



Monolithic stationary phases with incorporated fumed silica nanoparticles. Part I. Polymethacrylate-based monolithic column with incorporated bare fumed silica nanoparticles for hydrophilic interaction liquid chromatography



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ABSTRACT

Fumed silica nanoparticles (FSNPs), were incorporated for the first time into a polymethacrylate monolithic column containing glyceryl monomethacrylate (GMM) and ethylene dimethacrylate (EDMA) in order to develop a new monolithic column for hydrophilic interaction high performance liquid chromatography (HILIC). When compared to poly(GMM-EDMA) monolithic column without FSNPs, the same monolithic column with incorporated FSNPs yielded important effects on HILIC separations. The effects of monomers and FSNPs content of the polymerization mixture on the performance of the monolithic column were examined in details, and the optimized stationary phase was investigated over a wide range of mobile phase composition with polar acidic, weakly basic and neutral analytes including hydroxy benzoic acids, nucleotides, nucleosides, dimethylformamide, formamide and thiourea. The retention of these analytes was mainly controlled by hydrophilic interactions with the FSNPs and electrostatic repulsion from the negatively charged silica surface in the case of hydroxy benzoic acids and nucleotides. The electrostatic repulsion was minimized by decreasing the pH of the aqueous component of the mobile phase, which in turn enhanced the retention of acidic solutes. Nucleotides were best separated using step gradient elution at decreasing pH as well as ACN concentration in the mobile phase. Improved peak shape and faster analysis of nucleosides were attained by a fast linear gradient elution with a shallow decrease in the ACN content of the ACN-rich mobile phase. The run-to-run and column-to-column reproducibility were satisfactory. The percent relative standard deviations (%RSDs) for the retention times of tested solutes were lower than 2.5% under isocratic conditions and lower than 3.5 under gradient conditions.

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1. Introduction

Hydrophilic interaction chromatography (HILIC) has gained wide acceptance in the life sciences for solving important separation problems (for recent reviews see Refs. [1–3]). HILIC, which was first introduced by Alpert using bonded silica-based polar stationary phases [4], continues to employ silica-based bonded phases (for recent reviews see Refs. [5,6]). In addition, HILIC has been expanded to using polar polymeric stationary phases [2], including those based on monolithic approaches in both electro- and liquid phase separation techniques [7–9]. These trends were fac-

ilitated by the rapid developments of microparticles and porous monoliths in recent years (for recent reviews, see Refs. [10,11]). Currently, monolithic columns have proven to be good alternatives to particle-packed columns and have become an important niche in the development of HPLC column technology [7,10,12].

Despite the progress made in HILIC monoliths in the past decade [13], most of these stationary phases have been silica-based monoliths and to a much lesser extent organic polymer-based monoliths. This has been majorly due to the limited number of commercially available polar monomers and their low solubility in many organic porogens, which necessitated the inclusion of water and short alkyl chain alcohols [14,15]. To remedy this shortcoming, post hydrophilization of organic polymer monoliths have been introduced. This involved the covalent attachment of stratified polar layers [14] or grafting the polymeric surface with allyl groups through which polar groups are attached by click chemistry (for representative see Refs. [16,17]). In another thought, adding to the

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polymerization mixture of a relatively polar organic monolith a polar inorganic entity as a polar amendment would be very beneficial in order to realize a monolith that combines the favorable morphology of organic polymer-based monoliths with the polar character of an inorganic entity. More precisely, silica nanoparticles would be the choice for such polar inorganic amendment. The current investigation reports on such an approach using fumed silica nanoparticles as the inorganic polar amendment. The merit of such an approach resides in the fact that nano-entities as separation media yield stationary phases with more developed surfaces, and the smaller the size of the nanoparticles, the higher the specific surface area of the resulting materials. Also, nano-entities permit the achievement of widely differing selectivity because of the unique surface adsorption properties of nanoparticles (for recent reviews on this topic, see Refs. [18,19]).

Because of the nano size of their particles, nano-entities must be held on a good support media with favorable flow characteristics that can be readily prepared and confined in columns. Polymeric monoliths are the preferred support media for incorporating nano entities since they are readily prepared via in situ polymerization in columns and channels of all sizes, can be tailor made from many available monomers, can trap or incorporate covalently nano entities, and have very favorable flow characteristics. Recently, the sound characteristics of polymer-based monoliths (e.g., high permeability and through pores) have been exploited to design stationary phases of different selectivity than the simple nonpolar monolithic columns with alkyl ligands by incorporating into the nonpolar monoliths nano-entities (e.g., multiwalled carbon nanotubes MWCNTs) to the extent that these entities would not obstruct the porous structures of the original nonpolar monoliths or disrupt the realization of a mechanically stable monolithic structure [20]. The incorporated MWCNTs provided additional interactions, and in turn yielded different selectivity thus enhancing the overall separation. In another thought, the ideal flow characteristics of monoliths should make them suitable supports for nano entities that can afford distinct selectivity toward a wide range of solutes in the aim of realizing “nano entities-based stationary phases”. This was recently demonstrated by Mayadunne and El Rassi [20] by designing a blank monolith with no significant matrix interactions with the solutes of interest on which MWCNTs were immobilized to provide the chromatographic retention.

Fumed silica, which is produced by continuous flame hydrolysis of silicon tetrachloride in an oxyhydrogen gas flame at 1000° C, has the following characteristics: amorphous, highly dispersed, nonporous, and hydrophilic [21,22]. Since fumed silica consists basically of nanoparticles, it is then referred to as fumed silica nanoparticles (FSNPs). The FSNPs used in this study is commercially available under the trade name of Aerosil 200 and has an average primary particle size of ~12 nm and $S_{BET} = 200 \pm 25 \text{ m}^2/\text{g}$ (for more detailed characteristics see the bulletin [21]). In a recent report, FSNPs were incorporated into hydrophilic poly(ethylene glycol)-based monoliths in a spin-tip format in the aim of mechanically stabilizing the monolithic structure. The spin-tip columns thus obtained were amino-functionalized and further used as adsorbents for the immobilization of concanavalin A for lectin affinity chromatography purification of ovalbumin [23]. The present research report demonstrated, for the first time, the use of FSNPs incorporated into a polymer based monolithic columns to enhance the separation performance of HILIC-HPLC of small polar organic and bio-molecules.

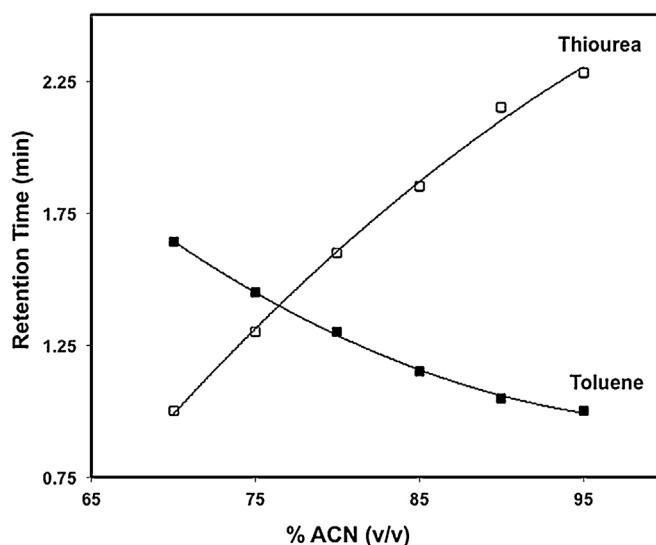


Fig. 1. Retention times of toluene and thiourea vs. %ACN (v/v) in the mobile phase. Column, 10 cm × 4.6 mm at 3.1 mg fumed silica/cm; mobile phase, water at various %ACN (v/v); detection wavelength, 254 nm; flow rate, 1.0 mL/min.

2. Experimental

2.1. Instrumentation

HPLC experiments were performed on a binary gradient system consisting of a constaMetric Model 3500 solvent delivery system (LDC Analytical, Rivera Beach, FL, USA) coupled with a constaMetric pump Model III metering pump from Milton Roy, LDC division (Riviera Beach, FL, USA), a Spectra 100 variable wavelength detector from Thermo Separation Products (Waltham, MA, USA) and a high pressure injection valve Model 7125 from IDEX/Rheodyne (Rohnert Park, CA, USA). Chromatograms were recorded with PowerChrom software version 2.5.4 from eDAQ (Denistone East, NSW, Australia).

A Branson 1510 ultrasonic cleaner was from Branson Ultrasonic Corp. (Danbury, CT, USA). A water bath equipped with a Fisher Scientific Isotemp 2100 immersion circulator was from Thermo Fischer Scientific (Waltham, MA, USA).

2.2. Chemicals and materials

Fumed silica nanoparticles (Aerosil-200) were gifted by Evonik Industries (Parsippany, NJ, USA). Hydroxybenzoic acids, nucleosides, nucleotides, 2,2'-azobis(isobutyronitrile) (AIBN), ethylene glycol dimethacrylate (EDMA), polyethylene glycol 1000 (PEG1000) were purchased from Sigma-Aldrich (Milwaukee, WI, USA). Glyceryl monomethacrylate (GMM) was from Monomer-Polymer and Dajac Labs (Trevose, PA, USA). Stainless steel tubing of 4.6 mm id was obtained from Alltech Associates (Deerfield, IL, USA). Acetonitrile (ACN) and methanol were purchased from Pharmco Aaper (Brookfield, CT, USA).

2.3. Preparation of FSNPs incorporated monolithic columns

The monolithic stationary phases were directly synthesized by *in-situ* polymerization within the stainless steel column tube. The method was similar to the one described by Mayadunne and El Rassi for the incorporation of carbon nanotubes in poly(glyceryl monomethacrylate-ethylene glycol dimethacrylate) abbreviated as poly(GMM-EDMA) [20]. GMM 25.27 wt%, EDMA 14.2 wt%, FSNPs at varying wt% (0.35–2.7 wt%) were added to the binary porogenic solvent consisting of cyclohexanol 28.8–29.98 wt% and dodecanol 28.8–29.98 wt% in order to complete to a total of 100 wt% with

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