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Packing characteristics of winged shaped polymer fiber supports for preparative chromatography

Johannes Winderl, Tamara Spies, Jürgen Hubbuch*

Institute of Engineering in Life Sciences, Section IV: Biomolecular Separation Engineering, Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany

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ABSTRACT

Polymer fibers have been identified as a promising alternative support material for liquid chromatography. Area enhanced fibers may overcome the shortcomings of conventional fiber supports with respect to binding capacity and packing efficiency. One type of area enhanced fiber supports are winged shaped microfibers, which have a more than tenfold higher surface area than round fibers, and can be manufactured via inexpensive, conventional extrusion techniques.

In the present study, the packing characteristics of native and grafted winged shaped fiber supports have been investigated. A suspension based packing technique was used to pack short winged shaped polyamide 6 (PA6) fibers into small laboratory scale columns. Low column-to-column variabilities in porosities, plate heights, axial dispersion coefficients, and peak asymmetries were observed. Peak asymmetries were within typical ranges of preparative columns, and plate heights were at the lower end of those reported for other fiber supports. Packing density was found to be the main parameter that affected column performance. Lower packing densities were associated with lower plate heights, while increases in bed height resulted in more symmetric peak shapes. Packing density was also found to have a strong impact on the performance of poly (glycidyl methacrylate) (PGMA) grafted and sulfonated (SO_3^-) winged shaped PA6 fibers. Higher packing densities resulted in higher dynamic binding capacities (DBCs), but led to a decrease in capacity utilization and resolution. A comparison to conventional perfusive and diffusive adsorbents revealed that under optimized packing conditions such adsorbents can achieve a better resolution than conventional adsorbents at high mobile phase velocities.

Overall, these results suggest, that winged shaped fibers have strong potential as supports for preparative chromatography. Further improvements may be possible via adjustments in the fiber dimensions.

1. Introduction

Chromatographic unit operations account for a large fraction of the manufacturing costs of biotherapeutics. One of the main reasons for this is the high cost of chromatography adsorbents, which make up a significant portion of the material costs for chromatography steps [1]. This is due to both the cost of the support materials, and the costs for modification and functionalization of the support materials. While a wide range of modification and functionalization schemes have been developed [2], and applied to different supports, only certain types of supports have been found to be suitable for preparative protein chromatography [3]. At present the majority of chromatographic processes are performed in packed beds filled with adsorbents that are prepared from spherical, porous supports. Typically, these are reused for multiple cycles, which necessitates a costly, thorough evaluation of both the cleaning and storage protocols, and the performance degradation after continued use.

Polymeric fibers have been identified as alternative chromatography supports [4] with several possible advantages over conventional supports [5,6]. Most of these materials are non-porous or have a very limited porosity such that the majority of binding sites are accessible via convection. This reduces diffusional limitations for large biomolecules and favors fast mass transfer. This characteristic is also accompanied by low resistance to flow, which allows for operations at higher flow rates, together with faster mass transfer rates that can enable higher overall productivity. As fibers originate from the textile industry, technologies for mass production exist, which lead to very low costs of these materials. The costs of synthetic fibers are estimated to be up to 50 times lower than the costs of conventional support materials such as agarose [7]

* Corresponding author at: Fritz-Haber-Weg 2, 76131 Karlsruhe, Germany.
E-mail addresses: johannes.winderl@kit.edu (J. Winderl), tamara.spies@student.kit.edu (T. Spies), juergen.hubbuch@kit.edu (J. Hubbuch).
URL: http://mab.blt.kit.edu/english/ (J. Hubbuch).

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or silica [5]. Consequently, fibers are good candidates for use as low-cost disposables and could be suitable materials for high productivity and cost-efficient bioseparations. This may help to meet current challenges in downstream processing, which call for more productivity and lower costs of downstream operations.

Despite the many possible benefits of fibers, research into fibers and fiber-based adsorbents has mostly been restricted to analytical applications. A main reason for this lies in the low specific surface area of conventional non-porous textile fibers with typical diameters on the order of several micrometers [8,18]. These give rise to fibers and fiber-based adsorbents with rather low binding capacities. Recently, several studies have investigated how this problem might be solved. One approach lies in the use of porous fibers with internal hydrogel structures [9,10]. While this increases the specific surface area for protein binding, it may also increase mass transfer resistances. An alternative approach is to use nanofibers [11,12], which can be produced in the form of non-woven discs via techniques such as electrospinning [8]. Challenges with this approach are the limited mechanical stability of the non-woven fiber discs, which may require further treatment [13] and the low speed of electrospinning in comparison to conventional mechanical fiber-spinning techniques [8]. A third approach is the use of surface shaped fibers. These can be produced by conventional techniques and possess significantly increased cross-sectional areas compared to conventional fibers with equivalent diameters and circular cross-sections. Different cross-sections have been developed, such as capillary-channeled polymer (C-CP) fibers [14] or winged shaped fibers [15]. In recent studies it has been demonstrated that with appropriate surface modification techniques, fiber-based adsorbents with high dynamic binding capacities at short residence times can be prepared from such fibers [7,16,17].

The second challenge for the use of fibers is the packing of such materials. Suitable packing techniques must ensure a good efficiency of the packed bed, and must be reproducible and scalable. To make use of the cost benefit of fibers, they should be combinable with surface modification steps and be easy to integrate into current packing equipment. For preparative applications pressure drop constraints (typically less than 2–3 bars) also have to be satisfied. In addition, the packing conditions, for instance packing density and bed height, have to be optimized in order to achieve the best column performance. The choice of a suitable packing technique for fibers is influenced by various factors, but depends predominantly on the fiber manufacturing process, the resulting fiber structure and the techniques that are used for fiber modification and functionalization. Both suspension based packing techniques [19] and dry packing techniques [20,21] have been described in the literature. For conventional round fibers, however, comparatively low packing efficiencies have been reported [23], even under optimized packing conditions [22].

Area enhanced shaped fibers may have better packing characteristics due to their different structure. However, there are only a few studies that have reported on the packing of area enhanced shaped fibers. Only C-CP fibers have been investigated in detail. Marcus et al. used filaments of capillary channeled fibers and aligned them in a colinear fashion to prepare C-CP fiber columns [14]. The packing reproducibility [24] and the impact of packing conditions such as column diameter, column length and fiber packing density on packing efficiency [25–28] and on the dynamic binding capacity of proteins [29] on different types of native C-CP fibers have been evaluated. The packing technique was reproducible [24]. Plate heights of 6 mm for thyroglobulin [28] and 0.9 mm for uracil [26] have been reported. However these studies focused on HPLC applications and high packing densities were used, which may not be feasible for preparative operations with low pressure drop requirements.

The packing characteristics of other types of area enhanced shaped fibers such as winged shaped microfibers have hardly been explored. These fibers have a more than tenfold higher surface area than round fibers and a higher surface area than C-CP fibers, and they can be manufactured via inexpensive, conventional extrusion techniques [15]. Schwellenbach et al. recently prepared strong cation-exchange fiber based adsorbents from short-cut winged shaped polyethylene terephthalate (PET) fibers [17]. A dry packing technique was used to pack the fiber-based adsorbents. Plate heights of 0.1 cm and peak asymmetries of 1.8 were reported for acetone on a reference fiber-based adsorbent with optimized grafting parameters. However only the fiber-based adsorbent was investigated, and the packing characteristics of native winged fibers or the impact of different packing conditions and grafting on packing efficiency were not explored. Other packing techniques, such as suspension based packing techniques, which could be performed with conventional packing equipment directly after suspension based surface modification, still have to be evaluated.

In the present study the packing characteristics of native winged shaped polyamide 6 (PA6) fibers were investigated. We evaluated if slurry based packing can be performed with such fibers and if this is reproducible. We characterized the resulting fiber beds and studied the impact of packing density and bed height on the column performance in terms of porosities, plate heights, axial dispersion coefficients and peak asymmetries. In addition, we assessed if and how grafting impacts the column performance, both in terms of column efficiency, but also in terms of dynamic binding capacity and resolving power.

2. Experimental

2.1. Materials

2.1.1. Chemicals, buffers and proteins

Sodium dihydrogen phosphate, sodium chloride (NaCl), hydrogen chloride (HCl), sodium hydroxide (NaOH), ethanol (EtOH), methanol (MeOH), isopropanol (IPA) and acetone were purchased from Merck (Darmstadt, Germany). Ammonium sulfate was from AppliChem (Darmstadt, Germany). Sodium phosphate, lysozyme from chicken egg white (Lys, no. L6876), cytochrome c from bovine heart (Cyt c, no. 30398), and dextran with an average molecular mass of 2000 kDa (D2000) from *Leuconostoc* spp. were obtained from Sigma-Aldrich (St. Louis, MO, USA). The monoclonal antibody (mAb) was a CHO-derived IgG from a known industrial manufacturer. All buffers were prepared with ultra-pure (UP) water (Purelab Ultra, Elga LabWater, High Wycombe, UK). The pH was adjusted with HCl or NaOH as needed. Prior to usage the buffers were filtered through 0.2 μm cellulose acetate (CA) membrane filters (Sartorius, Göttingen, Germany) and degassed via sonication. All proteins and tracers were dissolved in the appropriate buffers as needed and filtered through 0.2 μm CA syringe filters (Sartorius, Göttingen, Germany). Buffer exchange for the mAb was performed with PD-10 desalting columns (GE Healthcare, Little Chalfont, UK). The protein concentration of protein solutions was verified photometrically with a NanoDrop2000c UV-Vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

2.1.2. Stationary phases

Area enhanced shaped polymer fibers with different cross-sectional shapes were obtained for this study. Each fiber type was sourced in the smallest available linear density and characterized with respect to morphology and specific surface area (SSA).

Winged shaped fibers (3 deniers per filament (dpf)) were selected for this study due to their high specific surface area (SSA) in comparison to round fibers, but also in comparison to other types of area enhanced shaped polymer fibers. The fibers were acquired from Allasso Industries (Raleigh, NC, USA) and were made from