Talanta 89 (2012) 433–440

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

A new 14-membered tetraazamacrocycle-bonded silica stationary phase for reversed-phase high-performance liquid chromatography

Lijun He[a,*], Mingliang Zhang[a], Wenjie Zhao[a,b], Jie Liu[a], Xiuming Jiang[a], Shusheng Zhang[b], Lingbo Qu[a]

[a] School of Chemistry and Chemical Engineering, Henan University of Technology, Zhengzhou 450001, China
[b] Department of Chemistry, Zhengzhou University, Zhengzhou 450001, China

ARTICLE INFO

Article history:
Received 11 September 2011
Received in revised form
19 December 2011
Accepted 21 December 2011
Available online 27 December 2011

Keywords:
14-Membered-tetraazamacrocycle
Stationary phase
High-performance liquid chromatography
Linear solvation energy relationship
Retention property

ABSTRACT

A new high-performance liquid chromatography stationary phase has been prepared by covalently bonding 14-membered tetraazamacrocycle to silica gel using γ-chloropropyltrimethoxylsilane as coupling agent. The structure of the new material was characterized by infrared spectroscopy and elemental analysis. With 32 solutes including aromatic and aliphatic compounds, the linear solvation energy relationship method was successfully used to chromatographically evaluate the new phase in reversed phase mode. The retention property of the new phase shows evident similarity with that of ODS stationary phase, as well as distinctive, unique retention characteristics. The separations of *n*-alkylbenzene, carbamate and organophosphorus pesticides with diversified functional groups as well as phenolic compounds demonstrate that in addition to hydrophobic interaction, dipole–dipole interaction and hydrogen bonding interaction plus acid–base equilibrium could also be simultaneously offered by this new stationary phase, as a result excellent chromatographic performances are guaranteed.

1. Introduction

Distinguished by its outstanding ability to form complexes with various guest molecules, the macrocyclic polyamine or azamacrocycle, has drawn immense attention in complex chemistry, artificial catalyst, supramolecular chemistry, *etc.* Its selective recognition, depending on the type and arrangement of binding sites, is crucial for the form of mononuclear and binuclear complexes with a great diversity of cations and anions, such as metal ions, catechol, amino acid and so on [1]. Tetraazamacrocycle, since its first introduction by Curtis [2,3], has been proven of great interest in biological and medical science, due to not only its intrinsic structural properties, but also its recognition toward guest molecules plus fluorescence and luminescence performance [4,5]. In view of its function as strong receptors, a diversity of action mechanisms, including hydrophobic, hydrogen bonding, π–π stacking and dipole–dipole interaction could be offered.

Though many azamacrocycles have been synthesized, very limited effort has been made into their applications to chromatography, especially to stationary phase (SP). In the field of capillary electrophoresis, the macrocyclic polyamines had been reported as additive and for the covalent modification of silica capillary for the improvement of separation of positive, negative and neutral analytes [6–9]. Zielinska [10] had utilized a series of macrocyclic polyamines as receptors for sensitive potentiometric detection of organic acids in high-performance liquid chromatography (HPLC). Shinbo group [11,12] had described their study on the employment of macrocyclic polyamine in the preparation of SP for revered-phase (RP) HPLC in hope of separating some naphthalene derivatives, the results revealed that the macrocycle-bonded material did outshine the common ODS and phenyl-SP. A perhydro-26-membered hexaazamacrocyle (bis-p-xylyl-BISDIEN, L^1)-bonded silica SP (L^1GlySil) described in our previous work [13] has been proven multifunctional, qualified for operation in both RP and normal-phase HPLC for separation of many kinds of analytes. Although specific selectivity of plenty solutes has been accomplished on RP-HPLC SPs modified by different functional group such as cyclodextrin [14–16], calixarene [17–19] and artificial membranes [20,21], the development of new chromatographic supports capable of very specific interactions is always desirable. It will be reasonable to assume that bonding macrocyclic polyamine to silica gel may bring about certain changes to the retention mechanism of the SP, resulting in the combination of the unique ability of polyamine with the advantages of ODS SP, *i.e.* incorporated mechanism involving hydrophobic, hydrogen bonding, π–π and dipole–dipole interaction.

Because of the great influence exerted by the intermolecular interactions among the SP, the analytes and the mobile phase (MP) on the retention mechanism, it is beneficial and necessary to adopt a qualitative and quantitative approach to evaluate

* Corresponding author.
E-mail address: lijunhe@haut.edu.cn (L. He).

0039-9140/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2011.12.057

the interactions. The linear solvation energy relationships (LSER), extensively applied and studied in the evaluation of SPs [22–28], is an efficient methodology suitable for assessment of retention mechanism of RP-HPLC. Through LSER study, contribution of individual intermolecular interaction governing the chromatographic process could be visualized, thus a more exhaustive chemical insight could be obtained. The retention relationship is expressed as follows:

$$\log k = c + rR_2 + s\pi^{H_2} + a\Sigma\alpha^{H_2} + b\Sigma\beta^{H_2} + vV_x \quad (1)$$

Each parameter denotes corresponding intermolecular interaction, c is the intercept, R_2 is the excess molar refraction, π^{H_2} is solute dipolarity/polarizability, $\Sigma\alpha^{H_2}$ and $\Sigma\beta^{H_2}$ are the solute overall hydrogen bond donor (HBD) acidity and solute hydrogen bond acceptor (HBA) basicity respectively, V is the McGowan characteristic volume. The coefficients r, s, a, b and v are characteristics of the HPLC system, *i.e.* a particular RP-HPLC column with a specified composition of MP.

The present paper for the first time reported the preparation of a new HPLC SP by covalently bonding 5, 7, 7, 12, 14, 14-hexamethyl-1, 4, 8, 11-tetraazacyclotetradecane (Me_6[14]aneN_4) to silica, elucidation of its retention property along with the interpretation of whose chemical origins by LSER study with a set of 32 solutes, and furthermore, its chromatographic behavior including methylene selectivity and separation of carbamate and organophosphorus pesticides as well as phenolic compounds under RP-HPLC conditions was illustrated.

2. Experimental

2.1. Chemicals

Silica (particle diameter: 5 μm, pore size: 90 Å, surface area: 220 $m^2\,g^{-1}$) was purchased from Lanzhou Institute of Chemical Physics, Chinese Academy of Science (Lanzhou, China). γ-Chloropropyltrimethoxysilane was purchased from Sinopharm Group Chemical Reagent Co. Ltd. (Shanghai, China). Triethylamine and ethylenediamine were obtained from Tianjin Chemical Reagent Co. Ltd. (Tianjin, China). The other reagents of analytical grade were purchased from various manufacturers, all the solvents were dried prior to use. Carbamate pesticides (methomyl, carbofuran, isoprocarb, carbaryl and fenobucarb) and organophosphorus pesticides (sumithion, fenthion, parathion, phoxim and chlorpyrifos) (Fig. 1) were purchased from Pesticide Research Institute (Shanghai, China) and Agro-Environmental Protection Institution (Beijing, China), respectively. The other analytes (Table 1) of analytical grade or better were obtained from different origins. All analytes were dissolved in pure methanol. Doubly distilled water and HPLC grade methanol and acetonitrile were used.

2.2. Preparation of Me_6[14]aneN_4-bonded stationary phase and column packing

2.2.1. Sililation of silica gel

Silica was drenched in aqueous hydrochloric acid solution (v/v = 1/1, HCl (aq.) wt.%, 37%) for 24 h, then rinsed with water, dried under vacuum at 120 °C for 12 h. Activated silica (5 g) was placed in 150 mL of anhydrous toluene in a flask with a reflux condenser and a gas inlet. After the addition of 5 mL of γ-chloropropyltrimethoxysilane and triethylamine at catalytic level (*ca.* 0.5 mL), the mixture was magnetically stirred and refluxed for 24 h in an argon atmosphere. Then the mixture was cooled to room temperature and filtered, the filtrate was washed successively with toluene, acetone, methanol and water (50 mL × 2 for each). The product, γ-chloropropyltrimethoxy-sililated silica (CPS) was dried under vacuum at 100 °C for 12 h.

2.2.2. Synthesis of Me_6[14]aneN_4

Me_6[14]aneN_4 was synthesized according to the reported procedures [29,30] with slight modifications. Ethylenediamine (5 g) was added to 70 mL of acetone at −10 °C, to which a acetone solution (50 mL) of 11 g of $HClO_4$ (wt.%, 70%) was added dropwise in 2 h. After 24 h-reaction, the white crystal of 5, 7, 7, 12, 14, 14-hexamethyl-1, 4, 8, 11-tetraazacyclotetradiene diperchlorate (Me_6[14]eneN_4·2$HClO_4$) precipitated, which was filtered and washed successively with acetone and water (10 mL × 3), dried under vacuum, yield *ca.* 25 g (64%, based on ethylenediamine). The IR spectrum has $\nu_{C=N}$ at 1666 cm^{-1} and ClO_4^- bands at 1100 cm^{-1} and 625 cm^{-1}(KBr disc). (Caution: due to the potential danger of perchloric acid and its salt, it is strongly recommended that precaution measures be taken to ensure personal safety.)

Me_6[14]eneN_4·2$HClO_4$ (9.3 g) was immersed in 150 mL of methanol at 60 °C, to which 3 g of $NaBH_4$ was added in small portions over a period of 30 min. Then the mixture was refluxed for 2 h. Methanol was distilled under vacuum until the mixture became cloudy, to which sodium hydroxide (2 mol L^{-1}) was added to adjust the pH to highly basic. The white deposit was filtered and washed by water, dissolved in methanol. Excessive concentrated hydrochloric acid was added to maximize the precipitation. The resulting salt was filtered and washed with cold methanol. This hydrochloride was dissolved in water, KOH was added till pH 13; extracted by chloroform (50 mL × 2). The organic layers were combined and dried by K_2CO_3. Removal of chloroform and recrystallization from ethanol/water gave Me_6[14]aneN_4, yield *ca.* 3 g (55%). The IR spectrum has ν_{NH} at 3238 cm^{-1}. ^{1}HMNR (400 MHz, $CDCl_3$): δ1.08 (m, 3H), δ1.09 (m, 6H), δ1.42 (m, 2H), δ2.31 (m, 1H), δ2.51–2.87 (m, 4H), δ1.43–2.26 (broad s, 2H).

2.2.3. Preparation of Me_6[14]aneN_4-bonded stationary phase

Me_6[14]aneN_4 (0.75 g), triethylamine (as catalyst, 0.3 mL) and CPS (4 g) were placed in a flask containing 150 mL of dimethyl formamide. The mixture was heated at 85 °C with stirring for 24 h in an argon atmosphere. The obtained product was filtered and washed twice with DMF, DMF/methanol (v/v = 50/50) and methanol in turn. The Me_6[14]aneN_4-bonded material (Me_6[14]aneN_4CPS) was dried under vacuum at 90 °C for 12 h before packing and characterization. The whole process for preparation of Me_6[14]aneN_4CPS was displayed in Fig. 2.

The prepared material was dispersed in tetrachloromethane and packed into stainless steel tube column (250 mm × 4.6 mm I.D.) using methanol as propulsive solvent by slurry packing technique.

2.3. Apparatus and chromatographic conditions

The carbon, hydrogen and nitrogen contents of CPS and Me_6[14]aneN_4CPS were determined by elemental analysis (EA) at 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 ^{1}HNMR spectra were measured using DPX-400 (Bruker, Ettlingen, Germany).

All chromatographic tests were carried out on a Shimadzu system (Shimadzu, Kyoto, Japan) 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 7725i injector with 20 μL sample loop (Rheodyne, Rohnert Park, CA, USA) was employed. All the solutes were analyzed at room temperature at a flow rate of 1.0 mL min^{-1} with UV detection wavelengths at 254 nm and/or 290 nm. A Shimadzu VP-ODS column (250 mm × 4.6 mm I.D.,

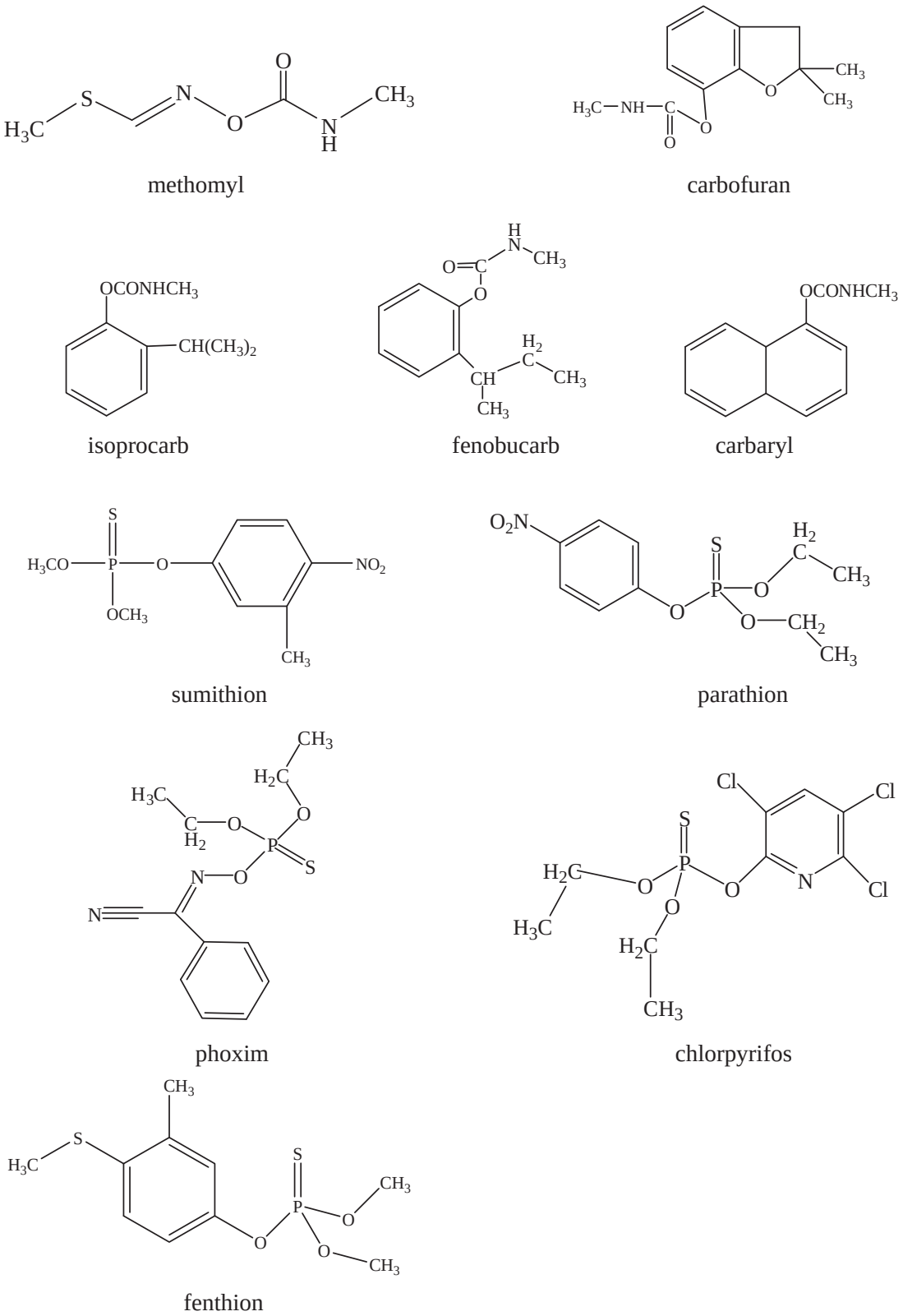

Fig. 1. Structure of organic pesticides used.

end-capped) was used to compare the chromatographic performance. Mobile phases were degassed ultrasonically prior to use. Dead time was determined by the signal of methanol.

Performance of the multiple regression analysis between log *k* and all the solute descriptors and other integrant data analysis were *via* Microsoft Excel software and OriginPro 8.0.

3. Results and discussion

3.1. Characterization of Me_6[14]aneN_4CPS

Comparison between IR spectrograms of CPS and Me_6[14]aneN_4CPS showed that a new absorption of the secondary

Table 1
Solute descriptors of the compounds studied.

No.	Compounds	R_2	π^{H_2}	$\Sigma\alpha^{H_2}$	$\Sigma\beta^{H_2}$	V_x
1	Benzaldehyde	0.820	1.00	0.00	0.39	0.8730
2	Nitrobenzene	0.871	1.11	0.00	0.28	0.8906
3	Naphthalene	1.340	0.92	0.00	0.20	1.0854
4	Biphenyl	1.360	0.99	0.00	0.26	1.3420
5	Aniline	0.955	0.96	0.26	0.41	0.8162
6	Toluene	0.601	0.52	0.00	0.14	0.8573
7	4-Nitrotoulene	0.870	1.11	0.00	0.28	1.0315
8	Anisole	0.708	0.75	0.00	0.29	0.9160
9	Bromobenzene	0.882	0.73	0.00	0.09	0.8914
10	Chlorobenzene	0.718	0.65	0.00	0.07	0.8388
11	2-Nitroaniline	1.180	1.37	0.30	0.36	0.9904
12	3-Nitroaniline	1.200	1.71	0.40	0.35	0.9904
13	4-Nitroaniline	1.220	1.91	0.42	0.38	0.9904
14	Diphenylamine	1.585	0.88	0.10	0.57	1.4240
15	Pyridine	0.631	0.84	0.00	0.52	0.6753
16	Benzophenone	1.447	1.50	0.00	0.50	1.4810
17	2-Chlorophenol	0.853	0.88	0.32	0.31	0.8975
18	2,4-Dimethylphenol	0.843	0.80	0.53	0.39	1.0569
19	Ethylbenzene	0.613	0.50	0.00	0.15	0.9982
20	*n*-Propylbenzene	0.604	0.50	0.00	0.15	1.1391
21	*n*-Butylbenzene	0.600	0.51	0.00	0.15	1.2800
22	Iodobenzene	1.188	0.82	0.00	0.12	0.9750
23	2-Nitrotoluene	0.866	1.11	0.00	0.27	1.0320
24	Acetophenone	0.818	1.01	0.00	0.48	1.0139
25	Cyclohexanone	0.403	0.86	0.00	0.56	0.8610
26	Cyclopentanone	0.373	0.86	0.00	0.52	0.7202
27	N-methylaniline	0.948	0.90	0.17	0.43	0.9571
28	N,N-dimethylaniline	0.957	0.84	0.00	0.42	1.0980
29	2-Chloroaniline	1.033	0.92	0.25	0.31	0.9390
30	4-Cresol	0.820	0.87	0.57	0.32	0.9160
31	3-Cresol	0.822	0.88	0.57	0.34	0.9160
32	2-Cresol	0.840	0.86	0.52	0.30	0.9160

Values of No. 1–18 were obtained from Ref. [24] and values of No. 19–32 were obtained from Ref. [25].

amine appeared at 3238 cm^{-1} on Me_6[14]aneN$_4$CPS, this fact qualitatively indicated the successful covalent immobilization of Me_6[14]aneN$_4$ to CPS. The rise of nitrogen content and carbon content from zero (CPS) to 0.370 (Me_6[14]aneN$_4$CPS) and from 3.941 (CPS) to 6.000 (Me_6[14]aneN$_4$CPS), respectively, was demonstrated by the elemental analysis, whereby a quantitative verification of the immobilization was acquired. The bonding amount of the new phase is *ca.* 1.510 μmol·m^{-2}, based on the carbon content.

Table 2
Variance–covariance matrix of solute descriptors used in LSER equation.

	R_2	π^{H_2}	$\Sigma\alpha^{H_2}$	$\Sigma\beta^{H_2}$	V_x
R_2	1	0.528	0.221	0.080	0.579
π^{H_2}		1	0.387	0.386	0.053
$\Sigma\alpha^{H_2}$			1	0.214	−0.047
$\Sigma\beta^{H_2}$				1	−0.030
V_x					1

3.2. LSER analysis

To minimize the limitation of LSER induced by potential covariance of solute descriptors, it is indispensable to examine the issue of cross-correlations between R_2, π^{H_2}, $\Sigma\alpha^{H_2}$, $\Sigma\beta^{H_2}$ and V_x. Table 2 shows the variance-covariance matrix of the solute descriptors, no occurrence of strong correlation between each pair is found. Nevertheless, a minute correlation is exhibited by R_2 and π^{H_2}, this phenomenon, previously noted by Gritti and Reta [28,31], is ascribed to their sensitivity to the presence of π-electron, since they both reflect the solute polarizability. Additionally, R_2 seems to be weakly coupled to V_x, the possible explanation could be that the larger volume of the solute is, the more double bonds it contains, as inferred by Gritti [28]. The correlation coefficients are always less

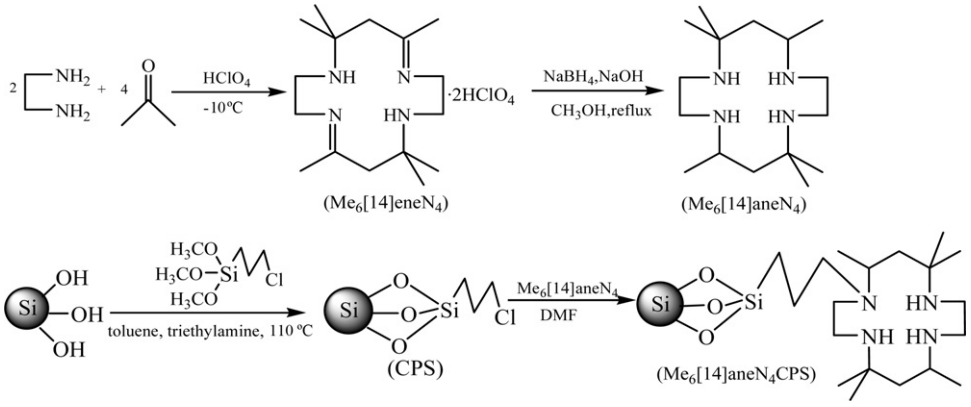

Fig. 2. Scheme for preparation of Me_6[14]aneN$_4$CPS.

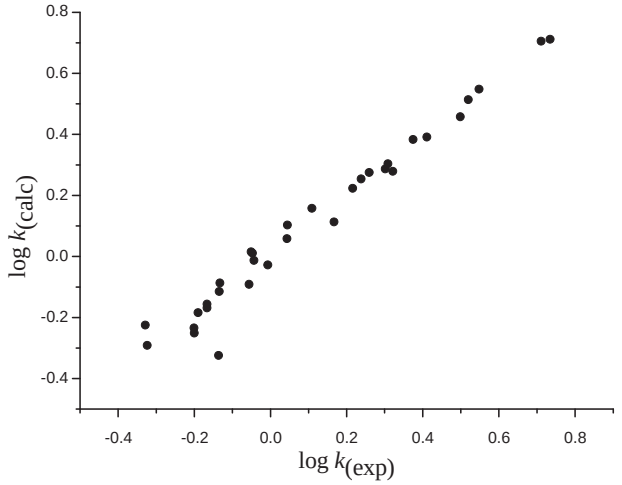

Fig. 3. Plot depicting $\log k_{(calc)}$ *versus* $\log k_{(exp)}$ with 55/45 (v/v) methanol/water.

than 0.6 at 95% confidence level, the choice of solutes is thereby appropriate to conduct LSER study.

The ODS SPs have been extensively and thoroughly investigated by many authors [31–36]. Outcomes of these applications of the LSER equation display evident similarity between each other in methanol–water MPs. The LSER regression coefficients of Me_6[14]aneN_4CPS SP are listed in Table 3 in comparison with that of a referred ODS SP, which was characterized in closest system conditions. The inter-correlation coefficients of Me_6[14]aneN_4CPS between observed $\log k_{(exp)}$ and the corresponding calculated $\log k_{(calc)}$ are shown in Fig. 3, from which good fits are observed. However, it should be noted that aniline has been less successfully modeled. The deviation of its $\log k_{(calc)}$ from $\log k_{(exp)}$ may lie in the polar amino group (—NH_2), whose ionized form (NH_3^+) in water media could adversely impact certain descriptor. However, aniline does not seriously interfere with the whole LSER study. In fact, the relatively high correlation coefficients (>0.94) were obtained on the equation of Me_6[14]aneN_4CPS SP.

In order to describe the differences in specific interactions involved in the SP and MP, the negative *s*, *a* and *b* coefficients reveal the higher affinity of the methanol–water MP for the solute concerning the dipolarity/polarizability and hydrogen bonding interactions; contrarily, positive coefficients stand for stronger molecular interactions in SP than in MP.

As a measure for combination of cavity formation and dispersive interactions, the positive *v* coefficient of Me_6[14]aneN_4CPS is at similar magnitude to that of ODS. In accordance with observed results of conventional ODS, the *v* values rise with the decrease of methanol content in MP, emphasizing the hydrophobic character of the Me_6[14]aneN_4CPS phase. Thus it is reliable to conclude that, in spite of the absence of long alkyl chain, Me_6[14]aneN_4CPS is more hydrophobic than MP, thanks to the hydrophobic cavity of macrocycle and six peripheral methyl arms.

The change of Me_6[14]aneN_4CPS's *r* value, referring to the difference between SP and MP to interact with solute *n*- and π-electrons, is reverse from that of ODS. It becomes positively smaller and smaller with the decrease of methanol content, possibly due to the lack of bulky alky chain on Me_6[14]aneN_4CPS, notwithstanding the presence of six methyl groups, hence polarizability of Me_6[14]aneN_4CPS is more susceptible to suppression by higher polarizability of MP, produced by the increase of water content. The positive *r* values obtained in all MP compositions suggest that electron-involved interactions in SP are stronger than that in MP. Comparison between values and magnitudes of *v* and *r* coefficients can doubtlessly tell that *v* is a much more critical factor that impacts the retention of solute than *r*.

The *s* coefficient, which reflects the discrepancy in SP and MP dipolar interaction with solute, ascends with the increasing methanol content in MP, and exiguously exceeds zero at high organic concentration (85% MeOH, data excluded) on Me_6[14]aneN_4CPS phase. However, *s* value keeps rather small, either negative or positive, the fact may be attributed to the slight dipolarity/polarizability difference between MP and SP triggered by the substantial sorption of strongly polar molecules, *i.e.* methanol and water, into the SP [32]. Dipolarity/polarizability of Me_6[14]aneN_4CPS consequently, in coordination of amino groups, approaches that of MP. As a result, the discrimination of a solute between SP and MP would be obscure, hereby a small negative *s* coefficient is observed. Similar to a butylimidazolium-based SP reported by Stalcup and coworkers [33,34], a small negative *s* can be treated as indication of higher dipolarity/polarizability of Me_6[14]aneN_4CPS over ODS, which can be explicated by the chemical nature of the amino groups of Me_6[14]aneN_4 and free silanol groups on the surface of silica gel [26].

The *b* and *a* coefficients are measures of the difference between SP and MP in HBD acidity and HBA basicity respectively. Although both Me_6[14]aneN_4CPS and ODS phases possess negative *b* coefficients, the smaller *b* value on Me_6[14]aneN_4CPS indicates its stronger HBD ability over ODS in all MP conditions. The dissimilarity is likely the consequence of the amino group as a stronger hydrogen bond donor and higher MP sorption capacity of Me_6[14]aneN_4CPS than ODS. The values of *b* on both phases mount up with the decrease of organic content as water is a strong HBD acid. The more water molecules are sorbed into SP, the more vigorous the hydrogen bonding interaction between solutes and sorbed water molecules as well as between solutes and the residual silanol groups will be, accordingly an enhanced HBD acidity ensued [26]. The *a* coefficients on Me_6[14]aneN_4CPS phase in all MP conditions keep larger than that on ODS, indicating Me_6[14]aneN_4CPS is less basic than ODS, and maintain almost immobile in all MP compositions.

By summary of the LSER results and interpretations above, a conclusion can be reached that this new Me_6[14]aneN_4-bonded material possesses obvious RP-HPLC retention properties, involvements of hydrogen bonding and dipole–dipole interaction have synergic contribution to the retention of solutes.

3.3. Chromatographic behavior of Me_6[14]aneN_4CPS stationary phase

3.3.1. Methylene selectivity

Methylene selectivity, which depends on the hydrophobic interaction between the SP and MP, has been a frequently studied property to characterize RP-HPLC SP. To understand the effect of different MP compositions and different type of organic modifiers on the methylene selectivity, both methanol and acetonitrile were applied to elute *n*-alkylbenzenes, including benzene, toluene, ethylbenzene, propylbenzene and butylbenzene by varying MP composition. The $\log k$ of each analyte was plotted *versus* concentration of corresponding organic modifier. As displayed in Fig. 4, for given MP, the increasing $\log k$ means an increase of carbon numbers in alkyl chain; for all the MPs, the $\log k$ ascends with the increase of water content. These two instances are consistent with observations for conventional RP-HPLC SPs. The bigger slope of the plots and greater selectivity factor α (*e.g.* ethylbenzene to toluene) in Fig. 4a than that in Fig. 4b indicates methanol as organic modifier favors higher methylene selectivity than acetontitrile. This finding is supported by results from literatures [35,36]. The baseline separations of 5 *n*-alkylbenzenes using both

Table 3
System coefficients obtained with two SPs in varying MP compositions.

Column	Eluent, MeOH–water (%, v/v)	*c*	*r*	*s*	*a*	*b*	*v*	*R*	*n*	*SE*	*F*
Me_6[14]aneN_4CPS	55:45	−0.66	0.07	−0.09	−0.64	−0.80	1.17	0.971	32	0.05	175
	65:35	−0.72	0.08	−0.07	−0.65	−0.56	0.89	0.952	32	0.06	102
	75:25	−0.78	0.11	−0.01	−0.67	−0.39	0.51	0.942	32	0.05	84
Nucleosil-C18[a]	55:45	0.11	0.22	−0.48	−0.43	−1.48	1.55	0.994	35	0.04	523
	65:35	0.13	0.20	−0.34	−0.37	−1.13	1.10	0.992	35	0.05	351
	75:25	0.09	0.15	−0.28	−0.29	−0.77	0.76	0.991	32	0.04	299

R: overall correlation coefficient, *n*: number of solutes, SE: standard error in the estimate, *F*: statistic.
[a] Values obtained from Ref. [22,32], bonding amount: 2.03 μmol m^{-2}.

methanol and acetontitrile as organic modifier are accomplished, and corresponding chromatograms are shown in Fig. 5.

3.3.2. Selected separation of organic pesticides

Five carbamate pesticides containing polar group were selected for chromatographic separation to gain deeper insight of the chemical sense of LSER results. A chromatogram of their complete separation on Me_6[14]aneN_4CPS phase with 50% methanol content is exhibited in Fig. 6. The elution order of 5 carbamate pesticides is methomyl < carbofuran < isoprocarb < carbaryl < fenobucarb and does not change in all studied methanol compositions. The elution order is not entirely consistent with that on ODS phase, where carbaryl was eluted ahead of isoprocarb. Accordingly it can be inferred that the separation of 5 carbamate pesticides is decisively governed by hydrophobic interaction (*v*). In addition, the successful separation was achieved indispensably with contributions of other interactions, including dipole–dipole interaction (*s*) and hydrogen bonding interaction (*a* and *b*). However, the separation of 5 carbamate pesticides could not be achieved on Me_6[14]aneN_4CPS phase at any employed acetonitrile content level.

Another set of 5 organophosphorus pesticides with diverse functional groups was investigated as well. Fig. 7 is a typical HPLC chromatogram of their separation on Me_6[14]aneN_4CPS phase with

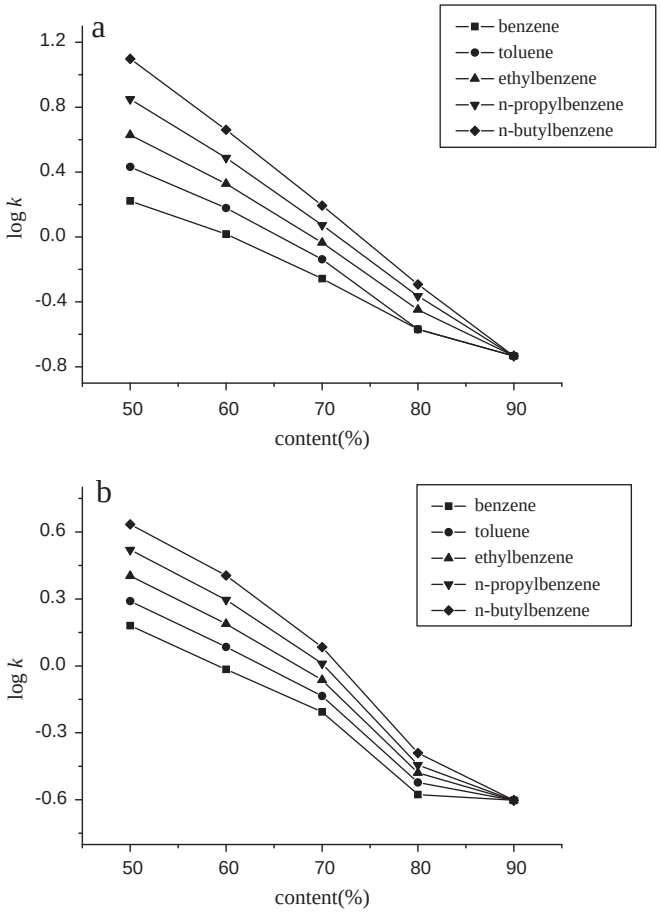

Fig. 4. Effect of different methanol (a) and acetonitrile (b) contents on log *k* of *n*-alkylbenzenes. Chromatographic conditions: column: Me_6[14]aneN_4CPS; flow rate: 1 mL min^{-1}; UV at 254 nm.

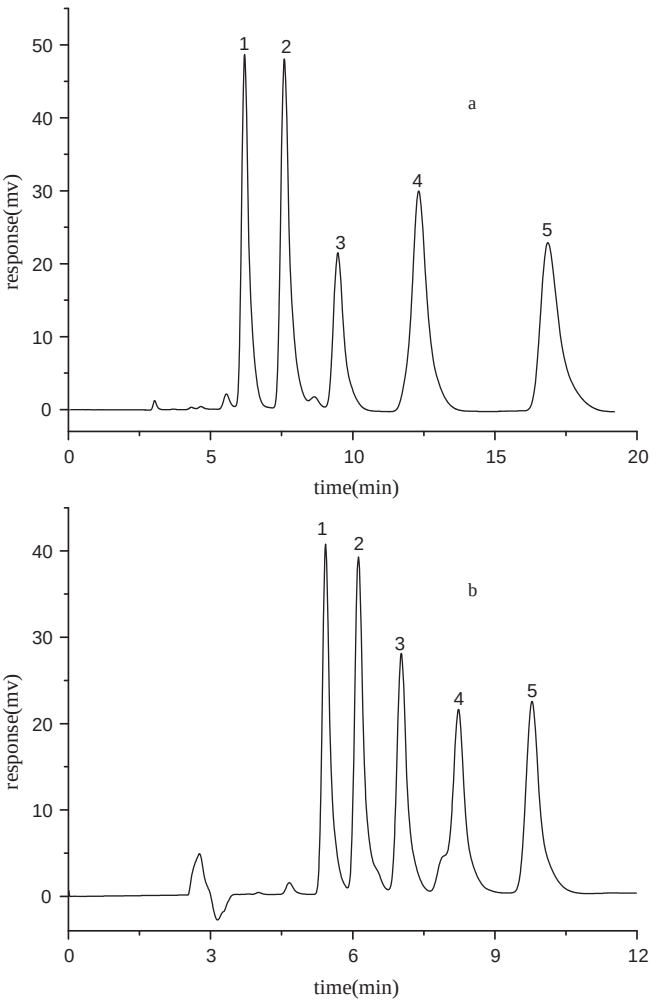

Fig. 5. Chromatogram of separation of benzene (1), toluene (2), ethylbenzene (3), *n*-propylbenzene (4) and *n*-butylbenzene (5). Chromatographic conditions: MP: (a) methanol/water (60/40, v/v), (b) acetonitrile/water (60/40, v/v), identical other chromatographic conditions with that of Fig. 4. (a): $\alpha_{ethylbenzene/toluene}$ = 1.41, $\alpha_{n\text{-}propylbenzene/ethylbenzene}$ = 1.44, $\alpha_{n\text{-}butylbenzene/n\text{-}propylbenzene}$ = 1.49 and (b): $\alpha_{ethylbenzene/toluene}$ = 1.27, $\alpha_{n\text{-}propylbenzene/ethylbenzene}$ = 1.28, $\alpha_{n\text{-}butylbenzene/n\text{-}propylbenzene}$ = 1.28. (Protuberance at the foot of peak 4 in (b) was caused by impurity.)

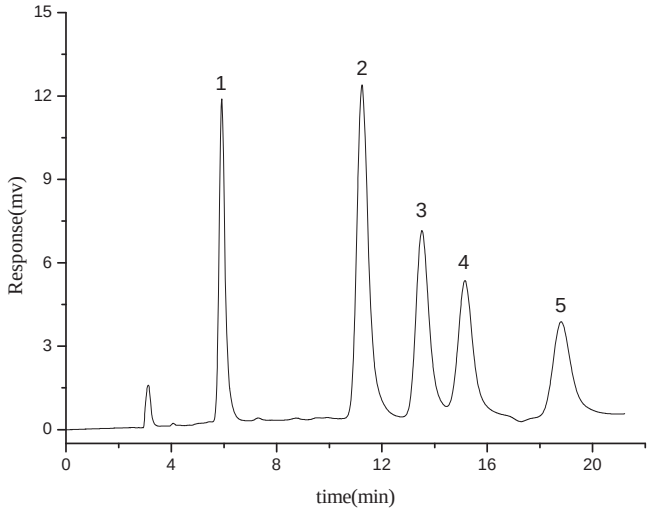

Fig. 6. Chromatogram of separation of carbamate pesticides composed of methomyl (1), carbofuran (2), isoprocarb (3), carbaryl (4) and fenobucarb (5). Chromatographic conditions: MP: methanol/water (50/50, v/v), identical other chromatographic conditions with that of Fig. 4.

50% aqueous acetonitrile. The elution order of anlytes have connection with their structures. Retention times of the analytes with ethoxyl group (parathion, phoxim, chlorpyrifos) are longer than that of ones with methoxyl group (sumithion, fenthion), which can be ascribed to the stronger hydrophobicity of ethoxyl group than that of methoxyl group. The elution orders of the former three and latter two are parathion < phoxim < chlorpyrifos and sumithion <fenthion, respectively. However, special attention should be paid to the elution order of fenthion and parathion, which was different from the case on ODS phase. The possible reason is that Me_6[14]aneN_4CPS phase interacts with solutes simultaneously through hydrophobic, dipole–dipole and hydrogen bonding interactions, while the retention of solutes on ODS phase is mainly governed by hydrophobic interaction. It is worthy to note that, in contrast to the attempted separation of carbamate pesticides with aqueous acetonitrile, separation of organophosphorus pesticides with aqueous methanol of different content levels as eluent led to a failure. For polar carbamate pesticides, the impact of methanol on their separation is greater than that of acetonitrile. Methanol has stronger hydrogen bond donating and accepting abilities than

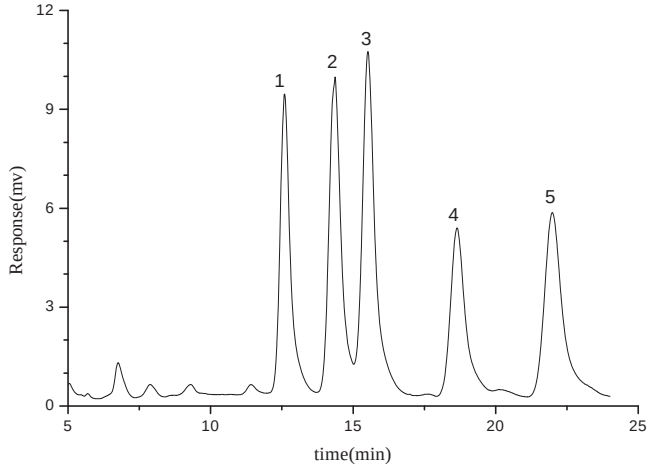

Fig. 7. Chromatogram of separation of organophosphorus pesticides composed of sumithion (1), fenthion (2), parathion (3), phoxim (4) and chlorpyrifos (5). Chromatographic conditions: MP: acetonitrile/water (50/50, v/v), identical other chromatographic conditions with that of Fig. 4.

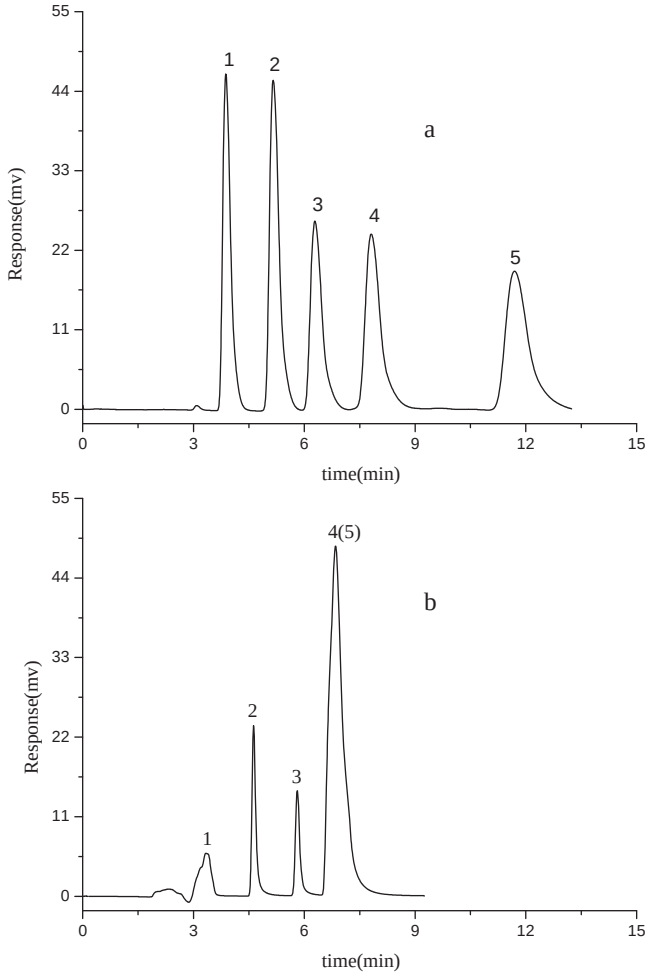

Fig. 8. Chromatogram of separation of phenolic compounds composed of resorcinol (1), phenol (2), 2-cresol (3), 2,4-xylenol (4) and 2-nitrophenol (5). Chromatographic conditions: column: (a) Me_6[14]aneN_4CPS and (b) VP-ODS; MP: methanol/water (50/50, v/v), identical other chromatographic conditions with that of Fig. 4.

acetonitrile, which can result in an improved separation selectivity for polar carbamate pesticides. For organophosphorus pesticides, the selectivity will be enhanced when acetonitrile is used, which can be attributed to the lipophilicity of acetonitrile, thereby the hydrophobic interaction will be intensified [37].

3.3.3. Chromatographic performance on phenolic compounds

Attempts to separation of phenolic compounds had also been made to examine the chromatographic performance of Me_6[14]aneN_4CPS on another set of polar compounds. To elucidate the selectivity and retention mechanism of new phase, 5 phenolic compounds including resorcinol, phenol, 2-cresol, 2,4-xylenol and 2-nitrophenol were selected for evaluation. Obvious disaccord is observed between the retention order of analytes on Me_6[14]aneN_4CPS phase and that on ODS phase (Fig. 8), as highlighted by 2-nitrophenol. Participations of hydroxyl group in acid–base equilibrium with amino groups on Me_6[14]aneN_4CPS is amplified by the strong electron-attracting nitro group, thus the retention of 2-nitrophenol is greatly enhanced. The electronic effect is more remarkable when eluting 2,4-dinitrophenol, whose retention time is close to 25 min on Me_6[14]aneN_4CPS phase. Involvement of acid–base equilibrium in the separation mechanism had also been reported on L^1GlySil SP for separation of three nitrophenols (2-, 3- and 4-nitrophenol) [13], whose retention order was according to the order of electronic effect between amino groups

on L^1GlySil and hydroxyl group of nitrophenol. Based on this finding, it can be deduced that the existence of nitro group significantly alters the dominating role of hydrophobic interaction involved in the retention mechanism of Me_6[14]aneN_4CPS-MP system, consequently, the selectivity of 2-nitrophenol and 2,4-xylenol is greatly improved. From Fig. 8a, the baseline separation of 5 phenolic compounds is achieved on Me_6[14]aneN_4CPS with methanol/water (v/v = 50/50). However, the peaks in Fig. 8b obtained on ODS reveals poor selectivity toward 2,4-xylenol and 2-nitrophenol, which is co-eluted under the same mobile phase conditions.

4. Conclusion

A new stationary phase modified by 14-membered tetraazamacrocycle has been successfully prepared and characterized. Besides its unique features, retention property of this new material in RP-HPLC mode was found similar to that of ODS phases by LSER study. Investigation into the retention behavior toward successful separations of *n*-alkylbenzene, two classes of pesticides and phenolic compounds clearly demonstrated the hydrophobic character of Me_6[14]aneN_4CPS, and the multiple-function mechanism, such as hydrogen bonding interaction, dipolar-dipolar interaction and acid–base equilibrium provided by it. In conclusion, the new Me_6[14]aneN_4CPS shows considerable prospect for separation of polar compound with improved selectivity, especially for phenolic compounds, due its multi-model retention properties. The amino groups on Me_6[14]aneN_4CPS could shield free silanol groups of silica support through hydrogen bonding interaction. Thus, it is expected the new stationary phase would be valuable in separation of basic compounds such as amines, and the further study is in progress.

Acknowledgments

Financial support from the National Natural Science Foundation of China (No. 20905020 and 20875083), Plan for Scientific Innovation Talent of Henan Province and Major Research and Public Service Tender of Henan Province are gratefully acknowledged.

References

[1] J.L. Sessler, P.A. Gale, W.S. Cho, Anion Receptor Chemistry, RSC Publishing, UK, 2006.
[2] N.F. Curtis, J. Chem. Soc. (1960) 4409.
[3] M. Blight, N.F. Curtis, J. Chem. Soc. (1962) 3016.
[4] H. Aneetha, Y.H. Lai, S.C. Lin, K. Paneerselvam, T.H. Lu, C.S. Chung, J. Chem. Soc. Dalton Trans. (1999) 2885.
[5] A. Gualandi, L. Cerisoli, H. Stoeckli-Evans, D. Savoia, J. Org. Chem. 76 (2011) 3399.
[6] C.Y. Liu, T.H. Chen, T.K. Misra, J. Chromatogr. A 1154 (2007) 407.
[7] Y.C. Wang, Z.R. Zeng, C.H. Xie, N. Guan, E.Q. Fu, J.K. Cheng, Chromatographia 54 (2001) 475.
[8] S. Hu, E. Fu, P.C.H. Li, J. Chromatogr. A 844 (1999) 439.
[9] N. Guan, Z.R. Zeng, Y.C. Wang, E.Q.Fu, J.K. Cheng, Anal. Chim. Acta 418 (2000) 145.
[10] D. Zielinska, A. Gil, M. Pietraszkiewicz, O. Pietraszkiewicz, D. Van de Vijve, L.J. Nagels, Anal. Chim. Acta 523 (2004) 177.
[11] T. Shinbo, Y. Sudo, Y. Shimabukuro, Y. Shimabukuro, T. Kanamori, T. Masuoka, T. Iwatsubo, A. Yamasaki, K. Ogasawara, K. Mizoguchi, J. Chromatogr. A 803 (1998) 95.
[12] T. Shinbo, Y. Sudo, Y. Shimabukuro, T. Kanamori, T. Iwatsubo, Y. Nagawa, K. Hiratani, J. Chromatogr. A 977 (2000) 61.
[13] L.J. He, J. Zhang, Y.J. Sun, J. Liu, X.M. Jiang, L.B. Qu, J. Chromatogr. A 1217 (2010) 5971.
[14] Y.Y Zhao, Z.M Guo, Y.P Zhang, X.Y Xue, Q. Xu, X.L Li, X.M Liang, Y.K Zhang, Talanta 78 (2009) 916.
[15] J. Zhao, D. Tan, S.K. Thamarai Chelvia, E.L. Yong, H. Kee Lee, Y.H Gong, Talanta 83 (2010) 286.
[16] Z.B. Zhang, M.H. Wu, R.A. Wu, J. Dong, J.J.Ou, H.F. Zou, Anal. Chem. 83 (2011) 3616.
[17] K. Hu, W.J. Zhao, F.Y Wen, J.W Liu, X.L Zhao, Z.H Xu, B.L Niu, B.X Ye, Y.J Wu, S.S. Zhang, Talanta 85 (2011) 317.
[18] S. Erdemir, M. Yilmaz, Talanta 82 (2010) 1240.
[19] L.S Li, M. Liu, S.L Da, Y.Q. Feng, Talanta 62 (2004) 643.
[20] W. Yu, B.X. Yuan, X.L. Deng, L.C. He, Y.Y. Zhang, Q.D. Han, Anal. Biochem. 339 (2005) 198.
[21] S. Han, S.M. Martin, J. Membr. Sci. 367 (2011) 1.
[22] M.H. Abraham, M. Roses, C.F. Poole, S.K. Poole, J. Phys. Org. Chem. 10 (1997) 358.
[23] P.B. Ogden, J.W. Coym, J. Chromatogr. A 1218 (2011) 2936.
[24] F.Z. Oumada, M. Roses, E. Bosch, M.H. Abraham, Anal. Chim. Acta 382 (1999) 301.
[25] S.K. Poole, C.F. Poole, J. Chromatogr. A 845 (1999) 381.
[26] L. Szepesy, V. Hada, Chromatographia 54 (2001) 99.
[27] M. Vitha, P.W. Carr, J. Chromatogr. A 1126 (2006) 143.
[28] F. Gritti, G. Felix, M.F. Achard, F. Hardouin, J. Chromatogr. A 922 (2001) 51.
[29] N.F. Curtis, R.W. Hay, Chem. Commun. (1966) 524.
[30] A.M. Tait, D.H. Busch, Inorg. Synth. 18 (1978) 2.
[31] M. Reta, P.W. Carr, P.C. Sadek, S.C. Rutan, Anal. Chem. 71 (1999) 3484.
[32] A. Kaibara, M. Hirose, T. Nakagawa, Chromatographia 29 (1990) 551.
[33] Y. Sun, B. Cabovska, C.E. Evans, T.H. Ridgway, A.M. Stalcup, Anal. Bioanal. Chem. 382 (2005) 728.
[34] Y. Sun, A.M. Stalcup, J. Chromatogr. A 1126 (2006) 276.
[35] Á. Sándi, L. Szepesy, J. Chromatogr. A 845 (1999) 113.
[36] J.H. Park, P.W. Carr, M.H. Abraham, R.W. Taft, R.M. Doherty, M.J. Kamlet, Chromatographia 25 (1988) 373.
[37] F. Chan, L.S. Yeung, R. LoBrutto, Y.V. Kazakevich, J. Chromatogr. A 1082 (2005) 158.