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A mechanistic model of ion-exchange chromatography on polymer fiber stationary phases

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ABSTRACT

Fibers are prominent among novel stationary phase supports for preparative chromatography. Several recent studies have highlighted the potential of fiber-based adsorbents for high productivity downstream processing in both batch and continuous mode, but so far the development of these materials and of processes employing these materials has solely been based on experimental data. In this study we assessed whether mechanistic modeling can be performed on fiber-based adsorbents. With a column randomly filled with short cut hydrogel grafted anion exchange fibers, we tested whether tracer, linear gradient elution, and breakthrough data could be reproduced by mechanistic models. Successful modeling was achieved for all of the considered experiments, for both non-retained and retained molecules. For the fibers used in this study the best results were obtained with a transport-dispersive model in combination with a steric mass action isotherm. This approach accurately accounted for the convection and dispersion of non-retained tracers, and the breakthrough and elution behaviors of three different proteins with sizes ranging from 6 to 160 kDa were accurately modeled, with simulation results closely resembling the experimental data. The estimated model parameters were plausible both from their physical meaning, and from an analysis of the underlying model assumptions. Parameters were determined within good confidence levels; the average confidence estimate was below 7% for confidence levels of 95%. This shows that fiber-based adsorbents can be modeled mechanistically, which will be valuable for the future design and evaluation of these novel materials and for the development of processes employing such materials.

1. Introduction

Chromatography is the main unit operation for the purification of biological products at preparative scale. At present, the majority of such unit operations are performed in packed beds filled with porous and spherical adsorbent particles. These are prepared from inorganic base materials or natural or synthetic polymers such as agarose and dextran or polystyrene and polymethacrylate [1]. While these stationary phase materials offer high binding capacities and high separation efficiencies, there are several disadvantages [2] with respect to preparative scale bioseparations. As most binding sites in these materials are located within the adsorbent particles, they are only accessible via diffusion. This results in diffusional limitations, particularly for larger molecules with low diffusivity, such as proteins. Packed beds filled with such stationary phase materials feature high packing densities and high pressure drops. When taken together, these properties limit the range of feasible operational flow rates and bed heights, and thus also limit the throughput of processes involving these materials.

Higher titers in upstream processing, overall increasing demand for biopharmaceuticals, and tightening cost constraints necessitate increasing both the throughput and the productivity of downstream processes. At the same time, regulatory requirements call for a better process and quality understanding. To overcome the limitations of conventional adsorbents, several alternative stationary phases with improved flow and/or mass transfer properties have been developed and commercialized, such as pellicular or gigaporous beads, monoliths and membrane adsorbers.

Another approach, which has been proposed early on [3] and has regained interest recently [4–10], lies in the use of polymeric fibers as chromatographic supports. They can be prepared from various base polymers, including natural and synthetic ones, and are available in different formats, i.e. different shapes, lengths, and structures. They can be arranged in different ways for use as a

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chromatography matrix [4], ranging from randomly packed short fibers [6,11,12] to aligned fibers [13,14] to non-woven fiber mats [7], and woven fabrics [15]. Fibers are major intermediate products of the textile industry, and various technologies for mass production and surface modification exist. This results in very low manufacturing costs for fibers, which are potentially much lower than the costs for other stationary phases [4]. Recent advances in the fabrication of high surface area fibers and in surface modification protocols have made it possible to prepare fiber-based adsorbents with high capacities and low pressure drops at high flow rates. Several experimental studies have highlighted the potential of such materials for high throughput and high productivity downstream processing in both batch [6–8] and continuous mode [9].

Despite these promising reports, all studies on fibers as chromatographic supports have been based on experimental data only. To the best of our knowledge, no attempts have been made to model chromatographic processes on fiber stationary phases. For other types of stationary phases a wide variety of mechanistic models have been developed [16,17]. Different models have been compared and criteria for selecting the appropriate modeling depth [18] and work flows for the determination of model parameters have been put forward [16]. Mechanistic modeling has been shown to be valuable for process optimization [19], process characterization [20,21] and process scale up [22,23], as well as the optimization of the adsorbent structure itself [24,25]. A validated mechanistic model for fiber-based adsorbents would therefore be of great use; it would generate an understanding of the relevant transport and binding mechanisms, and thereby support the ongoing development and evaluation of these novel materials.

In this study we assessed whether mechanistic modeling can be performed on fiber-based adsorbents. First, we performed characterization and efficiency experiments on hydrogel grafted anion-exchange fibers in order to develop a mechanistic model. Then, we tested whether the proposed model can accurately describe the convection and dispersion of non-retained molecules. Next, the applicability of the model towards simulating the binding, breakthrough, and elution of differently sized proteins was evaluated. Finally, we assessed whether the model parameters can be identified with good confidence and compared the proposed model with alternative models.

2. Theory

In this study an experimental investigation was conducted in order to determine the porosities and transport properties of a column filled with randomly-packed fibers (cf. Sections 4.1 and 4.2). The fibers were short cut, shaped, hydrogel grafted strong anion exchange fibers (cf. Section 3.1.2). Based on the results of these experiments, the following mechanistic model was developed for a column that is randomly packed with fibers.

2.1. Model assumptions

In order to define the model parameters, a few assumptions needed to be made. When the column was randomly packed with fibers as described in Section 3.2.2., no peak fronting or major peak tailing was observed during efficiency testing. Because of this, the column was assumed to be radially homogeneous, without major cavities and with no influence from wall effects. As the column inlet pressure was typically below 4 bars, the compressibility of the mobile phase was neglected and the mobile phase velocity was considered to be constant. The fibers were assumed to be porous and uniform in size. We assumed that both the grafted hydrogel layer and the support phase can contribute to the porosity of the fibers. The pores between the fibers were considered to be uniform in size. The axial dispersion coefficient was considered to be independent of the axial position inside the column or the solute concentration. Finally, we made the general assumptions that the partial molar volumes of the sample components are the same in the mobile and the stationary phase, that the solvent is not adsorbed, that no thermal effects are present, and that the column is operated under constant conditions [17].

2.2. Transport dispersive model

A transport-dispersive model (TDM) was used to describe the macroscopic mass transport through the fiber column. The mobile phase was divided into the interstitial volume between the fibers with concentration c_i of component i and the pore volume within the fibers with concentration $c_{p,i}$ of component i, with respect to $i = 1, \ldots, N$ components. The fraction of the interstitial volume V_{int} with respect to the total column volume V is represented by the interstitial porosity ε, and the fraction of the pore volume within the fibers with respect to the total fiber volume is represented by the fiber porosity ε_f. The overall column porosity results from the sum of the interstitial volume between the fibers and the pore volume within the fibers, as depicted in Eq. (1):

$$\varepsilon_t = \varepsilon + \varepsilon_f(1-\varepsilon) \tag{1}$$

The rate of change of the interstitial concentration $c_i(x, t)$ of component i at position x in a column with length L is described by Eq. (2):

$$\frac{\partial c_i}{\partial t}(x,t) = -u_{\text{int}}(t)\frac{\partial c_i}{\partial x}(x,t) + D_{ax}\frac{\partial^2 c_i}{\partial x^2}(x,t) - \frac{1-\varepsilon}{\varepsilon}k_{eff,i}a_f(c_i(x,t) - c_{p,i}(x,t)) \quad \forall i \tag{2}$$

The first term in Eq. (2) accounts for the change in concentration due to convective mass transport along the column with an average interstitial velocity u_{int}. Peak broadening effects due to axial diffusion and hydrodynamic dispersion are modeled as dispersion in space with an axial dispersion coefficient D_{ax}. The last term in Eq. (2) describes the concentration exchange between the interstitial volume and the volume of the fibers. It considers the differences in concentrations and volumes and depends on the specific surface area (SSA) of the fibers a_f and a component-specific effective mass transfer coefficient $k_{eff,i}$, which lumps contributions of external film and internal pore diffusion processes. For the column inlet and outlet Danckwerts boundary conditions were used, as shown in Eqs. (3) and (4), where $c_{in,i}(t)$ is the injected concentration of component i at the column inlet at time t:

$$\frac{\partial c_i}{\partial x}(0,t) = \frac{u_{\text{int}}(t)}{D_{ax}}(c_i(0,t) - c_{in,i}(t)) \quad \forall i \tag{3}$$

$$\frac{\partial c_i}{\partial x}(L,t) = 0 \quad \forall i \tag{4}$$

The concentration of component i within the fiber pores $c_{p,i}$ depends on the fiber porosity ε_f, and its rate of change is influenced by exchange with the interstitial phase and stationary phase, as depicted in Eq. (5):

$$\frac{\partial c_{p,i}}{\partial t}(x,t) = \frac{k_{eff,i}a_f}{\varepsilon_f}(c_i(x,t) - c_{p,i}(x,t)) - \frac{1-\varepsilon_f}{\varepsilon_f}\frac{\partial q_i}{\partial t}(x,t) \quad \forall i \tag{5}$$

2.3. Steric mass action isotherm

The concentration exchange with the stationary phase was described with the steric mass action (SMA) isotherm [26]. The SMA isotherm is a commonly used semimechanistic isotherm for ion-exchange chromatography, and its applicability for adsorbents with