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Polyethylenimine attached-poly(3-chloro-2-hydroxypropyl methacrylate-co-ethylene dimethacrylate) monosized-porous microspheres as a new separation medium for polar compounds

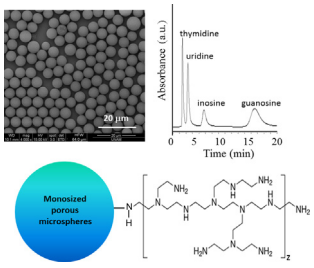
Bekir Çelebi [a], Aslıhan Gökaltun [a], Esra Arman [a], Oğulcan A. Evirgen [a], Ali Tuncel [a,b,*]

[a] Hacettepe University, Chemical Engineering Department, Ankara, Turkey
[b] Hacettepe University, Division of Nanotechnology and Nanomedicine, Ankara, Turkey

HIGHLIGHTS

- Chloropropyl functionalized reactive, hydrophilic polymer beads were newly synthesized.
- A macromolecular ligand, polyethylenimine was attached onto the hydrophilic beads.
- Polyethylenimine attached beads were used as stationary medium in HILIC mode.
- Polar compounds and biomolecules compounds were successfully separated in micro-LC.
- Theoretical plate numbers up to 23,000 plates/m were achieved.

GRAPHICAL ABSTRACT



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ABSTRACT

Monosized-porous and hydrophilic polymer microspheres with reactive chlorine functionality were first obtained in the form of poly(3-chloro-2-hydroxypropyl methacrylate-co-ethylene dimethacrylate), poly(HPMA-Cl-co-EDMA) copolymer by a new seeded polymerization. A macromolecular ligand, polyethylenimine (PEI) was covalently attached onto the poly(HPMA-Cl-co-EDMA) microspheres via a single stage reaction between 3-chloro-2-hydroxypropyl and amine groups. PEI attached-poly(HPMA-Cl-co-EDMA) microspheres were slurry packed into the microbore columns and used as stationary phase in hydrophilic interaction chromatography (HILIC) mode. The separation of various compounds, nucleosides, polar organics and peptides was successfully performed. The results showed that hydrophilic poly(HPMA-Cl-co-EDMA) microspheres are a new, alternative starting material for the synthesis of stationary media with different functionalities for various liquid chromatographic applications.

Abbreviations: HPMA-Cl, 3-chloro-2-hydroxypropyl methacrylate; EDMA, ethylene dimethacrylate; PEI, polyethylenimine; HILIC, hydrophilic interaction chromatography; GMA, glycidyl methacrylate; CMS, chloromethylstyrene; DVB, divinylbenzene; EB, ethylbenzene; Et-OH, ethanol; THF, tetrahydrofuran; ACN, acetonitrile; PVP K-30, poly(vinyl pyrrolidone); SLS, Sodium lauryl sulfate; PVA, polyvinylalcohol; AIBN, 2,2′-azobisizobutyronitrile; BPO, benzoyl peroxide; ISEC, inverse-size exclusion chromatography; AAc, acetic acid; TEA, triethylamine; THMAM, trishydroxymethyaminomethane; SPE, 3-(2-(N-methacryloyloxyethyl)-N,N-dimethyl-ammonium)propane sulfonate; DHBA, 3,4-dihydroxybenzoic acid; HTMA, 2-hydroxyl-3-[4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl] propyl 2-methylacrylate; VT, vinyl tetrazole.

* Corresponding author at: Hacettepe University, Chemical Engineering Department, Ankara, Turkey. Fax: +90 312 299 21 24.
E-mail address: atuncel@hacettepe.edu.tr (A. Tuncel).

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1. Introduction

The term HILIC was proposed by Alpert in 1990 for a chromatographic method involving the interaction of analytes with a polar, hydrophilic stationary phase and their elution with a relatively apolar binary eluent containing water as the stronger eluting component [1,2]. Various silica based stationary phases with different ligands were developed for the separation of polar compounds in HILIC mode [3–9]. The chromatographic characterization of different particulate and monolithic HILIC supports were performed in terms of hydrophilicity, charge, structural selectivity and separation efficiency [10–15].

Among the polymer-based stationary phases, monosized-porous poly(glycidyl methacrylate-co-ethylene dimethacrylate), poly(GMA-co-EDMA) or poly(chloromethylstyrene-co-divinylbenzene), poly(CMS-co-DVB) microspheres were preferred [11–15]. In the case of monosized microspheres, the coefficient of variation for size distribution is mostly lower than 5% [16,17]. Although poly(CMS-co-DVB) microspheres have a highly reactive chloromethyl functionality that can be used for the covalent attachment of various chromatographic ligands onto the microspheres, they are relatively hydrophobic in nature due to the presence of aromatic rings in their structure. Their hydrophobic character may cause undesired, non-specific interactions between plain chromatographic support and analytes separated in HILIC or ion-exchange modes. Due to their polymethacrylate structure, poly(GMA-co-EDMA) microspheres have more hydrophilic with respect to poly(CMS-co-DVB) microspheres and have a reactive epoxypropyl group that can be also used for the covalent attachment of various ligands via simple, one-pot protocols. For this reason, monosized-porous poly(GMA-co-EDMA) based structures were more widely used as a starting material for the synthesis of ion-exchange and HILIC supports [12,15,18,19]. In this study, we synthesized monosized-porous poly(HPMA-Cl-co-EDMA) microspheres as a new reactive starting material appropriate for the preparation of various HILIC stationary phases. For this purpose, a new seeded polymerization was developed. Poly(HPMA-Cl-co-EDMA) microspheres are hydrophilic due to the hydroxyl functionality in their HPMA-Cl units. Hence, undesired hydrophobic interactions between analytes and chromatographic support should be lower with respect to the other hydrophobic supports used in HILIC applications. Their chloropropyl functionality allows the covalent attachment of various chromatographic ligands via one-pot routes.

Amine functionalized stationary phases both in particulate and monolithic forms were successfully used in HILIC [10,20,21]. PEI is a hydrophilic, polar macromolecular structure containing primary, secondary and tertiary amine groups. The studies using PEI as a chromatographic ligand on the newly developed stationary phases particularly for ion-exchange chromatography have been found in the literature [22–24]. Recently, chloromethyl functionalized monoliths with exchangeable chemistries were developed [28]. In these studies, PEI coated gold nanoparticles attached onto a poly(chloromethylstyrene-co-divinylbenzene) capillary monolith was successfully used in HILIC mode [25,26]. In our study, PEI was selected as a chromatographic ligand for the preparation of a new HILIC stationary phase based on poly(HPMA-Cl-co-EDMA) microspheres. PEI was covalently attached onto the poly(HPMA-Cl-co-EDMA) microspheres via a one-pot reaction between chloropropyl and amine functionalities. Its long chain (i.e. macromolecular) character also allows easier interaction with the analytes with respect to the chromatographic ligands in the form of small, single molecules. Then, the aim of this study is to prepare monosized-porous PEI carrying-poly(HPMA-Cl-co-EDMA) microspheres as a new stationary phase and to investigate the chromatographic behavior in HILIC mode in microbore liquid chromatography.

2. Experimental

2.1. Materials

3-Chloro-2-hydroxypropyl methacrylate (HPMA-Cl), and ethylene dimethacrylate (EDMA) were supplied from Aldrich Chem. Co., USA and used without further purification. Polyethylenimine (branched, average M_w ~25,000 by light scattering, average M_n ~10,000 by gel permeation chromatography, supplier's information) was obtained from Fluka Chemie, Germany. Ethylbenzene (EB), ethanol (Et-OH, HPLC grade), tetrahydrofuran (THF, HPLC grade), acetonitrile (ACN, HPLC grade) were also obtained from Aldrich. Glycidyl methacrylate (GMA), poly(vinyl pyrrolidone) (PVP K-30, M_w: 40,000) were obtained from Sigma Chemical Co., St. Louis, USA. Sodium lauryl sulfate (SLS) and polyvinylalcohol (PVA, 87–89% hydrolysed, molecular weight: 85,000–146,000 Da) were also supplied from Sigma. The initiator, 2,2′-azobisisobutyronitrile (AIBN) were obtained from Merck A.G. Darmstad, Germany and recystallized from methanol before use. Benzoyl peroxide (BPO) was also obtained from Merck and dried in vacuo at 30 °C. The nucleosides, thymidine, uridine, inosine, guanosine; the polar organics acrylamide, acrylic acid and *p*-toluic acid, and the peptides, Met-Leu, Gly-Ser, N-cbz-β-Ala-Val and albumin (from bovine serum, cat no: A2153) were obtained from Sigma and used as analytes in HILIC. Distilled-deionized (DDI) water (18.2 MΩ cm) obtained from a water-purification unit (Merck-Millipore Direct-Q3, Germany) was used in all experiments.

2.2. Synthesis and characterization of poly(HPMA-Cl-co-EDMA) microspheres

Poly(glycidyl methacrylate), [poly(GMA)] seed latex 2.1 μm in size was synthesized by dispersion polymerization according to the procedure given elsewhere [27]. Monosized-porous poly(HPMA-Cl-co-EDMA) microspheres were obtained by a newly developed seeded polymerization [28]. In the method, the seed microspheres were swollen by an organic agent (EB) acting as a porogen by preserving the monodispersity of microspheres in the aqueous emulsion medium. In the next stage, the organic agent containing seed microspheres were reswollen by a monomer phase containing functional monomer (HPMA-Cl), crosslinking agent (EDMA) and initiator (BPO). Finally, the monomer phase was polymerized in the swollen seed microspheres for obtaining monosized-porous microspheres in the aqueous continuous medium.

In the first stage of the synthesis, the organic agent, EB (2.5 mL) was emulsified in water (50 mL) including SLS (0.125 g) by ultrasonication for 8 min. The aqueous dispersion containing poly(GMA) seed microspheres 2.1 μm in size (approximately 4 mL, solid content: 0.3 g) was added and sonicated for 4 min. The emulsion was stirred (22 °C, 4 h) for the swelling of seed microspheres by the organic phase. In the next stage, the monomer phase containing main monomer, HPMA-Cl (2.5 mL), the crosslinking agent, EDMA (2.5 mL) and the initiator, BPO (0.3 g) was emulsified in water (50 mL) including SLS (0.125 g) by ultrasonication for 12 min. The monomer emulsion was then mixed with the aqueous dispersion of organic agent-swollen seed microspheres. The resulting emulsion was magnetically stirred for 24 h, at room temperature for swelling of seed microspheres with the monomer phase. An aqueous solution (10 mL) of PVA (0.8 g) was added and the resulting emulsion was transferred into a sealed, pyrex® polymerization reactor, at room temperature. The reactor was heated to 80 °C in a temperature-controlled shaking water bath (Memmert, Germany).