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Dipyridine modified silica—A novel multi-interaction stationary phase for high performance liquid chromatography

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ABSTRACT

A novel multi-interaction stationary phase based on 4,4′-dipyridine modified silica was synthesized and characterized, by infrared spectra, X-ray photoelectron spectroscopy and elemental analysis. Mechanism involved in the chromatographic separation is the multi-interaction including π–π, hydrophobic, hydrogen-bonding, electrostatic and anion-exchange interactions. Based on these interactions, polycyclic aromatic hydrocarbons and phenols were successfully separated respectively in reversed-phase chromatography; inorganic and organic anions were also separated individually in anion-exchange chromatography by using the same column. Furthermore, the simultaneous separation of neutral organics, inorganic and organic anions was obtained on this stationary phase with the appropriate mobile phase. Therefore, such stationary phase has the characteristics of multi-interaction mechanism and multi-modal separation, and has potential application on complex samples.

1. Introduction

HPLC, benefited mostly from plentiful stationary phases with good performances, exhibits excellent advantages in the separation and analysis of complex samples. Separation mechanism of the stationary phase influences its chromatographic performance directly. For traditional stationary phases, the main separation mechanism is often single, such as hydrophobic interaction for separating nonpolar compounds on reversed-phase packing, and ion-exchange interaction for separating ions on ion-exchange packing. While some complex samples of pharmacology, environment and food are often mixtures of compounds with different properties [1], such as non-ionic, ionic analytes, which cannot be separated simultaneously on the stationary phase possessing single or simple separation mechanism [2].

Multimode chromatography provided a flexible and versatile method for the simultaneous separation of mixed classes of analytes, especially ions and organic compounds [2]. Currently existent multimode chromatography is obtained through the following way: (1) using mixed-bed columns which contain both reversed-phase and ion-exchange particles [3]; and (2) coupling a multifunction ligand by covalent bond to the surface of a support [2]. Because of its fantastic chromatographic performance, multimode chromatography has been widely used in biopolymer separations for the purification of proteins [4–6], analysis of drugs and drug metabolites [3], and so on. A few groups have devoted their effort to the research of multi-mode chromatography and some achievements have been obtained [2,7–17]. Wongyai [2] prepared phenylpropanolamine bonded silica which displayed both anion-exchange and reversed-phase characteristic, and quantitative determination of vitamin B_{12} in multivitamin tablets was performed by it [8]. Thompson and co-workers [7] synthesized 8-quinolinol silica, on which the simultaneous separation of hydrophilic inorganic anions and hydrophobic PAHs, as well as single-injection separation of organics, metal ions and anions were obtained [9]. Chiou and co-workers [10] resigned Fullerene C_{60}-cryptand resin and applied it as a multifunctional packing to separate anions, metal cations and aromatic compounds. Jiang et al. [11] prepared poly(1-allylimidazole)-grafted silica as the stationary phase in multimode chromatography.

Some other stationary phases having similar performances like imidazole [12,13], pyridine [14,15] and phenylamine [16,17] functional silicas were also studied by researchers. Most of the stationary phases mentioned above took advantages of the functional groups with charged or chargeable moieties. Electrostatic attractive or repulsive interaction was used for the separation of ionic analytes with ion-exchange conditions [7,12,14,17]. Inorganic ions like iodate, bromate, chloride, bromide, nitrate, iodide and

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thiocyanate got well separation on these stationary phases. Because of the extraordinary physicochemical properties of the functional molecules, some other coexistent interactions were also investigated with reversed-phase condition [2,13] and normal-phase condition [15]. Neutral, basic and acid organic compounds were also separated. Phenylaminopropyl silica [16] was used to separate phenol and its nine methyl-, chloro- and nitro-substituted derivatives. A multiple retention mechanism including hydrogen-bonding, hydrophobic, electrostatic and π–π interactions was suggested for the separation. Everything has two sides, these phases had some disadvantages such as lower column efficiency, poor peak symmetry, long retention time and so on.

In this work, 4,4′-dipyridine modified silica was synthesized and investigated as a novel multi-interaction stationary phase for HPLC. It can be applied as the reversed-phase stationary phase for the separation of PAHs and phenols, as well as the anion-exchange stationary phase for the separation of inorganic anions and organic anions. The simultaneous separation of neutral organics, inorganic and organic anions on this stationary phase further indicates its multi-interaction characteristic and its analytical prospect for complex samples. Furthermore, it has some advantages such as higher column efficiency, better peak symmetry and shorter retention time over some phases with similar performance.

2. Experimental

2.1. Reagents

Spherical silica porous particles of 5 μm, with an average pore diameter of 8 nm and a specific surface area (BET) of 390 $m^2\ g^{-1}$, which were made in our laboratory, were used as the support. 3-Chlorpropyltrimethoxysilane (98%) was obtained from Qufu Chenguang Fine Chemical Co. (Shandong, China). 4,4′-Dipyridine (98%) was purchased from Shanghai Jingchun Industry Co. (Shanghai, China). Organics including benzene, PAHs and phenols used in reversed-phase chromatographic tests as well as inorganic or organic salts including potassium iodate, potassium bromate, potassium bromide, potassium nitrate, sodium iodide, potassium thiocyanate, potassium chloride and sodium dihydrogen phosphate used in anion-exchange chromatographic tests were analytical reagents. Toluene was dried by reflux distillation with sodium before use. All the other compounds used in experiments were analytical grade and were used without further purification.

2.2. Preparation of stationary phase

4,4′-Dipyridine modified silica stationary phase was synthesized by the two step reaction. In the first step, 27.0 g silica was dried under vacuum at 135 °C for 20 h to remove the adsorbed water. Then, the activated silica was placed in a 500 mL reaction flask containing 250 mL of toluene. Under stirring, 25 mL of 3-chloropropyltrimethoxysilane were added into this mixture. The mixture was refluxed for 26 h, and then the reaction was stopped. The modified silica was cooled to room temperature, transferred to a vacuum glass filter, and 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 12 h.

In the second step, the chloropropyl groups on SilprCl reacted with 4,4′-dipyridine. Briefly, 3.0 g of SilprCl were placed in a 100 mL reaction flask containing 30 mL of *N*,*N*′-dimethylformamide. Then, a large excess of 4,4′-pyridine (3.0 g) was added into the mixture. The reaction mixture was stirred under nitrogen at 140 °C for 72 h. Then the reaction was stopped and the modified silica was washed with ethanol, water and methanol in turn. The 4,4′-dipyridine modified silica (SilprDipy) 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). X-ray Photoelectron Spectroscopy (XPS) was used to evaluate the modified silica. The XPS spectra were recorded with an Escalab 210 Axis Ultra photoelectron spectrometer (VG Scientific, UK) using an Mg K_a excitation souse. The carbon, hydrogen and nitrogen contents of SilprCl and SilprDipy were determined by elemental analysis performed on an Elementar Vario EL cube (Hanau, Germany). The average concentration of chloropropyl groups and 4,4′-dipyridinium groups bonded onto silica can be calculated through the carbon content of SilprCl and nitrogen content of SilprDipy, respectively. The specific surface area (BET) of silica, SilprCl and SilprDipy was 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 column (150 × 4.6 mm I.D.) was made from stainless-steel tube and was downward packed, with slurry of SilprDipy in ethanol and hexane as the propulsive solvent, under a constant packing pressure of 50 MPa (6752B-100, Beijing, China). Mobile phases were filtered through a 0.45 μm nylon membrane filter. All reversed-phase chromatographic tests used methanol-water as the mobile phase with UV detection at 254 nm. The separation of inorganic anions was accomplished by using KCl solution as the mobile phase with UV detection at 210 nm. The separation of organic anions and the simultaneous separation of neutral organics, inorganic and organic anions were obtained by using methanol–NaH_2PO_4 solution as the mobile phase, with UV detection at 200 nm in all pH of a 50 mmol L^{-1} KCl eluent was modulated from 3.0 to 6.0 with hydrochloric acid and potassium hydroxide, using a calibrated Sartorius PB-10 pH meter (Goettingen, Germany). All tests were performed at about 25 °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. Characterizations

SilprCl was prepared after silica reacted with 3-chloropropyltrimethoxysilane, then the chloropropyl groups reacted with 4,4′-dipyridine to produce SilprDipy. The synthesized process is schematically described in Fig. 1.

In the FTIR spectra of SilprCl and SilprDipy (shown in Fig. 2), bands around 1110 cm^{-1} are attributed to the stretching vibrations of the siloxane (Si–O) groups of the silica backbone. Broad bands around 3448 cm^{-1} are attributed to the stretching vibrations of O–H bonds of residual silanol groups on the surface of silica. Bands appeared at 2920 cm^{-1} and 2850 cm^{-1} in the spectrum of SilprCl are atrributed to aliphatic C–H stretching. In addition, the bands at 700 cm^{-1} are attributed to the stretching vibrations of C–Cl groups of SilprCl. In the spectrum of SilprDipy, bands at 2920 cm^{-1} and 2850 cm^{-1} are also attributed to aliphatic and aromatic C–H stretching. Meanwhile, two new bands appeared at 1645 cm^{-1} and 1415 cm^{-1} respectively in the spectrum of SilprDipy are due to the presence of the 4,4′-dipyridinium, and they are attributed to the aromatic C=C or C=N stretching vibration modes.

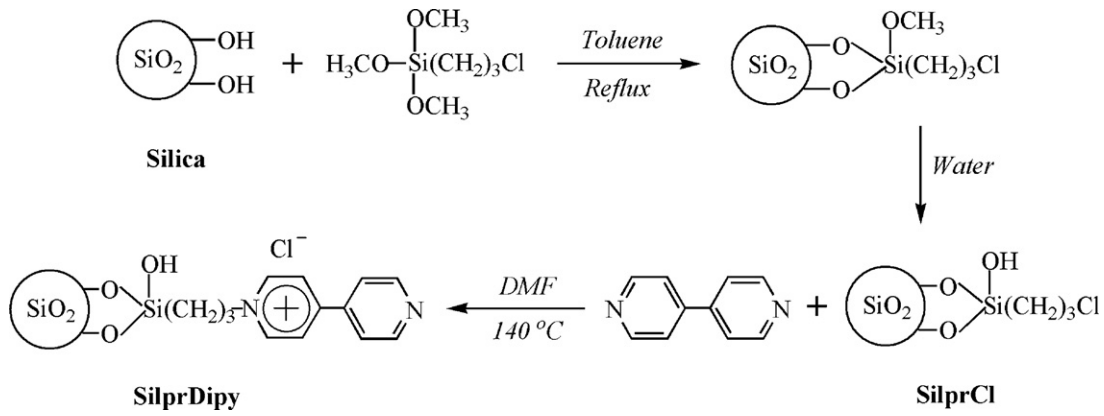

Fig. 1. Synthesized process of 4,4′-dipyridine modified silica stationary phase.

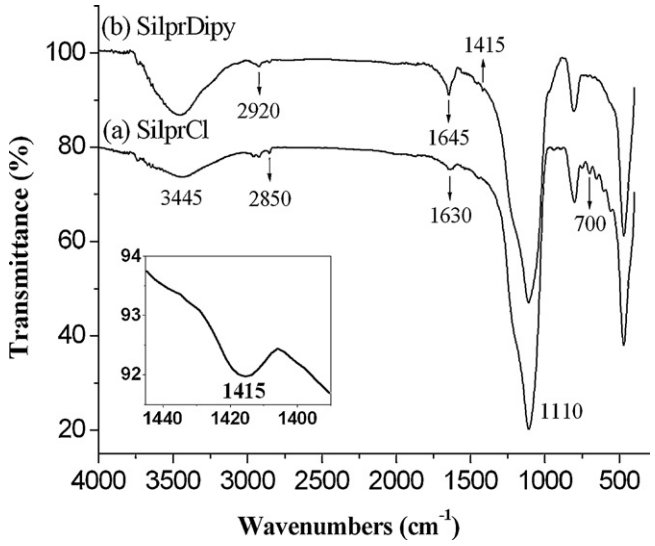

Fig. 2. FT-IR spectra of (a) SilprCl and (b) SilprDipy.

The XPS spectra of SilprCl and SilprDipy are shown in Fig. 3. Except the peaks at 532.3 eV (O 1s) and 102.8 eV (Si 2p) sourced from the matrix of SiO_2, the peak at 285.0 eV is from C 1s in the XPS spectra of SilprCl and SilprDipy. The peaks at 401.9 eV (N1 1s) and 399.2 eV (N2 1s) are from nitrogen of the pyridinium cations and nitrogen of the neutral pyridine groups, respectively. The peak of N 1s in the XPS spectrum of SilprDipy evidently shows that 4,4′-dipyridine was attached onto the silica surface.

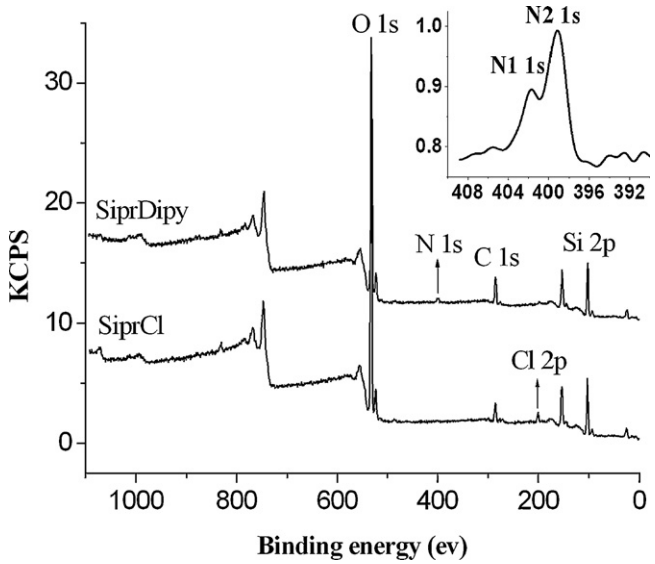

Fig. 3. XPS spectra of SilprCl and SilprDipy.

The elemental contents were C 5.21%, H 1.26% for SilprCl and were C 8.22%, H 1.27% and N 1.31% for SilprDipy. From the carbon content of SilprCl, the chloropropyl groups attached onto the silica was calculated as 4.47 μmol m^{-2} for SilprCl. From the nitrogen content of SilprDipy, the average concentration of 4,4′-dipyridinium groups attached onto the silica was calculated as 1.55 μmol m^{-2} for SilprDipy. The calculation formulas of the surface coverage are as follows:Coverage of chloropropyl groups (μmol m^{-2}) $= \frac{C\% \times 10^4}{36 \times S_1} = 4.47$, Coverage of 4, 4′ − dipyridinium groups (μmol m^{-2}) $= \frac{N\% \times 10^4}{28 \times S_2} = 1.55$, 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 (324 m^2 g^{-1}) and SilprDipy (301 m^2 g^{-1}), respectively.

3.2. Chromatographic separations

3.2.1. Separations of PAHs and phenols in reversed-phase chromatography

Pyridinium cations and neutral pyridine groups of SilprDipy can take part in the π–π interaction with the π conjugative systems of organic molecules. PAHs are common molecules containing the π conjugative system. As shown in Fig. 4, a test mixture of PAHs containing benzene, naphthalene, fluorene, anthracene, fluoranthene and 1,2-benzophenanthrene is separated baselinely on SilprDipy with methanol–water (85:15, v/v) as mobile phase. With the increase of the π conjugative system in six PAHs, the retentions increase. The effect of methanol content on retention (log *k*) of six PAHs on SilprDipy was investigated. As can be seen from Fig. 5, with the decrease of methanol content from 95% to 70% (v/v) in methanol–water mobile phase, the retentions of six PAHs increase, which shows that hydrophobic interaction may exist between PAHs and SilprDipy. So, the separation of PAHs on SilprDipy mainly results from the comprehensive mechanism of hydrophobic and π–π interactions.

Phenols were also used to investigate the chromatographic performance of SilprDipy in the reversed-phase chromatography. Methanol–NaAc (60:40, v/v) with different pH was used as the mobile phase to investigate the electrostatic interaction in the separation of phenols on SilprDipy. As can be seen in Fig. 6, retention factors of phenols with lower pK_a (nitrophenol) vary greatly with pH values from 3.2 to 6.4, while variations for phenols with higher pK_a (phenol, *p*-chlorophenol) are slight. Dissociation degrees of phenols with lower pK_a increased with the increase of the pH

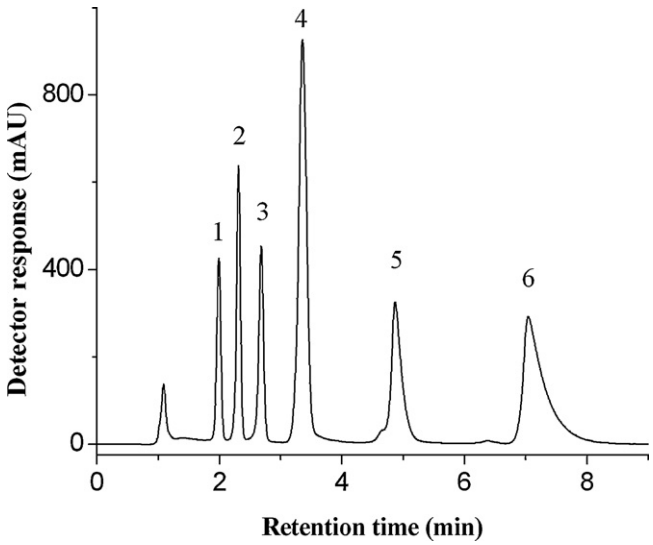

Fig. 4. Separation of test mixture of benzene (1), naphthalene (2), fluorene (3), anthracene (4), fluoranthene (5) and 1,2-benzophenanthrene (6). Chromatographic conditions: SilprDipy column (150 × 4.6 mm I.D.), mobile phase: methanol–water (85:15, v/v), flow-rate: 1 mL min^{-1}, injection volume: 20 μL and detection: UV at 254 nm.

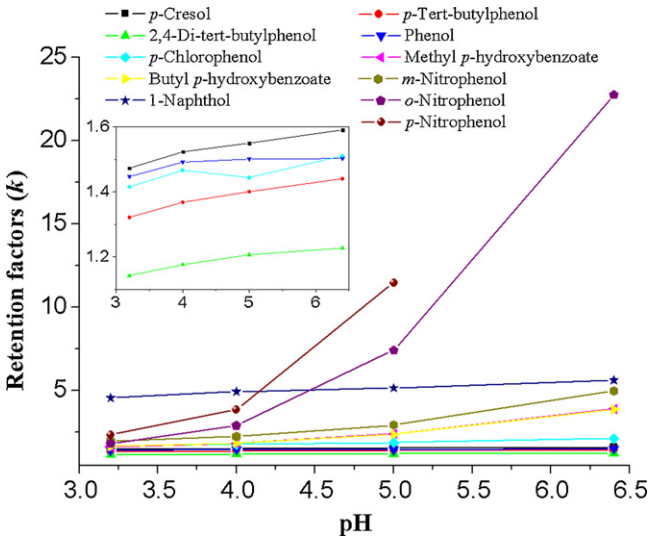

Fig. 6. The effect of pH of eluent on retention factors (k) of phenols. Chromatographic conditions: mobile phase: methanol-5 mmol L^{-1} NaAc with different pH (60:40, v/v), other conditions are the same as in Fig. 4.

in mobile phase. The dissociated electronegative solutes brought stronger interactions with the positive stationary phase and the retention factors were increased. Phenols with larger pK_a were almost nondissociated, and the retentions were hardly changed. So the electrostatic interaction may be involved in the separation of phenols, especially for some phenols with larger pK_a. The test mixture composed of phenol (pK_a, 10.00), *p*-chlorophenol (pK_a, 9.38), butyl *p*-hydroxybenzoate (pK_a, 8.37), methyl *p*-hydroxybenzoate (pK_a, 8.17), *m*-nitrophenol (pK_a, 8.00) and 1-naphthol (pK_a, 9.45) was separated on SilprDipy. The chromatogram of six phenols with methanol-water (70:30, v/v) as mobile phase was shown in Fig. 7. It seems that the retention order of phenols is related to their pK_a values, which is influential for the electrostatic interaction. With the reduction of pK_a, the electronegativity of phenols increases, resulting in increasing retention of phenols except for 1-naphthol. In addition, 1-naphthol with ten π-electron conjugated system has stronger π–π interaction with SilprDipy than other five phenols with six π-electron systems in molecular structure, and 1-naphthol is more hydrophobic. Thus, comparing 1-naphthol with other phenols having little pK_a, longer retention time of 1-naphthol on SilprDipy may result from stronger π–π and hydrophobic interactions. Overall, hydrophobic, π–π and electrostatic interactions are involved in the separation of phenols on SilprDipy in reversed-phase chromatography.

3.2.2. *Separations of inorganic and organic anions in anion-exchange chromatography*

Using KCl solution as the mobile phase, SilprDipy can also be applied as an anion-exchange stationary phase. Besides anion-exchange capacity of the pyridinium cation bonding chloropropyl groups, the neutral pyridine group can be protonated and additional anion-exchange sites occur, increasing the anion-exchange

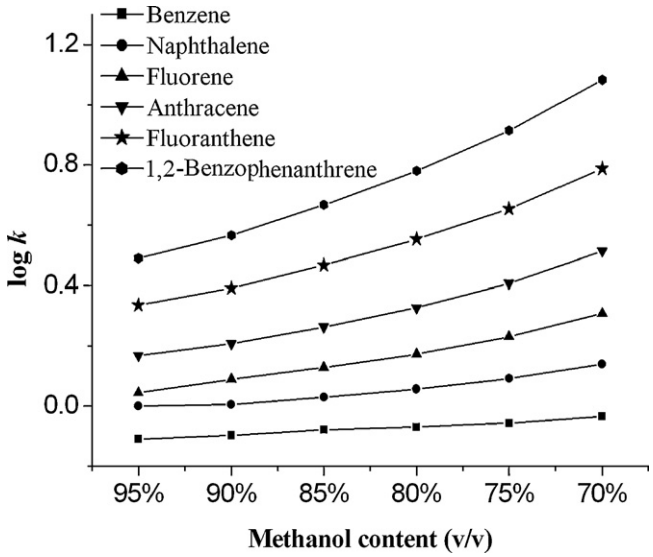

Fig. 5. The effect of methanol content on retention (log k) of benzene (1), naphthalene (2), fluorene (3), anthracene (4), fluoranthene (5) and 1,2-benzophenanthrene (6). Chromatographic conditions: mobile phase: methanol–water (v/v), other conditions are the same as in Fig. 4.

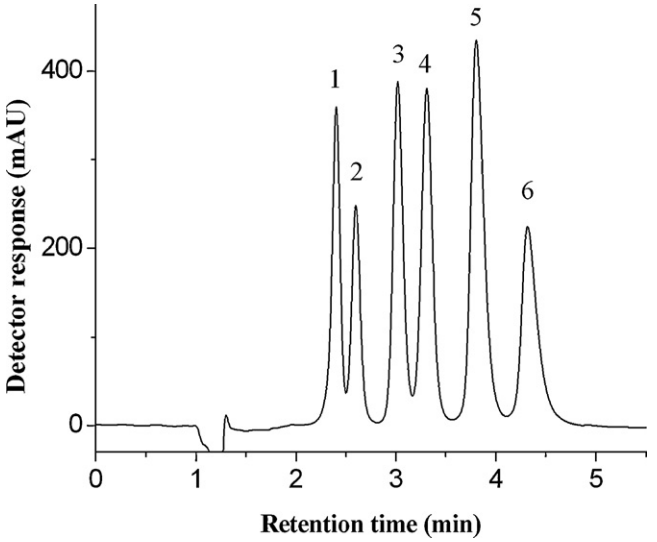

Fig. 7. Separation of test mixture of phenol (1), *p*-chlorophenol (2), butyl *p*-hydroxybenzoate (3), methyl *p*-hydroxybenzoate (4), *m*-nitrophenol (5) and 1-naphthol (6). Chromatographic conditions: mobile phase: methanol-water (70:30, v/v), other conditions are the same as in Fig. 4.

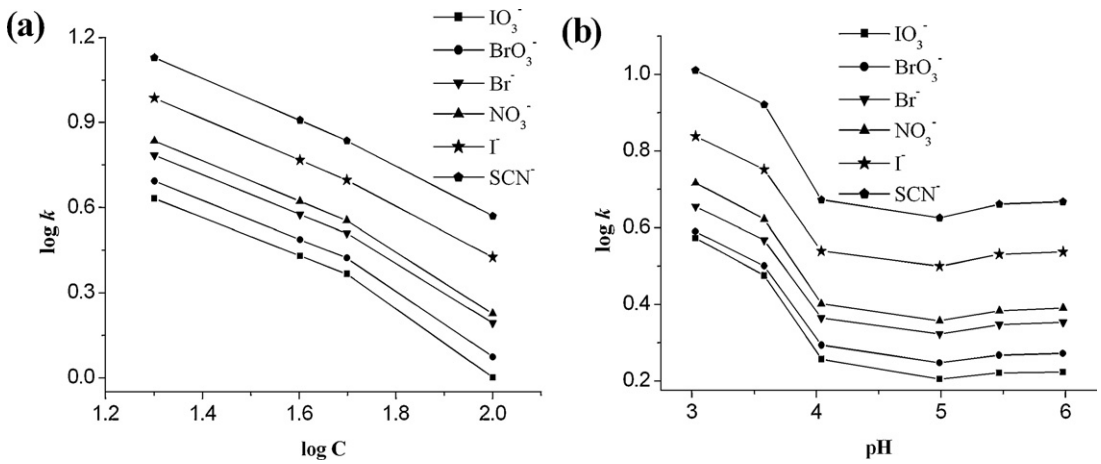

Fig. 8. (a) Effect of KCl concentration (log C) on retention (log *k*) of inorganic anions; (b) effect of pH on retention (log *k*) of inorganic anions. Inorganic anions include iodate (1), bromate (2), bromide (3), nitrate (4), iodide (5) and thiocyanate (6). Chromatographic conditions: mobile phase: pH of KCl solution is 5.8 in (a), 50 mmol L^{-1} KCl solution in (b), detection: UV at 210 nm, other conditions are the same as in Fig. 4.

characteristic of SilprDipy. Common inorganic anions composed of IO_3^-, BrO_3^-, Br^-, NO_3^-, I^- and SCN^- were used to investigate the anion-exchange characteristic of SilprDipy in anion-exchange chromatography. The effect of the concentration of eluent varied from 20 to 100 mmol L^{-1} (pH = 5.8) was examined. As shown in Fig. 8(a), it is obvious that retentions of inorganic anions decrease with the increase of concentration of eluent. The linearity of log *k*-log C is also shown.

At the same time, the effect of pH of the mobile phase on the retention was also examined. pH of a 50 mmol L^{-1} KCl eluent was modulated from 3.0 to 6.0. The pH of mobile phase influences on the protonation of pyridine nitrogen in bonded groups, which affects the retention of anions. The pK_a value of the bonded groups (1-propyl-4,4′-bipyridinium chloride) is about 3.60. When the pH of mobile phase is higher than pK_a + 1, pyridine nitrogen in bonded groups does not be protonated. As shown in Fig. 8(b), the retention factors (*k*) are almost unchanged when the pH is from 6.0 to 4.6. When the pH of mobile phase is lower than pK_a + 1, pyridine nitrogen in bonded groups is protonated. The retention factors (*k*) increase slightly with pH in mobile phase from 4.6 to 4.0, and increase greatly from 4.0 to 3.0. The reason may be that the higher acidity accelerates the protonation of neutral pyridine groups and the electropositivity of SilprDipy, inducing stronger retention of anions. It is indicated that pH of mobile phase can regulate the anion-exchange strength of SilprDipy to obtain appropriate retentions and better separation; thereby SilprDipy presents an adjustable anion-exchange characteristic in anion-exchange chromatography. Fig. 9 shows the chromatographic separation of six inorganic anions, and the column efficiency is 52,000–54,000 plates/meter for the fore five anions and 28,000 (SCN^-) plates/meter.

An organic anions mixture of *p*-aminobenzoic acid (pK_a, 4.87), sodium benzoate (pK_a, 4.19), *p*-anilinesulfonic acid (pK_a, 3.24), potassium hydrogen phthalate (pK_{a1}, 2.89; pK_{a2}, 5.51) and sodium salicylate (pK_{a1}, 2.97; pK_{a2}, 12.38) was used to invesigate the performance of SilprDipy. Under the appropriate mobile phase condition, organic anions can be separated well on SilprDipy. The chromatogram of five organic anions is shown in Fig. 10, with methanol-30 mmol L^{-1} NaH_2PO_4 at pH 4.6 (45:55, v/v) as the mobile phase. Except for sodium salicylate, the retention order of four organic anions is closely related to their pK_a. With the decrease of pK_a values, their retentions increase. The results indicate that the separation mechanism of organic anions on SilprDipy includes the anion-exchange interaction. Although sodium salicylate has higher pK_a than that of potassium hydrogen phthalate, it has stronger retention than potassium hydrogen phthalate and the reason may be the difference of their hydrophobic interaction with SilprDipy. The octanol/water partition coefficient (logP) is often used as a measurement of molecular hydrophobicity. Sodium salicylate (logP, 2.06) is much more hydrophobic than potassium hydrogen phthalate (logP, 0.81). The more hydrophobic compound corresponds to the more hydrophobic interaction with SilprDipy. Moreover, the increase of methanol in mobile phase can decrease the retentions of organic anions. So, the hydrophobic interaction is workable in the separation of organic anions on SilprDipy. Based on the above result, the mechanism may mainly contain π–π, hydrophobic and anion-exchange interactions in separation of organic anions.

3.2.3. Simultaneous separation of neutral organics, inorganic and organic anions

Above separation experiments demonstrate SilprDipy can provide the multiple interactions. So, the simultaneous separation of neutral organics, inorganic and organic anions on SilprDipy was investigated. In Fig. 11, a test mixture of phenol, naphthalene, 1-naphthol, fluorene, IO_3^-, I^-, potassium hydrogen phthalate and sodium salicylate was separated in one run, with methanol-

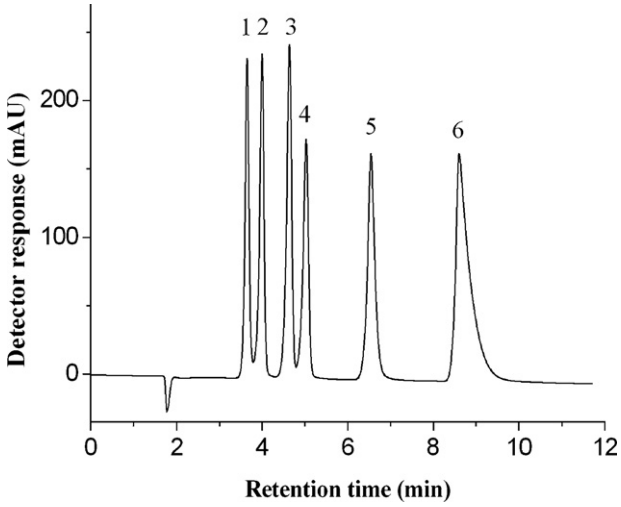

Fig. 9. Separation of test mixture of iodate (61 mg kg^{-1}) (1), bromate (115 mg kg^{-1}) (2), bromide (20 mg kg^{-1}) (3), nitrate (9.2 mg kg^{-1}) (4), iodide (37 mg kg^{-1}) (5) and thiocyanate (45 mg kg^{-1}) (6). Chromatographic conditions: mobile phase: 50 mmol L^{-1} KCl solution at pH 5.5, other conditions are the same as in Fig. 8.

Table 1
The chromatographic parameters of dipyridine modified silica and some other stationary phases having similar bonded groups.

Stationary phases	Column efficiency (N/m)	Resolution (R_s)	Peak symmetry (A_s)	Retention factor (k)
Dipyridine modified silica	51,800-54,300[a], 28,300 (SCN^-) 31,200-43,000[b]	1.80-5.83[a] 2.66-5.78[b]	0.75-3.12[a] 0.98-1.49[b]	2.33-6.85[a] 0.85-3.53[b]
Imidazolium-based stationary phase	37,000-45,500[c] 20,700-32,700[d] [12] 19,100-54,200[e] [13]	1.45-3.99[c] 1.76-3.02[d] [12] 1.74-6.38[e] [13]	0.6-1.7[c] 1.5-1.7[d] [12] 1.54-4.76[e] [13]	7.0-12[c] 8.3-16[d] [12] 2.3-8.0[e] [13]
Pyridine-based stationary phase	20,500-22,200[f] 29,800-33,800[g] [15]	1.20-1.24[f] 2.3-9.3[g] [15]	1.29-1.38[f] 1.3-1.7[g] [15]	7.7-13[f] 0.7-2.0[g] [15]
Phenylamine-based stationary phase	–	–	–	0.98-2.32[h] [16]

[a] 50 mmol L^{-1} KCl solution, pH = 5.5.
[b] Reversed-phase conditions, methanol–water (85:15, v/v).
[c] Eluent conditions, 20 mmol L^{-1} KH_2PO_4, pH = 4.6.
[d] Eluent conditions, 20 mmol L^{-1} NaAc, pH = 4.6.
[e] Eluent conditions, 200 mmol L^{-1} KH_2PO_4, pH = 3.4.
[f] Eluent conditions, 2.5 mmol L^{-1} phthalate buffer, pH = 4.2.
[g] Eormal phase conditions, hexane-dichloromethane (95:5, v/v).[h]Reversed-phase conditions, acetonitrile-5 mmol L^{-1} NaAc (50:50, v/v).

30 mmol L^{-1} NaH_2PO_4 at pH 4.6 (55:45, v/v) as the mobile phase. Comparing their retention time, the retention of neutral organics is the least, inorganic anions are secondary, and organic anions are the strongest. The interaction between neutral organics and SilprDipy is mainly π–π and hydrophobic interactions. For inorganic anions, anion-exchange should be the main interaction. Meanwhile, π–π, hydrophobic and anion-exchange interactions result in the strongest retention of organic anions on SilprDipy. The simultaneous separation of several classes of analytes further demonstrates the multi-interaction characteristic of SilprDipy, including π–π, hydrophobic, electrostatic, anion-exchange, and other interactions.

3.3. Comparison between the prepared stationary phase and the phases having similar bonded groups

To fully evaluate the chromatographic performances of the prepared stationary phase, some other stationary phases having similar bonded groups like imidazole [12,13], pyridine [14,15], phenylamine [2,16,17] and quinolinol [7,11] were compared with it on their separation versatility and some chromatographic parameters. The chromatographic parameters of these phases were summarized in Table 1.

Dipyridine modified silica has the most similar functional group with propylpyridinium modified silica [15] and HILIC Polar-Pyridine [14]. However, it is advantageous over other two phases in the chromatographic separation. It can provide stronger retentions of inorganic anions to separate more anions and has obviously higher column efficiency than propylpyridinium modified silica. HILIC Polar-Pyridine can only separate anions under the acidic condition, but cannot separate anions under the neutral condition. Because this protonated phase at acidic condition can only provide anion-exchange mechanism. While dipyridine modified silica can separate anions at either neutral or acidic condition for having anion-exchange site. Although a microcolumn (150 × 0.53 mm I.D.) packed with HILIC Polar-Pyridine was used in the separation of anions, dipyridine modified silica column (150 × 4.6 mm I.D.) obtained better resolution of iodate and bromate and smaller plate height (20 < 60 μm). *N*-Methylimidazolium silica [12] and dipyridine modified silica have the same particle diameter (5 μm), but the higher column efficiency and the more symmetric peaks of inorganic anions except for thiocyanate were obtained on dipyridine

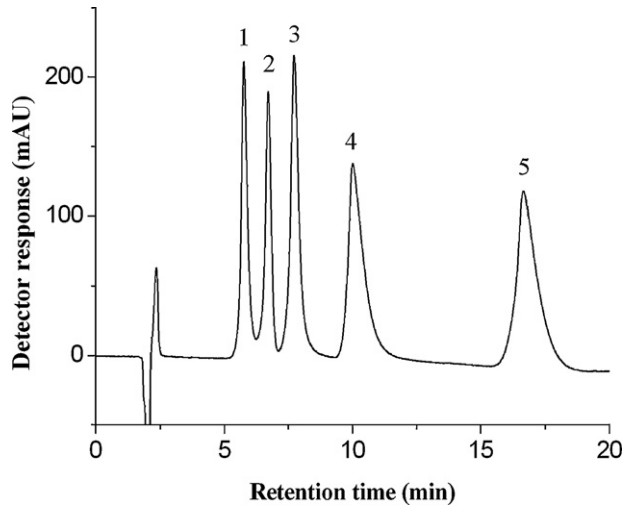

Fig. 10. Separation of organic anions mixture of *p*-aminobenzoic acid (74 mg kg^{-1}) (1), sodium benzoate (81 mg kg^{-1}) (2), *p*-anilinesulfonic acid (105 mg kg^{-1}) (3), potassium hydrogen phthalate (148 mg kg^{-1}) (4) and sodium salicylate (157 mg kg^{-1}) (5). Chromatographic conditions: mobile phase: methanol-30 mmol L^{-1} NaH_2PO_4 at pH 4.6 (45:55, v/v), detection: UV at 200 nm, other conditions are the same as in Fig. 8.

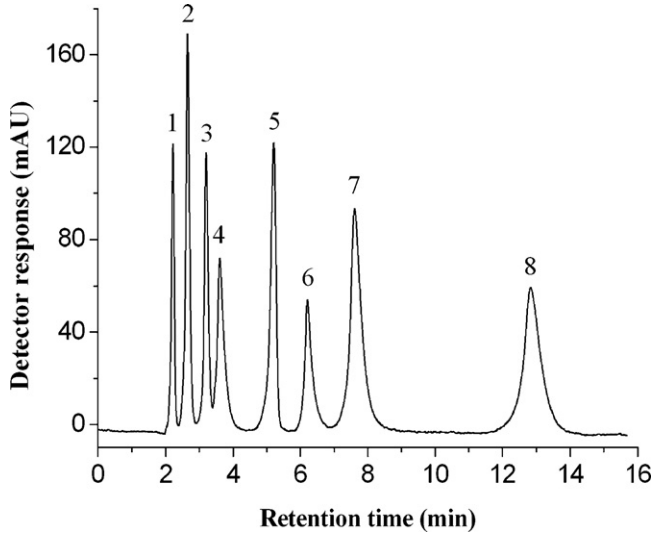

Fig. 11. Separation of test mixture of phenol (1), naphthalene (2), 1-naphthol (3), fluorene (4), IO_3^- (5), I^- (6), potassium hydrogen phthalate (7) and sodium salicylate (8). Chromatographic conditions: mobile phase: methanol-30 mmol L^{-1} NaH_2PO_4 at pH 4.6 (55:45, v/v), detection: UV at 200 nm, other conditions are the same as in Fig. 4.

modified silica. Although the column length of dipyridine modified silica (150 × 4.6 mm I.D.) is shorter than *N*-methylimidazolium silica (200 × 4.6 mm I.D.), it has stronger retention of anions and can separate more organic anions. pH of mobile phase has stronger influence on the retention of anions on dipyridine modified silica than that on *N*-methylimidazolium silica, which helps to obtain good separation by regulating pH of mobile phase. Compared with imidazolium functionalized silica [13], it also has better column efficiency and peak symmetry of anions. Compared with phenylaminopropyl bonded silica [17], it has similar column efficiency but it has better resolution for the separation of common inorganic anions (BrO_3^-, Br^-, NO_3^-, I^- and SCN^-). For the separation of phenols, phenylaminopropyl bonded silica [16] may has stronger separation but the separation of 10 phenols needed 45 min. While dipyridine modified silica separated 8 phenols within 16 min. This indicated it provided a higher analysis efficiency. Compared with 8-quinolinol silica [7], it has different selectivity for anions and PAHs, and the retention of PAHs such as naphthalene is weaker than inorganic anions. The main reason is that the anion-exchange interaction is stronger than π–π interaction on it. Relatively weak π–π interaction is beneficial to the separation of mixture of PAHs and anions. Because strong π–π and hydrophobic interactions can lead to too long retention time of some PAHs. At the same time, the retention of anions on it can be regulated by changing concentration and pH of mobile phase to obtain good separation with PAHs. For simultaneous separation of anions and PAHs on two stationary phases, better resolution and shorter retention time were obtained on dipyridine modified silica. Besides, 8-quinolinol silica [11] with quinoline and amino had complicated mechanism for two mixed functional groups. While dipyridine modified silica with single functional group provided clear mechanism and the effect on retention from every interaction can also be regulated easily. In conclusion, although dipyridine modified silica had some similar performance with these phases reported, it provided some advantages such as higher column efficiency, shorter analysis time, larger resolution, better separation and better peak symmetry over the others.

4. Conclusions

The preparation and characterization of a novel multi-interaction stationary phase based on 4,4′-dipyridine modified silica are described. Different series of analytes including PAHs, phenols, inorganic and organic anions were successfully separated on this multi-interaction stationary phase respectively. Furthermore, the simultaneous separation of different species including neutral organics, inorganic and organic anions was obtained with appropriate eluent. The multi-interaction mechanism including π–π, hydrophobic, electrostatic and anion-exchange interactions is involved in the chromatographic separation. With such characteristics of multi-interaction mechanism and multi-modal separation, this stationary phase has a promising application in the analyses of complex samples.

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