



# New reversed-phase/anion-exchange/hydrophilic interaction mixed-mode stationary phase based on dendritic polymer-modified porous silica



Yun Li, Jiajia Yang, Jing Jin, Xiaoli Sun, Longxing Wang, Jiping Chen\*

Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, Liaoning 116023, PR China

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## ABSTRACT

A novel dendritic polymer-modified silica (DPS) stationary phase was prepared by a divergent synthesis scheme starting from propylamine on silica by consecutive amine-epoxy reactions with 1,4-butanedioldiglycidyl ether and aniline. Both elemental analysis and infrared spectra data shows the successful growth of dendritic polymer on silica particles. The carbon and nitrogen contents increased with an increasing number of reaction cycles and achieved 25.2% and 2.1% (w/w) after 11 reaction cycles. The combination of a phenyl ring with a quaternary ammonium, or a tertiary amine at the branch point along with embedded polar functionalities (including ether and hydroxyl groups) in the branch, generated hydrophobic, electrostatic, as well as hydrophilic interactive domains. Depending on solute structure and mobile phase composition, the DPS stationary phase provided multiple retention mechanisms, including reversed phase (RP), anion-exchange (AEX), and hydrophilic interactions. The RP capability achieved separation of polycyclic aromatic hydrocarbons. Basic, neutral and acidic molecules were well separated under RP/AEX mixed mode. Effective separation of small polar compounds (such as nucleobases and nucleosides) was also obtained under hydrophilic interaction liquid chromatography (HILIC) mode.

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## 1. Introduction

Reversed-phase chromatography (RPC) is by far the most widely used mode in high performance liquid chromatography (HPLC) [1,2]. The most common ligands for RP stationary phase include alkyl (C4, C8, C18) and phenyl, which are hydrophobic and retain non-polar analytes effectively. However, these non-polar phases are not suitable for retention and resolution of ionic or polar analytes (e.g. pharmaceuticals or nucleotides) [3]. To solve this problem, mixed-mode chromatography or hydrophilic interaction chromatography (HILIC) has emerged as a valuable alternative or complement to RPC [4,5].

A typical mixed mode stationary phase has both RP and ion-exchange (IEX) functionalities, which facilitates rather independent control of retention for ionizable and neutral analytes. According to the combination of functional groups, it can be classified into three main groups by Liu et al., RP/anion-exchange (AEX) [6] and RP/cation-exchange (CEX) bimodal [7], and RP/AEX/CEX trimodal phases [8]. According to the arrangement of functional

groups, bimodal phases are classified into four categories: blend bed (I), mixed ligand (II), “embedded” (III), and “tipped” (IV) phases. For both I and II, the difference in hydrolytic stability between RP and IEX ligands usually exists, thus leading to an undesirable drift in selectivity [6,9]. This problem does not exist for both III and IV categories because they are prepared by functionalization of silica supports with single silyl ligands containing both RP and IEX domains simultaneously [10,11]. Smadja et al. [10] prepared a RP/strong anion-exchange (SAX) mixed-mode stationary phase with a quaternary ammonium group embedded between an octadecyl and a propyl chain. Several RP/weak anion-exchange (WAX) materials have been prepared by immobilizing a selector (based on an alkyl chain tipped with a cyclic tertiary amine) onto thiol-modified silica gel [12–14]. Type IV materials are generally more hydrophilic due to the IEX terminus. Based on such an alkyl chain with an IEX terminus, many manufacturers have introduced RP/IEX mixed-mode columns, such as the RP/WAX and RP/weak cation-exchange mixed-mode columns from Dionex [7,11]. However, the information about nature of functional groups is usually proprietary.

Initially investigated by Alpert [15], HILIC continues to grow in interest due to its ability to analyze ionic or polar compounds, such as pharmaceuticals, carbohydrates and peptides [16–18]. Several

\* Corresponding author. Tel.: +86 411 84379562; fax: +86 411 8437 9562.  
E-mail address: [chenjp@dicp.ac.cn](mailto:chenjp@dicp.ac.cn) (J. Chen).

reviews of HILIC have been published [19–21]. Retention in HILIC is believed to be caused by a complex multimodal mechanism involving the retention of analytes between the bulk mobile phase and a water-rich layer immobilized on the stationary phase surface. For this reason, the HILIC mechanism of retention strongly depends on the chemistry of the stationary phase, of the analytes, and of the mobile phase [22–26]. Therefore, any polar functionality that is able to form water-rich regions on the surface of packing material can be used for HILIC application. Various polar functionalities for HILIC include silanols, neutral hydrophilic moieties (e.g. hydroxyl, cyano, amide), as well as anionic (e.g. amino), cationic (e.g. carboxylate), zwitterionic (e.g. sulfoalkylbetaine and phosphorylcholine) and amphoteric moieties. Among them, the anionic and cationic phases are also called HILIC/AEX and HILIC/CEX mixed modes, respectively [27,28]. Recently, a new AEX/RP/HILIC trimode stationary phase was also developed based on 1-(2-acryloyloxyundecyl)-3-methylimidazolium bromide ionic liquid-grafted silica [29]. Due to their unique column chemistries, mixed-mode stationary phases can achieve multiple separation capabilities, including RP, IEX, ion-exclusion chromatography, and HILIC, depending on the chromatographic conditions and the analyte characteristics.

Dendritic polymers, including dendrimers and hyperbranched polymers, are globular macromolecules that are characterized both by a highly branched structure, in which all bonds converge to a focal point or core, and a multiplicity of reactive chain-ends [30]. Hyperbranched polymers that allow imperfectly branched or irregular structures are easily prepared and can be a good alternative to dendrimers. These macromolecules display obviously many excellent advantages (e.g. good solubility and extremely high density of functional groups) and are good candidates for the design of single site catalyst, efficient adsorbents and sensors [31]. Modified particles with a hyperbranched structure are also attractive for HPLC applications, such as affinity chromatography [32], size exclusion chromatography [33], and ion-exchange chromatography [34].

In this work, a novel reversed-phase/anion-exchange/hydrophilic interaction mixed-mode stationary phase was developed based on dendritic polymer-modified silica (DPS) particles. We tested in detail its retention properties, including hydrophobic, electrostatic, as well as hydrophilic interactions, using a series of test compounds. Its applications in RP, RP/AEX, and HILIC chromatographic modes are also demonstrated.

## 2. Experimental

### 2.1. Chemicals and materials

The reagents for growing of the hyperbranched molecule on silica surface, (3-aminopropyl)triethoxysilane (APTES, 98%), aniline (99.5%) and 1,4-butanedioldiglycidyl ether (BDDE, 98%) were purchased from J&K Scientific (Beijing, China). The test analytes under different chromatographic modes were as following: ultrapure uracil and thiourea from Aladdin (Shanghai, China), pyridine (>99.5%) from Alfa Aesar (Ward Hill, MA, USA), analytical-grade toluene, benzene, N,N-dimethylformamide and formamide from Beijing Chemical Works (Beijing, China), acrylamide (ultrapure) and N,N-dimethylenbisacrylamide (>99%) from Amresco LLC (Solon, OH, USA), naphthalene, anthracene, phenanthrene, pyrene, benzopyrene, and benzoperylene from Fluka (Buchs, Switzerland), uridine (99%), cytosine (99%), guanosine (98%), benzoic acid (98%), salicylic acid (99%) from J&K Scientific, cytidine and guanine (>98%) from Tokyo Chemical Industry Co. (Tokyo, Japan), ketoprofen (>98%) and amitriptyline hydrochloride (>98%) from Sigma-Aldrich (Milwaukee, WI, USA). HPLC-grade methanol and acetonitrile were supplied by Fisher Scientific (Pittsburgh, PA, USA). The water used in all experiments was deionized (DI) water from a Milli-Q

purification system (Billerica, MA, USA). The reagents used to prepare the buffers were all of analytical grade and obtained from Tianjin Kermel Chemical Reagent Co. (Tianjin, China), including ammonium formate, formic acid, sodium dihydrogen phosphate and sodium hydroxide. The pH of the buffer solutions was adjusted with a Mettler Toledo MPC 227 Dual Purpose Conductivity/pH/T Meter (Columbus, OH, USA). Spherical silica gel (5  $\mu\text{m}$  particle size, 300  $\text{\AA}$  pore size, 150  $\text{m}^2 \text{g}^{-1}$  surface area) was purchased from Fuji Silysia Chemical (Aichi, Japan).

### 2.2. Preparation of the DPS particles

Fig. 1 shows the synthesis route to DPS silica particles. At first, the silica particles were soaked overnight in 3 M HCl, rinsed with DI water, and then dried at 160  $^{\circ}\text{C}$  for 6 h. Secondly, the activated silica particles (4 g) were aminated by reaction with APTES (3 mL) in dry toluene (100 mL) for 12 h at 110  $^{\circ}\text{C}$  under nitrogen atmosphere. After the reaction, the silica particles were filtered, washed successively with toluene, methanol and acetone, and dried at 70  $^{\circ}\text{C}$  overnight under vacuum. Then, the DPS particles were prepared by a dendrimer synthesis reaction, which involved alternate reactions with BDDE at the  $(n + 0.5)$ th generation and aniline at the  $n$ th generation ( $G_n$ ). The first generation dendrimer was prepared as follows. (a) Reaction with BDDE for the 0.5th generation. Aminated silica particles were stirred with BDDE in water (100 mL, 0.3 M) at 80  $^{\circ}\text{C}$  for 2 h. After filtration through a glass filter, the product was thoroughly washed with 100 mL water. (b) Reaction with aniline for the first generation. Epoxy-terminated silica particles were reacted with aniline in methanol/ $\text{H}_2\text{O}$  (100 mL, 0.3 M) at 80  $^{\circ}\text{C}$  for 2 h. After filtration, the product was washed and purified with 100 mL of methanol. The higher generations were prepared by repeating the two steps described above, the concentration of BDDE and aniline being adjusted as required. Finally, the obtained silica particles were dried at 70  $^{\circ}\text{C}$  overnight under vacuum. A schematic illustration of the G4 dendritic polymer on silica surface was also given in Fig. 1.

### 2.3. Characterization of the DPS particles

Elemental analyses were carried out on a Vario EL III CHNOS Elemental Analyzer (Hanau, Germany). The Fourier transform infrared (FT-IR) spectra were recorded on a Perkin Elmer Spectrum GX spectrometer (Waltham, MA, USA) in the range  $\nu = 4000\text{--}400 \text{ cm}^{-1}$ .

### 2.4. Chromatographic evaluation for the DPS phase

The obtained silica particles were slurry-packed into a stainless steel column (150 mm  $\times$  4.6 mm I.D.) using 20 mL of methanol as slurry solvent and a HY-HPLC-S packing pump (Hydrosys Technology, Beijing, China) under a pressure of 40 MPa. Chromatographic analyses were performed with an Agilent 1200 series LC system (Santa Clara, CA, USA) consisting of a quaternary pump, on-line degasser, autosampler, column thermostat and diode array detector. All chromatographic separations of the test analytes were carried out on the DPS column (5  $\mu\text{m}$ , 150 mm  $\times$  4.6 mm I.D.).

## 3. Results and discussion

### 3.1. Characterization of the DPS phase

According to Fig. 1, the resulting dendritic polymer is composed of repeating branch units that contain phenyl ring, quaternary ammonium (or amine) and hydroxyl groups. Therefore, the elemental analysis on carbon, hydrogen and nitrogen can be used to identify the chemical composition of the silica phase. As shown in Table 1, the successive growth of dendritic polymers on silica

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