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A facile and efficient strategy to enhance hydrophilicity of zwitterionic sulfoalkylbetaine type monoliths

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ABSTRACT

In order to prepare zwitterionic HILIC monolithic columns with high polarity, the highly hydrophilic monomer *N*,*N*-dimethyl-*N*-acryloyloxyethyl-*N*-(3-sulfopropyl)ammonium betaine (SPDA) and crosslinker *N*,*N*′-methylenebisacrylamide (MBA) were employed for developing a novel sulfoalkylbetaine type stationary phase. The polymerization parameters were systematically optimized in order to obtain a satisfactory performance for column permeability, mechanical stability, hydrophilicity, efficiency and selectivity. Compared to the previously reported poly(*N*,*N*-dimethyl-*N*-methacryloxyethyl-*N*-(3-sulfopropyl)ammonium betaine-*co*-ethylene dimethacrylate) (poly(SPE-*co*-EDMA)) monolith and the poly(SPDA-*co*-EDMA) monolith that we developed, a significantly enhanced hydrophilicity was obtained on the poly(SPDA-*co*-MBA) monolithic column, illustrated by the lowered critical composition of the mobile phase corresponding to the transition from the HILIC to the RP mode. Excellent permeability, reproducibility and stability were achieved on this optimized poly(SPDA-*co*-MBA) monolith. A column efficiency of 70,000 plates/m was obtained for the analysis of bases at a linear velocity of 1.95 mm/s. As expected, by studying the influence of mobile phase pH and salt concentration on their retention, a weak electrostatic repulsion interaction for negatively charged analytes was also observed at low organic solvent content on the poly(SPDA-*co*-MBA) monolithic column. The final optimized poly(SPDA-*co*-MBA) monolith exhibited good selectivity for a series of polar compounds, such as phenols, bases, benzoic acid derivatives, small peptides, urea and allantoin.

1. Introduction

Hydrophilic interaction chromatography (HILIC), which was first termed by Alpert [1], represents an increasingly popular separation mode due to its complementary selectivity to reversed-phase liquid chromatography, and the fact that it also has good compatibility with LC–MS and 2D-HPLC [2]. A variety of silica-based or polymer-based polar stationary phases, such as non-modified bare silica [3], amino- [4], amide- [5], cyano- [6,7], carbamate- [8], diol- [9,10], and cyclodextrins [11], have all been successfully used as HILIC stationary phases. In order to promote hydrophilic interactions between very polar compounds and a hydrophilic stationary phase, eluents containing a high content of water miscible organic solvent (e.g., ACN and MeOH) are normally chosen as mobile phase modifier for HILIC separations. However, the relatively narrow mobile phase range only provides the chromatographer with a small degree of freedom in the optimization of mobile phase composition. Additionally, the limited solubility of polar analytes in a very high-organic, low-aqueous mobile phase (>85% organic) remains a challenge. In order to counteract these problems, highly hydrophilic stationary phases are preferred, which can increase the retention of polar analytes, and further allow the use of mobile phases with a higher water content. Great efforts have been directed toward the development of novel highly hydrophilic packed columns [12] and monolithic columns [13].

Monoliths are of interest in the development of novel HIILC column technology because of their high permeability, low resistance to mass transfer, and fast and simple preparation within micro- or nano-formats [14,15]. Based on their functionality, HILIC stationary phases can be classified into three categories, neutral, charged and zwitterionic. Among these three categories, zwitterionic phases exhibit peculiar selectivity since they can take advantage of

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weak electrostatic interactions between charged analytes and zwitterionic functional groups combined with the high efficiency and selectivity of hydrophilic interaction [16–18]. In the past 10 years, two commercial zwitterionic HILIC packed columns ZIC-HILIC [19] and ZIC-pHILIC [20], possessing both strongly basic quaternary ammonium groups and strongly acidic sulfonic acid groups separated by a short alkyl spacer, have been successfully developed by SeQuant. SeQuant subsequently introduced their ZIC-cHILIC packed column [21,22], which has both a positively charged quaternary ammonium group and a negatively charged phosphate group with an opposite spatial charge arrangement to that of sulfoalkylbetaine. Corresponding zwitterionic HILIC monolithic columns have also been successfully developed [23,24]. For example, we have successfully developed a sulfoalkylbetaine type [23] and phosphorylcholine type [24] HILIC monolithic column by the thermal co-polymerization of *N*,*N*-dimethyl-*N*-methacryloxyethyl-*N*-(3-sulfopropyl)ammonium betaine (SPE) or 2-methacryloyloxyethyl phosphorylcholine (MPC) with ethylene dimethacrylate (EDMA) within 100 μm I.D. capillaries. It was observed that the critical composition of the mobile phase corresponding to the transition from the HILIC to the RP mode, which is considered as a guide to the polarity of a HILIC stationary phase [25,26], is around 60% ACN in water on both of these monolithic columns. A similar critical composition was observed on several other reported zwitterionic HILIC monolithic columns [27–29]. A possible method to increase the polarity of the zwitterionic HILIC monolithic column is to use a more hydrophilic monomer or crosslinker. For example, 2-(methacryloyloxy)ethyltrimethylammonium methyl sulfate (META) or 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) were co-polymerized with pentaerythritol triacrylate (PETA) in order to prepare either a poly(META-*co*-PETA) monolithic column [30] or a poly(AMPS-*co*-PETA) monolithic column [31] with the critical composition for the transition from HILIC to RP mode now around 20% of ACN. Unfortunately, the poor solubility of the highly hydrophilic monomers in the commonly used porogens was often a challenge. Previously, we developed a highly hydrophilic zwitterionic monolithic column by the thermal co-polymerization of 1-(3-sulfopropyl)-4-vinylpyridinium-betaine (4-SPV) and *N*,*N*′-methylenebisacrylamide (MBA) whose critical composition was around 30% ACN [32]. However, poor column-to-column reproducibility was obtained because the initial polymerization mixture did not completely dissolve and therefore only the supernatant liquid was introduced into the capillaries for the polymerization process.

In this study, a more polar monomer *N*,*N*-dimethyl-*N*-acryloyloxyethyl-*N*-(3-sulfopropyl)ammonium betaine (SPDA) and the more hydrophilic crosslinker MBA were employed in order to improve the polarity of the zwitterionic sulfoalkylbetaine type HILIC monolithic columns. Two novel zwitterionic HILIC monolithic columns, i.e. poly(SPDA-*co*-EDMA) and poly(SPDA-*co*-MBA) were prepared by the one-step in situ co-polymerization of corresponding monomer and crosslinker within 100 μm I.D. capillaries. The composition of the polymerization mixture was systematically optimized in order to obtain satisfactory performance for column permeability, efficiency and separation. The polarity of the poly(SPDA-*co*-MBA) monolithic column was compared with that of the poly(SPE-*co*-EDMA) monolithic column and the poly(SPDA-*co*-EDMA) monolithic column by investigating the influence of ACN on the retention factors of a test mixture consisting of thiourea, uracil and toluene. The effects of pH and salt concentration on the retention of charged analytes were also studied in order to confirm any electrostatic interaction. Other chromatographic and physical behaviors of this novel monolith were also evaluated using scanning electron microscopy and micro-HPLC. The final optimized highly hydrophilic zwitterionic monolithic column was applied to the separation of neutral, acidic and basic analytes in HILIC mode.

2. Experimental

2.1. Reagents and materials

The hydrophilic monomers (SPDA and SPE) were kindly gifted by Raschig GmbH (Ludwigshafen, Germany) whilst the Gly-Trp-Met-OH was kindly donated by Professor Hua Xiang from China Pharmaceutical University. EDMA, azobisisobutyronitrile (AIBN), 3-methacryloxypropyltrimethoxysilane (γ-MAPS), sodium hydroxide, ammonium formate, ammonium hydroxide, formic acid and all test compounds, including benzoic acid (B), 2-hydroxybenzoic acid (2-HB), 4-hydroxybenzoic acid (4-HB), 3,5-dihydroxybenzoic acid (3,5-DHB), 2,4-dihydroxybenzoic acid (2,4-DHB), 2,6-dihydroxybenzoic acid (2,6-DHB), 3,4,5-trihydroxybenzoic acid (3,4,5-THB), phenol, *p*-aminophenol, hydroquinone, pyrogallic acid, phloroglucinol, toluene, thiourea, thymine, uracil, adenine, cytosine, MBA, Gly-Leu and Gly-Gly-Gly were all purchased from Aladdin Chemical (Shanghai, China). Leu-Leu and Ala-Tyr were obtained from Sigma–Aldrich (Shanghai, China). HPLC-grade MeOH and ACN were obtained from Merck (Shanghai, China). The fused silica capillaries with a dimension of 100 μm I.D. (375 μm O.D.) were purchased from Yongnian Optic Fiber Plant (Hebei, China). Distilled water was filtered through a 2 μm membrane before experiments.

Cosmetic product was purchased from local market and pretreated following [33]. In brief, about 0.5 g sample and 0.5 g diatomaceous earth were placed into a 50 mL beaker. 10 mL *n*-heptane was added, and the mixture was stirred for 5 min using a magnetic stirrer. The organic phase was discarded, and the inorganic phase was extracted two more times. The residue was dried completely at 60 °C in a constant temperature oven. 3 mL water and 7 mL acetonitrile were added to the dried residue, stirred for 3 min, allowed to stand for 10 min, and then the solution layer was moved to a 10 mL centrifuge tube and centrifuged at 10,000 rpm for 10 min under 0 °C. All extracts was moved to a 25 mL volumetric flask and diluted with acetonitrile to volume. Prior to injection, the extract was filtered through a 0.22 μm filter.

2.2. Instruments

All micro-HPLC experiments were carried out on a self-assembled HPLC system which consisted of a Shimadzu SPD-15C UV detector (Kyoto, Japan) with a lab-made on-column detection cell, a four-port Valco injection valve with 20 nL internal loop (Houston, TX, USA) and a DiNa-S nano single pump (Tokyo, Japan). Data acquisition and date handling were performed using Unimicro Trisep™ Workstation 2003 (Shanghai, China). All chromatograms were converted into .txt files and then redrawn using Microcal Origin 8.5. A water bath was used for thermal initiated co-polymerization. All SEM experiments were carried out using an ultra-high resolution Hitachi S-4800 SEM (Tokyo, Japan) at an acceleration voltage of 3 kV.

2.3. Preparation of the monolithic column

In order to provide anchoring sites for the bulk polymer, all capillaries were pre-treated with the bi-functional reagent γ-MAPS prior to polymerization according to a previously reported method [23]. Various amounts of the functional monomers (SPDA or SPE), the crosslinkers (EDMA or MBA), the initiator (AIBN, ~1%, w/w, in terms of the monomers) and the porogens (methanol and/or water) were weighed into a 1.5 mL screw-cap vial and then ultrasonicated into a homogenous solution. After ultrasonication and purging for