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Novel reversed-phase high-performance liquid chromatography stationary phase with oligo(ethylene glycol) "click" to silica

Zhimou Guo [a], Yanfang Liu [a], Junyan Xu [a,b], Qing Xu [a], Xingya Xue [a], Feifang Zhang [a], Yanxiong Ke [a,b], Xinmiao Liang [a,b,*], Aiwen Lei [c,**]

[a] *Dalian Institute of Chemical Physics, Graduate School of the Chinese Academy of Sciences, Chinese Academy of Sciences, Dalian 116023, China*
[b] *School of Pharmacy, East China University of Science and Technology, 130 Meilong Road, Shanghai 200237, China*
[c] *College of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430072, China*

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Abstract

Oligo(ethylene glycol) (OEG) covalently bonded silica was prepared by using click chemistry and employed as a stationary phase for reversed-phase high-performance liquid chromatography. The column efficiency and effect of organic modifier content on retention were investigated. The separation selectivity was also studied with phenyl compounds and an actual sample of natural products. The results indicated that the stationary phase possessed good separation efficiency and separation selectivity in RP-HPLC mode. Moreover, the stationary phase showed good complementary separation selectivity to the C18 stationary phase. The OEG stationary phase had "clustering" function for "homologous component" in the separation of natural products.

Keywords: Oligo(ethylene glycol); Stationary phase; Click chemistry; High-performance liquid chromatography

1. Introduction

RP-HPLC with a silica-based C18 stationary phase is the most popular and efficient chromatographic mode. However, C18 for RP-HPLC cannot resolve all the separation problems, especially for separations of very complex samples in the fields of proteomics, metabolomics, pharmaceutical study and natural products [1]. Therefore, it is desirable to develop complementary stationary phases for RP-HPLC to supplement the ordinary C18. In recent years, many novel stationary phases for RP-HPLC were provided to resolve the separation problems [2–4]. Most of these stationary phases could be divided into two types with either modified C18 [3,5–13] or novel molecules as functionalities [14–18]. The modified C18 stationary phases are the polar-embedded C18, polar-endcapped C18 [7] and polar-headed C18 [11]. Enhanced selectivity could be obtained by the modified approach due to the multiple interactions brought by the introduced polar groups [7]. However, C18 was also the major domain in these modified C18 ligands and the complementary function in the separation selectivity was limited. Another approach employing functionalities with absolute different structures from C18 also has attracted much attention. For example, since Glennon and co-workers developed bonded calix[4]arene tetraester stationary phases [14,19,20], calixarene stationary phases were studied intensively and proven to be a useful RP-HPLC stationary phases [15,21–25]. These novel stationary phases possess complementary selectivity compared to C18 due to the unique molecular structures. In recent years, a novel RP-HPLC stationary phase with poly(ethylene glycol) (PEG) as functionality was introduced and attracted increasing attention [26–29].

Herein, we proposed a novel oligo(ethylene glycol) (OEG) stationary phase synthesized by using click chemistry [30,31] (Fig. 1). Besides hydrophobic interaction, OEG can provide some other interactions, such as hydrogen bonding, dipole–dipole interaction, etc. In addition, the OEG could form a helix-like conformation in the organic-aqueous media [32,33]. These properties of OEG may bring different separation selectivity from C18. Furthermore, different from the PEG molecule, OEG has a similar molecular structure (a flexible chain-molecule) and similar chain length to C18, which will be beneficial to form favorable adsorbent geometry on the ordinary

* Corresponding author at: Dalian Institute of Chemical Physics, Graduate School of the Chinese Academy of Sciences, Chinese Academy of Sciences, Dalian 116023, China. Tel.: +86 411 84379519; fax: +86 411 84379539.
** Corresponding author. Tel.: +86 27 68754672; fax: +86 27 68754672.
E-mail addresses: liangxm@dicp.ac.cn (X. Liang), aiwenlei@whu.edu.cn (A. Lei).

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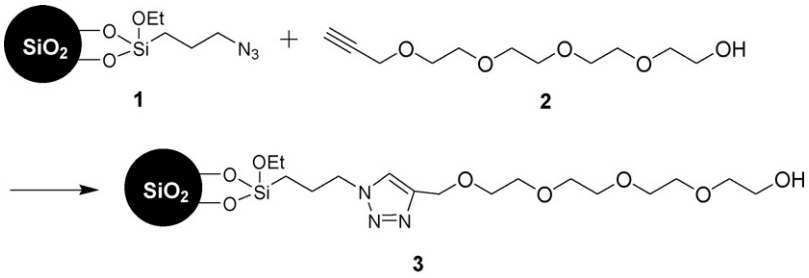

Fig. 1. The synthetic procedure for preparation of "click oligo(ethylene glycol)" stationary phase via click chemistry. Reagents and conditions: *O*-propargyltetra(ethylene glycol), (CH_3OH/H_2O, 1/1, v/v), $CuSO_4$ (5 mol%), sodium ascorbate (15 mol%), RT.

mesoporous silica surface [34]. In this communication, we present an example of an OEG stationary phase and demonstrate its potential as a complementary RP-HPLC stationary phase to C18.

2. Experimental

2.1. Chemicals

Spherical silica (5 μm particle size; 10 nm pore size; 270 m^2/g surface area) was purchased from Fuji Silysia Chemical (Japan). 3-Chloropropyltriethoxysilane was obtained from ABCR (USA). Tetra(ethylene glycol) and propargyl bromide were purchased from Acros (USA). Sodium azide was obtained from Tianjin Chemical Reagents (China). Compounds used as test probes were obtained from different commercial sources. Acetonitrile of HPLC purity was purchased from Fisher (USA). Water was purified by a Milli-Q water-purification system (USA). All other reagents were analytical grade and used without purification.

2.2. Synthesis of the stationary phases

The synthetic procedures used in the preparation of the stationary phases were shown in Fig. 1. Intermediates azide-silica (**1**) [31] and *O*-propargyltetra(ethylene glycol) (**2**) [35] were synthesized according to the literature. The general procedures are described in brief as follows: To the solution of *O*-propargyltetra(ethylene glycol) (1.4 g) in H_2O/CH_3OH (v/v, 1:1, 40 mL) was added $CuSO_4$ (the solution of 0.075 g $CuSO_4$ in 10 mL H_2O) and sodium ascorbate (the solution of 0.24 g in 10 mL H_2O). Azide-silica (3.0 g) was added into the mixture with slow stirring. The mixture was stirred at room temperature for 60 h and then was filtered to afford the solid products. The crude product was washed with water (100 mL), methanol (100 mL), 0.1 mol L^{-1} EDTA solution (400 mL), water (100 mL) and acetone (200 mL) in succession. The solid product was dried at 60 °C for 24 h to afford the "click oligo(ethylene glycol)" stationary phase (Click OEG) **(3)**.

2.3. HPLC

The Click OEG was slurry-packed into 150 mm × 4.6 mm I.D. stainless steel columns with toluene–acetone (v/v, 1:1) as slurry-solvent and methanol as propulsion solvent.

The chromatographic system consisted of a Waters 2695 HPLC pump and a Waters 2996 photodiode array detection (DAD) system (USA). For chromatographic evaluations, the column temperature was held constant at 30 °C. The mobile phases consisted of acetonitrile (ACN) and water, with formic acid as additive. Mobile phase A (water with 0.1% formic acid) and mobile phase B (ACN with 0.1% formic acid) were prepared by the addition of formic acid to water or ACN. The test probes were dissolved in methanol to about 1 mg L^{-1} concentration. The mixture of phenyl compounds was prepared by mixing the solution of test probes. Actual sample of natural products was a component extracted from the water-extracts of *Lamiophlomis rotata* with *n*-butanol.

3. Results and discussion

The Click OEG was prepared according to the "click strategy", in which the functionalized molecule immobilized on azide-silica through 1,3-cycloaddition between the alkyne group and the azide group. In the immobilization process of alkyne-oligo(ethylene glycol), only the alkyne group on the oligo(ethylene glycol) reacts with the azide group on the silica. Therefore, the multi-anchor caused by the hydroxyl group on oligo(ethylene glycol) could be avoided and the resulting materials would bear a flexible chain-ligand. The completion of the immobilization process could be characterized by FT-IR [36]. The distinct peak at 2010 cm^{-1} (azide groups) in the IR spectra of azide-silica disappeared on the IR spectra of Click OEG. The surface concentration of oligo(ethylene glycol) on the silica was calculated from the results of elemental analysis. The carbon content was 5.996% for azide-silica **1** and 10.810% for Click OEG **3**. The surface concentrations of the azide group and OEG on silica were 2.203 and 1.476 μmol m^{-2}, respectively.

The chromatographic behaviors of Click OEG were investigated with phenyl compounds as test probes. As shown in Fig. 2, Click OEG column demonstrated good column efficiency. The peak symmetry is 1.15 and column efficiency is 65,000 plates m^{-1} calculated from naphthalene on the chromatogram. It is known that ACN–water binary solution is the commonly used mobile phase system in ordinary RP-HPLC. Increasing the ACN content would lead to a decrease in the retention time. In this study, the effect of ACN content on the retention behavior was investigated by varying the percentage of ACN in the mobile phase while keeping the additive formic acid constant at 0.1%