Journal of Chromatography A, 1214 (2008) 71–80

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Chromatographic behavior of a polyclonal antibody mixture on a strong cation exchanger column. Part II: Adsorption modelling

Nicola Forrer, Alessandro Butté, Massimo Morbidelli*

Institute for Chemical and Bioengineering, Department of Chemistry and Applied Biosciences, ETH Zurich, 8093 Zurich, Switzerland

ARTICLE INFO

Article history:
Available online 17 October 2008

Keywords:
Polyclonal IgG
Regression
Multi-component pore model
Prediction
Concentration profiles

ABSTRACT

The adsorption of a polyclonal IgG mixture on a strong cation exchanger column is characterized using a detailed multi-component pore model. This model is explicit in all transport parameters and includes salt dependent isotherms. As discussed in the first part of this work, the IgG mixture can be simplified by considering two pseudo-variants only. Linear gradient experiments are used to fit the salt dependent adsorption isotherms and the mass transport parameters for the two pseudo-variants. Using the model, breakthrough curves are predicted with good accuracy. The model is also implemented to visualize the axial and radial concentration profiles of the two pseudo-variants in the column during a loading experiment.

1. Introduction

In Part I of this work [1], the adsorption of the polyclonal antibody (PAb) mixture on a strong cation exchanger column was discussed. The adsorption isotherm and mass transport parameters have been estimated using a suitable combination of experiments, namely pulse injections under non-adsorbing conditions, linear gradient elutions and frontal analysis. In particular, short-cut methods have been used to estimate the important fluid- and thermodynamic parameters, such as the measurement of the HETP values to estimate the transport coefficients or the use of the Yamamoto method to estimate the adsorption isotherm under diluted conditions. It was found that the pore accessibility of the PAb is very small (only 28% of the total particle volume) and, as a consequence, the mass transport inside the particle pores is very hindered. In addition to this, the isotherm was found to be a strong function of the ionic strength, which made it estimation paricularly difficult.

The previous analysis has revealed the complex behavior of the PAb mixture in conventional cation exchange supports. Short-cut methods can be hardly used to estimate all the physicochemical parameters involved in the process. In fact, the nature of the adsorption isotherm and the severe mass transport limitations are making the system extremely sensitive to small errors in the parameters, so that their use in a numerical model would lead to largely inaccurate model forecasts. For this reason, it is often preferable to directly regress the parameters using the numerical model.

Two main approaches can be found in the literature to carry out the regression of the parameters needed to simulate the behavior of a chromatographic column: regression of batch uptake experiments or regression of elution profiles. In the first approach, the isotherm is determined directly from batch adsorption data and the mass transport is found by regressing protein uptake experiments. This approach has been widely used for the adsorption of proteins on ion exchanger materials [2–11]. Another option for the determination of the mass transport parameters is to use microscopic techniques to visualize the protein front moving to the center of the particles as the adsorption takes place. This can be done either by confocal microscopy [12–14] or by light microscopy [15]. However, these so called “off-line” methods have the disadvantage of measuring the adsorption parameters in a different fluid dynamic environment than that of the chromatographic column. For example effects like packing compression by the solvent flow, happening in a chromatographic column, can not be seen by the “off-line” methods. These effect can become very important for systems that are very sensible to pore diffusion. This limitation can be avoided when using the so called “on-line” methods. Here the isotherm and the mass transport parameters are found by regression of elution profiles. This method has also been widely used for the adsorption of proteins on ion-exchange columns [16–21].

For the simulation of the adsorption of large molecules like proteins, a very comprehensive model is needed. The most complete model present in literature is the so-called general rate model (GRM), where the concentration distribution of the different solutes

* Corresponding author.
E-mail address: morbidelli@chem.ethz.ch (M. Morbidelli).

0021-9673/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2008.10.047

in both the axial and the particle radial directions is accounted for [22]. Due to its complexity and the large number of differential equations involved, this model has gained importance in the last years due to the strong increase of computational power of the modern computers. It must be pointed out that the use of this model is mandatory in the presence of dominant mass transport resistances, as discussed by Kaczmarski and Antos [23].

In this work, the parameter regression of the elution profiles is applied for a complete characterization (i.e. under diluted and overloaded conditions) of the adsorption of a polyclonal IgG on a preparative strong cation exchanger column. Note that the aim of this work is not only to estimate the relevant parameters to run the model simulation, but to use the model as a tool for the understanding of the adsorption process. As it will be discussed in the following, through the model only it is possible to capture the full complexity of the system and the behaviors observed in the first part of this work. The use of mathematical modelling is also essential for process design and optimization. This has become especially important after the launch of the PAT initiative [24] by the FDA.

As described in Part I of this work, although the original polyclonal antibody mixture is made by a very large number of different antibodies, it can be approximated by considering only two so-called pseudo-variants, which in the following will be considered as single components. In order to simplify the regression procedure, the experiments were designed in such a way that as few parameter as possible are fitted together. The rationale behind the proposed regression procedure will be discussed in this work.

2. Model development

The preparative separation of large molecules like proteins involve complex adsorption mechanism and slow mass transfer [22]. In order to achieve an accurate prediction of the elution profile a complete model is needed, which includes all contributions to the mass transport in the chromatographic column. The GRM is accounting for the concentration changes along the column axis and the particle radius. In this regard, the following assumptions are made:

- transport is taking place by convection and diffusion in the mobile phase, i.e. in the inter-particle voids; transport is purely by diffusion in the intra-particle voids, the so-called stagnant phase;
- packing is uniform. Therefore, all porosities are constant;
- there is no concentration gradient along the column radius;
- particles have spherical symmetry;
- transport inside the particle is due to diffusion in the liquid phase only. Solid diffusion is not accounted for;
- the adsorption process is always at equilibrium. Adsorption kinetics is neglected.

Considering the previous set of assumption, a model consisting of two sets of mass balance equations, for the mobile and stagnant liquid phase, respectively, can be written. It is oft convenient to write the equations in dimensionless form. The mass balance for the i-th component in the mobile phase is

$$\frac{\partial c_i}{\partial \tau} + \frac{\partial c_i}{\partial \eta} + \varepsilon_{p,i}\frac{(1-\varepsilon_b)}{\varepsilon_b} St_i[c_i - c_{p,i}(\rho = 1)] = \frac{1}{Pe_{ax,i}}\frac{\partial^2 c_i}{\partial \eta^2} \quad (1)$$

where c_i is the concentration of the i-th component, $\tau = (tu_{int})/L$ is the dimensionless time, $\eta = z/L$ the dimensionless axial position, $\varepsilon_{p,i}$ the particle porosity accessible to the component i, ε_b is the column bed porosity, St_i is the Stanton number of component i, $c_{p,i}(\rho = 1)$ the concentration of the i-th component at the particle surface and $Pe_{ax,i}$ the axial Peclet number of component i.

The corresponding mass balance for the stagnant liquid in the particle pores can be written as

$$\frac{\partial c_{p,i}}{\partial \tau} + \frac{(1-\varepsilon_{p,i})}{\varepsilon_{p,i}}\frac{\partial q_i}{\partial \tau} = \frac{1}{Pe_i}\frac{1}{\rho^2}\frac{\partial}{\partial \rho}\left(\rho^2 \frac{\partial c_{p,i}}{\partial \rho}\right) \quad (2)$$

where $c_{p,i}$ is the pore concentration of the i-th component, q_i the corresponding concentration in the solid phase, Pe_i the Peclet number of component i and $\rho = r/R_p$ is the dimensionless radial position . Note that according to the previous definition, $\tau = 1$ corresponds to the retention time of a tracer totally excluded from the pores. The dimensionless numbers are defined as

Axial Peclet number:

$$Pe_{ax,i} = \frac{u_{int}/L}{D_{ax,i}/L^2} = \frac{t_{ax}}{t_{conv}} \quad (3)$$

Peclet number:

$$Pe_i = \frac{u_{int}/L}{D_{p,i}/R_p^2} = \frac{t_{pore}}{t_{conv}} \quad (4)$$

Sherwood number:

$$Sh_i = \frac{k_{f,i}/R}{D_{p,i}/R_p^2} = \frac{t_{pore}}{t_{film}} \quad (5)$$

Stanton number:

$$St_i = 3\frac{Sh_i}{Pe_i} = 3\frac{k_{f,i}/R_p}{u_{int}/L} = \frac{t_{conv}}{t_{film}} \quad (6)$$

where t_{ax}, t_{conv}, t_{pore} and t_{film} are the characteristic times for axial diffusion, convection, pore diffusion and film mass transfer, respectively.

The initial and boundary conditions for the mass balance equations in the mobile (Eq. (1)) and in the stagnant (Eq. (2)) phases can be then written as [25]

$$\tau = 0, \qquad c_i = c_i(0, \eta)$$

and

$$\eta = 0, \qquad \frac{\partial c_i}{\partial \eta} = Pe_{ax,i}(c_i - c_{f,i})$$

$$\eta = 1, \qquad \frac{\partial c_i}{\partial \eta} = 0$$

For Eq. (2) the initial and boundary conditions become:

$$\tau = 0, \qquad c_{p,i} = c_{p,i}(0, \rho)$$

and

$$\rho = 0, \qquad \frac{\partial c_{p,i}}{\partial \rho} = 0$$

$$\rho = 1, \qquad \frac{\partial c_{p,i}}{\partial \rho} = Sh_i[c_i - c_{p,i}(\rho = 1)]$$

For the salt, the same lumped mass balance equation as shown by Melter et al. [29] has been used in this work.

Due to its complexity, the general rate model has no analytical solution. Numerical methods have to be applied. In this work the finite difference method is used to solve the original system of partial differential equations. This method consist in transforming the space derivatives in difference equations over a small discretization interval [26,27]. The interval is achieved discretizing the space (radial and axial) coordinate. In this work, 9 grid points along the particle radius and 99 along the column axis are used. The final system of equations (ODEs) then consists of 9x99 ordinary differential equations per solute. The numerical code has been written