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A novel two-zone protein uptake model for affinity chromatography and its application to the description of elution band profiles of proteins fused to a family 9 cellulose binding module affinity tag

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Abstract

A novel two-zone model (TZM) is presented to describe the rate of solute uptake by the stationary phase of a sorption-type chromatography column. The TZM divides the porous stationary-phase particle into an inner protein-free core and an outer protein-containing zone where intraparticle transport is limited by pore diffusion and binding follows Langmuir theory. The TZM and the classic pore-diffusion model (PDM) of chromatography are applied to the prediction of stationary-phase uptake and elution bands within a cellulose-based affinity chromatography column designed to selectively purify proteins genetically labelled with a CBM9 (family 9 cellulose binding module) affinity tag. Under both linear and nonlinear loading conditions, the TZM closely matches rates of protein uptake within the stationary phase particles as measured by confocal laser scanning microscopy, while the PDM deviates from experiment in the linear-binding region. As a result, the TZM is shown to provide improved predictions of product breakthrough, including elution behavior from a bacterial lysate feed.

Keywords: Protein purification; Affinity chromatography; Fusion tag; Cellulose binding module; Pore diffusion model; Confocal laser scanning microscopy

1. Introduction

Due to its exquisite binding selectivity, affinity chromatography is finding increasingly widespread use in the purification of natural and recombinant protein products at the manufacturing scale [1]. The large-scale capture and affinity purification of monoclonal antibodies on immobilized protein A columns is the most widely used and thoroughly studied application of industrial affinity chromatography [2], but many other important applications exist, including the purification of human tissue plasminogen from blood plasma using immobilized lysine [3] and the purification of ATP-dependent kinases and NAD+-dependent dehydrogenases using immobilized 5′-AMP [4].

The power of affinity separations can be extended to proteins with no known binding partner through recombinant DNA technology, which enables production of a target protein as a recombinant fusion to an N- or C-terminal affinity tag possessing a highly specific binding partner that can be immobilized to form a stable affinity chromatography media. A number of affinity tag technologies are commercially available, including the glutathione *S*-transferase (GST) tag [5,6], the calmodulin binding peptide tag [7–9], the streptavidin tag [10,11], the FLAG peptide tag [12,13] and the polyhistidine tag, which permits selective capture and purification of the fusion protein on an immobilized metal affinity chromatography column [13–15].

Intraparticle mass transport, most notably the rate of diffusion within the pore liquid of the stationary phase, typically limits protein uptake and controls band broadening in adsorptive modes of protein chromatography, including affinity chromatography, where the binding interaction with the stationary phase is strong [16,17]. A number of models have therefore been

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developed to describe intraparticle mass transport inside sorbent particles, with the pore diffusion model (PDM) finding the most widespread use (e.g. [18]). The PDM assumes that the overall rate of protein uptake is proportional to the concentration gradient in the pore liquid, permitting simplification of the general rate model of chromatography by eliminating concentration gradients in the hydrodynamic film surrounding each sorbent particle and by establishing local equilibrium of the sorbate at each radial position within the sorbent particle. Assuming for the moment that surface diffusion effects are negligible, the rate of protein uptake within the porous sorbent particle is given by the PDM through the relation

$$\varepsilon_p \frac{dc_i}{dt} = \frac{1}{r^2}\frac{d}{dr}\left(\varepsilon_p D_p r^2 \frac{dc_i}{dr}\right) - \frac{dq_i}{dt} \quad (1)$$

where when sorbate equilibrium is described by the Langmuir isotherm,

$$\frac{dq_i}{dt} = \frac{dq_i}{dc_i}\frac{dc_i}{dt} = \frac{q_i^{sat} K_{a_i}}{(1 + K_{a_i} c_i)^2}\frac{dc_i}{dt} \quad (2)$$

Thus knowledge of the stationary phase porosity ε_p, the saturation capacity of the sorbent q_i^{sat} (kg m^{-3}), and the Langmuir equilibrium binding constant K_{a_i} (M^{-1}), permits estimation of solute c_i and sorbate q_i concentration profiles within the stationary phase as a function of time. Mass transfer within an interstitial volume element of the column is given by the column continuity equation

$$\frac{dC_i}{dt} = D_L \frac{d^2 C_i}{dz^2} - u_0 \frac{dC_i}{dz} - \frac{(1-\varepsilon)}{\varepsilon}\frac{d\bar{s}_i}{dt} \quad (3)$$

where C_i is the concentration of protein i in the interstitial mobile-phase liquid, ε is the interstitial void fraction of the column, D_L is the axial dispersion coefficient (m^2 s^{-1}), u_0 is the interstitial velocity of the mobile phase (m s^{-1}), z is the axial positional vector, and $\bar{s}_i$ is the average protein concentration within the stationary phase particles of uniform radius r_p, given by

$$\bar{s}_i = \frac{3}{r_p^3}\int_0^{r_p} (\varepsilon_p c_i + q_i) r^2 dr = \frac{3}{r_p^3}\int_0^{r_p} s_i(r,t) r^2 dr \quad (4)$$

The boundary conditions for solving Eq. (1) of the PDM are given by

$$c_i(r = r_p, t) = C_i \quad (5)$$

$$\frac{dc_i(r=0,t)}{dr} = 0 \quad (6)$$

where C_i is given by solution of Eq. (3) at time t. The boundary condition at $r = 0$ given by Eq. (6) is generally applied in all continuous models of chromatography. However, the full implications of its use are not always appreciated. In particular, since c_i and q_i are both specified by Eq. (1) to be continuous functions of r and t, the application of Eq. (6) necessarily leads to a physically improbable model prediction that both $c_i(r = 0, t)$ and $q_i(r = 0, t)$ become nonzero immediately upon contact of the stationary phase particle with the mobile phase liquid, where $c_i(r_p, t = 0) = C_i^0$. Eq. (6) has nevertheless been extensively applied to the modeling of many different forms of adsorptive chromatography [19–21], including various forms of affinity chromatography [16,22–26].

A more general and physically realistic model for protein uptake within a porous stationary phase would predict for sufficiently short contact times a region within the interior of the sorbent particle that contains no protein, while both c_i and q_i would be nonzero and increase with r in the outer shell of the particle. The protein-containing zone would then be predicted to increase with time at the expense of the protein-free zone. This two-zone behavior has been observed in confocal laser scanning microscopy (CLSM) studies of protein uptake in porous chromatography particles, particularly when there is strong interaction between the sorbate and the sorbent, as is typically observed in the affinity chromatography systems [27–30]. It is reminiscent of the classic shrinking-core model of diffusion-controlled chemical reaction engineering first proposed by Weisz and Goodwin [31]. However, that model assumes that local sorbate equilibrium is defined by the rectangular isotherm and thereby predicts an infinitely steep concentration gradient at the core radius r_c separating the protein-free inner core from the sorbent-saturated outer shell of the porous particle [32].

Here we describe a generalized two-zone model for protein uptake in a porous sorbent particle that relaxes the rectangular-isotherm approximation of the traditional shrinking-core model to allow for simultaneous intraparticle mass transport and sorbent loading within the outer zone of the porous particle and thus, the presence of concentration gradients within the shell region. The model is applied to the description of elution band profiles for fusion proteins tagged at their N-terminus with TmXyn10ACBM9-2 (henceforth referred to as CBM9), the C-terminal family 9 carbohydrate-binding module of xylanase 10A from *Thermotoga maritima* [33]. In a previous paper [34], we introduced the CBM9 affinity tag and demonstrated its application in the affinity purification of recombinant proteins from *E. coli* using an inexpensive, commercially available cellulosic resin, Perloza™ MT100. CBM9 binds specifically and tightly to the reducing ends of both insoluble cellulose and simple soluble sugars, including glucose [35]. These unique binding properties allow for selective binding of CBM9-tagged fusion proteins to a porous cellulose sorbent particle and quantitative elution using 1 M glucose. Perloza™ MT100, a highly porous, beaded cellulosic resin sells for ca. $35 per liter of resin. The extraordinary low cost of this matrix, combined with its high static binding capacity for CBM9-tagged fusion proteins (10 μmol g^{-1} dry resin), offer considerable economic advantages over other commercially available affinity tag technologies. In this work, we fuse CBM9 to the N-terminus of the green fluorescent protein (GFP) from the jellyfish *Aquorin victoria* [36,37], and use the natural fluorescence of GFP as a direct and convenient means to track our fusion protein and validate our model. CLSM is used to measure temporally and radially resolved CMB9-GFP concentration profiles inside the Perloza™ MT100 sorbent particle, permitting tracking of r_c and intraparticle mass transport of protein in the outer zone of the particle. Because GFP of CBM9-GFP fluoresces naturally, uptake artifacts associated with competi-