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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

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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).

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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.,