



Synthesis and characterization of phosphodiester stationary bonded phases for liquid chromatography



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ARTICLE INFO

Article history:

Received 16 February 2015

Received in revised form

21 April 2015

Accepted 24 April 2015

Available online 5 May 2015

Keywords:

Phospho-ester bonded stationary phase

Liquid chromatography

Stationary phase surface characterization

Polar embedded stationary phase

ABSTRACT

The new type of stationary bonded phases for liquid chromatography with immobilized artificial membrane properties was synthesized. Based on the modification of diol-bonded silica gel the phosphodiester stationary phases were obtained. The structures of synthesized material were confirmed by different physico-chemical techniques such as elemental analysis, infrared spectroscopy (FTIR), ^{13}C CP/MAS NMR and in reversed phase and hydrophilic interaction liquid chromatography, where hydrophobic and polar compounds were sufficiently separated. To present the influence of phosphate group on the retention properties, obtained material were compared with Diol-Ester C12 stationary phase.

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1. Introduction

Some of the liquid chromatographic analyses, especially those that separate polar compounds, needs the application of water-rich mobile phases (more than 85% water or a buffer). In such condition the performance of hydrophobic stationary phases indicates that the bonded ligands might be collapsing. This problem could be solved by two ways. One way is increasing of organic content in the mobile phase which should improve solvation and bring bonded ligands back to the original conformation but it strongly reduces the retention. Another idea is the application of stationary phases with incorporated polar groups mixed with the original alkyl ligands (polar embedded stationary phases) or a stationary phase that contain some polar groups attached during endcapping procedure (polar end-capped stationary phases) [1–4]. The main advantage of polar embedded and polar endcapped stationary phases is, that it may be applied in both, reversed phase chromatography (RP HPLC) and hydrophilic interactions liquid chromatography (HILIC) [5,6]. It is a result of water adsorption on polar functional groups. The stable solvated structure is formed, which was called “hydrolytic pillow” [7,8].

One of the method increasing the polarity of hydrophobic adsorbent is the synthesis of *N*-acylamide stationary phases [7,9]. These types of adsorbents consist of a hydrophobic alkyl ligand (e.g. C12 or C18) bonded to aminopropyl silica with using amide bond.

Such structure of the stationary phase offers better solvation and thus better stability and efficiency in water-rich mobile phases [10].

Another material that may be called polar embedded/polar endcapped stationary phase is *immobilized artificial membrane* (IAM) [11,12]. Due to specific surface properties IAM bonded phase closely mimics the interaction of analytes with biological membranes. It is caused by combinations of possible hydrophobic, ion pairing, and hydrogen bondings [13,14].

One of the new material, which simulate the biologic membrane and possess polar embedded groups, is the *N,O*-dialkyl phosphoramidate stationary phases [15,16]. Physico-chemical properties of those adsorbents allow using them in separation of both hydrophobic and polar substances, in RP HPLC and HILIC. Changing the length of main chain in wider range enabled to obtain chromatographic materials with different hydrophobic-polar properties.

Polar embedded stationary phase that contain phosphate and cholesterol group (called PCP) were also synthesized [14]. This material was applied in reversed phase chromatography for separation of hydrophobic compound, providing good shape selectivity.

Another chromatographic material that may be included in both polar embedded and polar endcapped stationary phases is the group of ester bonded stationary phases [17]. These material is a modification of diol-bonded silica with long chain carboxylic acid. It provide to ester bond creation, similar to lipids. Ester bonded stationary phases were successfully applied in RP HPLC.

The goal of current work was to synthesize a new generation of phosphodiester stationary phases for liquid chromatography which possess properties similar to the cell membrane due to the structure that imitate phospholipids. The idea of the study is to

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obtain material that is able to separate polar compounds in no buffered mobile phases with neutral pH. Based on the previous experiences with the synthesis of *N,O*-dialkyl phosphoramidate stationary phase, the currently prepared stationary phase was obtained during chemical modification of diol-bonded silica gel. The structure of the synthesized chemically bonded ligands was confirmed by different instrumental techniques, such as elemental analysis, solid state NMR and FT-IR spectroscopy. The potential application of synthesized materials for chromatographic analyses in reversed phase chromatography and HILIC was presented below.

2. Experimental

2.1. Instruments

The liquid chromatograph was a Shimadzu Prominence system (Tokyo, Japan) equipped with ternary gradient pump (LC-20AD), diode array detector (SPD-M20A), an autosampler (SIL-20A), and a column thermostat (CTO-10AS VP). Data were collected using LabSolution software.

The surface coverage density of alkylsilyl ligands (α_{RP}) was calculated on the basis of the carbon percentage determined on a Model 240 CHN analyzer (Perkin-Elmer, Norwalk, USA).

Solid state NMR measurements were performed on a Bruker Avance III 700 MHz (Karlsruhe, Germany). The ^{29}Si and ^{13}C CP/MAS NMR spectra were obtained with rotation frequency 8 kHz, pulse time 2 ms, acquisition time 0.01643 s, and relaxation time 6 s. All spectra were externally referenced with liquid tetramethylsilane (TMS) and the chemical shifts (δ) were given in parts per million (ppm).

Adsorbents were packed using laboratory-made apparatus equipped with Haskel packing pump (Burbank, CA, USA) into $125 \times 4.6 \text{ mm}^2$ i.d. stainless steel columns using the slurry method. About 1.5 g of the modified silica was made into the slurry with 15 ml of chloroform and placed into the packing apparatus. Methanol has been used as a packing pressurizing solvent during the filling process. Columns were packed under a constant pressure of 40 MPa.

2.2. Materials

As a support for the synthesis the silica gel Kromasil 300 (Akzo Nobel, Bohus, Sweden) were used with particle size 5 μm , and pore diameter 300 Å.

Reagents for the stationary phase synthesis: (3-glicidoxypyl)trimethoxysilane, decanol, octadecanol, and phosphoryl chloride were purchased from Alfa Aesar (Karlsruhe, Germany). Organic solvents used during synthesis: toluene, methanol and hexane were ACS. Grade was purchased from Avantor Performance Materials (Center Valley, PA, USA).

Two different mobile phase systems were used in the measurements: methanol–water and acetonitrile–water. Organic solvents (methanol and acetonitrile) were high-purity “for HPLC” gradient grade from Sigma-Aldrich (St. Louis, MO, USA). Water was purified using a Milli-Q system (Millipore, El Paso, TX, USA) in our laboratory.

The standard test compounds: aniline, phenol, homologs of benzene and PAHs were obtained from Sigma-Aldrich (St. Louis, MO, USA). The concentration of tests compounds was in the range of 10–40 $\mu\text{g/ml}$. The injection volumes were in the range of 2–10 μl .

2.3. Methods

Silanol activity (SA_G) and the hydrophobicity (H_G) of the stationary phases were determined according to the method described by Galushko [18] and commonly used for stationary phases

characterization [19]. To determine these factors the analysis of aniline, phenol, benzene and toluene retention was done using a mobile phase containing 60% methanol in water. The silanol activity SA_G and hydrophobicity H_G can be calculated as follows using solute retention factors k :

$$SA_G = 1 + 3 \left[\left(k_{\text{aniline}}/k_{\text{phenol}} \right) - 1 \right] \quad (1)$$

$$H_G = (k_{\text{toluene}} + k_{\text{benzene}})/2 \quad (2)$$

In all methods the flow rate was 1 ml/min. Measurement were carried at 298 K. Column dead volumes were measured using thiourea as a marker.

2.4. Synthesis procedure

2.4.1. Diol silica gel

Before the chemical modification of bare silica gel, a sample of adsorbent was placed in a specially designed glass reactor protecting against the contact of the reagents with the external environment. Silica gel was treated at 180 °C under vacuum (10^{-2} Pa) for 10 h in order to remove physically adsorbed water. Then, the temperature was decreased to 90 °C and (3-glicidoxypyl)trimethoxysilane was added. After 12 h, the reaction products were washed out with toluene, methanol and hexane, and dried.

In the second step, the synthesized material was treated with 1% sulfuric acid to hydrolyze the epoxide ring. After the hydrolysis, the diol-bonded stationary phase was washed in water and methanol. Synthesized material was dried under vacuum.

2.4.2. Phosphodiester phases: Diol-P-10 and Diol-P-C18

The diol-bonded silica gel was placed in a glass reactor and heated up to 100 °C during 10 h. Further, dried support was modified using phosphoryl chloride and proper alcohol: decanol and dodecanol, to obtain Diol-P-C10 and Diol-P-C18, respectively. Dried support was suspended in dry toluene. Next, the solution of phosphoryl chloride and alcohol was added. Reactions were carried out with addition of triethylamine at 65 °C during 12 h under reflux. The reaction products were washed out with toluene, methanol and hexane. Synthesized material was dried under vacuum.

3. Results and discussion

3.1. Elemental analysis

Synthesized adsorbents were a subject of elemental analysis. Table 1 presents results of chemical modification of the silica gel surface, i.e. content of carbon, hydrogen after each bonding reaction, determined by elemental analysis. It allows the calculation of bonded ligands coverage density (α_{RP}) on the silica surface. Calculation was performed using Berendsen equation [20]:

$$\alpha_{RP}^I = \frac{10^6 P_C}{1200 n_C - P_C (M_1 - n_X)} \cdot \frac{1}{S_{\text{BET}}} \left[\mu\text{mol/m}^2 \right] \quad (3)$$

where

Table 1
Physico-chemical characterization of prepared stationary phases.

Property	Abbreviation	Diol	Diol-P-C10	Diol-P-C18
Carbon percentage [%]	P_C	1.023	2.610	2.114
Hydrogen percentage [%]	P_H	0.621	0.827	0.729
Coverage density [$\mu\text{mol/m}^2$]	α_{RP}	1.32	1.27	0.47

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