Talanta 131 (2015) 684–692

Contents lists available at ScienceDirect

Talanta

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

Synthesis and characterization of ester-bonded stationary phases for liquid chromatography

Szymon Bocian [a], Alicja Nowaczyk [b], Bogusław Buszewski [a,*]

[a] Chair of Environmental Chemistry & Bioanalytics, Faculty of Chemistry, Nicolaus Copernicus University, Gagarin 7 st., 87-100 Torun, Poland
[b] Department of Organic Chemistry, Faculty of Pharmacy, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University, Jurasza 2, 85-094 Bydgoszcz, Poland

ARTICLE INFO

Article history:
Received 23 June 2014
Received in revised form
17 July 2014
Accepted 21 July 2014
Available online 21 August 2014

Keywords:
Ester bonded stationary phase
Liquid chromatography
Stationary phase 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 cholesterol-ester and alkyl-ester 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 chromatography. Synthesized stationary phases were characterized and visualized by computer modeling that indicates the regions of potential hydrophobic and polar interactions. Synthesized material possess residual hydroxyl groups that reduce the hydrophobicity of the material and causes better stability at high water concentration. Due to surface properties these materials are became polar embedded stationary phases.

1. Introduction

Some of the liquid chromatographic analyses needs the application of water-rich mobile phases (more than 85% water or a buffer). In such condition the performance of hydrophobic stationary phases indicate that the bonded ligands might be collapsing. This problem could be solved by increasing of organic content in the mobile phase which should improve solvation and bring bonded ligands back to the original conformation. To avoid this procedure, which reduces the retention and selectivity of the separation, some manufacturers introduce stationary phases incorporated polar groups mixed with the original alkyl ligands (polar embedded stationary phases). Another possibility is to add some polar groups during endcapping procedure (polar end-capped stationary phases) [1]. This produces variation in the bonding.

One of the method increasing the polarity of hydrophobic adsorbent is the synthesis of *N*-acylamide stationary phases [2,3]. 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 [4].

In the group of hydrophobic/hydrophilic phases the cholesterol-bonded material has to be mentioned [5,6]. The first attempt was described by Siouffi's [7]. This methodology relays on the reaction of the terminal amine group of bonded aminopropyl silica with cholesteryl chloroformate. In the same time Buszewski et al. [8], had presented independently the methodology of cholesterol bonded phase synthesis during modification of amino-propyl intermediate deposition. This material offers specific properties and it can be successfully used to separate mixtures by both chromatographic systems: reversed phase and normal-phase [9–13]. Unfortunately they are not so popular than other stationary phases.

Since the late 60's the knowledge of the silica gel modification was developed and led to the synthesis of so-called “dedicated” stationary phases. Coupling the skills with physicochemical studies the nature of the interactions of analytes with the surface of stationary phase enabled design of the new generation chemically bonded stationary phases. The molecular modeling also contributed to the development of a new generation of chromatographic packing. The consequence of making a progress in this area is the introduction of new types of stationary phases which are characterized by a variety of properties. An example can be materials mimic biological membranes. Simplified methods of such chemically bonded stationary phase are shown in Fig. 1.

In 1989, Pidgeon et al. [14] first described the stationary bonded phases for liquid chromatography that mimics the phospholipids bilayer of cell membranes. It was consisted of phospholipids analogs monolayer covalently bonded to the silica surface.

* Corresponding author. Tel.: +48 56 611 43 08; fax. +48 56 611 48 37.
E-mail address: bbusz@chem.umk.pl (B. Buszewski).

http://dx.doi.org/10.1016/j.talanta.2014.07.069
0039-9140/

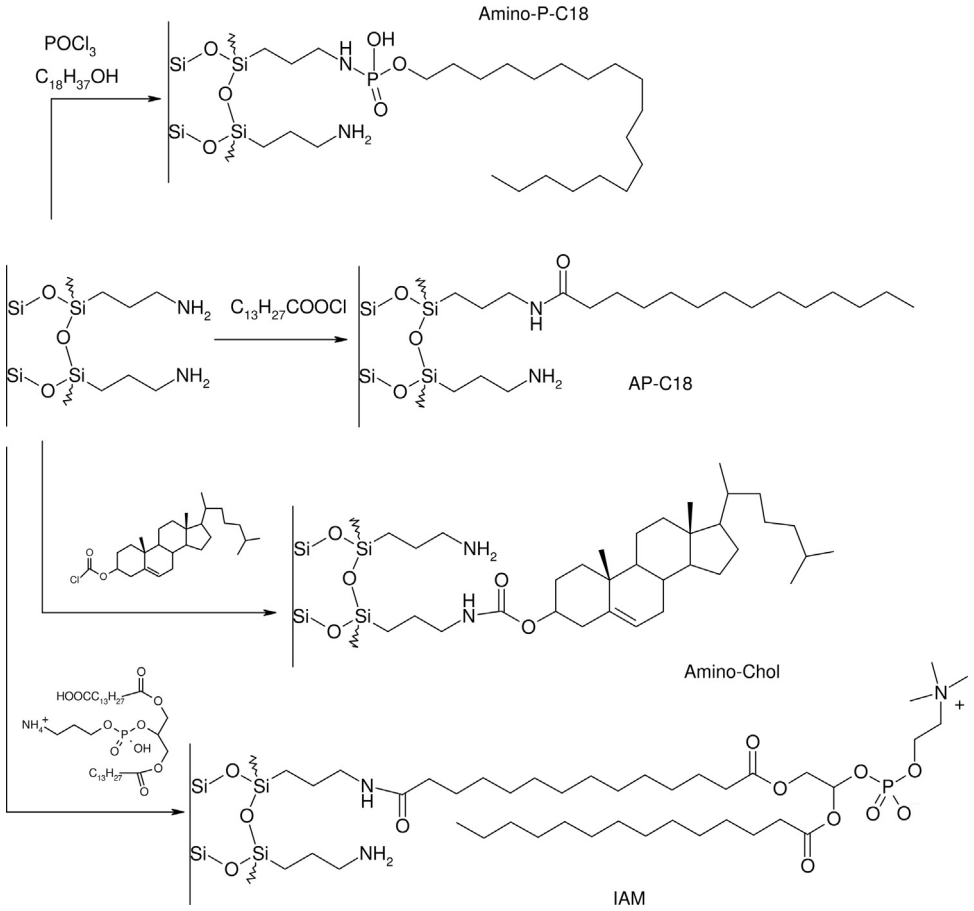

Fig. 1. Simplified methods of the chemically bonded stationary phases, which mimics the cell membrane properties.

This material was called *immobilized artificial membranes* (IAMs) [15,16]. IAM bonded phase more closely mimics the interaction of analytes with biological membranes rather than the classical octadecyl stationary phase. It is caused by combinations of possible hydrophobic, ion pairing, and hydrogen bondings [17,18].

One of the newest material, which simulate the biologic membrane is the *N*,*O*-dialkyl phosphoramidate stationary phase [19,20]. Physico-chemical properties of those adsorbents allow using them in separation of both hydrophobic and polar substances. Changing the length of main chain in wider range enabled to obtain chromatographic materials with different hydrophobic-polar properties.

The goal of our work was to synthesize a new generation of ester-bonded stationary phases for liquid chromatography which possess properties similar to the cell membrane. 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 elemental analysis, solid state NMR and FT-IR spectroscopy. The potential application of synthesized material for chromatographic analyses 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 degree of coverage of the surface by 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, aquisition 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 × 4.6 mm 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-glicidoxypropyl)trimethoxysilane, dodecanoyl chloride, and cholesteryl chloroformate were purchased form Alfa Aesar (Karlsruhe, Germany). Organic solvents used during synthesis: toluene, methanol and