Journal of Chromatography A, 1410 (2015) 200–209

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Polyethylenimine modified poly(ethylene terephthalate) capillary channeled-polymer fibers for anion exchange chromatography of proteins

Liuwei Jiang, Yi Jin, R. Kenneth Marcus*

Clemson University, Department of Chemistry, Biosystems Research Complex, Clemson, SC 29634, United States

ARTICLE INFO

Article history:
Received 22 May 2015
Received in revised form 23 July 2015
Accepted 25 July 2015
Available online 29 July 2015

Keywords:
Capillary-channeled polymer
Fiber
Surface modification
Polyethylenimine
Anion exchange
Protein

ABSTRACT

Native poly(ethylene terephthalate) (PET) capillary-channeled polymer (C-CP) fibers have been previously studied as stationary phases for reversed phase and affinity protein separations. In this study, surface modified PET C-CP fibers were evaluated for the anion exchange separation of proteins. The native PET C-CP fibers were aminated using polyethylenimine (PEI) followed by a 1,4-butanediol diglycidyl ether (BUDGE) cross-linking step. Subsequent PEI/BUDGE treatments can be employed to further develop the polyamine layer on the fiber surfaces. The PEI densities of the modified fibers were quantified through the ninhydrin reaction, yielding values of 0.43–0.89 μmol g^{-1}. The surface modification impact on column permeability was found to be 0.66×10^{-11} to 1.33×10^{-11} m^2, depending on the modification time and conditions. The dynamic binding capacities of the modified fiber media were determined to be 1.99–8.54 mg mL^{-1} bed volume, at linear velocities of 88–438 cm min^{-1} using bovine serum albumin as the model protein. It was found that increasing the mobile phase linear velocity (up to 438 cm min^{-1}) had no effect on the separation quality for a synthetic protein mixture, reflecting the lack of van Deemter C-term effects for the C-CP fiber phase. The low-cost, easy modification method and the capability of fast protein separation illustrate great potential in the use of PEI/BUDGE-modified PET C-CP fibers for high-throughput protein separation and downstream processing.

1. Introduction

The development and use of protein therapeutics is experiencing phenomenal growth [1]. Over the last decade, the productivity of the protein upstream processes have been greatly improved, further relegating downstream processing as the bottleneck limiting the throughput of current bio-manufacturing [2–5]. The development of high efficiency, low-cost stationary phases for protein separation on the analytical and preparative scales thus continues to be an area of high interest. Capillary-channeled polymer fibers (C-CP fibers) have been extensively studied in this laboratory for bio-macromolecule separations [6,7]. C-CP fibers are manufactured by melt-extruding commodity polymers, such as poly(ethylene terephthalate) (polyester, PET), polyamide (nylon 6) or polypropylene (PP) [8]. These base polymers provide chemical and physical stability over a wide range of the chromatographic conditions used in protein separations. C-CP fibers are unique in shape, with eight capillary channels extending to the whole length of the fiber that gives the fiber ~3× larger surface area then the circular cross-sectional fibers with the same diameters (30–50 μm) [8]. When packed in columns, C-CP fibers self-align and yield a monolith-like structure with 1–5 μm open, parallel channels. These channels provide C-CP fiber packed columns excellent fluid transportation property and low mass transfer resistance, in comparison to the traditional monolithic columns and microsphere packed columns [9]. This allows for the direct loading of complex and viscous feedstock, such as cell fermentation broths, for target molecule capture. It is believed that the high column permeability, low mass transfer resistance and the low cost of the C-CP fiber chromatography columns make them promising choices for fast protein separations at various scales [7,10].

C-CP fiber-packed columns have been used for protein separations in different chromatographic modes, including reversed-phase (RP), ion-exchange (IEC), and hydrophilic-interaction (HIC) as dictated by the surface chemistry of the native fibers. In that regard, and of relevance here, nylon 6 has a polyamide backbone, with end groups that vary in ionic character as a function of pH. As such, that material has been applied previously

* Corresponding author.
E-mail address: marcusr@clemson.edu (R.K. Marcus).

http://dx.doi.org/10.1016/j.chroma.2015.07.102
0021-9673/

for HIC, mixed-mode, and cation exchange (CEX) protein separations [11–13]. However, the binding selectivity of the native C-CP fibers limits the development of the practical applications of C-CP fiber chromatography media. Therefore, various surface modification strategies, including physical adsorption and covalent coupling chemistries, have been shown to provide C-CP fibers with a variety of practical functionalities. For example, very robust adsorption of protein A to the surface of PP C-CP fibers has been demonstrated for IgG capture [14]. Likewise, a completely new family of ligands has been developed based on the high affinity for aliphatic chains for the hydrophobic PP fiber surfaces. Lipid tethered ligands (LTLs) having a variety of capture head groups readily adsorb at room temperature, and are immune from elution/leakage from the column under any sort of conditions common to biomolecule separations [14–17]. An example of covalent coupling involves the use of ethylenediamine as a means of activating PET C-CP fibers, yielding a surface rich with primary amines. These amines are then readily modified with capture ligands such as biotin for protein affinity separations [15].

Ion-exchange chromatography is a widely used technique in the bio-separations, including protein downstream processing, because of the use of solvent conditions that do not affect large amounts of protein denaturation [18,19]. Bio-molecules have ionizable chemical moieties that retain them on the charged IEC stationary phase. When the environmental conditions are changed (e.g., pH, ionic strength), the retained bio-molecules are sequentially eluted from the stationary phase and the separation is then realized. An amine-rich polymer, polyethylenimine (PEI), has been widely used in the chemical modifications of chromatography stationary phases for anion exchange (AEX) separation due to its low-cost and easy availability. PEI has been adsorbed on inorganic supports, including silicas [20,21], zirconia [22,23], graphite [24], and hydroxyapatite [25], followed by cross-linking for ion-exchange chromatography. On organic supports, PEI is usually reacted with surface anchor groups and covalently bound onto the surface. PEI has been used for the modifications of monoliths [26,27], cryogels [28–30], membranes [31], polymer resins [32], and open-tubular capillaries [33,34] for protein separations.

In this study, PEI has been used for the surface modification of PET C-CP fibers to affect an AEX stationary phase. 1,4-Butanediol diglycidyl ether (BUDGE) was used to cross-link the PEI entities, creating better-ordered and greater density phases [32]. While the primary goal of the effort was to affect greater selectivity than previous found with nylon 6, the on-column modification could not compromise the hydrodynamic advantages inherent to the C-CP fiber format. The accessible amine densities, column permeability, protein dynamic binding capacity, protein IEC performance of the PEI-modified PET C-CP fiber columns were investigated as a function of different surface modification conditions. To the best of our knowledge, this is the first study of using PEI modification of PET to affect a stationary phase medium for AEX protein separations. The results presented here suggest that there is a great deal of practical advantage to combining the enhanced selectivity and capacity of the PEI phase and the previously demonstrated throughput and yield characteristics of C-CP fiber columns [7,35].

2. Experimental methods

2.1. Materials

Unless otherwise specified, chemicals were purchased from commercially available sources and used without further purification. Polyethylenimine (PEI, MW 10,000, 99%) was purchased from Polysciences, Inc. (Warrington, PA). Dimethyl sulfoxide (DMSO, ACS grade), pyridine (99%), Tris base (99.8%) were purchased from VWR (Atlanta, GA). 1,4-Butanediol diglycidyl ether (BUDGE, 95%) and all proteins were purchased from Sigma–Aldrich (St. Louis, MO). All HPLC solvents were purchased from EMD (Billerica, MA). Deionized water (DI-H_2O) was obtained from a Milli-Q water system.

2.2. Preparation of PET C-CP fiber microbore columns

The C-CP fiber microbore columns were prepared following previously reported methods [10,36]. PET C-CP fibers were obtained from the Clemson University School of Materials Science and Engineering. In this study, 450 PET fibers were pulled through polyether ether ketone tubing (PEEK, 0.762 mm i.d., IDEX Health & Science LLC, Oak Harbor, WA). After packing, the columns were mounted on a Dionex Ultimate 3000 HPLC system (LPG-3400SD Quaternary pump, MWD-3000 UV–vis absorbance detector, Thermo Fisher Scientific Inc., Sunnyvale, CA) and washed with acetonitrile then deionized water until a stable baseline was observed at 216 nm. Once assembled and cleaned, the microbore columns could be stored in ambient conditions and cut to appropriate lengths prior to surface modifications.

2.3. Single modification of PET C-CP microbore column with polyethylenimine

PET C-CP fibers were packed in columns before modifications. It is easier to pack the fibers before modifications as the fibers may become twisted or folded during the modification process, which would result in inhomogeneous column packing after the fact. Before modifications, PET C-CP columns were mounted on a HPLC pump and washed with DMSO at 0.5 mL min^{-1} for 10 min. The columns were then placed in a column heater assembly and connected to a syringe pump that contained the reactive PEI solution. The column heater was set to 100 °C, and solutions of 15% PEI in DMSO were continuously pumped through the columns at a flow rate of 0.6 mL h^{-1} for 2 h, 3 h or 4 h. As such, the surface modification chemistries were varied from 1 to 4 h. The resulting columns were designated as PET-PEI-2 h, PET-PEI-3 h, and PET-PEI-4 h. After the modifications, the columns were brought up to ambient temperature and mounted on the HPLC to be washed with DMSO and water until a stable baseline at 216 nm was observed; reflective of removal of unreacted/unbound PEI.

2.4. Multiple modification repetitions of PET C-CP fibers with polyethylenimine and 1,4-butanediol diglycidyl ether

Before modifications, PET C-CP columns were mounted on a HPLC pump and washed with DMSO at 0.5 mL min^{-1} for 5 min. Different from the simple modification described above, the inclusion of BUDGE cross-linking and multiple repetitions were undertaken under static conditions. In the initial PEI modification step, 10 column volumes (CV) of 15% PEI in DMSO were pumped through the column. Then, the columns were sealed at both ends and placed in an oven at 100 °C for 20 min. After the initial PEI modification, the columns were cooled to room temperature and washed with DMSO at 0.5 mL min^{-1} for 5 min. For the BUDGE modification, 10 CV of 15% BUDGE in DMSO were pumped through the column, the columns re-sealed, and placed in an oven at 100 °C for 20 min. The columns were washed with DMSO at 0.5 mL min^{-1} for 5 min between each modification to remove the unreacted PEI or BUDGE. For the column designated PET-PEI/BUDGE #1, the column was first modified by PEI, followed by BUDGE cross-linking, and an additional PEI application. For the column designated PET-PEI/BUDGE #2, BUDGE and PEI modifications were repeated once beyond that of PET-PEI/BUDGE #1. Finally, for the column designated PET-PEI/BUDGE