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Robust naphthyl methacrylate monolithic column for high performance liquid chromatography of a wide range of solutes

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ABSTRACT

An organic monolithic column based on the co-polymerization of 2-naphthyl methacrylate (NAPM) as the functional monomer and trimethylolpropane trimethacrylate (TRIM) as the crosslinker was introduced for high performance reversed-phase liquid chromatography (RPC). The co-polymerization was performed *in situ* in a stainless steel column of 4.6 mm i.d. in the presence of a ternary porogen consisting of 1-dodecanol and cyclohexanol. This monolithic column (referred to as naphthyl methacrylate monolithic column or NMM column) showed high mechanical stability at relatively high mobile phase flow velocity indicating that the column has excellent hydrodynamic characteristics. To characterize the NMM column, different probe molecules including alkyl benzenes, and aniline, benzene, toluene and phenol derivatives were chromatographed on the column and the results in terms of *k*, selectivity and plate counts were compared to those obtained on an octadecyl silica (ODS) column in order to assess the presence of π–π and hydrophobic interactions on the NMM column under otherwise the same elution conditions. The NMM column offered additional π–π interactions with aromatic molecules in addition to hydrophobic interactions under RPC elution conditions. Run-to-run and column-to-column reproducibility of solute *k* values were evaluated, and percent relative standard deviation of <1% and ~2–3.5%, respectively, were obtained. Six standard proteins were readily separated on the NMM column using shallow (30 min at 1.0 mL/min), steep (10 min at 1.0 mL/min) and ultra steep (1 min at 3.0 mL/min) linear gradient elution at increasing ACN concentration in the mobile phase using a 10 cm × 4.6 mm i.d. column in case of shallow and steep linear gradients and a 3 cm × 4.6 mm i.d. column for ultra steep linear gradient.

1. Introduction

Since its early stages of development, high performance reversed-phase chromatography (RPC) using nonpolar stationary phases has been practiced primarily with surface bound n-alkyl chain ligands and predominantly with n-octadecyl (i.e., C18) bound surfaces [1]. In search for different selectivity stationary phases, other nonpolar sorbents were introduced and in particular surfaces bearing, among other things, aromatic ligands, e.g., phenyl, naphthyl, anthryl, pyrenyl [2–5] and pentafluorophenyl [6]. These aromatic surfaces offer additional π–π interactions apart from their hydrophobicity [7–9]. This combined feature of stationary phases with surface bound aromatic ligands has been exploited in the separation of various kinds of aromatic compounds [2,10–12]. π–π interactions are based on the fact that aromatic stationary phases are π-electrons rich (i.e., soft Lewis bases) [13], which can then associate with solutes that are relatively π-electrons deficient (i.e., soft Lewis acids) such as aromatic compounds with electron withdrawing substituents (i.e., deactivating substituents) thus leading to π–π interactions that are considered as a type of electron donor–electron acceptor interactions between the stationary phase and the solutes [9].

Monolithic columns are currently witnessing increased use in liquid phase separation techniques (for recent reviews, see [14,15]) due to their distinctive characteristic features, including relatively high permeability, rapid solute mass transfer through the network of mesopores that are interconnected with large flow through pores and the readily tailor made surface chemistry (for a recent review, see [16]). Despite the major progress made in monolithic columns for liquid phase separations, organic monolithic columns with phenyl ligands for HPLC separations have been mostly based on the traditional poly(styrene-co-divinylbenzene) monolith [17–20] and its variants such as poly(p-methylstyrene-co-1,2-bis(p-vinylphenyl)ethane) [21], poly(phenyl acrylate-co-1,4-phenylene diacrylate) [22] and

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poly(styrene-divinylbenzene-methacrylic acid) [23]. Therefore, it is the aim of this research report to further the development of monolithic columns with aromatic ligands by optimizing a recently developed naphthyl monolithic (NMM) column with surface bound naphthyl ligands, which was introduced by Karenga and El Rassi for CEC separations of aromatic compounds *via* π–π and hydrophobic interactions [7]. This NMM monolith is derived from the co-polymerization of 2-naphthylmethacrylate (NAPM) as the functional monomer and trimethylolpropane trimethacrylate (TRIM) as the crosslinker. As will be discussed below, the basic composition of the polymerization mixture was altered and tailored to suit HPLC use.

2. Experimental

2.1. Instrumentation and procedures

HPLC experiments were performed on an in-house assembled instrument consisting of a quaternary solvent delivery system Model Q-grad pump from Lab Alliance (State College, PA, USA), a Model Spectromonitor 3100 UV–vis variable wavelength detector from Milton Roy, LDC Division (Riviera Beach, FL, USA), and a Rheodyne high pressure injection valve Model 7010 from IDEX Health & Science LLC (Rohnert Park, CA, USA) equipped with a 20 μL injection loop. The chromatograms were occasionally recorded with a C-R5A integrator from Shimadzu (Kyoto, Japan). Otherwise, data collection was made by Clarity Chromatography Station v3.0.06.589 (Data Apex, Prague, The Czech Republic) and the chromatographic data were processed by OriginPro v8.5.1 (Origin Lab Corp., Northampton, MA, USA). During the experiment, the entire HPLC system including the pump, injector, column and detector was kept at ambient temperature. The UV detector was set at 214 nm, and a constant flow rate was maintained at 1.00 mL/min, unless otherwise mentioned. The number of theoretical plates of a column (N) was calculated based on peak width at inflection point.

An HPLC solvent delivery system Model M-45 from Waters Associates (Milford, MA, USA) was used to wash the monolithic column from porogens and unreacted monomers. Constant pressure pump from Shandon Southern Products Ltd. (Cheshire, UK) was used to transfer the monolithic mold at high pressures into the HPLC column. Water bath Model Isotemp 105 from Fischer scientific (Pittsburgh, PA, USA) was used to carry out the polymerization reactions at specified temperatures.

Standard solutions of various compounds were prepared within the concentration range of 2.0–5.0 μg/L and were stored in a lab refrigerator at 4 °C. Protein samples were prepared in the concentration range of 0.02–0.05 mg/mL. Water was thoroughly filtered through a 0.45 μm membrane (Millipore, Bedford, MA, USA) before use to remove micro-particulates.

2.2. Reagents and materials

Stainless steel tubing of 4.6 mm i.d. was obtained from Alltech Associates (Deerfield, IL, USA). Columns were made with Swagelok end fittings purchased from Crawford Fitting Co. (Solon, OH, USA). Octadecyl silica (ODS) UltraSphere™ column, 5-μm average particle diameter, 4.5 cm × 4.6 mm i.d. was obtained from Beckman Coulter Inc. (Brea, CA, USA). Methacrylol chloride, 2-naphthol, diethyl ether (analytical grade), triethylamine (TEA), 1-dodecanol, 2,2′azobis(isobutyronitrile) (AIBN), TRIM, cyclohexanol, alkylbenzenes (ABs), chlorophenols, aniline, benzene, toluene, their derivative compounds and all other test standard solutes were purchased from Sigma-Aldrich (Milwaukee, WI, USA). Nitrobenzene, ethyl acetate (HPLC grade) and chloroform (GR grade) were obtained from Fisher Scientific Co. (Fair lawn, NJ, USA). Benzaldehyde was obtained from Mallinckrodt (St. Louis, MO, USA). Acetonitrile (HPLC grade), methanol (analytical grade), isopropyl alcohol (IPA) and hexanes (GR grade) were obtained from Pharmco-Aaper (Brookfield, CT, USA). Standard proteins such as bovine milk β-lactoglobulin A, horse skeletal muscle myoglobin, equine heart cytochrome C, chicken egg white lysozyme, bovine pancreas ribonuclease A and α-chymotrypsinogen A were obtained from Sigma (St. Louis, MO, USA).

2.3. Preparation of monolith

2.3.1. Monomer synthesis

The monomer NAPM was synthesized according to a previously published procedure [24] and characterized by ^{1}H NMR and ^{13}C NMR. Firstly in a 500 mL round bottom flask 10 g of 2-naphthol (69.4 mmol) dissolved in 150 mL of diethyl ether, 14.5 mL of TEA (104.0 mmol) were added and constantly stirred by a magnetic stirrer. To this solution, 8.0 mL of methacrylol chloride (81.9 mmol) dissolved in 100 mL of diethyl ether were added drop-wise for about 1 h *via* dropping funnel while the reaction is kept at 0 °C, the mixture was continuously stirred for another 24 h at room temperature. The resulting precipitate was filtered off and the solvent was evaporated *in vacuo* to get white colored powder. This powder was again dissolved in little amount of chloroform and purified by column chromatography on a silica gel of bed dimensions 50 cm × 3 cm, using 10% (v/v) ethyl acetate in hexanes as the mobile phase. The obtained fractions were concentrated *in vacuo* and the white solid thus obtained showed a peak purity of 98.12% (m/z 212.2) as analyzed by GC–MS. This purified white solid was characterized by ^{1}H and ^{13}C NMR. In ^{1}H NMR (400 MHz, chloroform-d), the following signals characteristic of the product NAPM were observed: δ 7.90–7.83 (m, 2H), 7.83–7.79 (m, 1H), 7.60 (d, J = 2.1 Hz, 1H), 7.49 (dtd, J = 12.7, 6.8, 3.4 Hz, 2H), 7.28 (d, J = 2.3 Hz, 1H), 6.41 (s, 1H), 5.80 (s, 1H), 2.11 (s, 3H). The ^{13}C NMR (chloroform-d) produced the following unique signals for the NAPM product: δ 166.26, 148.77, 136.12, 127.97, 127.56, 125.86, 121.40, 118.76, 18.66.

2.3.2. In situ *polymerization*

The reaction mixture for the NMM column was prepared as follows: The reactants were weighed into a clean glass vials with 4.7 g of total polymeric solution comprising 13.46% (w/w) NAPM as the functional monomer, 16.50% (w/w) TRIM as the crosslinker, the porogenic solvents cyclohexanol, 1-dodecanol and water in the proportions of 53.87% (w/w), 13.64% (w/w) and 2.53% (w/w), respectively. The mixture was gently shaken in a water bath at 40 °C to facilitate the dissolution of NAPM, vigorously mixed in a vortex mixture and sonicated for about 20 min. Thereafter, the polymerization mixture containing AIBN (1% w/w with respect to monomers) was introduced into a stainless steel column (250 mm × 4.6 mm i.d.) with fittings at both column ends, which were then sealed with end plugs. Thereafter, the polymerization was allowed to proceed at 60 °C for 12 h in a water bath. The end plugs were removed and the column was washed with acetonitrile to remove any traces of unreacted monomers and the porogenic solvents. Following, the column was equilibrated with IPA and then using the same solvent, a packing pressure at 8000 psi was used to transfer this 'mold' into a 100 mm × 4.6 mm i.d. stainless steel column. Usually, upon monolith formation a contraction may take place at both ends of the mold, and most often at the column inlet end. The high-pressure transfer to a shorter column will result in a total filling of the shorter column thus removing any column contraction or voids, which could form during the polymerization process in the mold column by making the transferred portion of the monolith to the shorter column tightly compressed and in turn void free. Thereafter, the column was conditioned for 1 h with acetonitrile prior to the chromatographic tests.