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Determination of adsorption isotherm parameters for minor whey proteins by gradient elution preparative liquid chromatography

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ABSTRACT

Ion-Exchange Chromatography (IEC) techniques have been extensively investigated in protein purification processes, due to the more selective and milder separation steps. To date, existing studies of minor whey proteins fractionation in IEC have primarily been conducted as batch uptake studies, which require more experimental search space, time and materials. In this work, the selected resin's (SP Sepharose FF) equilibrium and dynamic binding capacity were first investigated. Next, adsorption of the pure binary mixture of lactoperoxidase and lactoferrin was studied to calibrate steric mass action (SMA) model using a simplified approach with data from single column experiments. The calibrated model was then verified by performing factorial-design based experiments for various process operating conditions assessing process performance on a larger bed height column. The model predicted results demonstrated a realistic agreement with the experiments providing reproducible column elution profile and reduced experimental work. Finally, whey protein isolate was used to evaluate model parameters in real conditions. Results obtained herein are suitable for future large scale applications.

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1. Introduction

Lactoperoxdiase and lactoferrin are two minor whey proteins having unique properties for nutritional, biological, and food ingredient applications [1]. Hence, they exemplify the potential for commercial exploitation of minor, bioactive milk protein products [2–4]. Extraction of proteins from dairy fluids by techniques such as chromatography [5–7], precipitation and membrane-based technologies, mainly ultrafiltration and diafiltration [8–12] has been extensively studied over the last few decades. However, precipitation and membrane techniques may result in undesirable consequences on changes in native structure affecting functional properties of the whey proteins. Therefore, there has been an increasing interest in liquid chromatographic processes because of many advantages reported for this technology, such as the very rapid rate of association between target proteins and functional groups; short processing times; ease of scale-up and operation without the need for lengthy column packing procedures; no heat-treatments, extremes of pH, or chemical pre-treatment that could compromise protein structure and functionality among others [13]. Over the last few decades ion-exchange chromatographic techniques have been used to purify lactoperoxidase and lactoferrin [2,14–16]. For example, Fee and Chand [17] have extensively investigated the use of cation exchange chromatography for the capture of lactoperoxidase and lactoferrin from raw milk while Noppe et al. [18] were able to purify lactoferrin from skim milk using macro-porous monolithic column.

In spite of the promise of ion-exchange chromatography for effective separation and purification of proteins in many analytical, preparative and process applications, purification of high-value proteins in bio-pharmaceutical industry is still a challenging task as the existing separation methods are not applicable for large-scale operation due to inadequate understanding of the mechanistic features affecting protein adsorption. This represents an obstacle to the development of design of large-scale chromatographic separation processes for applications estimated to average above $200 million per pharmaceutical product other than based on an empirical basis [18]. The approaches for the determination of isotherm parameters solely based on batch experiments require extensive experimental work. Consequently, downstream process development incurs large overhead costs. Besides, the batch experiments do not essentially produce parameters that could be used directly to obtain precise predictions of industrial process development. Hence there is a great interest in developing more efficient and straightforward methods to recover pure protein fractions. A more cost-effective process can be accomplished through further

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Nomenclature

$c_{p,i}$	concentration of protein i in the pores of the adsorbent (M)
$c_{p,salt}$	salt concentration in the pores of the adsorbent (M)
c_i	protein concentration i in the mobile phase (M)
c_{prot}	Concentration of protein i in stock solution (M)
c_{salt}	salt concentration in buffer (M)
c_0	initial concentrations of salt and protein at column inlet (M)
$c_{a,s}$	salt concentration at gradient begin (M)
$c_{e,s}$	salt concentration at gradient end (M)
D_{ax}	axial dispersion coefficient (mm^2/s)
$k_{eff,i}$	effective mass transfer coefficient of protein i (m/s)
$k_{ads,i}$	adsorption coefficient of protein i in the SMA isotherm
$k_{des,i}$	desorption coefficient of protein i in the SMA isotherm
$k_{eq,i}$	equilibrium coefficient of protein i
L	length of the column (mm)
q_i	protein concentration i on the adsorbent phase (M)
r_p	particle radius (mm)
u_{int}	interstitial velocity of the fluid (mm/s)
ε_c	column voidage
ε_p	particle voidage
ε_t	total voidage
$1-\varepsilon_t/\varepsilon_t$	phase ratio
Λ	total ionic capacity (M)
ν_i	characteristic charge of protein i in SMA isotherm
σ_i	steric factor of protein i in the SMA isotherm

development of knowledge of its fundamentals. A quantitative understanding of protein adsorption would, therefore, overcome the obstacle to the development of large-scale chromatographic separation processes other than based on an empirical basis [19]. It would enable to simulate the process efficiently as a tool for modern process development strategies. By this approach, the need for labor-intensive experiments is reduced, and hence, not only shortens the time for development from laboratory to production scale but also reduces the overall cost for development.

The aim of this study is to develop a model-based method to determine steric mass action (SMA) adsorption isotherm parameters of two minor whey proteins, lactoperoxidase and lactoferrin. The isotherm parameters were determined for lactoperoxidase and lactoferrin at pH 6.7 on a 1 mL column pre-packed with the strong cation-exchange adsorbent, SP Sepharose FF. A Fast Protein Liquid Chromatography (FPLC) system was coupled to the experimental system for analysis. Subsequently, the quality of the results was evaluated on a pre-packed 4.7 mL column. Moreover, this study also incorporated the prediction of the model with respect to the target protein recovery from whey protein isolate.

2. Theory

2.1. Transport-dispersive model

A mathematical model involving convective and dispersive transport, mass transfer resistances and equations describing sorption in ion-exchange chromatography is described below. Details on the equations and the execution of their solution can be found elsewhere [20–22]. On column level, concentration change for the ith component with respect to the time and position, is described by:

$$\frac{\partial c_i}{\partial t} = -u_{int}\frac{\partial c_i}{\partial x} + D_{ax}\frac{\partial^2 c_i}{\partial x^2} - \frac{1-\varepsilon_c}{\varepsilon_c}\cdot\frac{3}{r_p}k_{eff,i}[c_i - c_{p,i}] \quad (1)$$

The first term on the right hand side represents the convective transport through the column while the second and third term represents respectively the dispersive transport and the mass transfer to the particle surface. The symbol u_{int} indicates the interstitial velocity, ε_c the column voidage, r_p the particle radius, D_{ax} the axial dispersion representing combined effect of dispersion and diffusive processes, and $k_{eff,i}$ epitomize combined effect of both the internal and external mass transfer resistances in one lumped film diffusion coefficient. Likewise, on particle level, concentration change for the ith component is expressed by:

$$\frac{\partial c_{p,i}}{\partial t} = \frac{3}{\varepsilon_p r_p}k_{eff,i}[c_i - c_{p,i}] - \frac{1-\varepsilon_p}{\varepsilon_p}\frac{\partial q_i}{\partial t} \quad (2)$$

where q_i denotes the concentration of component i within the particle and ε_p the particle voidage. The first term on right hand side describes adsorption and desorption processes on particle level, i.e. the interaction between mobile and particle bound phase. The expression $\partial q_i/\partial t$ employs an isotherm equation described in the next section.

The boundary conditions used are of Danckwert's type conditions [23]:

$$\frac{\partial c_i}{\partial x}(0,t) = \frac{u_{int}}{D_{ax}}(c_i(0,t) - c_{inj}(t)) \quad (3)$$

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

Method of lines was used to convert the partial differential equations (PDEs) into a system of ordinary differential equations (ODEs). The resultant ODEs along with Danckwert's boundary conditions are then solved in Matlab R2014a (The Mathworks, Natick, ME, USA).

2.2. Steric mass action isotherm

The steric mass action (SMA) isotherm [24] is a frequently used isotherm in IEC when one or more macromolecules with steric hindrance are involved as the case of proteins studied here. SMA isotherm is capable in replicating the influence of counter-ions on the retention behavior of protein species through the use of the proteins' characteristic charges, ν_i and the average number of adsorbent binding sites of the proteins based on the assumption of a monovalent salt counter-ion. SMA isotherm also considers column properties such as total ionic capacity of adsorbent, Λ, steric effect of the proteins, σ_i, as well as the average number of shielded biding sites on adsorbent surface. The dynamic SMA isotherm is given in Eq. (5):

$$\frac{\partial q_i}{\partial t} = k_{ads,i}\left[\Lambda - \sum_{j=1}^{k}(\upsilon_i - \sigma_i)q_i\right]^{\upsilon_i} c_i - k_{des,i}c_{salt}^{\upsilon_i}q_i \quad (5)$$

where q_i and c_i represent the concentration of the protein i in adsorbed and in solution, respectively, c_{salt} is the salt concentration of the solution, and $k_{ads,i}$ and $k_{des,i}$ are respectively the adsorption and desorption coefficients. Assuming attainment of rapid equilibrium ($\partial q_i/\partial t = 0$), Eq. (5) rearranges to:

$$k_{i,eq} = \left(\frac{q_i}{c_i}\right)\left(\frac{c_{salt}}{\Lambda - \sum_{i=2}^{n}(\nu_i + \sigma_i)q_i}\right)^{\nu_i} \quad (6)$$

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