chromatography

As a weak basic group, benzimidazole can be protonated to form anion-exchange sites on SilprBim in appropriate mobile phase. In order to eliminate the effect of pH on concentration and valence of eluting ion in mobile phase, KCl solution as a strong electrolyte was used as the mobile phase to evaluate SilprBim by anion-exchange chromatography. Inorganic anions including IO_3^-, BrO_3^-, Br^-, NO_3^-, I^- and SCN^- were used to investigate the anion-exchange characteristic of SilprBim. The effect of KCl concentration on the retentions of inorganic anions was examined from 100 to 250 mmol L^{-1} (pH, 5.6). As can be seen in Fig. 9(a), retention of inorganic anions obviously decreased with the increase of KCl concentration. It is easy to understand that the retention of anions is reduced with the increase of elution strength of mobile phase.

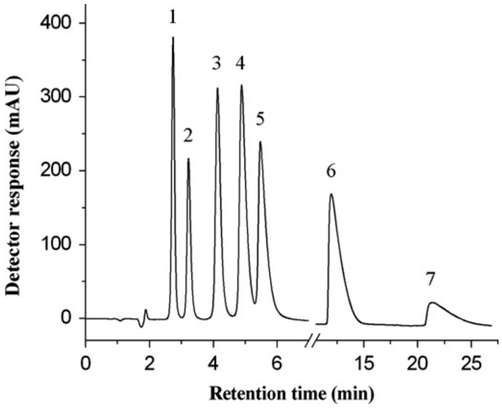

Fig. 8. Separation of test mixture of phenol (1), *p*-chlorophenol (2), methyl *p*-hydroxybenzoate (3), *m*-nitrophenol (4), 1-naphthol (5), *o*-nitrophenol (6) and *p*-nitrophenol (7). Chromatographic conditions: mobile phase: methanol–water (60:40, v/v), other conditions are the same as in Fig. 4.

At the same time, the effect of pH of the mobile phase on the retentions of inorganic anions was also examined. pH of a mobile phase (175 mmol L^{-1} KCl solution) was modulated from 7.0 to 2.5. As shown in Fig. 9(b), the retentions of inorganic anions change slightly from 7.0 to 4.5 of pH, but the retentions increase from 4.5 to 2.5 of pH. Without considering the effect of different pH values on elution ability of KCl solution, the retentions of anions only depend on the anion-exchange strength of SilprBim. From 7.0 to 4.5 of pH in mobile phase, the degree of protonation of benzimidazolium groups changes a little, so anion-exchange strength of SilprBim is almost the same. When pH was decreased from 4.5 to 2.5 in mobile phase, the higher acidity accelerates protonation of benzimidazolium groups on stationary phase, so anion-exchange strength of SilprBim is enhanced. It is particularly obvious that the retentions of inorganic anions increase greatly from 4.0 to 3.0 of pH, while the retentions increase slowly from 4.5 to 4.0 and from 3.0 to 2.5 of pH. This phenomenon might show that SilprBim began to be protonated at pH from 4.5 to 4.0 and mostly protonated at pH from 4.0 to 3.0, after that reached to the end of protonation at pH from 3.0 to 2.5. Therefore, appropriate retentions and better separation can be obtained through varying pH values of mobile phase.

Not only the concentration but also pH of mobile phase influenced the retentions of inorganic anions on SilprBim. As shown in Fig. 10, the separation of six common inorganic anions with short retention time, high resolution and column efficiency is achieved, and chromatographic parameters are shown in Table 1.

3.3.6. Separation of organic anions by anion-exchange chromatography

Organic anions having π-electron conjugated system in molecular structure and weak hydrophobicity were used to investigate anion-exchange, π–π and hydrophobic characteristic of SilprBim, with methanol–KH_2PO_4 solution as eluent. The retention factors (k) of organic anions including *p*-aminobenzoic acid (pK_a, 4.87), benzoic acid (pK_a, 4.19), *p*-anilinesulfonic acid (pK_a, 3.24), sodium *p*-toluenesulfonate (pK_a, 1.7), phthalic acid monopotassium salt (pK_{a1}, 2.95; pK_{a2}, 5.51) and sodium salicylate (pK_a, 2.97) were

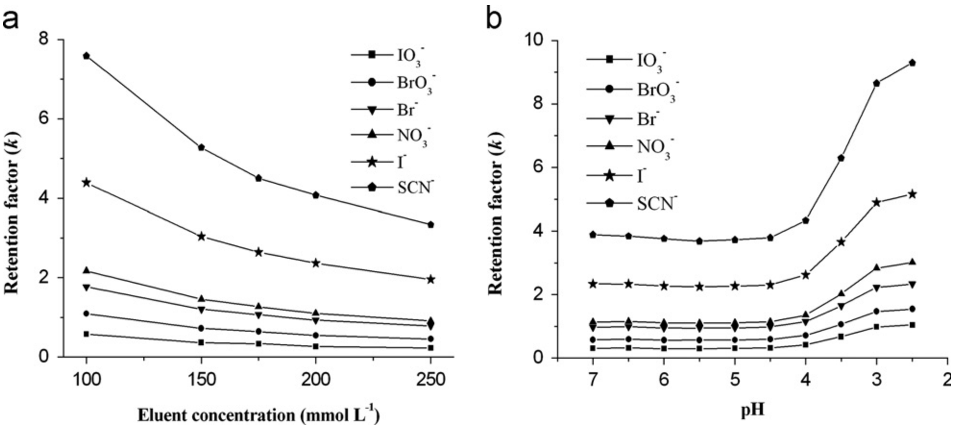

Fig. 9. Effect of KCl concentration and pH on retention factors (k) of inorganic anions including iodate (1), bromate (2), bromide (3), nitrate (4), iodide (5) and thiocyanate (6). Chromatographic conditions: mobile phase: pH of KCl solution is 5.6 in (a), 175 mmol L^{-1} KCl solution in (b), detection: UV at 210 nm, other conditions are the same as in Fig. 4.

investigated by changing the methanol content and concentration of KH_2PO_4 in mobile phase, respectively. As shown in Fig. 11(a), the retention factors of organic anions decrease with the increase of concentration of KH_2PO_4 in mobile phase (20% (v/v) methanol in KH_2PO_4 solution), which indicates the existence of the anion-exchange interaction in the separation process. The anion-exchange interaction was also reflected in the relationship of retention factors and pK_a, and the organic anions with the smaller pK_a had the greater retention factors except for sodium *p*-toluenesulfonate. At the same time, the retention factors of organic anions decrease with the increase of methanol content in mobile phase (different contents of methanol in 200 mmol L^{-1} KH_2PO_4 solution) (shown in Fig. 11(b)). So π–π and weak hydrophobic interactions may take part in the separation of organic anions on SilprBim in addition to anion-exchange . In Fig. 11(b), the retention of sodium salicylate is more sensitive to methanol content than other organic anions, for its highest hydrophobicity (log *P*, 2.06). The chromatogram of an organic anions mixture containing *p*-aminobenzoic acid, benzoic acid, *p*-anilinesulfonic acid, phthalic acid monopotassium salt and sodium salicylate is shown in Fig. 12, using 30% (v/v) methanol in 200 mmol L^{-1} KH_2PO_4 solution at pH 5.7 as a mobile phase.

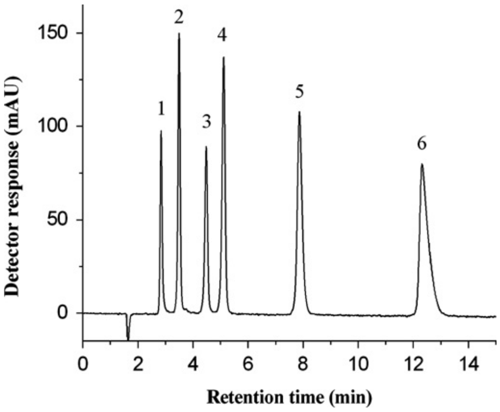

Fig. 10. Separation of test mixture of iodate (75 mg kg^{-1}) (1), bromated (117 mg kg^{-1}) (2), bromide (24 mg kg^{-1}) (3), nitrate (12 mg kg^{-1}) (4), iodide (36 mg kg^{-1}) (5) and thiocyanate (62 mg kg^{-1}) (6). Chromatographic conditions: mobile phase: 175 mmol L^{-1} KCl solution at pH 3.5, other conditions are the same as in Fig. 9.

Table 1
Chromatographic parameters obtained for separation of some anions on SilprBim column under anion-exchange chromatographic conditions in Fig. 10.

Anions	***N*** **(m^{-1})**	***R*$_s$**	***A*$_s$**	***k***
Iodate	51,300	–	1.23	0.68
Bromate	63,700	4.75	0.99	1.06
Bromide	65,100	6.10	0.90	1.65
Nitrate	67,000	3.30	0.93	2.02
Iodide	67,200	10.65	1.32	3.66
Thiocyanate	52,600	10.26	2.44	6.30

Under ion-exchange chromatographic conditions, SilprBim exhibited the anion-exchange characteristic. According to the effect of mobile phase on retention of anions, SilprBim possessed adjustable anion-exchange strength and could separate inorganic anions with high resolution and column efficiency. Furthermore, some organic anions were also separated by multiple interactions such as π–π, anion-exchange and weak hydrophobic interactions.

3.3.7. *Simultaneous separation of neutral organic compounds and anions*

A test mixture of neutral organic compounds and anions containing *p*-aminophenol, bromate, benzene, nitrate, toluene, iodide, benzoic acid, thiocyanate, *p*-anilinesulfonic acid, phthalic acid monopotassium salt and sodium salicylate was used to investigate chromatographic performance of SilprBim column. As shown in Fig. 13(a), these analytes are well separated simultaneously on SilprBim column. In order to compare with the performance of SilprBim column, a Kromasil C_{18} column was used to separate the same test mixture. The optimal separation of this

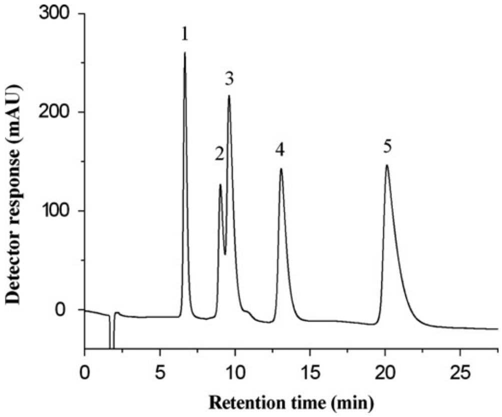

Fig. 12. Separation of test mixture of *p*-aminobenzoic acid (1), benzoic acid (2), *p*-anilinesulfonic acid (3), phthalic acid monopotassium salt (4) and sodium salicylate (5). Chromatographic conditions: mobile phase: 30% (v/v) methanol in 200 mmol L^{-1} KH_2PO_4 solution at pH 5.7, other conditions are the same as in Fig. 11.

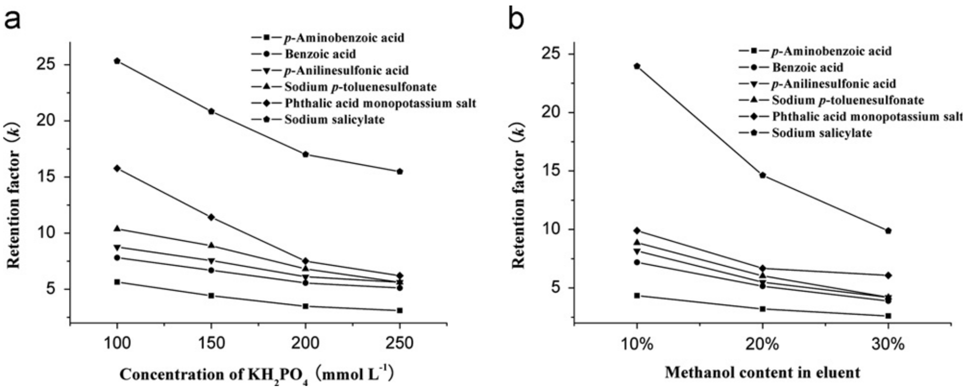

Fig. 11. Retention factors of organic anions including *p*-aminobenzoic acid (1), benzoic acid (2), *p*-anilinesulfonic acid (3), sodium *p*-toluenesulfonate (4), phthalic acid monopotassium salt (5) and sodium salicylate (6). Chromatographic conditions: mobile phase: 20% (v/v) methanol in different concentrations of KH_2PO_4 solution (pH 5.7) in (a); different contents of methanol in 200 mmol L^{-1} KH_2PO_4 solution (pH 5.7) in (b), detection: UV at 200 nm, other conditions are the same as in Fig. 4.

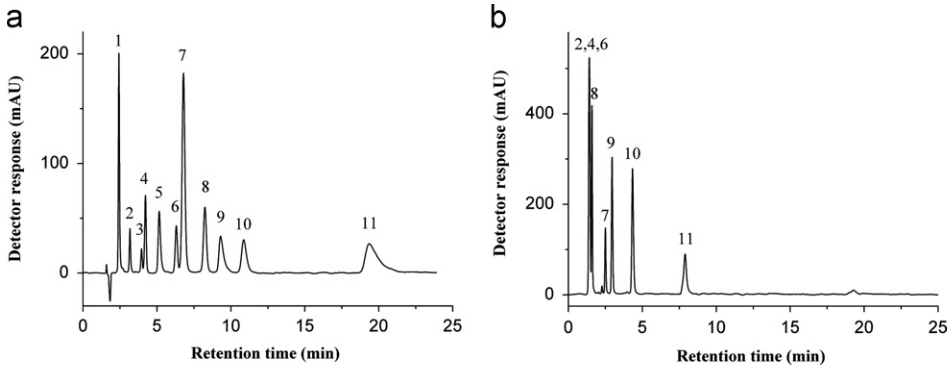

Fig. 13. Separation of test mixture of *p*-aminophenol (1), bromate (2), benzene (3), nitrate (4), toluene (5), iodide (6), benzoic acid (7), thiocyanate (8), *p*-anilinesulfonic acid (9), phthalic acid monopotassium salt (10) and sodium salicylate (11). Chromatographic conditions: mobile phase: 200 mmol L^{-1} KCl solution–methanol (70:30, v/v) in (a); mobile phase: 200 mmol L^{-1} KH_2PO_4 solution–methanol (70:30, v/v) in (b); detection: UV at 210 nm, other conditions are the same as in Fig. 4.

test mixture on C_{18} column is shown in Fig. 13(b). C_{18} column was poor for the separation of inorganic anions. The bromate, nitrate and iodide present a chromatographic peak. In addition, C_{18} column provided too strong retention for neutral aromatic compounds, and *p*-aminophenol, benzene and toluene cannot be eluted even after 60 min. 8-Quinolinol silica gel was an anion-exchange stationary phase, the resolution of the inorganic anions on it was poor [11]. An Alltech mixed-mode C_8/anion-exchange column separated five inorganic anions, phenanthrene, pyrene and chrysene [11], but the resolution was worse than that on SilprBim column. SilprBim was also superior for the separation of organic anions in neutral compounds. So, the new stationary phase is promising for the simultaneous separation of anions and organic compounds.

4. Conclusions

A novel mixed-mode stationary phase based on benzimidazole modified silica was synthesized and evaluated with reversed-phase and anion-exchange modes in HPLC. Hydrophobic organic compounds including PAHs, phenols, phthalates and anilines, and some inorganic or organic anions got baseline separations by reversed-phase and anion-exchange chromatography, respectively. It seemed that multiple interactions including π–π, hydrophobic, hydrogen bond, electrostatic and anion-exchange interactions were involved in the chromatographic separation. The new stationary phase has potential application to the separation of complex analytes with different physicochemical properties.

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