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A multiple-function stationary phase based on perhydro-26-membered hexaazamacrocycle for high-performance liquid chromatography

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ABSTRACT

A perhydro-26-membered hexaazamacrocycle-based silica (L^1GlySil) stationary phase for high-performance liquid chromatography (HPLC) was prepared using 3-glycidoxypropyltrimethoxysilane as coupling reagent. The structure of new material was characterized by infrared spectroscopy, elemental analysis and thermogravimetric analysis. The chromatographic performance and retention mechanism of the new phase were evaluated in reversed-phase (RP) and normal-phase (NP) modes using different solute probes including aromatic compounds, organophosphorus pesticides, carbamate pesticides and phenols. The results showed that L^1GlySil was a sort of multimode-bonded stationary phase with excellent chromatographic properties. The new phase could provide various action sites for different solutes, such as hydrophobic, hydrogen bonding, π–π, dipole–dipole interactions and acid–base equilibrium. The presence of phenyl rings, secondary amino groups and alkyl linkers in the resulting material made it suitable for the separation of above-mentioned analytes by multimode retention mechanisms.

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1. Introduction

Macrocyclic polyamines, with similar structures to crown ether, are noted for their ability to selectively form complexes with anions and cations [1,2]. Complexing properties of macrocyclic receptors depend mainly on the type and arrangement of binding site. Macrocyclic polyamines have been demonstrated to be strong receptors with catechol, polyanions and so on, which probably associate with the amine and amide hydrogen atoms packed in the macrocyclic cavities via hydrogen bonding [3]. Based on their strong interactions with these guest molecules, macrocyclic polyamine was used either as an additive in capillary electrophoresis or to covalently modify the fused-silica capillary for enhancement of separation of positive, neutral and negative guest molecules [4]. Liu group demonstrated that the 24-, 28- and 32-membered macrocyclic polyamines were highly selective as the receptor for anions, such as oxyanions, polycarboxylates, carbohydrates and polyphosphates in the capillary electrochromatographic separation on a bonded-phase capillary column. The selectivity of the separation could be attributed to anion complexation, anion exchange and reversal of the electroosmotic flow provided by the wall-bonded functional groups [5–7]. The stationary phase bonded with macrocyclic dioxopolyamine (1,4,7,10-tetraazacyclotridecane-11,13-dione) was prepared in open-tubular capillary electrochromatography and showed effective separations of nitrophenol and aminophenol isomers, and biogenic monoamine neurotransmitters in comparison with untreated capillaries [8].

The retention of solutes on the stationary phase may involve a variety of interaction mechanisms, such as hydrophobic, π–π, hydrogen bonding and charge-transfer interactions in high-performance liquid chromatography (HPLC). As a result, the stationary phases utilizing a multimode retention mechanism have been proposed to achieve the desired improvement in the selectivity of separation of specific solutes and can offer more potential than classical reversed-phase (RP) or normal-phase (NP) chromatography [9–11]. Hydrophobic effect is responsible for the separation of solutes on alkyl silica-based stationary phase in RP-HPLC. The selectivity of RP separations can be improved by introduction of phenyl group into bonded molecules providing π–π interactions between the stationary phase and solutes. An additional change in the selectivity of the stationary phase can be obtained by introducing polar amino group into the bonded phase. The amino group is responsible for dipole–dipole and hydrogen bonding interactions [12–14].

26-Membered hexaazamacrocycle (bis-p-xylyl-BISDIEN Schiff base, L) is a typical macrocyclic polyamine compound and has been widely used as metal ligand in metalloenzyme [15,16]. It has distinct interaction sites as a chromatographic ligand, for instance hydrophobic alkyl chains (hydrophobic interaction), phenyl rings (π–π interaction), polar amino groups (hydrogen bonding and dipole–dipole interaction). It was anticipated that the phenyl rings,

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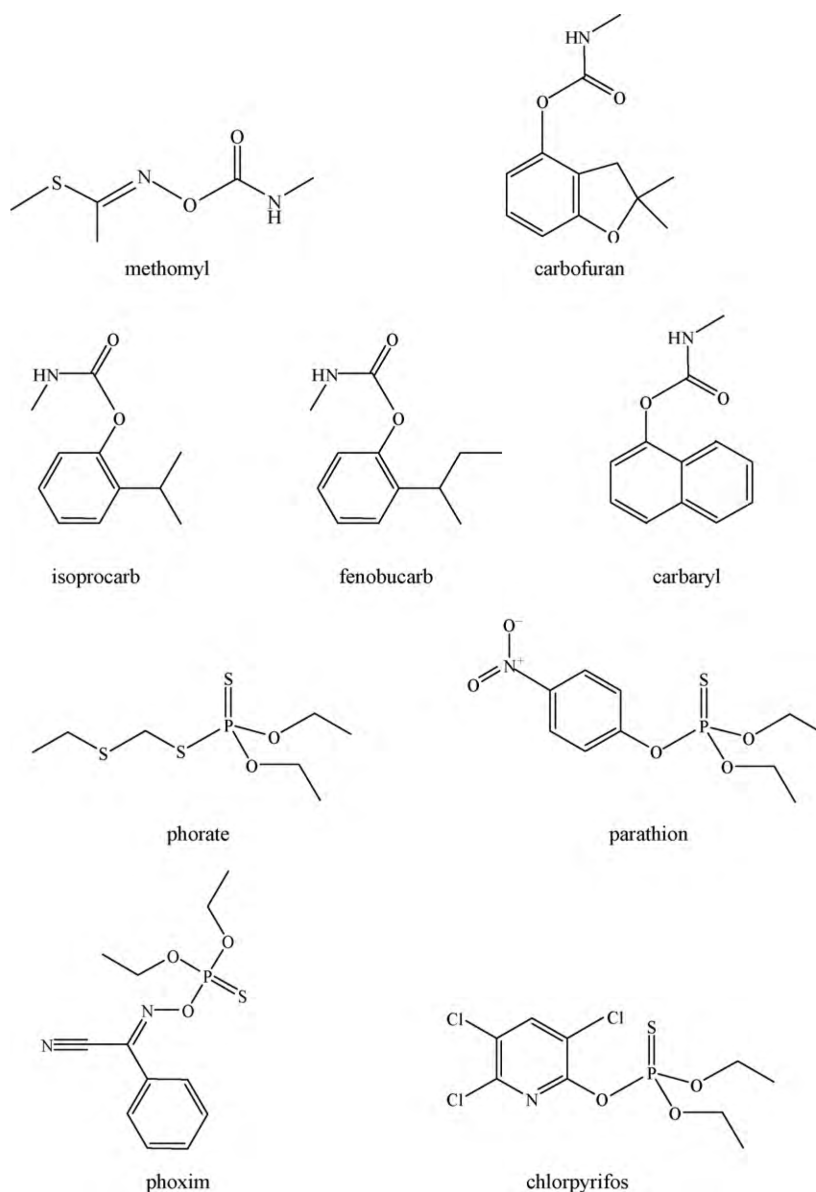

Fig. 1. Structures of carbamate and organophosphorus pesticides.

amino groups and alkyl chains in this polyazamacrocyclic compound might serve to increase selectivity of chromatographic separation. It would be interesting to associate L as stationary phase with HPLC. The paper showed for the first time the use of perhydro-26-membered hexaazamacrocycle (L^1) attached on the silica surface for separation of aromatic compounds, phenols, organophosphorus and carbamate pesticides in HPLC. As a consequence of its distinct structure binding sites, the bonded material was a true multimode phase that, depending on the elution conditions and the characteristics of the selected solutes, could be operated in a variety of chromatographic modes, such as RP and NP mode.

2. Experimental

2.1. Apparatus and reagents

Silica having diameter 5 μm, pore size 90 Å, and surface area 300 $m^2\,g^{-1}$ was purchased from Lanzhou Institute of Chemical Physics, Chinese Academy of Science (Lanzhou, China). 3-Glycidoxypropyltrimethoxysilane and toluene were purchased from Sinopharm Group Chemical Reagent Co., Ltd. (Shanghai, China). Triethylamine was obtained from Tianjin Kermel Chemical Reagent Co., Ltd. (Tianjin, China). Diethylenetriamine and 1,4-phthalaldehyde were purchased from Alfa Aesar (Ward Hill, USA). Phorate, parathion, phoxim and chlorpyifos were bought from Agro-Environmental Protection Institution (Beijing, China) and methomyl, carbofuran, isoprocarb, fenobucarb and carbaryl were purchased from Pesticide Research Institute (Shanghai, China) and their structures are listed in Fig. 1. Toluene was distilled and dried over sodium before use. HPLC grade methanol (Luoyang Haohua Chemical Reagent Co., Ltd., Luoyang, China) was used. Doubly distilled water was used throughout this work. Other reagents used in the experiment were analytical grade.

The carbon, hydrogen and nitrogen contents of each compound were determined by elemental analysis (EA) at a Flash EA 1112 elemental analyzer (Thermo, Waltham, USA). The Infrared spectra were recorded on a Prestige-21 spectrometer (Shimadzu, Kyoto, Japan) at 4000–400 cm^{-1}. The ^1H NMR spectra were measured using DPX-400 (Bruker, Ettlingen, Germany). ESI-MS of analyte was

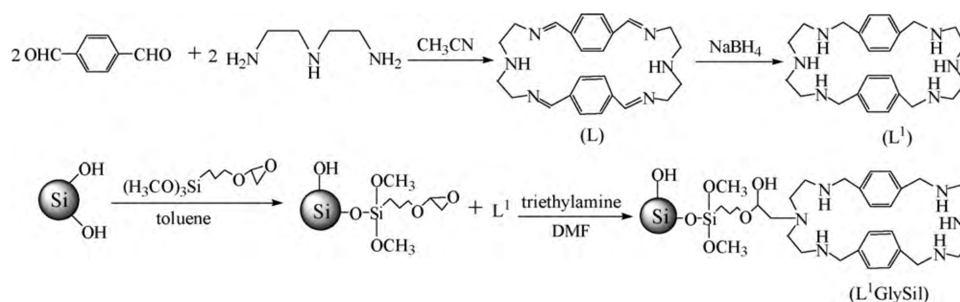

Fig. 2. Schemes demonstrating the synthesis of L¹GlySil.

determined using 6310 ion trap LC/MS system (Agilent, Santa Clara, USA). The thermogravimetric and differential curves were obtained on a Shimadzu DT-40 thermal analyzer with temperature programming rate of 10 °C min^{-1}.

2.2. Preparation of L^1 functionalized silica

2.2.1. Sililation of silica gel

Silica was immersed in hydrochloric acid/water solution (v/v = 1/1) for 24 h and then washed with water and dried under vacuum at 120 °C for 8 h. In a round-bottomed flask equipped with a reflux condenser and a gas inlet valve, 5.0 g activated silica was dispersed in toluene. After the addition of 5 mL 3-glycidoxypropyltrimethoxysilane and 0.3 mL triethylamine (as a catalyst), the mixture was stirred and refluxed for 24 h under a nitrogen atmosphere. After refluxing, the product was cooled to room temperature and washed successively with toluene, acetone, methanol and acetone/water (v/v = 1/1). The 3-glycidoxypropyltrimethoxysilane silica (GlySil) was dried under vacuum at 100 °C for 12 h.

2.2.2. Synthesis of L^1

26-Membered hexaazamacrocycle (L) was synthesized according to the reported procedure [17]. An acetonitrile solution (150 mL) of diethylenetriamine (0.009 mol) was added dropwise to a stirred acetonitrile solution (100 mL) of 1,4-phthalaldehyde (0.009 mol) at room temperature, and the mixture was stirred for 24 h continuously. Then the resulting precipitate was filtered and washed with ether, then obtained white powder L was dried under vacuum at 30 °C for 12 h. The yields of L are 85%. L was characterized by MS, FTIR, ^1H NMR and EA and the results were as follows: ESI-MS *m/z* $[M+H]^+$ 403.2; ^1H NMR (400 MHz, $CDCl_3$) δ 1.853 (m, 2H), 3.009 (m, 8H), 3.802 (m, 8H), 7.554 (s, 8H), 8.315 (d, 4H); FTIR (cm^{-1}) 3444.9, 3304, 2870, 2832, 2742, 1645, 1568, 1447, 1333, 1300, 1130, 837, 795, 513; Anal. Calcd for $C_{24}H_{30}N_6$ C, 71.61%, N, 20.88%, H, 7.51%, Found C, 71.33%, N 20.69%, H, 7.56%.

In a reaction flask, 2.5 g sodium borohydride was slowly added to methanol solution (300 mL) of 1.5 g L under magnetic stirring at 45 °C, the mixture was stirred for 1 h continuously. After stirring, the solution was cooled to room temperature and concentrated in rotary evaporator at 45 °C. The resulting product was extracted with water/dichloromethane. The remaining dichloromethane was removed by heating the organic phase to 30 °C. 10 mL HBr and 100 mL ethanol were added to the obtained product to produce L^1·6HBr. L^1·6HBr was neutralized by addition of NaOH solution, and then L^1 was extracted with dichloromethane again. The resulting solution was concentrated in rotary evaporator to yield L^1.The obtained product was dried under vacuum at 30 °C for 12 h and the yields are 50%. L^1 was characterized by FTIR, ^1H NMR and EA and the results were as follows: ^1H NMR (400 MHz, $CDCl_3$) δ 1.758 (broad s, 6H), 2.782 (m, 16H), 3.759 (s, 8H), 7.266 (s, 8H); FTIR (cm^{-1}) 3337, 3248, 2868, 2801, 2355, 2320, 1449, 1331, 1240, 1105, 828, 772; Anal. Calcd for $C_{24}H_{38}N_6$ C, 70.20%, N, 20.47%, H, 9.33%, Found C, 70.32%, N, 20.28%, H, 9.33%.

2.2.3. Preparation of L^1 functionalized silica

In a round-bottomed flask equipped with a magnetic stirrer and a gas inlet valve, 4.0 g GlySil was added to dimethylformamide (DMF) solution of 0.65 g L^1 under magnetic stirring, then 0.3 mL triethylamine was dropped as catalyst. The mixture was refluxed under nitrogen atmosphere at 85 °C for 24 h. The obtained product was washed twice with DMF, DMF/methanol (v/v = 40/10), DMF/methanol (v/v =25/25) and DMF/methanol (v/v = 10/40) in turn. The L^1 bonded silica (L^1GlySil) stationary phase was dried under vacuum at 90 °C for 12 h prior to packing or characterization by FTIR, EA and thermogravimetric analysis.

A schematic diagram of the synthetic procedure for preparation of L^1GlySil is listed in Fig. 2.

2.3. Chromatographic evaluation

The L^1GlySil were slurry packed into stainless steel tube column (250 mm × 4.6 mm I.D.) using tetrachloromethane as slurry solvent and methanol as propulsive solvent. A CGY-100B pneumatic pump (Bejing Fusiyuan Mechanical Processing Factory, Beijing, China) was used with packing pressure of 50 Mpa.

All chromatographic tests were performed on a Shimadzu system equipped with a LC-10AT vp plus pump, a SPD-10A vp plus UV–vis detector and CBM-10A vp plus chromatographic station. A Rheodyne (Cotati, CA, USA) 7725i injector with 20 μL sample loop was used. Separations were carried out with a L^1GlySil column (250 mm × 4.6 mm I.D., 5 μm). All the test mixtures were analyzed at room temperature at a flow rate of 1.0 mL min^{-1} with UV detection wavelength at 254 or 230 nm. Mobile phase was filtered through a 0.45-μm nylon membrane filter and was degassed ultrasonically prior to use. Dead time was the signal of mobile phase.

3. Results and discussion

3.1. Characterizations

Examination of the elemental analysis of GlySil and L^1GlySil showed the successful immobilization of L^1 on silica surface. In GlySil: C 7.65%, H 1.38%, In L^1GlySil; C 9.21%, H 2.25%, N 0.48%. The bonding amount of L^1GlySil stationary phase was about 0.054 mmol g^{-1}, which was calculated from the carbon content.

The materials were also analyzed by FTIR. Compared with activated silica, the spectrum of L^1GlySil showed that –OH group (3446 cm^{-1}) decreased using bending vibration of Si–O–Si (473 cm^{-1}) as internal reference peak. This reduction of free silanol group indicated a successful covalent modification of the silica surface. The band at 1450–1650 cm^{-1} in spectrum of L^1GlySil is associated to the stretching vibration of benzene. The bands at 2925

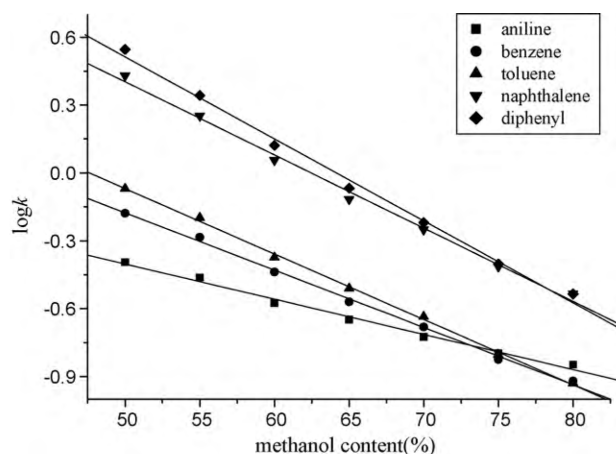

Fig. 3. Effect of methanol content on log k of aromatic compounds. Chromatographic conditions: L^1GlySil column, mobile phase: different methanol contents, flow rate, 1 mL min^{-1}, UV at 254 nm.

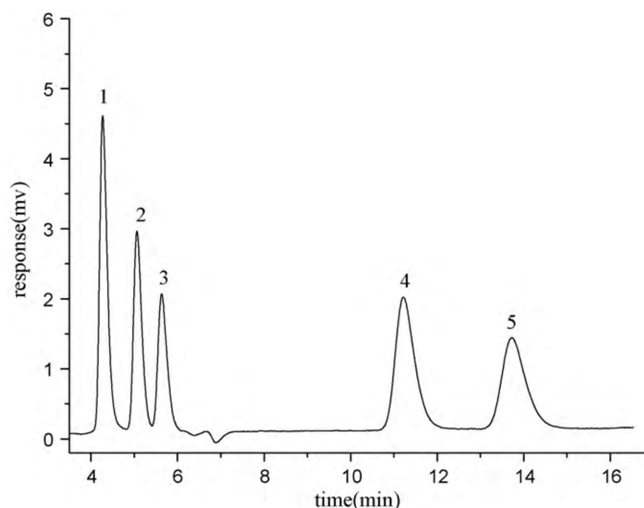

Fig. 4. Separation of aniline (1), benzene (2), toluene (3), naphthalene (4) and biphenyl (5). Chromatographic conditions: mobile phase: methanol–water (50/50, v/v), other chromatographic conditions are the same as in Fig. 3.

and 2850 cm^{-1} in the L^1GlySil spectrum are due to the stretching vibration of $-CH_2-$ groups.

The thermogravimetric curve can give information on the material thermal stability and also confirm the amount of the immobilized compound. The mass loss of L^1GlySil phase occurred in the range from 260 to 566 °C and the decomposition temperature of phase is 472.9 °C, which indicated the stationary phase had good thermal stability below 260 °C.

3.2. Chromatographic separations

3.2.1. Separation of aromatic compounds

To understand the role of hydrophobic interaction in the retention of solutes, five aromatic compounds including aniline, benzene, toluene, naphthalene and biphenyl were firstly chosen as test probes for investigations on retention behavior of L^1GlySil stationary phase in RP-HPLC. The effect of content of methanol in the mobile phase on the retention factors (k) of analytes was evaluated. As shown in Fig. 3, log k of five aromatic compounds tend to decline and have favorable linearity with increase of methanol content. For a small amount of methanol in the mobile phase, the retention order of analytes on L^1GlySil was the same as that on ODS. The five peaks were aniline, benzene, toluene, naphthalene and biphenyl, respectively, and their retentions followed the order of hydrophobicity of the solutes (the log $K_{O/W}$ values were listed in Table 1). These results accorded with RP separation mechanism and also indicated the hydrophobic interaction between the L^1GlySil stationary phase and aromatic compounds played an important role in the separation.

For large ratio of methanol, the retention order of analytes changed and the five peaks were toluene, benzene, aniline, biphenyl and naphthalene using 80/20 (v/v) methanol/water as eluent. The hydrogen bonding interaction between amino groups of aniline and that of L^1GlySil resulted in the stronger retention of aniline than benzene and toluene. In comparison with toluene, benzene was likely to form stronger induce dipole–dipole interaction with amino groups on L^1GlySil packing, and thus enhancing its retention. So it can be concluded that hydrogen bonding, induce dipole–dipole interactions as well as hydrophobic interaction were responsible for the retention of solutes in methanol-rich eluent. Although above-mentioned polar interactions were involved in the retention, five aromatic compounds can not be separated in NP-HPLC.

Fig. 4 is a chromatogram from an isocratic separation of five analytes on L^1GlySil column using 50/50 (v/v) methanol/water as eluent and five aromatic compounds can be effectively separated in RP-HPLC. The number of theoretical plates (N) of the column was above 14,000 per meter. After used for 2000 h, N of the column decreased about 10%.

Methanol and acetonitrile are the most common organic modifiers of mobile phase in RP-HPLC. Methanol has stronger H-bonding donating ability and less lipophilicity than acetonitrile. Acetonitrile is an electron rich organic modifier, which could suppress the π–π interactions between the analyte and the stationary phase. The selectivity and retention behaviors of L^1GlySil could be influenced using different organic modifiers owing to different adsorptions of the organic modifier on the adsorbent surface [19]. As shown in Fig. 5, with increasing acetonitrile content in the eluent the retentions of aromatic compounds first decrease then increase. The L^1GlySil phase seems to show hydrophilic interaction chromatographic mode using acetonitrile as organic modifier. But five aromatic compounds had similar retention and could not be separated in the whole content range tested.

Table 1
The log $K_{O/W}$ values of analytes studied [18].

Analyte	Log $K_{O/W}$
Aniline	0.9
Benzene	2.13
Toluene	2.73
Naphthalene	3.3
Biphenyl	3.98
Methomyl	0.6
Carbofuran	2.32
Isoprocarb	2.31
Fenobucarb	–
Carbaryl	2.36
o-Cresol	1.95
Phenol	1.46
1-Naphthol	2.85
3-Nitrophenol	2.00

3.2.2. Separation of carbamate pesticides

Carbamate pesticides, a kind of broad-spectrum pesticide, are derived from carbamic acid and possess polar groups. Separation of five carbamate pesticides, including methomyl, carbofuran, isoprocarb, fenobucarb and carbaryl, were examined on L^1GlySil column. In RP-HPLC, the influence of different methanol contents on k of carbamate pesticides was investigated. With the increase of the

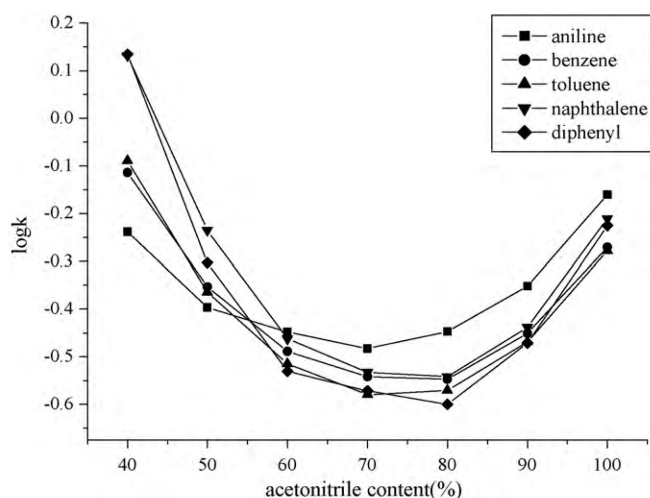

Fig. 5. Effect of acetonitrile content on the retention of aromatic compounds. Mobile phase: different acetonitrile contents, other chromatographic conditions are the same as in Fig. 3.

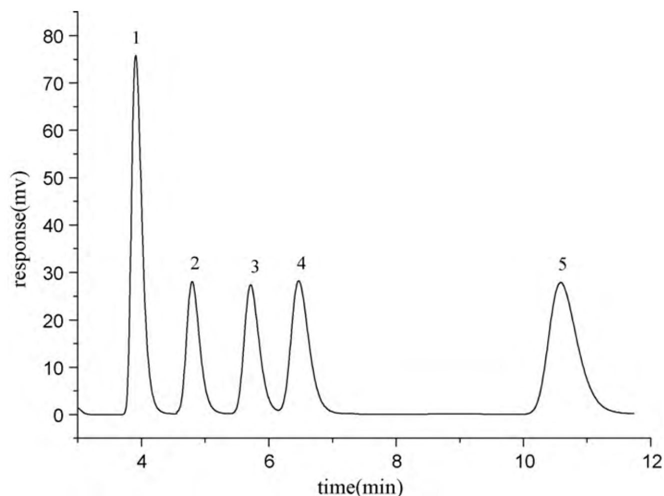

Fig. 7. Separation of carbamate pesticides composed of methomyl (1), carbofuran (2), isoprocarb (3), fenobucarb (4), carbaryl (5). Mobile phase: methanol–water (40/60, v/v), other chromatographic conditions are the same as in Fig. 3.

amount of methanol in the mobile phase, the log k of all compounds decreased and it could be deduced that the hydrophobic interaction was responsible for the retention of carbamate pesticides. As can be seen in Fig. 6, the retention and elution order are methomyl < carbofuran < isoprocarb < fenobucarb < carbaryl at low methanol content. This elution order, except carbaryl, was in agreement with the hydrophobicity of carbamate pesticides [20]. The selectivity of RP separations can be changed by introducing of phenyl groups into bonded molecules providing π–π interactions between analytes and stationary phase [13–14]. The π–π interaction between two phenyl groups of carbaryl and that of L^1GlySil could be responsible for the increase in retention of carbaryl. With increasing methanol content the selectivity of L^1GlySil column for solutes changed. Of all the carbamate pesticides, only carbaryl maintained the same elute order at all content of methanol and the elution order is carbofuran < fenobucarb < isoprocarb < methomyl < carbaryl with 90/10 (v/v) methanol/water. Among the four analytes (carbofuran, fenobucarb, isoprocarb and methomyl), methomyl has the strongest polarity (the log $K_{O/W}$ value was listed in Table 1) and different chromatographic behaviors on L^1GlySil column. Its retention was mainly ascribed to dipole–dipole and hydrogen bonding interactions, while the retentions of other three analytes were induced by hydrophobic and π–π interactions as well as hydrogen bonding interaction, which could be the reason that methomyl was eluted firstly at low-content methanol and eluted finally at high-content methanol.

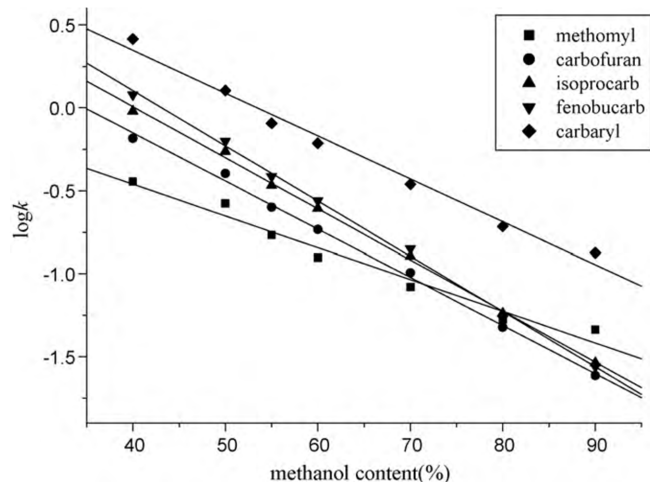

Fig. 6. Effect of different methanol contents on log k of carbamate pesticides. Mobile phase: different content methanols, other chromatographic conditions are the same as in Fig. 3.

These results indicated the L^1GlySil column contained typical RP chromatographic mechanism, meanwhile, the elution order of analytes depended on a number of complicated factors such as π–π, hydrogen bonding and dipole–dipole interactions. Five carbamate pesticides could be successfully separated on L^1GlySil column with 40/60 (v/v) methanol/water in 12 min and the result was excellent (Fig. 7). It should be indicated that the influence of the content of acetonitrile in the eluent on the rention of carbamate pesticides was similar to aromatic hydrocarbons, and five carbamate pesticides could also not be separated on L^1GlySil column with acetonitrile/water.

Compared with the aromatic hydrocarbons, carbamate pesticides possess stronger polarity and can form hydrogen bonding and dipole–dipole by carbamate groups with amino groups on L^1GlySil. Multiple-interaction increased the retention of carbamate pesticides and selectivity of L^1GlySil packing material (Table 2), thus separation of carbamate pesticides on L^1GlySil under NP condition was expected. Fig. 8 represents the separation of four carbamate pesticides using the mobile phase containing 60/40 (v/v) hexane/isopropyl alcohol. The peaks of fenobucarb and isoprocarb overlap due to their close polarity.

3.2.3. Separation of organophosphorus pesticides

L^1GlySil column was applied for the separation of organophosphorus pesticides including phorate, parathion, phoxim and chlorpyrifos. Figs. 9 and 10 show the typical chromatograms of organophosphorus pesticides on L^1GlySil column with 45/55 (v/v) methanol/water and 70/30 (v/v) hexane/isopropyl alcohol, respectively. In RP-HPLC, the elution order of four organophosphorus pesticides on L^1GlySil column was different from that on ODS column [21] and phorate were eluted before parathion and phoxim. The π–π interaction resulting from phenyl groups of the latter analytes and that of L^1GlySil could strengthen the hydrophobic interaction and was responsible for the increase in retention of parathion and phoxim. It was confirmed again that the retention mechanism of the new stationary phase in RP-HPLC was ascribed to multi-interaction, like π–π as well as hydrophobic interactions,

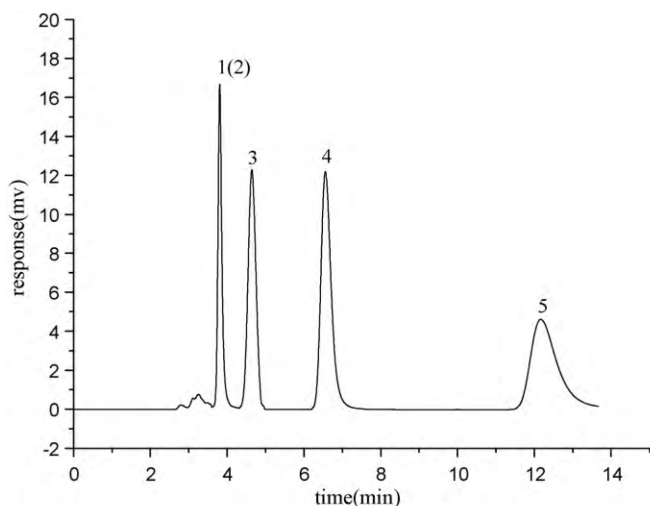

Fig. 8. Separation of carbamate pesticides composed of fenobucarb (1), isoprocarb (2), carbofuran (3), carbaryl (4), methomyl (5). Mobile phase: hexane–isopropyl alcohol (60/40, v/v), other chromatographic conditions are the same as in Fig. 3.

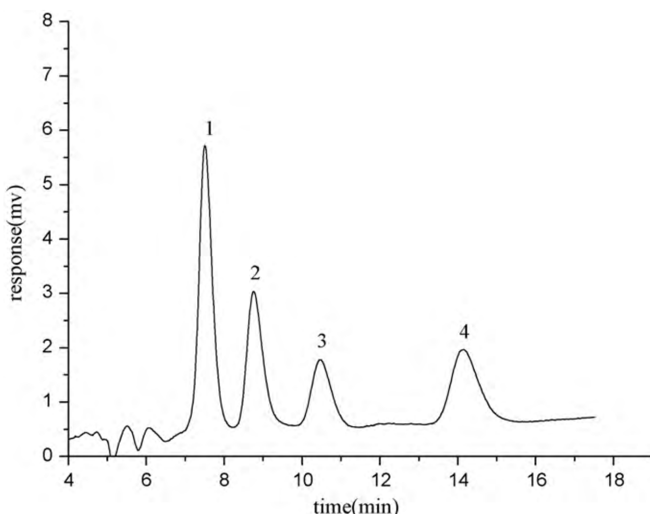

Fig. 9. Separation of organophosphorus pesticides composed of phorate (1), parathion (2), phoxim (3), chlorpyrifos (4). Mobile phase: methanol–water (45/55, v/v), other chromatographic conditions are the same as in Fig. 3.

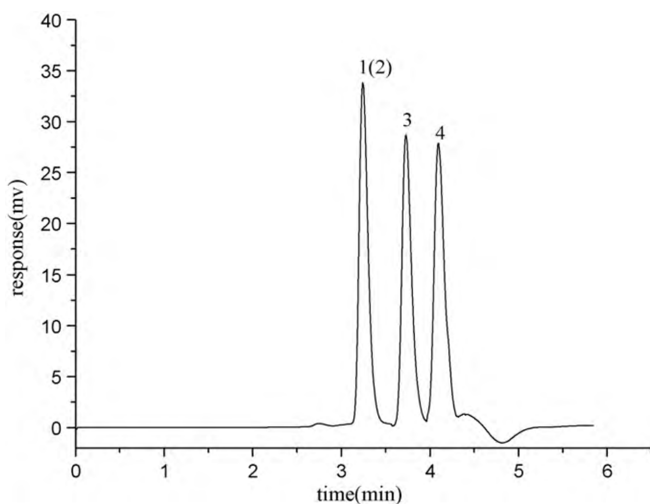

Fig. 10. Separation of organophosphorus pesticides composed of chlorpyrifos (1), phorate (2), phoxim (3), parathion (4). Mobile phase: hexane–isopropyl alcohol (70/30, v/v), other chromatographic conditions are the same as in Fig. 3.

Table 2
Comparison of k, α and R of analytes between RP- and NP-HPLC.

	RP-HPLC[a]			NP-HPLC[b]		
	k	α	R	k	α	R
Aniline	0.40	–	–	0.91	8.27	7.93
Benzene	0.66	1.65	2.30	0.04	/	/
Toluene	0.85	1.29	1.54	0.01	–	–
Naphthalene	2.69	3.16	9.17	0.11	/	/
Diphenyl	3.51	1.30	2.72	0.08	/	/
	RP-HPLC[c]			NP-HPLC[d]		
	k	α	R	k	α	R
Methomyl	0.36	–	–	3.05	2.58	6.55
Carbofuran	0.65	1.81	2.50	0.55	2.04	2.79
Isoprocarb	0.95	1.46	2.19	0.27	–	–
Fenobucarb	1.20	1.26	1.54	0.27	1.00	0
Carbaryl	2.61	2.18	5.99	1.18	2.15	4.30
	RP-HPLC[e]			NP-HPLC[f]		
	k	α	R	k	α	R
Phorate	1.40	–	–	0.08	–	–
Parathion	1.80	1.29	1.74	0.36	1.50	1.62
Phoxim	2.35	1.31	1.97	0.24	3.00	2.34
Chlorpyrifos	3.52	1.50	3.29	0.08	–	–
	RP-HPLC[a]			NP-HPLC[g]		
	k	α	R	k	α	R
Phenol	0.63	–	–	0.71	1.29	1.73
o-Cresol	0.80	1.27	1.38	0.55	–	–
1-Naphthol	3.57	4.46	5.28	0.99	1.39	2.67
3-Nitrophenol	4.01	1.12	1.16	1.70	1.71	4.54
Catechol	/	/	/	2.30	1.35	3.01
Resorcin	/	/	/	2.66	1.16	1.78
2-Nitrophenol	/	/	/	3.91	1.47	4.63

/: α and R cannot be calculated because compounds were not selected as analyte or the retentions of compounds were similar.

[a] Methanol–water (50/50, v/v), other chromatographic conditions are the same as in Fig. 3.

[b] Hexane/isopropyl alcohol (70/30, v/v), other chromatographic conditions are the same as in Fig. 3.

[c] Methanol–water (40/60, v/v), other chromatographic conditions are the same as in Fig. 3.

[d] Hexane/isopropyl alcohol (60/40, v/v), other chromatographic conditions are the same as in Fig. 3.

[e] Methanol–water (45/55, v/v), other chromatographic conditions are the same as in Fig. 3.

[f] Hexane/isopropyl alcohol (70/30, v/v), other chromatographic conditions are the same as in Fig. 3.

[g] Hexane/isopropyl alcohol (92.5/7.5)–methanol (80/20, v/v) , other chromatographic conditions are the same as in Fig. 3.

which resulted in the change of selectivity of L^1GlySil column for different types of solutes.

L^1GlySil may interact with organophosphorus pesticides by π–π and dipole–dipole interactions, which were expected to enhance the selectivity of the new phase for analytes in NP-HPLC. As shown in Fig. 10 and Table 2, the baseline separation of three organophosphorus pesticides including chlorpyrifos, phoxim and parathion could be achieved in 5 min, and phorate and chlorpyrifos was coeluted.

3.2.4. Separation of phenols

The unique structure of the attached macrocyclic molecules provided a multi-mode retention mechanism for solutes and can offer the possibility of strong interactions with phenols [22]. To demonstrate the special selectivity and retention mechanism of L^1GlySil for phenols, four representative phenols including phenol, o-cresol, 1-naphthol and 3-nitrophenol were used for the evaluation. The retention factors of phenols were listed in Table 2 under different

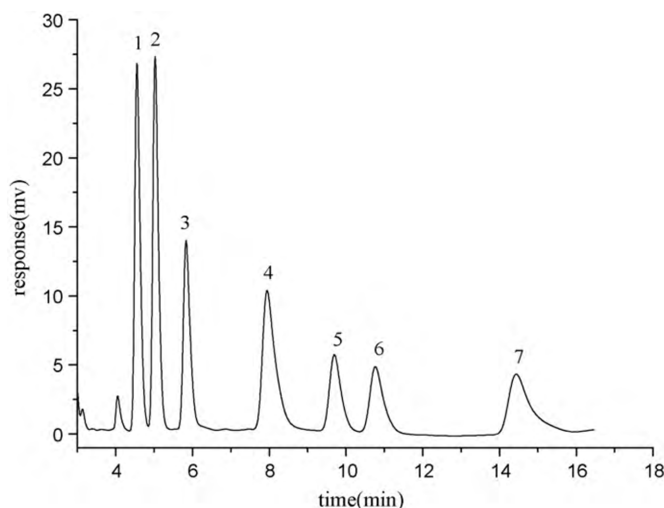

Fig. 11. Separation of phenols composed of o-cresol (1), phenol (2), 1-naphthol (3), 3-nitrophenol (4), catechol (5), resorcinol (6), 2-nitrophenol (7). Mobile phase: hexane/isopropyl alcohol (92.5/7.5)–methanol (80/20, v/v), UV at 230 nm, other chromatographic conditions are the same as in Fig. 3.

chromatographic conditions. The elution order of compounds on L^1GlySil phase was not in agreement with that on ODS phase in RP-HPLC. The retention of phenols increased with their hydrophobicity except 3-nitrophenol (the $\log K_{O/W}$ values were listed in Table 1). Solutes were firstly separated according to their hydrophobicity as usual in typical RP-HPLC. The strongest retention of L^1GlySil column for 3-nitrophenol, as compared with ODS column, was likely due to the fact that the acid–base equilibrium between the hydroxy group of 3-nitrophenol and the amino group of L^1GlySil played a significant role. The existence of electron-attracting group ($-NO_2$) causes the formation of acid–base equilibrium between the hydroxy group of nitrophenol and amino group of L^1 macrocycle.

The acid–base equilibrium could be also confirmed by the fact that 3-nitrophenol eluted finally among above four analytes in NP-HPLC. For investigating the role of the above-mentioned electronic effect in retention, other two nitrophenols including 2-nitrophenol and 4-nitrophenol were also used as analytes. The retention times of 3-nitrophenol, 2-nitrophenol and 4-nitrophenol are 7.9, 14.4 and more than 30 min, respectively. This electronic effect of three nitrophenols is followed the order of 4-nitrophenol > 2-nitrophenol > 3-nitrophenol, which is consistent with the retention order of three nitrophenols. The retention of 1-naphthol on L^1GlySil in NP-HPLC did not correspond with the polarity, which was due to the π–π interaction enhancing its retention. Besides above-mentioned interactions, hydrogen bonding and dipole–dipole interactions were responsible for the retention of solutes in NP-HPLC. According to the selectivity factor (α) of L^1GlySil for four phenols in Table 2, L^1GlySil in NP-HPLC showed higher selectivity for the phenol and o-cresol pair as well as the 1-naphthol and 3-nitrophenol pair than that in RP-HPLC, thus L^1GlySil phase could preferably be operated in NP-HPLC to separate the polar phenols. The capability of separating some other phenols was also investigated. Fig. 11 displays the baseline separation of 7 phenols on L^1GlySil under NP-HPLC condition.

4. Conclusions

The new promising stationary phase based on L^1 was prepared in this paper. Characterization using infrared spectroscopy, elemental analysis and thermogravimetric analysis proved that this macrocyclic polyamine molecule was covalently attached on the silica surface. The L^1GlySil phase could provide various action sites including hydrophobic, dipole–dipole, π–π, hydrogen-bonding interactions and acid–base equilibrium and presented a multi-mode retention mechanism for different types of analytes. Simultaneous interactions enabled the new phase to be operated in RP- and NP-HPLC with excellent chromatographic properties. To some extent, the L^1GlySil phase showed prospect for the separation of neutral aromatic compounds and polar pesticides and phenols.

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