Journal of Chromatography A, 1218 (2011) 2425–2431

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Methacrylate-based monolithic layers for planar chromatography of polymers

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ARTICLE INFO

Article history:
Available online 21 December 2010

Keywords:
Monoliths
Polymer sorbents
Planar chromatography
Synthetic polymers

ABSTRACT

A series of macroporous monolithic methacrylate-based materials was synthesized by *in situ* free radical UV-initiated copolymerization of functional monomers, such as glycidyl methacrylate (GMA), butyl methacrylate (BuMA), 2-aminoethyl methacrylate (AEMA), 2-hydroxyethyl methacrylate (HEMA) and 2-cyanoethyl methacrylate (CEMA), with crosslinking agent, namely, ethylene glycol dimethacrylate (EDMA). The materials obtained were applied as the stationary phases in simple and robust technique – planar chromatography (PLC). The method of separation layer fabrication representing macroporous polymer monolith bound to the specially prepared glass surface was developed and optimized. The GMA–EDMA and BuMA–EDMA matrixes were successfully applied for the separation of low molecular weight compounds (the mixture of several dies), as well as poly(vinylpyrrolidone) and polystyrene homopolymers of different molecular weights using reversed-phase mechanism. The materials based on copolymers AEMA–HEMA–EDMA and CEMA–HEMA–EDMA were used for normal-phase PLC separation of 2,4-dinitrophenyl amino acids and polystyrene standards.

1. Introduction

Nowadays, rigid macroporous copolymers synthesized by bulk method and known as *polymer monolithic materials* [1] are widely used as efficient sorbents for fast HPLC separations [2], high-speed affinity chromatography [3,4], capillary electrochromatography [5], gas chromatography [6], solid phase extraction [7], as well as high-throughput solid phases for flowing enzymatic reactors [8–10] and platforms for microarrays [11–13]. The arise of interest to polymer monoliths is stimulated by convection-controlled interphase mass exchange resulting from high permeability of such materials and dramatically elevating the speed of a process, their mechanical and chemical stability, as well as the easiness of monolith preparation. Enormous number of current publications are devoted to the various modes of chromatographic separations of biological objects (proteins, peptides, oligonucleotides, DNA, viruses) using monolithic sorbents [14–16]. However, the chromatography of synthetic macromolecules on monoliths is still not thoroughly investigated and, moreover, practically used.

The general separation technique widely applied for polymer analysis is size-exclusion or gel-permeation, chromatography (SEC or GPC, respectively) based on a difference of molecular size and, accordingly, different ability to diffuse into porous space of sorbent particle. This method allows determination molecular weight (M_w) and molecular weight distribution (MWD) of synthetic polymers. On the other hand, SEC does not enable to give any information on chemical composition of studied polymer. Therefore, the separation of macromolecules with close hydrodynamic radii but different composition seems to be not a possible task for this case. Usually, a modern analysis of synthetic polymers is carried out by a combination of SEC with adsorption modes of liquid chromatography (two-dimensional separation) [17–19]. As to the monolithic stationary phases with their high speed advantage, the development of separation methods for synthetic macromolecular compounds represents very important scientific and practical interest.

There are only a few early publications concerning polymer separation, namely, the chromatography on monolithic columns at gradient elution conditions of styrene oligomers [20] and some examples of polymers [20–23]. For example, Petro et al. used styrene–divinylbenzene, glycidyl methacrylate–ethylene dimethacrylate and dihydroxypropyl methacrylate–ethylene dimethacrylate monolithic columns for HPLC of commercial polystyrene, poly(methyl methacrylate), poly(vinyl acetate) and polybutadiene standards [20,21]. It was shown that monolithic columns provided fast and efficient determination of molecular weight parameters of synthetic polymers and constituted a viable, less expensive, much faster alternative to the more expensive and slower conventional packed columns.

Rapid and sufficiently robust method of polymer fractionation is a well-known planar chromatography (PLC) [24]. The first results on this topic were published by Inagaki et al. and Belenkii et al. [25,26]. The advantages of PLC based on a difference in adsorption energy of analytes are the simplicity of equipment, low cost and high speed of separation process. Additionally, planar chro-

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doi:10.1016/j.chroma.2010.12.061

matography can be considered as a rapid method for a selection of conditions for HPLC mode, namely, found at PLC eluent composition providing appropriate separation can be transferred to the column packed with a sorbent of the same chemistry.

The stationary phase in planar chromatography usually represents porous inorganic particles (silica gel) applied as a thin layer on a plate. The bead size of a sorbent and particle size distribution defines an efficiency of separation. The eluent flow is realized due to capillary forces influencing the diameter of interparticle channels.

In 2002, monolithic silica materials in a shape of thin layers were developed and offered for PLC. Such inorganic phase is characterized by bimodal pore size distribution that means its skeleton consists of large transport macropores with a diameter of 1–2 μm and a network of mesopores with size of a few nm. The advantage of such solid phase in PLC was proved by significant increase of adsorption capacity, speed and chromatographic efficiency [27].

Polymer monoliths have not yet found wide application in this separation format. The papers concerning planar separation of peptides and proteins using MALDI-TOF-MS detection and poly(butyl methacrylate-*co*-ethylene dimethacrylate) monoliths as separation of phases have to be mentioned here [28,29]. Recently, Woodward et al. [30] have demonstrated the ability of thin monolithic layers to be used in planar electrophoresis and pressurized electrochromatography for rapid separation of peptides and oligonucleotides.

The presented work describes the development of monolithic methacrylate-based layers with different surface functionality that allows application of different mechanisms of adsorption at PLC separations. The plates were tested in separation of substances of different classes and molecular masses. Thus, the general goal was the preparation of planar polymer monolithic supports of various functionalities and study of their behavior in separation of small compounds and synthetic polymers in different adsorption chromatographic modes.

2. Experimental

2.1. Materials

The microscope glass slides (75 mm × 25 mm, 1 mm thick) were obtained from MiniMed (St. Petersburg, Russia). Chromatographic chamber with dimensions 150 mm × 20 mm × 80 mm and glass capillaries for sample spotting were from Lenchrom (St. Petersburg, Russia).

2.2. Chemicals

Glycidyl methacrylate (GMA, 97% pure), ethylene glycol dimethacrylate (EDMA, 98% pure), butyl methacrylate (BuMA, 99% pure), 2-aminoethyl methacrylate (AEMA, 90% pure), 2-hydroxyethyl methacrylate (HEMA, 98% pure), (CyOH, 99% pure), 2-methoxy-2-phenylacetophenone (99% pure), *p*-aminoazobenzene (98% pure), 1-dodecanol (DoOH, 98% pure), 1,4-butandiol (1,4-BD, 99% pure), N-methyl-2-pyrrolidone (NMP, 99% pure), *p*-aminoazotoluene (97% pure), methyl red (ACS reagent), N-(2,4-dinitrophenyl)-DL-aspartic acid (DNP-aspartic acid), N-(2,4-dinitrophenyl)-L-leucine (DNP-leucine), N-(2,4-dinitrophenyl)-L-tryptophan (DNP-tryptophan), N-(2,4-dinitrophenyl)-β-alanine (DNP-alanine) were purchased from Sigma–Aldrich Rus (Moscow, Russia). 3-(Trimethoxysilyl)propyl methacrylate, polyethylene glycol with $M = 200$ (PEG-200, standard for GPC) were from Fluka AG (Buchs, Switzerland). 2-Hydroxy-2-methylpropiophenone (Darocur-1173, 97% pure), methanol (MeOH, 99.8% pure for liquid chromatography LiChrosolv®), tetrahydrofuran (THF, 99.9% pure for liquid chromatography LiChrosolv®), propan-2-ol (isopropyl alcohol, 99.8% pure for HPLC), were purchased from Merck KGaA (Darmstadt, Germany). 2-Cyanoethyl methacrylate (CEMA, 97% pure) was provided by Yarsintez (Yaroslavl', Russia). N,N-dimethylformamide (DMF, 99% pure), toluene (98% pure), acetone (98% pure), were purchased from Vekton (St. Petersburg, Russia). Acetonitrile (AcN, 99,95% pure), n-hexane (97% pure) were from Cryochrom (St. Petersburg, Russia).

2.3. Samples

Polystyrene (PS) samples with molecular masses (M_w) 154,000, 500,000 and 960,000 were purchased from Fluka AG (Buchs, Switzerland). The value of sample polydispersity was in a range of 1.02–1.05. The synthesized by free-radical polymerization and characterized samples of poly-N-vinylpyrrolidones (PVP) with molecular masses (M_w) 14,400, 94,700 and 1,065,000 were kindly donated by Dr. I.I. Gavrilova (IMC RAS).

2.4. Instruments

The Philips 125-W mercury lamp (Philips, Netherlands) of wide spectrum and constant intensity of irradiation was used for free-radical copolymerization of chosen monomers. The mean pore size and specific surface area were estimated using ThermoQuest Pascal 440 porosimeter (Rodano, Italy). The morphology of the polymer samples obtained was investigated with JEOL JSM-35 CF scanning electron microscope (Tokyo, Japan).

2.5. Methods

2.5.1. Preparation of monolithic layers for PLC

The glass plates were etched using paraffin mask with 11 M hydrochloric acid for 30 min to provide formation of operative cell on a glass slide surface. Fig. 1 represents a scheme of monolithic layer manufacturing. The size and depth of cells obtained were 60 mm × 20 mm and 200 μm, respectively. After that, the slides were washed with water, boiled with 0.1 M NaOH for 40 min, then with water again. The plates were dried at 100 °C for 1 h.

To introduce double bonds into the cell surface for further polymer layer preparation by triple free radical copolymerization, the plate was immersed into 15% toluene solution of 3-(trimethoxysilyl)propyl methacrylate. The reaction was allowed to proceed for 12 h at room temperature [31].

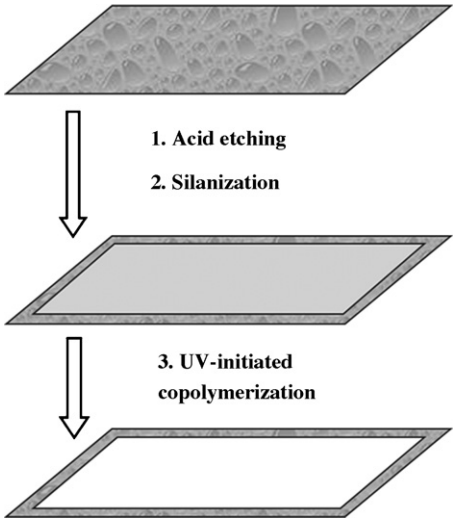

Fig. 1. Scheme of the monolithic layer fabrication.