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Nucleophilic substitution in preparation and surface modification of hypercrosslinked stationary phases

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ABSTRACT

Four linear diaminoalkanes (1,2-diaminoethane, 1,4-diaminobutane, 1,6-diaminohexane, and 1,8-diaminooctane) have been used to hypercrosslink poly(styrene-*co*-vinylbenzyl chloride-*co*-divinylbenzene) monolithic stationary phases by nucleophilic substitution reaction. The column efficiency of polymer monoliths improved with longer diaminoalkane with 1,8-diaminoctane providing the highest efficiency. The concentration of 1,8-diaminoctane, together with hypercrosslinking time and temperature has been optimized. To improve the permeability of prepared columns, the hypercrosslinking modification has been combined with an early termination of polymerization reaction and decrease in polymerization temperature. The optimal column has been prepared by a polymerization reaction for 2 h at 65 °C and hypercrosslinked in the presence of 3% 1,8-diaminooctane for 2 h at 95 °C. The repeatability study of the presented protocol provided relative standard deviation for nine columns prepared independently out of three individual polymerization mixtures in between 2.0–12.0% for retention factors and 1.5–6.5% for plate heights, respectively.

Further, we have modified residual chloromethyl groups with 2-aminoethanesulfonic acid (taurine) to prepare monolithic columns suitable for separation of small polar molecules in hydrophilic interaction chromatography. The highest retention of polar thiourea showed the column modified at 70 °C for 20 h. Taurine-modified hypercrosslinked column showed the minimum of van Deemter curve of 20 μm. The prepared column provided dual-retention mechanism, including hydrophilic interaction and reversed-phase liquid chromatography that can be controlled by the composition of the mobile phase. The prepared column has been successfully used for an isocratic separation of low-molecular phenolic acids.

1. Introduction

Monolithic stationary phases are already well established part of chromatographic stationary phases [1]. Based on chemical skeleton and internal structure, they are divided into inorganic silica-based and organic polymer-based counterparts [2]. Recently, scanning electron microscopy [3,4], dual beam electron microscopy [5], and serial block face electron microscopy [6,7] have been used to reconstruct an internal structure of polymer monoliths and to characterize flow dispersion in an associated pore size distribution. Characteristics as plate height, porosity, pore tortuosity, chord length, permeability, and homogeneity factor were obtained from models and correlated to experimental values [3–7]. Based on these studies, an improved column performance can be obtained by the preparation of monoliths with simultaneous reduction in the pore size and enhanced homogeneity of the monolith [5,8]. In their study about nanoscale structure and mechanical properties of polymer monoliths Laher et al. [9] showed that polymer monoliths swell differently in water and acetonitrile as a typical mobile phase components and that nanoscale mechanical properties of polymer monoliths shows smooth transition on length scale of approximately 1 μm [9].

Thanks to rigid nature of their internal structure inorganic silica monoliths perform very well in the separation of small molecules. On the other hand, the main application area of polymer monoliths is gradient elution of synthetic and natural polymers [10]. An adjustment of the polymerization mixture composition [11–13] or an early termination of polymerization reaction [14–16] have been introduced to prepare columns suitable for efficient isocratic separations of low molecular compounds. Yet another way how to prepare monolithic stationary phases for the separation of small molecules is a hypercrosslinking post-polymerization modification introduced several decades ago by Davankov [17,18]. In 2010, we introduced hypercrosslinking to an area of polymer-based

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monolithic stationary phases [19] and optimized hypercrosslinked monoliths for separation of low-molecular compounds [20]. We have also studied an effect of hypercrosslinking modification mixture composition on porous properties and efficiency of hypercrosslinked columns [21]. In spite of quite recent introduction to the field of polymer monoliths, hypercrosslinked monoliths have been already used in capillary liquid chromatography [22,23], capillary electrochromatography [24], modified with gold nano particles [25], grafted with zwitterion monomer [26], or used as a stationary phases in thin layer chromatography of peptides and proteins [27] and in ion-exchange reversed-phase mixed mode in the determination of genomic DNA methylation [28]. Hypercrosslinking modification was also used to introduce mesopores in high internal phase emulsion monoliths [29].

Direct chemical modification of residual chloromethyl groups with nucleophilic substitution is a simple and straightforward method for surface modification. In styrene-based monoliths, vinylbenzyl chloride is the preferred monomer for post-polymerization functionalization. In our work, a free radical initiator, 4,4′-azobis(4-cyanovaleric acid), was attached to the pore surface of hypercrosslinked monolith. Activated surface has been then grafted with zwitterion sulfobetaine monomer. Prepared monolithic capillary columns have been applied in one- and two-dimensional separation of small polar molecules in hydrophilic interaction chromatography [26]. Lv et al. [25] combined hypercrosslinked monoliths with gold nanoparticles functionalized with hydrophilic functionalities and attached through a layered architecture, to analyze nucleosides and peptides in hydrophilic interaction liquid chromatography.

Maya and Svec [23] used external crosslinkers to satisfactory crosslink swollen poly(styrene-*co*-divinylbenzene) monoliths by Friedel–Crafts alkylation. In this work, we used similar approach and tested the ability of nucleophilic substitution reaction to prepare hypercrosslinked stationary phases applicable in a capillary liquid chromatography of small molecules. For this, we have crosslinked the swollen poly(styrene-*co*-vinylbenzyl chloride-*co*-divinylbenzene) polymer by linear diaminoalkanes with various length of alkyl chain and optimized the reaction conditions including the concentration of diaminoalkane, reaction temperature and time. To further increase the applicability of prepared columns, we have modified residual chloromethyl groups with 2-aminoethanesulfonic (taurine) [30] and prepared monolithic columns suitable for separation of small polar molecules.

2. Experimental part

2.1. Materials

Styrene (99%), vinylbenzyl chloride (mixture of 3- and 4-isomers, 97%), divinylbenzene (80%, technical grade), 2,2′-azobisisobutyronitrile (98%), 1,2-diaminoethane, 1,4-diaminobutane, 1,6-diaminohexane, 1,8-diaminooctane, triethylamine, uracil, phenol, thiourea, 1-dodecanol, acetonitrile (HPLC grade), and tetrahydrofuran (HPLC grade) were all obtained from Sigma–Aldrich (Steinheim, Germany). 3-(Trimethoxysilyl)propyl methacrylate and alkylbenzenes (benzene, toluene, ethylbenzene, propylbenzene, butylbenzene, amylbenzene) were purchased from Fluka (Buchs, Switzerland). Polyimide-coated 320 μm I.D. fused-silica capillaries were purchased from Polymicro Technologies (Phoenix, AZ, USA).

2.2. Preparation of capillary columns

In order to improve the stability of organic polymer monolith inside the silica capillary, the inner wall was modified by 3-(trimethoxysilyl)propyl methacrylate as described previously [31]. Generic monoliths were prepared in capillaries using *in situ* radical polymerization of mixture containing 12% of styrene, 12% of vinylbenzyl chloride, and 16% of divinylbenzene dissolved in binary porogen solvent containing 18% toluene and 42% 1-dodecanol (all concentrations in w/w) [19,21,26]. The initiator used was 2,2′-azobisisobutyronitrile (AIBN) (1%, w/w, with respect to monomers). The polymerization mixtures were ultrasonicated for 10 min and filled into the vinylized capillaries. Both ends of the filled capillary were sealed with rubber stoppers and the capillary was placed in a water bath. The polymerization was carried out at 60–70 °C for 2 or 20 h. Both ends of the capillary were then cut to adjust its length, and the monolithic column was washed with acetonitrile.

To prepare hypercrosslinked monolithic stationary phases, the columns were flushed with tetrahydrofuran at a flow rate of 0.25 μl/min for 2 h. The solution of diaminoalkane (1,2-diaminoethane, 1,4-diaminobutane, 1,6-diaminohexane, 1,8-diaminooctane in tetrahydrofuran) was then pumped through the columns at a flow rate of 0.25 μl/min for 2 h. To improve solubility of 1,6-diaminohexane and 1,8-diaminooctane in tetrahydrofuran, the reaction mixtures were ultrasonicated for 20 min. The reaction was carried out at 80–120 °C for 1–4 h as shows Table 1 that summarizes all experimental conditions.

In order to further modify the surface of hypercrosslinked columns, the columns were first flushed for 2 h with 1 mol/L solution of taurine with an addition of equimolar triethylamine [30]. Then, the columns were sealed and nucleophilic substitution proceeded for 2, 6, or 20 h at 50–90 °C. After modification, the columns were flushed with water followed by mobile phase and used for further analysis.

2.3. Instrumentation

A modular micro liquid chromatograph was assembled from an LC10ADvp pump (Shimadzu, Kyoto, Japan), a micro valve injector with a 20-nL inner sampling loop (Valco, Houston, USA) controlled using an electronic actuator, a restrictor capillary inserted as a mobile phase flow splitter before the injector, a variable wavelength LCD 2083 UV detector operated at 214 nm, adapted for capillary electrophoresis with a 75 μm ID fused silica capillary flow-through cell (ECOM, Prague, Czech Republic), and a personal computer with a Clarity software (Data Apex, Prague, Czech Republic). Fused-silica capillary monolithic columns were fitted directly into the body of a micro-valve injector, with the end of the column connected to the detector using zero-volume fittings.

2.4. Characterization of prepared columns

Band broadening in chromatographic columns is described by van Deemter equation, Eq. (1), as the dependence of the height equivalent to theoretical plate, HETP, on the linear velocity of the mobile phase, u [32]:

$$\mathrm{HETP} = A + B/u + C \cdot u \quad (1)$$

where A is the eddy-diffusion, B is the longitudinal diffusion and C is the mass transfer resistance of the analyte between mobile and stationary phase. The mass transfer resistance (C-term) has been determined as a slope of linear regression fit of right-hand side of van Deemter curves for all prepared columns.

The flow resistance of prepared columns was characterized by the column permeability K_F (Darcy equation, Eq. (2)) [33]:

$$K_F = \frac{F_m \cdot \eta \cdot L}{\Delta p \cdot \pi \cdot r^2} \quad (2)$$