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Facile preparation of octadecyl monoliths with incorporated carbon nanotubes and neutral monoliths with coated carbon nanotubes stationary phases for HPLC of small and large molecules by hydrophobic and π–π interactions

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ABSTRACT

Two approaches for incorporating carbon nanotubes into monolithic columns for HPLC are described in this report. They pertain to the investigation of carbon nanotubes either (i) as entities to modulate solute retention on monolithic columns bearing well defined retentive ligands or (ii) as entities that constitute the stationary phase responsible for solute retention and separation. Approach (i) involved the incorporation of carbon nanotubes into octadecyl monolithic columns while approach (ii) concerns the preparation and evaluation of an ideal monolithic support and coating it with carbon nanotubes to yield a real "carbon nanotube stationary phase" for the HPLC separation of a wide range of solutes. First, an octadecyl monolithic column based on the *in situ* polymerization of octadecyl acrylate and trimethylolpropane trimethacrylate was optimized for use in HPLC separations of small and large solutes (e.g., proteins). To further modulate the retention and separation of proteins, small amounts of carbon nanotubes were incorporated into the octadecyl monolith column. In approach (ii), an inert, relatively polar monolith based on the *in situ* polymerization of glyceryl monomethacrylate (GMM) and ethylene glycol dimethacrylate (EDMA) proved to be the most suitable support for the preparation of "carbon nanotube stationary phase". This carbon nanotube "coated" monolith proved useful in the HPLC separation of a wide range of small solutes including enantiomers. In approach (ii), a more homogeneous incorporation of carbon nanotubes into the diol monolithic columns (i.e., GMM/EDMA) was achieved when hydroxyl functionalized carbon nanotubes were incorporated into the GMM/EDMA monolithic support. In addition, high power sonication for a short time enhanced further the homogeneity of the monolith incorporated with nanotubes. In all cases, nonpolar and π interactions were responsible for solute retention on the monolith incorporated carbon nanotubes.

1. Introduction

Monolithic columns are continuously attracting strong interest due to their unique characteristics such as high permeability [1,2] and excellent mass transfer properties that arise from their flow throughpores [3,4] in contrast with columns packed with microparticles that are characterized by slow diffusional mass transfer in the porous particles and large void space between the packed particles [5–8] which translate into a relatively increased band broadening. Polymer-based monolithic columns, which are the subject of this investigation, have evolved significantly in the last decade and have proven useful in the separation of a wide range of mixtures [9–14]. Although polymer-based monolithic columns can be readily prepared and confined in columns in all sizes, these media suffer from low surface area and in turn limited retention toward small solutes. However, their low surface areas make monolithic columns ideal for the efficient separations of biopolymers (e.g., proteins) using gradient elution in HPLC [6,15] or simply isocratic elution in capillary electrochromatography (CEC) [16,17].

Some of the characteristics of polymer-based monoliths, namely their high permeability and throughpores can be exploited to design stationary phases of different selectivities than the simple nonpolar monolithic columns with alkyl ligands by incorporating into their structure nano-entities to the extent that these entities would not obstruct the porous structures of the original

Abbreviations: AIBN, 2,2′-azobis(isobutyronitrile); BMA, butyl methacrylate; Dns, DL-dansyl; EDMA, ethylene glycol dimethacrylate; GMM, glyceryl monomethacrylate; MMA, methyl methacrylate; MWCNTs, multiwalled carbon nanotubes; ODA, octadecyl acrylate; TFA, trifluoroacetic acid; TRIM, trimethylolpropane trimethacrylate

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monoliths or disrupt the realization of a mechanically stable monolithic structure. Under these conditions, the incorporated nano entities may not increase significantly the surface area of the monolith but rather they would provide other interactions in the aim of achieving different selectivity that would compensate for the limited retention generally observed with small molecules. In another thought, the ideal flow characteristics of monoliths should make them suitable support for nano entities that can afford distinct selectivity toward a wide range of solutes with the aim of realizing "nano entities-based stationary phases".

A few nano entities, including carbon nanotubes and nanoparticles, fullerenes and nanodiamonds as well as metal oxide and gold nanoparticles have been reported in nanomaterials-based separation media for gas chromatography, HPLC, CEC, CE and microchip electrophoresis and the field has been reviewed recently in 2012 and 2013 by Speltini et al. [18], Nesterenko et al. [19] and by Pauwels and Van Schepdael [20]. As far as HPLC with organic-based polymer monolithic columns having incorporated carbon nanoparticles is concerned, only a few attempts have been made in this area (see Svec's recent review article [21]). After a brief study reported in 2005 by Li et al. [22] which involved nano-LC with carbon nanotubes entrapped into a monolithic capillary column made of poly(chloromethylstyrene-co-ethylene dimethacrylate), Chambers et al. [23] reported in 2011 the incorporation of carbon nanotubes in porous polymer monolithic capillary columns consisting of poly (glycidylmethacrylate-co-ethylene dimethacrylate) monoliths for the chromatographic separation of some alkylbenzenes. More recently, Arrura et al. [24,25] introduced monolithic cryopolymers with neutral and charged embedded nanoparticles for capillary liquid chromatography of proteins under a hydrophobic interaction chromatography mode as well as under ion exchange conditions. Although nonpolar polymer based monoliths allows the rapid separation of proteins by linear gradient elution HPLC, the nonpolar monolithic phases may benefit from fine tuning their selectivity by incorporating adequate amount of nano entities into their structures such as carbon nanotubes.

Thus, it is the aim of this investigation to optimize nonpolar octadecyl monolith for HPLC separations by adjusting the fabrication conditions of these monoliths and by further incorporating into their structure an adequate amount of carbon nanotubes. Also, reported here are some initial studies on the fabrication of a blank monolith "void" of strong interactions with the solutes of interest in the aim of functioning as an ideal support for realizing columns coated with "carbon nanotube stationary phases" for the HPLC of small solutes including some enantiomers.

2. Materials and methods

2.1. Apparatus

The HPLC setup consisted of a quaternary solvent delivery system Model Q-Grad pump from Lab Alliance (State College, PA, USA), a multiple solvent delivery system Model CM4000, and a Model SpectroMonitor 3100 UV–vis variable wavelength detector from Milton Roy, LDC division (Riviera Beach, FL, USA) and a Rheodyne injector Model 7010 (Cotati, CA, USA) equipped with a 20 μL loop. A constant pressure air-driven pump Model Shandon from Southern Products Limited (Cheshire, UK) was used for column packing. A Branson1510 ultrasonic cleaner from Branson Ultrasonic Corp. (Danbury, CT, USA). A water bath equipped with a Fisher Scientific Isotemp 2100 immersion circulator and a high power sonicator Model 50 Sonic Dismembrator were from Thermo Fischer Scientific (Waltham, MA, USA).

2.2. Reagents and materials

Multiwalled carbon nanotubes were purchased from Sun Innovation Inc. (Fremont, CA, USA). Alkylbenzenes, phenoxy acid herbicides, cyanobenzene derivatives, benzonitrile, aniline derivatives, trifluoroacetic acid (TFA), 2,2′-azobis(isobutyronitrile) (AIBN), octadecyl acrylate (ODA), butyl methacrylate (BMA), trimethylolpropane trimethacrylate (TRIM), ethylene glycol dimethacrylate (EDMA), ethylene glycol, methyl methacrylate (MMA), chlorophenols, 1-dodecanol, cyclohexanol, DL-dansyl (Dns) amino acids, were purchased from Sigma Aldrich (Milwaukee, WI, USA). Glyceryl monomethacrylate (GMM) was from Monomer–Polymer and Dajac Labs (Trevose, PA, USA). Egg white lysozyme, bovine serum albumin, ribonuclease A, ovalbumin, horse heart cytochrome C, bovine erythrocytes carbonic anhydrase, bovine milk β–lactoglobulin A and B and bovine milk α-lactalbumin were purchased from Sigma (St. Louis, MO, USA). HPLC grade acetonitrile and isopropyl alcohol were purchased from Pharmco Aaper (Brookfield, CT, USA). Stainless steel tubing of 4.6 mm id was obtained from Alltech Associates (Deerfield, IL, USA).

2.3. Preparation of monolithic columns

In all cases, and for the preparation of ODM based monoliths, polymerization mixtures consisting of 5.5 g each were prepared by weighing monomers and porogens as follows. 7-wt% ODA and 14.5-wt% TRIM were added to 78.5-wt% ternary porogen of cyclohexanol, ethylene glycol and water. While the % water was maintained constant at 3.2-wt%, cyclohexanol composition was decreased in the range of 54.2–50-wt% and that of ethylene glycol was increased in the range of 20.9–24.48-wt% so that the total porogen amounted in total to 78.5-wt%. All polymerization solutions for making the monoliths were vortexed for 1 min, sonicated at 40 °C for 15 min, purged with nitrogen for 5 min and introduced into stainless steel columns with dimensions of 25 cm × 4.6 mm I.D. that function as a mold for the monolith. Both column ends were plugged tightly and heated at 60 °C in a water bath for 15 h. The monolithic column was washed with acetonitrile for 30 min followed by isopropyl alcohol. The monolith was transferred from 25 cm mold to a shorter column of 10 cm × 4.6 mm I.D. by connecting the two columns with ¼ -union and passing isopropyl alcohol using a constant pressure pump starting at 6000 psi until the monolith was completely transferred. The transfer of monoliths from column-to-column has been performed in our laboratory for many HPLC column applications without noticeable adverse effect on column performance; for typical recent references see [26,27]. This is because by producing the initial monolithic mold in a longer column than the final column length, any shrinkage due to the formation of the monolith will be at the inlet of the mold. In the transfer process, the shorter column is connected to the bottom of the mold *via* a union. Under this condition, the final monolithic column is filled totally with the monolith without any void in its structure. Different amounts and types of MWCNTs were added to the ODM monolith as discussed later.

For the monoliths with retention characteristics due to the incorporated carbon nanotubes, polymerization mixtures of 6 g each were prepared by weighing monomers and porogens as described below. All the mixtures were first vortexed for 1 min, sonicated at 40 °C for 15 min, purged with nitrogen for 5 min and then introduced into a stainless steel column of dimensions 25 cm × 4.6 mm I.D. that functions as a mold for the monolith. Both column ends were plugged tightly with column end fittings and thereafter heated at 50–60 °C in a water bath for 15–20 h. The monolithic columns thus obtained were washed as stated in the preceding section. The monolith was transferred from 25 cm mold to a shorter column of 10 cm × 4.6 mm I.D. as explained above.