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Preparation and characterization of high capacity, strong cation-exchange fiber based adsorbents

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ABSTRACT

Motivated by the demand for more economical capture and polishing steps in downstream processing of protein therapeutics, a novel strong cation-exchange chromatography stationary phase based on polyethylene terephthalate (PET) high surface area short-cut fibers is presented. The fiber surface is modified by grafting glycidyl methacrylate (GMA) via surface-initiated atom transfer radical polymerization (SI-ATRP) and a subsequent derivatization leading to sulfonic acid groups. The obtained cation-exchange fibers have been characterized and compared to commercially available resin and membrane based adsorbers. High volumetric static binding capacities for lysozyme (90 mg/mL) and polyclonal human IgG (hIgG, 92 mg/mL) were found, suggesting an efficient multi-layer binding within the grafted hydrogel layer. A packed bed of randomly orientated fibers has been tested for packing efficiency, permeability and chromatographic performance. High dynamic binding capacities for lysozyme (50 mg/mL) and hIgG (54 mg/mL) were found nearly independent of the bed-residence time, revealing a fast mass-transport mechanism. Height equivalent to a theoretical plate (HETP) values in the order of 0.1 cm and a peak asymmetry factor (AF) of 1.8 have been determined by tracer experiments. Additionally inverse size-exclusion chromatography (iSEC) revealed a bimodal structure within the fiber bed, consisting of larger transport channels, formed by the voidage between the fibers, and a hydrogel layer with porous properties.

1. Introduction

Resin based packed bed chromatography is the most used unit operation in the downstream processing of protein therapeutics due to its high protein binding capacity and separation efficiency. Because porous resin based media is often accompanied by a high price, time and labor intensive validation and qualification, residence times up to 4 min and high pressure drops, the downstream processing tends to be one of the most costly aspects of modern bioprocessing [1–3]. As the biotechnology market is the fastest growing segment in the world pharmaceutical market, with major improvements in the upstream processing rising titers of biomolecules to several grams per liter within industrial applications, a high demand for improvements within the downstream processing is generated [4–6].

Replacing resin based packed bed adsorbents with media, like membrane adsorbers or monoliths, relying on fast mass-transport, is often discussed as a promising alternative to lower the pressure drops and residence times [7–10]. The use of membrane adsorbers has already been proven to be successful on a small scale and for polishing applications even on larger scales [11]. Flow rate independent operations and the potential for higher mass-transfer rates result in sufficient productivities.

To address issues like cost intensive validation and qualification of *pack bed* chromatography columns, many attempts have been made to further decrease the cost of chromatographic media to enable a disposable plug and play separation technology. In the course of these investigations low-cost materials have been investigated as possible chromatographic media. The demands for these materials include, besides the low price, a high surface area, high packed bed permeability, good flow uniformity and unrestricted surface access for large adsorbing species. Recent advances in the field of electrospun nanofibers allow the production of fabrics with properties comparable to those of membranes regarding voidage, pore size and specific surface areas. It could be demonstrated that binding capacity and permeability were high enough to generate competitive productivities, moreover the random deposition of nanofibers during the fabric formation encourages impeded flow and reduces channeling [12–15]. Other approaches make use of

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hydrogel functionalized composite fibers [16,17] or non-woven materials produced by extrusion [18].

Instead of using membrane-like fabrics, a bed of randomly orientated short-cut fibers instead of porous spherical particles has also been described. These early studies reported sufficient column packing efficiencies, but as the fibers possess no internal porosity the surface area is limited resulting in low binding capacities. Reducing the fiber dimension can lead to higher binding capacities but will also reduce the bed permeability resulting in lower productivities [19–21].

Recent advances in the production of fibers with shaped cross-sections based on extrusion processes [22] offer the possibility to produce high-surface fibers composed of various thermoplastic base materials without reducing the overall fiber dimension. Fiber extrusion additionally offers the advantage of mass-production at rather low cost. Their use as a stationary phase in HPLC [23,24] and as an affinity and ion-exchange medium [25–28] have been investigated. A general challenge, however, which has to be addressed, is the cost-efficient formulation of fiber bed formats useful for robust large scale operations.

This work makes use of commercially available high-surface area short cut fibers with a "winged" shaped cross-section produced by extrusion (Fig. 1). Cation-exchange functionalities are produced using SI-ATRP to graft a reactive hydrogel layer on the fiber surface followed by subsequent modification giving rise to sulfonic acid groups. This controlled grafting approach is used to produce favorable hydrogel structures to enhance the binding capacity and permeability of the fiber bed. The resulting fibers have been extensively characterized regarding their column packing efficiency, fiber bed structure, binding capacity and permeability and been compared to resin and membrane based commercial products with a similar hydrogel and ligand chemistry.

2. Experimental

2.1. Materials

2.1.1. Chemicals

2-Bromoisobutyrylbromide (2-BiBB, purity > 97%, Alfa Aesar), trimethylamine (TEA, purity > 99.5% Sigma Aldrich), poly(allyl amine) (PAA, 15 wt% in water, MW: 15,000 g/mol, Beckmann-Kenko GmbH), glycidyl methacrylate (GMA, purity > 97%, Sigma Aldrich), copper(I)bromide (CuBr, purity > 98%, Sigma Aldrich), l-ascorbic acid (AsAc, purity > 99%, Sigma Aldrich), 2,2′-bipyridine (bipy, purity > 98%, Alfa Aesar), dichloromethane (DCM, anhydrous purity 99.8%, Sigma Aldrich), 2-propanol (IPA, purity > 99.5%, Sigma Aldrich), hydrochloric acid (HCl, ACS reagent, 37%, Sigma Aldrich), sodium hydroxide (NaOH, purity > 97%, Sigma Aldrich), sodium chloride (NaCl, purity > 99.5%, Sigma Aldrich), acetic acid (AA, 100%, Roth), potassium dihydrogen phosphate (>99%, Roth), dipotassium hydrogen phosphate (purity > 99%, Roth), sodium sulfite (purity > 99.5%, Roth), tetrabutylammonium hydrogensulfate (TBABS, purity > 97% Sigma Aldrich), lysozyme (chicken egg white, dialyzed, lyophilized, Sigma Aldrich), myoglobin (equine skeletal muscle, purity 95–100%, Sigma Aldrich), bovine serum albumin (BSA, purity > 97%, Sigma Aldrich), pullulan (standard set, M_w 320–740,000 g/mol, Sigma Aldrich), polyclonal human immunoglobulin G (hIgG, Cytoglobin, Bayer). Monomers were freed of the inhibitor by passing over a column of neutral aluminum oxide. All other chemicals were used as received. Ultrapure (UP) water has been produced by an arium®pro ultra-pure water system (Sartorius Stedim Biotech GmbH). The monoclonal antibody (mAb1) has been kindly donated by Sartorius Stedim Biotech.

2.1.2. Stationary phases

Polyethylene terephthalate (PET) winged fibers (6 mm cut length, 3 deniers per filament (dpf)) have been purchased from Allasso Industries. As determined by BET nitrogen adsorption experiments (Gemini V – Surface Area and Pore Size Analyzer, Micromeritics) the fibers have a specific surface area of 2 m^2/g. SEM photographs reveal a diameter of about 15 μm.

Strong cation-exchange resins (Fractogel® EMD SO_3^- (M)) have been purchased from Merck Millipore. As stated by the supplier Fractogel® ion exchangers are cross-linked polymethacrylate resins with pore sizes of about 800 Å and a particle diameter between 48 and 60 μm grafted with polymer chains bearing sulfoisobutyl groups. Pre-packed columns (50 mm bed height, 5 mm diameter, 1.0 mL column volume) have been purchased from Atoll GmbH.

Strong cation-exchange membrane adsorbers (Sartobind S) have been kindly donated by Sartorius Stedim Biotech GmbH. The adsorber consists of a grafted hydrogel layer bearing sulfonic acid groups on a regenerated cross-linked cellulose membrane having a pore size between 3 and 5 μm as stated by the supplier. As determined by BET nitrogen adsorption experiments the membranes have a specific surface area of 1.1 m^2/g.

2.2. Preparation of strong cation-exchange fibers

The preparation of strong cation-exchange fibers is achieved via a four step sequence as shown in Fig. 2. In a first step the PET fibers were reacted with poly(allyl amine) to anchor a high density of amine groups on the surface without damaging the bulk material [29]. 50 mL of a solution containing 75 vol% UP-water, 25 vol% dioxane and 10 wt% PAA has been adjusted to a pH of 12.5 and heated to 85 °C before 1.0 g of PET fibers were added. The suspension has been heated for a predetermined time to reach the desired amine group density on the surface. Afterwards the fibers were filtered and washed with 200 mL of 0.1 m aqueous NaOH solution, 500 mL of UP-water and 200 mL ethanol before being dried at 60 °C for 6 h. The fibers were characterized regarding their weight loss and amine group density. The initiator immobilization was achieved by a protocol widely used in the literature [30]. 1.0 g of PET fibers with amine groups (PET-NH_2) were suspended in a solution containing 50 mL of DCM, 2 wt% 2-BiBB and 2 wt% TEA. The suspension was shaken at room temperature for 2 h before the fibers were filtered off and washed with copious amounts of 2-propanol, acetone and UP-water. The fibers (PET-Br) were characterized regarding their amine group density and initiator density.

The hydrogel layer was grafted on the PET-Br fibers by SI-ATRP. In a typical experiment 200 mL of a solution compromised of 50 vol% 2-propanol and 50 vol% UP-water containing GMA (2.5 g, 17.5 mmol, 170.0 eq.) is deoxygenated by purging with N_2 for 30 min before CuBr (15.0 mg, 104 μmol, 1.0 eq.), AsAc (40.0 mg, 227 μmol, 2.1 eq.) and bipy (100.0 mg, 640 μmol, 6.1 eq.) are added. The solution has been sonicated for 15 min under constant N_2 purging until a homogenous brown solution is formed. The PET-Br fibers were immersed in the polymerization solution at room temperature under oxygen-free conditions for a specific amount of time to control the grafting degree/chain length. The polymerization has been stopped by exposing the catalyst to air. The PET-GMA fibers were afterwards washed in copious amounts of water, 2-propanol and acetone to remove trace amounts of copper and non-covalently bound polymer and have been dried and weighed. To anchor sulfonic acid groups within the hydrogel layer the epoxide groups were reacted with sodium sulfite using a phase-transfer catalyst. 50 mL of a solution containing 18 wt% sodium sulfite, 5 wt% TBABS, 2 wt% dipotassium hydrogen phosphate and 75 wt% UP-water has been adjusted to pH 8 and heated to 85 °C before 1.0 g PET-GMA fibers were added. The suspension was agitated for