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Chromatography modelling to describe protein adsorption at bead level

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ABSTRACT

Chromatographic modelling can be used to describe and further understand the behaviour of biological species during their chromatography separation on adsorption resins. Current modelling approaches assume uniform rate parameters throughout the column. Software and hardware advances now allow us to consider what can be learnt from modelling at bead level, enabling simulation of heterogeneity in bead and packed bed structure due to design or due to changes during operation. In this paper, a model has been developed to simulate at bead level protein loading in 1.5 μl microfluidic columns. This model takes into account the heterogeneity in bead sizes and the spatial variations of the characteristics of a packed bed, such as bed void fraction and dispersion, thus offering a detailed description of the flow field and mass transfer phenomena. Simulations were shown to be in good agreement with published experimental data.

1. Introduction

Pharmaceutical companies frequently have limited time and material to make a full assessment of suitable resins for a given purification process so as to produce sufficient amount of material needed for clinical studies [1,2].

In recent years, scale-down systems based on the microplate format have been used for high throughput screening (HTS) studies so as to investigate separation conditions for therapeutic proteins [2–7]. Some of the systems currently used include batch binding microtiter plates, microtiter filter plates and chromatographic pipette tips. Their integration with robotic liquid dispensing systems has enabled high throughput studies.

In addition to these scale-down systems, microfluidic technologies have been used to provide a thorough investigation of separation conditions using even smaller amounts of protein [1,8–13]. A parallel architecture of microfluidic packed bed systems can be set up to investigate any number of parameters like adsorbent type, buffer solution, pH, flow rate, etc., which increase throughput. With the aid of these methods, microscopy may be used to visualise protein concentration profiles within the bed structure, whereas confocal microscopy may be used to observe within the bead itself, which would be otherwise difficult in larger scale systems [1].

Appropriate mass-transfer or kinetic models of the chromatographic separation may be used to convert the information provided by the HTS studies into quantitative predictions of column performance [4,14]. Current models like the equilibrium-dispersive model, the general rate model and the steric mass action (SMA) model have been developed based on an average bead size, thus ignoring bead size heterogeneity [15–25]. They use dispersion as a way to describe the movement of protein between beads in the liquid phase of the column and thin film theory to describe diffusion in the outer surface of the beads. They assume that there is no radial velocity and concentration variations, i.e., both profiles are considered flat, so only one spatial dimension is needed to be included in a chromatographic model.

The above assumptions introduce empirical equations with uniform rate parameters like dispersion and film mass transfer coefficients. Though physically unrealistic, they provide a simplified and accurate prediction of the separation of protein solutions in laboratory (1–40 ml) and manufacturing scale columns (~150 L) taking into account that any heterogeneity in concentration or velocity has been shown to be averaged out when the ratio of column to bead diameter is greater than 30 [16,17,26–28]. However, their use in microscale systems (~1.5–200 μl) is not applicable, as the ratio of column to bead diameter varies from 2 to 20. Thus spatial variations are not averaged out, making the transition from small to large scale unfeasible.

In this paper, a model has been developed to describe at bead level the hydrodynamics, mass transfer and adsorption/desorption kinetics of chromatographic processes. The need to use such models

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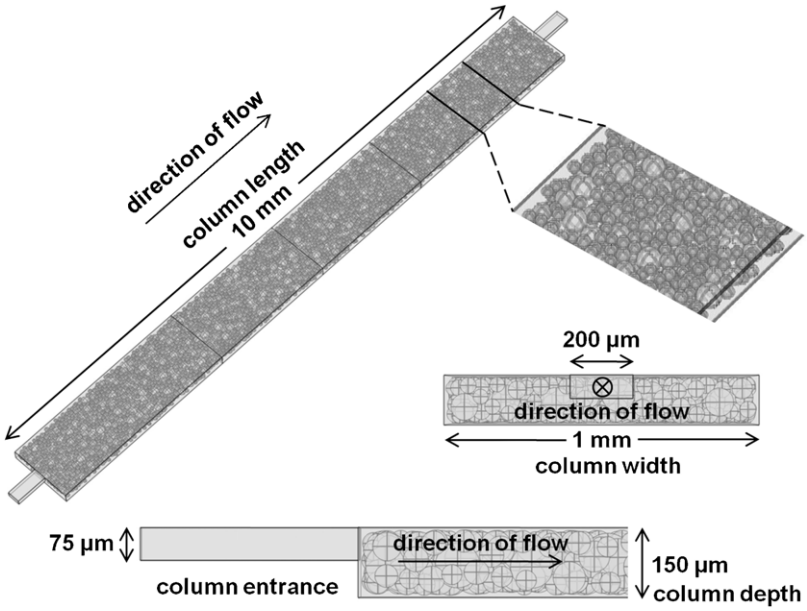

Fig. 1. Three-dimensional schematic view of the microfluidic column. The number of beads within the microfluidic column is around 4700 and their diameter is normally distributed between 40 and 100 μm (average bead diameter: 70 μm). The bed void fraction is 0.55.

derives from the inability of current models to capture the radial and concentration variations, which are dominant in microscale systems (microfluidic columns, microcolumns and microtips) used extensively in academia and industry. The validity of the model to simulate chromatographic processes at bead level has been demonstrated in 1.5 μl microfluidic columns by comparing simulated versus experimental chromatograms derived from the work of Shapiro et al. [1]. This type of modelling may provide an alternative future approach in predicting the performance of process scale separations using microscale data.

2. Materials and methods

2.1. Microfluidic column operation

Fig. 1 displays a schematic diagram of the microfluidic column. Its dimensions are 10 mm × 1 mm × 0.15 mm and it is packed with Sepharose™ FF beads (GE Healthcare, Uppsala, Sweden). The beads have been sieved since larger beads block the channels and smaller beads may block the entrance and exit of the microfluidic column [1]. Their diameter varies from 40 to 100 μm following normal distribution [1]. The bed void fraction is 0.55 [1].

The microfluidic column was equilibrated with five column volumes (CV) of 0.05 M sodium phosphate buffer pH 5.5 (start buffer). Lysozyme, 1 mg ml^{-1} (0.8 mg ml^{-1} unlabeled lysozyme and 0.2 mg ml^{-1} fluorescently labelled lysozyme in the same buffer), was pumped isocratically at linear velocities of 220 and 270 cm h^{-1} (5.33 and 6.67 μl min^{-1} respectively). Monitoring of the breakthrough was achieved by recording the average fluorescence intensity in the outlet channel every 3 s. The experimental breakthrough curves obtained at two different flow rates were compared to the corresponding simulated breakthrough curves in order to validate the model. Further details about the design, packing and operation of the microfluidic column are provided by Shapiro et al. [1].

2.2. Chromatographic modelling at bead level

The flow field of the mobile phase of the microfluidic column is provided by solving the continuity and Navier–Stokes equations for steady state incompressible flow [29]. The liquid inside the beads is assumed to be stagnant; therefore the continuity and Navier–Stokes equations are not solved inside the beads.

$$\nabla \cdot \vec{u} = 0 \tag{1}$$

$$-\nabla \cdot \mu(\nabla\vec{u} + (\nabla\vec{u})^T) + \rho\vec{u} \cdot \nabla\vec{u} + \nabla P - \rho \cdot \vec{g} = 0 \tag{2}$$

where P is the pressure, ρ is the density, μ is the viscosity $\vec{u}$ is the velocity and $\vec{g}$ is the accelaration due to gravity.

The term $-\nabla \cdot \mu(\nabla\vec{u} + (\nabla\vec{u})^T)$ describes the effect of viscous forces on the flow field, the term $\rho\vec{u} \cdot \nabla\vec{u}$ is the rate of momentum addition by convective acceleration, ∇P is the pressure gradient and $\rho \cdot \vec{g}$ is the gravity.

The protein solution is assumed to have water-like physical properties. A constant velocity and a zero reference pressure are imposed at the inlet and outlet of the microfluidic column respectively. No slip conditions are imposed in the surface of the beads and the walls of microfluidic column.

To lower the computer memory requirements of the overall simulation, the flow field is simulated first as the viscosity and densities of the protein solution are considered constant (relatively low protein concentration; no fouling present). The solved flow field is then saved and coupled with a mass transfer model, which describes protein convection/diffusion in the mobile phase of the microcolumn, diffusion in the liquid phase of the beads and adsorption onto the solid phase of the beads. In cases though, where the viscosity or the density of the feed solution is a function of protein concentration, both hydrodynamic and mass transfer models have to be solved simultaneously.

The model has the following assumptions: (i) the chromatographic separation is isothermal, (ii) the physical properties of the beads are constant and independent of the protein/salt concentration, (iii) the mass transfer within the beads is controlled by diffusion, (iv) the mass transfer parameters are concentration independent, (v) the salt is inert, (vi) the protein does not bind to the walls of the microfluidic column.

The mass transfer of protein in the mobile phase is described by the following equation:

$$\frac{\partial c_i}{\partial t} - D\nabla^2 c_i + \vec{u}\nabla c_i = 0 \tag{4}$$