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Covalent attachment of polymeric monolith to polyether ether ketone (PEEK) tubing

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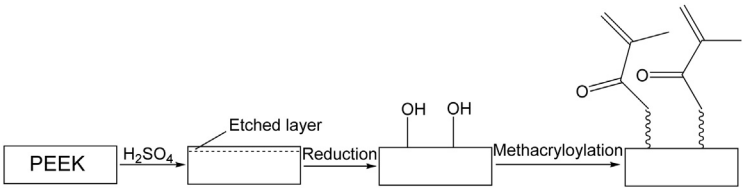
HIGHLIGHTS

- Reproducible polymeric monolithic columns with close to conventional LC dimensions.
- A three-step procedure for covalently anchoring the monolith to PEEK surface.
- The highest plate numbers beyond 30 000 plates per meter.

GRAPHICAL ABSTRACT



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ABSTRACT

A new method of reproducible preparation of vinylic polymeric monolithic columns with a key step of covalently anchoring the monolith to PEEK surface is described. In order to chemically attach the polymer monolith to the tube wall, methacrylate functional groups were introduced onto PEEK surface by a three-step procedure, including surface etching, surface reduction and surface methacryloylation. The chemical state of the modified tubing surface was characterized by attenuated total reflectance infrared (ATR-IR) spectroscopy. It was found that the etching step is the key to successfully modifying the PEEK tubing surface. Poly(styrene-*co*-divinylbenzene) monoliths were *in situ* synthesized by thermally initiated free radical copolymerization within the confines of surface-vinylized PEEK tubings of dimensions close to ones conventionally used in HPLC and UHPLC (1.6 mm internal diameter, 10.0–12.5 cm length). Adhesion test was done by measuring the operating pressure drop, which the prepared stationary phases can withstand. Good pressure resistance, up to 140 bar/10 cm (flow rate 0.5 mL min^{-1}, acetonitrile as a mobile phase), indicates strong bonding of monolith to the tubing wall. The monolithic material was proven to have a permeability of 1.7×10^{-14} m^2, applying acetonitrile–water 70:30 (v/v) as a mobile phase.

The column performance was reproducible from column to column and was evaluated via the isocratic separation of a series of alkylbenzenes in the reversed-phase mode (acetonitrile–water 70:30, v/v). The numbers of plates per meter at optimal flow rate were found to be between 26 000 and 32 000 for the different analytes.

1. Introduction

Approximately two decades ago, organic monolithic stationary phases were introduced by the pioneering works of Hjerten [1] and Svec [2] for use in high performance liquid chromatography. These single-piece polymeric materials possess high permeability due to the network of large canal-like pores which traverse their body, and offer acceptable separation efficiencies at considerably lower back pressures as compared to conventional packed particle columns. Development and application of monolithic media became a hot research area in separation

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science since the first emerging of these promising materials and is still full of dynamic activity [3,4].

Although organic monolithic stationary phases can be prepared in various sizes (micrometer scale [5,6] to centimeter scale [7]) and shapes [8], most of them are made within capillaries of inner diameters less than 200 μm. The main reason for this is that high-efficiency monolithic columns are difficult to fabricate in larger size scale. For one thing, the occurrence of exotherms and radial temperature gradients, during preparation of large-diameter monoliths, results in inhomogeneities in the pore structure [7]. For another, it is a key challenge to covalently bond large-diameter monoliths to column walls with sufficient strength to prevent the shrinkage of the monoliths during polymerization and chromatographic applications. These problems can be avoided if monoliths are synthesized within the confines of fused-silica capillaries with vinylized inner walls. However, capillary scale columns require dedicated chromatographic instruments, which most analytical laboratories do not have. This might limit the development and widespread application of monolithic media in many groups which only have conventional HPLC equipment. Therefore, it is highly interesting to develop monolithic columns with conventional or narrow-bore (1.0–2.0 mm) internal diameter (I.D.), which are compatible with the standard HPLC and the increasingly popular UHPLC systems (possibly with minor modification) [9]. Although rare, several research groups have made efforts to fabricate such polymeric monoliths utilizing different column housing materials, including silicosteel tubing of 1.0 mm I.D. [9,10], titanium tubing of 0.8 mm I.D. [11], and glass tube of 3.0 mm I.D [12,13]. One manufacturer now offers porous polymer monolithic columns with 4.6 mm I.D. Their properties and performance have been described in literature [14,15].

Polyether ether ketone (PEEK) is a semicrystalline high-performance thermoplastic possessing excellent thermal and mechanical properties and broad chemical resistance. Nowadays PEEK is extensively applied to fabricate items used in chromatography applications, including pump unions, column hardware, fittings, tubing etc. For many years, chromatography scientists had not tried to covalently attach polymeric monolithic stationary phase to PEEK surface until recently. The possible reason might have been that they considered PEEK material too chemically resistant to be modified for this attachment. Researchers from other fields, however, have already explored different wet chemical methods of PEEK modification, including sulfonation and nitration of the aromatic rings in the PEEK structure [16–18] and derivatization of the carbonyl group in the benzophenone segment of PEEK [19–21]. Since 2008, ProSwift® 1 mm I.D. monolithic columns are commercially available from Dionex, who developed these new columns by covalent attachment of poly(styrene-*co*-divinylbenzene) monoliths directly to the PEEK tubing. Later, a patent was issued, described a method of covalently attaching styrene-based monoliths to PEEK surfaces, starting from reducing the carbonyl groups of the PEEK surface [22]. In 2012, Shu et al. chemically anchored lauryl methacrylate-based monoliths to PEEK tubing of 1.0 mm I.D. via methacryloyl groups, starting from the sulfonation of the PEEK surface [23]. Recently, we have tried to prepare styrene-based monoliths in PEEK tubing using a surface modification method similar to that described in Ref. [22], but the results implied that the degree of the attachment of the monolith to PEEK surface was not sufficient to overcome the shrinkage (evaluated by SP factor, see below) of the monolith caused by mobile phases with high water content.

The aim of this work was to develop a scheme for modifying PEEK surface in order to improve the bond strength of PEEK tubing with polymeric monolith. In brief, vinylic anchoring groups were introduced onto PEEK surface by a three-step procedure, including surface etching, surface reduction and surface methacryloylation.

2. Material and methods

2.1. Materials

Sodium borohydride ($NaBH_4$, 99%), sodium bis(2-methoxyethoxy)aluminum hydride solution (Red-Al, ≥60 wt% in toluene), styrene (≥99%), divinylbenzene (DVB, 80%), aluminium oxide (activated, basic, Brockmann I), 2,2′-azobis(2-methylpropionitrile) (AIBN, 98%), 1-dodecanol (≥98%), toluene (anhydrous, 99.8%), acetone (≥99%), methanol (≥99.0%), tetrahydrofuran (THF, HPLC grade, ≥99.9%), dimethyl sulfoxide (DMSO, anhydrous, ≥99.9%), tetraethylene glycol dimethyl ether (tetraglyme, ≥99%), sulfuric acid (95–97%), methacrylic anhydride (94%), triethylamine (TEA, ≥99.5%), 4-(dimethylamino)pyridine (DMAP, ≥99%), 1,2-dichloroethane (DCE, anhydrous, 99.8%), thiourea (≥99.0%), benzene (anhydrous, 99.8%), ethylbenzene (≥99.0%), propylbenzene (analytical standard), butylbenzene (analytical standard) and pentylbenzene (analytical standard) were purchased from Sigma–Aldrich (Germany). 2-isocyanatoethyl methacrylate (IEM, >98.0%), 2,6-di-*tert*-butyl-p-cresol (BHT, >99.0%) and dibutyltin dilaurate (DBTDL) were obtained from TCI EUROPE N.V. (Belgium). Bovine serum albumine (BSA) was purchased from GE Healthcare (United Kingdom). Papain was obtained from Boehringer Mannheim GmbH (Germany) and trypsin from Corning (USA). Acetonitrile (HPLC grade, ≥99.9%) was purchased from Lab–Scan (Poland). Deionized Type I water obtained by a Milli-Q Advantage A10 system (Millipore, Bedford, USA) was used throughout the experiments. The monomers styrene and DVB were purified by passage through a bed of basic aluminium oxide to remove the inhibitors before use. PEEK tubing (1/8″ O.D. × 1.59 mm I.D.), PEEK reducing union (1/8″ to 1/16″), PEEK ferrule (1/8″) and PEEK nut (1/8″) were purchased from VICI AG International (Switzerland).

2.2. Instrumentation

IR spectra were recorded on a Nicolet 6700 FT-IR spectrometer with the “Smart Splitpea” diamond micro-ATR accessory (Thermo Fisher Scientific Inc., United States) described in detail in Ref. [24].

The morphology of the produced poly(styrene-*co*-divinylbenzene) monoliths was investigated by scanning electron microscope (SEM) EVO MA15 (ZEISS, Germany).

Nitrogen adsorption experiments were realized with an ASAP 2020 physisorption analyzer (Micromeritics, United States).

For the chromatographic studies, an Agilent 1200 HPLC system was used, comprising of a vacuum degasser, a quaternary pump, a well-plate autosampler, a column thermostat and a diode array detector (Waldbronn, Germany). Columns of 100–125 mm length, at a constant temperature of 298 K were used for all chromatographic separations. The injection volume was 0.5 μL and the detection wavelength was 210 nm. Separation of low molecular weight analytes was carried out in reversed phase mode using acetonitrile–water 70:30 (v/v) as mobile phase at linear velocity 0.65 mm s^{-1}. Benzene and alkylbenzenes were dissolved in the mobile phase at typical concentrations of 150 μg mL^{-1}, thiourea was dissolved in the mobile phase at a concentration of 10 μg mL^{-1}. Separation of proteins (in concentrations 1–3 mg mL^{-1}) was carried out with a linear gradient of acetonitrile in water from 28% to 48% (v/v) in 18 min (mobile phase linear velocity 2.5 mm s^{-1}). Both acetonitrile and water contained 0.1% (v/v) trifluoroacetic acid.

The van Deemter coefficients were obtained by fitting the plate heights to the van Deemter equation $H = A + B/u + Cu$.