Journal of Chromatography A, 1388 (2015) 133–140

Contents lists available at ScienceDirect

Journal of Chromatography A

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

A polar-embedded C30 stationary phase: Preparation and evaluation☆

Mingliang Zhang[a,b], Wenpeng Mai[c], Liang Zhao[a], Yong Guo[a], Hongdeng Qiu[a,*]

[a] *Key Laboratory of Chemistry of Northwestern Plant Resources and Key Laboratory for Natural Medicine of Gansu Province, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou 730000, China*

[b] *University of Chinese Academy of Sciences, Chinese Academy of Sciences, Beijing 100049, China*

[c] *School of Chemistry and Chemical Engineering, Henan University of Technology, Zhengzhou 450001, China*

ARTICLE INFO

Article history:
Received 11 November 2014
Received in revised form 6 February 2015
Accepted 7 February 2015
Available online 16 February 2015

Keywords:
Polar-embedded
Carbamate
Triacontyl chain
Shape selectivity
Reversed-phase liquid chromatography

ABSTRACT

A novel polar-embedded C30 stationary phase has been synthesized and characterized. The polar carbamate group was generated homogeneously *in situ* by the catalytic reaction between isocyanate and primary alcohol. The simple one-pot synthetic strategy provided an efficient and effective strategy for modification of silica spheres. Efficiency, selectivity and silanol activity of the resulting column were characterized in detail with different classes of analytes that included Standard Reference Materials (SRM) 870, SRM 869b and SRM 1647e, alkylbenzene congeners, as well as polar-substituted aromatics. The polar-embedded C30 stationary phase was found to exhibit excellent shape selectivity.

1. Introduction

The development of new alkyl bonded stationary phase is an important aspect of the expansion of reversed-phase liquid chromatography (RPLC), which is currently the most popular mode in high-performance LC. Among the alkyl-modified silica materials, octadecyl (C18) stationary phase has doubtlessly received the most attention thus far, due to its excellent comprehensive performance and well-studied characteristics. According to the difference in the surface chemistry and preparation methods, C18-modified silica can be classified into monomeric and polymeric C18 stationary phases [1–4]. Although C18 stationary phases are applicable to a wide range of separation problems, there is one drawback related to their performance, the “de-wetting” phenomenon in highly aqueous mobile phase, which tends to result in partial or complete loss of chromatographic retention [5,6]. Another problem encountered by C18 stationary phases is the broadened peak shapes and peak tailing for alkaline solutes, due to the unfavorable hydrogen bonding and ion-exchange interactions between solutes and residual silanols [7,8]. To alleviate these deleterious effects, additives such as traditional tertiary amines [9] or ionic liquids [10,11] can be used in the mobile phase. Alternatively, polar-embedded alkyl stationary phases have been developed to achieve reproducible separation of basic solutes of lower hydrophobicity in high water content mobile phase [12]. As its name suggests, the ligand in polar-embedded alkyl stationary phase is a combination of a hydrophobic aliphatic chain, including C18 (longest), C16, C14, C12 and C8, and a hydrophilic polar group, such as amide [13], carbamate [14], urea [15] and imidazolium [16–18].

Triacontyl (C30) stationary phase in LC was first introduced by Sander et al. for separation of carotenoids [19], which has later evolved into a powerful stationary phase after its successful application to resolutions of PAH, fullerene and vitamins A and E isomers [20,21]. Besides the exceedingly high molecular shape recognition for structural isomers, another distinctive advantage of C30 stationary phase is its compatibility with highly aqueous mobile phase. The unique characteristics of C30 stationary phase have been attributed to higher alkyl chain conformational order, as evidenced by solid ^{13}C nuclear magnetic resonance (NMR) [22] and infrared spectroscopy (IR) [23,24] studies. As with the classification of C18 stationary phases, C30 stationary phases can also be classified into monomeric or polymeric [25]. In contrast to the wide selection of polar-embedded C18 stationary phases, polar-embedded C30 stationary phase are not yet commercially available. It will thus be of interest to explore the potential of such C30 stationary phases. In order to obtain such materials, one can take advantage of the derivatives of triacontane. Since 1-triacontylamine and 1-triacontoic acid are commercially unavailable or extremely expensive, urea and amide groups are inapplicable. Carbamate seems a practical choice, as reflected by the successful example

---

☆ Presented at the 8th International Conference on Countercurrent Chromatography - CCC 2014, 23 - 25 July 2014, Uxbridge, United Kingdom.

* Corresponding author. Tel.: +86 931 4968877; fax: +86 931 8277088.
E-mail address: hdqiu@licp.cas.cn (H. Qiu).

http://dx.doi.org/10.1016/j.chroma.2015.02.023

in literature [14], where the carbamate-embedded ligand was produced using 1-alkylamine and chloroformate. The only viable choice is triacontanol, a natural product from beeswax. Moreover, evidence shows that alkanethiol can readily react with isocyanatopropylsilane to produce thiocarbamate-embedded alkyl stationary phase [26]. It is also well known that polysaccharide-based chiral stationary phase can be effectively modified by isocyanate [27] and polyurethane are being produced at industrial level *via* the reaction between polyisocyanate and polyol. Consequently, we believe that triacontanol is a candidate for preparation of carbamate-embedded C30 stationary phase.

To validate the feasibility of preparing carbamate-embedded alkyl stationary phase *via* aliphatic alcohol and isocyanate, herein we present the preparation of such silica material through simple catalytic reaction between triacontanol and isocyanate and subsequent immobilization. As RP stationary phase, the new material was evaluated with several key sets of interest, including Standard Reference Materials (SRM) 869b, 870 and 1647e, as well as two sets of diversely substituted aromatics. The results were comparatively studied with commercial C18 and C30 stationary phases to facilitate a better understanding of the LC behaviors of the new stationary phase.

2. Experimental

2.1. Reagents and materials

Ultrapure spherical porous silica Type 1 (diameter: 5 μm, pore size: 100 Å, surface area: 400 m^2 g^{-1}) and silica Type 2 (diameter: 5 μm, pore size: 100 Å, surface area: 450 m^2 g^{-1}), from Lanzhou Institute of Chemical Physics (Lanzhou, China) and Daiso Co., Ltd. (Osaka, Japan) respectively, were used. Triacontanol (99%) and octadecanol (99%) were purchased from Nitritopper Biotechnology Co., Ltd. (Changsha, China) and recrystallized twice from *n*-hexane and checked by ^{1}H NMR prior to use. γ-Isocyanatopropyltriethoxysilane (ICPTES) (98%) and triethylenediamine (DABCO) (99%, recrystallized from cold methanol) were purchased from Sun Chemical Technology Co., Ltd. (Shanghai, China). Standard Reference Material (SRM) 869b, column selectivity test mixture for liquid chromatography, and SRM 1647e, priority pollutant polycyclic aromatic hydrocarbons (PAHs), were obtained from the Standard Reference Materials Program (NIST, Gaithersburg, MD, USA); another column test mixture (CTM) with a composition same to SRM 870 was prepared in acetonitrile (MeCN) by individual solute of highest purity available. Single PAH solute, alkylbenzenes and other solutes were supplied by J&K Chemical (Beijing, China). Ultrapure water (18.3 MΩ at 25 °C) was produced by a Milipore Direct Q 3UV unit; MeCN, methanol (MeOH), ethanol (EtOH) and tetrahydrofuran (THF) of HPLC grade were used. Analytical grade solvents for syntheses were dried and distilled prior to use.

2.2. Preparation of polar-embedded C30 stationary phase

Firstly, the customized silane was synthesized. In N_2 or Ar atmosphere, triacontanol (6 mmol) and DABCO (0.3 mmol) were dissolved in 30 mL of hot toluene (50 °C), to which was added dropwise a solution of ICPTES (6 mmol) in 20 mL of toluene over 1 h. Then the solution was heated to reflux for 24 h. If desired, the intermediate C30-Carbamate could be readily isolated *via* precipitation from mother liquor. Recrystallization from dry *n*-hexane afforded silane sample as a sticky power with pale color (yields > 93%), which was pure enough for NMR characterizations. Prolonged storage of this intermediate was not recommended due to hydrolysis of ethoxy groups. ^{1}H NMR (400 MHz, $CDCl_3$) 4.03 (2 H, s), 3.82 (6 H, q), 3.17 (2 H, d), 1.61 (4 H, d), 1.37–1.14 (63 H, m), 0.88 (3 H, t), 0.67–0.58 (2 H, m). ^{13}C NMR (101 MHz, $CDCl_3$) 64.87, 58.46, 43.37, 31.95, 29.92–29.43, 29.37, 29.12, 25.93, 23.35, 22.71, 18.29, 14.12, 7.66 (see Fig. S1A and B).

Then the modification of silica was performed as follows: a slurry of Type 1 silica spheres (freshly activated and ultrasonically degassed, 3 g) in 20 mL of toluene was added to the solution of silane under mechanic stirring. The mixture was refluxed for 24 h. Finally the mixture was filtered by membrane and thoroughly washed by boiling xylene and THF, then hot THF/water (v/v = 2/1) and EtOH (100 mL for each). The obtained silica gel was suspended in 100 mL of boiling THF to remove the last trace of adsorbed silane molecules. Again the silica was collected by filtration and washed thoroughly by hot THF and MeOH (150 mL for each). The final silica material, Sil-Carbamate-C30 (Sil-CBM-C30), was dried in vacuum at 60 °C overnight and stored in a brown vial with stopper before any further usage and characterization. The illustrative scheme was given in Fig. 1.

The identical synthetic procedure, excepting substituting toluene with xylenes, was repeated for Type 2 silica.

The identical synthetic procedure, except substituting toluene with xylenes, was adopted in the case of octadecanol, the modified silica was nominated as Sil-Carbamate-C18 (Sil-CBM-C18).

2.3. Apparatus

The NMR spectra were recorded on a Varian INOVA-400M instrument (Varian, USA) using tetramethylsilane as reference. The carbon, hydrogen and nitrogen contents of the silica support were determined by elemental analysis using a Vario EL III elementat analyzer (Hanau, Germany). The infrared (IR) spectra were acquired upon a Brucker IFS120HR Fourier-transform spectrometer (Ettlingen, Germany). Thermogravimetric analyses (TGA) were conducted on a Seiko Exstar 6000 TG/DTA 6200 thermal analyzer (Chiba, Japan) in static air from 35 to 800 °C with a heating rate of 10 °C min^{-1}.

The stationary phase (2.0 g) was suspended in tetrachloromethane (50 mL) and slurry-packed into stainless steel column (150 mm × 4.6 mm I.D.) using MeOH as propellant solvent at a constant liquid pressure of 55 MPa until a constant outlet flow of MeOH was achieved (measured by stopwatch and cylinder). All the HPLC tests were run on a Shimadzu Essentia system (Kyoto, Japan) composed of LC-15C binary pumps, SPD-15C UV-Vis detector, CTO-15C column oven and a Rheodyne 7725i injector with 20 μL sample loop (Cotati, CA, USA). The column temperature was controlled by a glycol/water bath with a circulator having a heating and cooling system. The column was evaluated at flow rate of 1 mL min^{-1}, the UV detection wavelength was 254 nm. MeOH, MeCN and water were filtered through 0.45 μm membrane and ultrasonically degassed prior to use. The analytes were dissolved in MeCN and stored in refrigerator. A Shimadzu WondaSil C18-WR column (diameter: 5 μm, pore size: 100 Å, pore volume: 1.05 mL g^{-1}, surface area: 450 m^2 g^{-1}, carbon loading: 14%, end-capped, 150 mm × 4.6 mm I.D.) was used as reference column. The dead time of each column was determined by the signal of pure water.

3. Results and discussion

3.1. Preparation and characterization of the silica materials

The proposed strategy for immobilization of triacontyl carbamate onto silica sphere was an efficient one-pot reaction comprised of two steps. In the first step, the primary alcohol, albeit less reactive than amino analog toward isocyanate, had been transformed almost quantitatively under the catalysis of DABCO, which is the most powerful tertiary amine catalyst employed in the