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High efficiency polyethylene glycol diacrylate monoliths for reversed-phase capillary liquid chromatography of small molecules

Pankaj Aggarwal[a], John S. Lawson[b], H. Dennis Tolley[b], Milton L. Lee[a,*]

[a] Department of Chemistry and Biochemistry, Brigham Young University, Provo, UT 84602, USA
[b] Department of Statistics, Brigham Young University, Provo, UT 84602, USA

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ABSTRACT

Highly cross-linked monolithic networks (i.e., polyethylene glycol diacrylate, PEGDA) synthesized from monomers containing varying ethylene oxide chain lengths were fabricated inside fused silica capillary columns for use in liquid chromatography (LC) of small molecules. Tergitol was used as a surfactant porogen in combination with other typical organic liquid porogens. Column performance was correlated with quantitative descriptors of the physical/chemical properties of the monomers and porogens using a statistical model. Solubility and viscosity values of the components were identified as important predictors of monolith morphology and efficiency. The chromatographic retention mechanism was determined to be principally reversed-phase (RP) with additional hydrogen bonding between the polar groups of the analytes and the ethylene oxide groups embedded in the monolith structure. The fabricated monolithic columns were evaluated under RPLC conditions using phenols, hydroxy benzoic acids, and alkyl parabens as test compounds. Isocratic elution of hydroxy benzoic acids at a linear velocity of 0.04 cm/s using a PEGDA-700 monolith gave chromatographic peaks with little tailing (i.e., tailing factor < 1.28). The chromatographic efficiency measured for a non-retained compound (uracil) using this column was 186,000 plates/m when corrected for injector dead volume. High resolution gradient separations of selected pharmaceutical compounds and phenylurea herbicides were achieved in less than 18 min. Optimized monoliths synthesized from all four crosslinking monomers exhibited high permeability and demonstrated little swelling or shrinking in different polarity solvents. Column preparation was highly reproducible, with relative standard deviation (RSD) values less than 2.1%, based on retention times of the phenol standards (3 different columns).

1. Introduction

Chromatographic performance in liquid chromatography (LC) is governed by a variety of factors including stationary phase morphology (i.e., bed structure, skeletal dimensions and pore size distribution) [1]. The most important factor among these is the bed morphology, as it strongly influences diffusion of analyte into and out of the pores (i.e., resistance to mass transfer of analyte between stationary and mobile phases) [2–5]. In order to obtain high column efficiencies for particle packed columns, approaches have been developed to minimize the resistance to mass transfer, such as use of sub-2 μm particles [6–9] or core–shell particles [10,11]. However, this improvement in chromatographic performance comes with low column permeability and high back pressure. The availability of UHPLC pumps capable of operating at 1000–1200 bar provides the means to deliver the mobile phase at high pressures [7,12]; however, lower pressure pumping would be much more desirable.

Monolithic columns were introduced in the early 1990s as a low pressure alternative to particle packed columns [13,14]. An additional advantage of monolithic stationary phases compared to particle packed columns is that through-pore size and skeletal dimensions (analogous to particle diameter in packed columns) can be varied nonlinearly [15]. This offers the potential to engineer monolithic stationary phases with high porosities and thin skeletal sizes to reduce the resistance to mass transfer (i.e., improve chromatographic efficiency) without decreasing column permeability. Other attractive advantages of monolithic columns, such as easy preparation, wide selectivity, and applicability to most LC separation modes, have been illustrated in a number of recent excellent review articles [1,15–21].

Organic polymer monoliths typically exhibit agglomerated, inter-adhered globular morphologies intertwined with through-pores. Since they have relatively low surface areas and can be

* Corresponding author. Tel.: +1 801 422 2135.
E-mail address: milton_lee@byu.edu (M.L. Lee).

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synthesized from biocompatible monomers, they have proven advantageous for separation of large bio-molecules such as proteins, peptides, and oligonucleotides [22–26]. We have previously shown that polyethylene glycol diacrylate (PEGDA) monoliths are well-suited for separation of proteins and peptides under hydrophobic interaction chromatography (HIC) conditions [24,27]. This is due in large part to the low total mesopore volume in the polymer backbone, which severely limits diffusion of biomacromolecules into the stagnant mobile phase in the swollen monolith gel structure [28,29]. The surface areas for organic polymer monoliths generally range from single m^2/g to tens of m^2/g [30,31]. Therefore, radial mass transfer for large molecules is dominated by mobile phase convection because of the large through pore size in the monolith, with little contribution from diffusion into and in the stationary phase [32]. On the other hand, organic polymer monoliths have proven to be relatively ineffective for separation of small molecules because of high gel porosity and low mesopore volume [33–35].

Several new approaches have been reported for fabricating organic monoliths with larger surface areas to improve their separation performance for small molecules. These include copolymerization of stearyl methacrylate with several dimethacrylate crosslinkers differing in chain length and branching fragments [30], use of higher polymerization temperature [36], post-polymerization hyper-cross linking [31,37,38], early termination of the polymerization reaction [34,39,40] and addition of nanostructures such as carbon nanotubes and fullerenes [41]. All of these methods lead to higher crosslinking density with resulting higher surface area (e.g., an increase in surface area from 21 m^2/g to 424 m^2/g was reported by Urban et al. [38]), a major factor leading to improved column performance. Another straightforward approach to obtain highly crosslinked monolithic structures is to use a high concentration of crosslinking monomer in a multi-monomer system or use a single monomer crosslinker [34,42,43]. Our previous work has demonstrated the advantages associated with single monomer synthesis, including better mechanical stability, improved reproducibility, simpler optimization of polymerization conditions and, particularly important in this study, higher surface area [24,44–46]. Several moderately efficient RPLC monolithic columns have been synthesized using single diacrylate or dimethacrylate crosslinkers (e.g., pentaerythritol diacrylate monostearate [44] and neopentyl glycol dimethacrylate [46]), and demonstrated for separation of small alkyl benzene and alkyl paraben molecules.

In this work, we describe the fabrication and application of monoliths prepared from PEGDA monomers for RPLC of small molecules. The PEGDA monoliths were demonstrated for separation of phenols, hydroxy benzoic acids, alkyl parabens, pharmaceutical compounds (i.e., non-steroidal anti-inflammatory drugs) and acidic herbicides (i.e., phenylurea derivatives). The fabrication conditions were optimized using statistical principles with column efficiency as the guiding parameter. To our knowledge, this is the first study that demonstrates a quantitative correlation between physical/chemical properties of the monomer solution constituents and column efficiency, leading to the rational selection of porogens.

2. Experimental

2.1. Chemicals and reagents

The reagents, 2,2-dimethoxy-2-phenyl-acetophenone (DMPA, 99%), 3-(trimethoxysilyl)propyl methacrylate (TPM, 98%) and poly(ethyleneglycol) diacrylate (PEGDA, Mn 258, 302, 575, and 700), were purchased from Sigma–Aldrich (St Louis, MO, USA). All porogenic solvents and chemicals were HPLC or analytical reagent grade, respectively, and were used as received. Tergitol surfactants T-15-S-12, T-15-S-15, and T-15-S-20, also used as co-porogens, were obtained from Dow Chemical, Midland, MI, USA. The standard compounds, phenol, catechol, resorcinol, pyrogallol, benzoic acid, 2-hydroxy benzoic acid, 3-hydroxy benzoic acid, 3,4-dihydroxy benzoic acid, 2,4-dihydroxy benzoic acid and 3,4,5-trihydroxybenzoic acid were obtained from Sigma–Aldrich. Methyl paraben, ethyl paraben, propyl paraben and butyl paraben were purchased from Fluka (Buchs, Switzerland). Mixtures of phenylurea herbicides (i.e., isoproturon, monuron, monolinuron, diuron and linuron) and pharmaceutical compounds (i.e., paracetamol, ibuprofen, aspirin and indomethacin) were purchased from Sigma–Aldrich.

2.2. Polymer monolith preparation

Monoliths were synthesized inside pre-treated UV transparent Teflon-coated capillaries (150 μm i.d.). The inner surface of the capillary was functionalized by (1) flushing with ethanol and HPLC grade water to clean the surface, (2) etching the inner surface with 1 M NaOH at 120 °C for 3 h followed by leaching with 1 M HCl for 3 h at 110 °C, (3) rinsing with water and ethanol followed by drying with nitrogen purge at 110 °C overnight in a GC oven, (4) treating the capillary with a 15% solution of TPM in dry toluene overnight at room temperature to introduce diacrylate groups and (5) washing the column with toluene and acetone to remove residual TPM followed by drying with nitrogen purge at room temperature.

Monomer solutions were prepared in 1-dram (4 mL) glass vials by admixing initiator, monomer, and porogen solvents (Table 1). The solution was vortexed and then degassed by sonicating for 2 min if non-volatile solvents were used as porogens. For volatile porogens, the solution was only vortexed to prevent vaporization of porogen. A section of the surface treated capillary was cut and filled with monomer solution using helium gas pressure. One end of the capillary was left empty for on-column UV detection. After introducing the reagent solution, the capillary was sealed with rubber septa at both ends and placed directly under a PRX 1000–20 Exposure Unit UV lamp (390 ± 15 nm, 1000 W, TAMARACK Scientific, Corona, CA, USA). Columns were exposed to a light intensity of 1 mW/cm^2 for a time period of 5 min. The light intensity was measured using an ACCU-CAL^{tm}-30 UV intensity meter (DYMAX, Torrington, CT, USA). Monoliths obtained after exposing with UV light were flushed with methanol and then water until stable pressure readings were obtained. An FEI Helios Nanolab 600 dual-beam scanning electron microscope (SEM) (Hillsboro, OR) was used to provide visual images of the monolith surface structures. SEM images were collected from 0.5 cm long monolithic columns coated with a conducting gold layer to overcome charging of the samples.

2.3. Capillary liquid chromatography

The capillary liquid chromatography system was an Ultimate 3000 high-pressure gradient LC system (Dionex, Sunnyvale, CA, USA) equipped with an FLM-3300 nanoflow manager (1:1000 spilt ratio). The system was operated with Chromeleon software. A zero dead-volume nanoViper (Thermofisher Scientific, Sunnyvale, CA, USA) loop having a volume of 1 μL was used as sample loop. The sample injection volume was kept constant at 30 nL for all experiments using time-gated injection by switching the injection valve at a specific time interval for each mobile phase flow rate. The dead volume of the system was determined to be 21–35 nL, depending on the flow rate [47]. This dead volume dependence on flow rate can be attributed to exponential peak tailing that occurs at low flow rates and low injection volumes, commonly employed in capillary LC. On-column detection was performed using a Crystal 100