



Combined Yamamoto approach for simultaneous estimation of adsorption isotherm and kinetic parameters in ion-exchange chromatography



Matthias Rüdert¹, Florian Gillet, Stefanie Heege, Julian Hitzler, Bernd Kalbfuss, Bertrand Guélat*

Novartis Pharma AG, Biologics Process R&D, Klybeckstrasse 141, 4057 Basel, Switzerland

ARTICLE INFO

Article history:

Received 17 April 2015

Received in revised form 22 July 2015

Accepted 10 August 2015

Available online 17 August 2015

Keywords:

Chromatography

Ion-exchange

Protein

Adsorption equilibrium

Mass transfer

Parameter estimation

ABSTRACT

Application of model-based design is appealing to support the development of protein chromatography in the biopharmaceutical industry. However, the required efforts for parameter estimation are frequently perceived as time-consuming and expensive. In order to speed-up this work, a new parameter estimation approach for modelling ion-exchange chromatography in linear conditions was developed. It aims at reducing the time and protein demand for the model calibration. The method combines the estimation of kinetic and thermodynamic parameters based on the simultaneous variation of the gradient slope and the residence time in a set of five linear gradient elutions. The parameters are estimated from a Yamamoto plot and a gradient-adjusted Van Deemter plot. The combined approach increases the information extracted per experiment compared to the individual methods. As a proof of concept, the combined approach was successfully applied for a monoclonal antibody on a cation-exchanger and for a Fc-fusion protein on an anion-exchange resin. The individual parameter estimations for the mAb confirmed that the new approach maintained the accuracy of the usual Yamamoto and Van Deemter plots. In the second case, offline size-exclusion chromatography was performed in order to estimate the thermodynamic parameters of an impurity (high molecular weight species) simultaneously with the main product. Finally, the parameters obtained from the combined approach were used in a lumped kinetic model to simulate the chromatography runs. The simulated chromatograms obtained for a wide range of gradient lengths and residence times showed only small deviations compared to the experimental data.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Chromatographic separations in downstream processing of biopharmaceuticals build the back-bone of the purification and are critical for reducing the levels of product related variants, host-cell proteins, DNA and viral particles [1]. Different modes of chromatography have been successfully applied to preparative purifications, with ion-exchange chromatography (IEC) being one of the most frequently used tools. IEC is popular because of its high binding capacity, good resolution of different species, the chemically mild

conditions and the variety of possible process conditions [1]. However, the search for the best combination of resin and process conditions may be challenging. Chromatographic performance of IEC is affected by numerous parameters including the resin itself, the pH, the salt concentration, the buffer composition, the flow rate and the resin load. High-throughput formats have been proposed for the initial screening of resins and conditions [2,3]. Batch adsorption experiments on automated liquid pipetting platforms in multi-well formats allow screening large parameter ranges in short time.

The further optimization of chromatographic steps generally aims at improving the process performance (yield, purity, productivity, etc.) by varying the gradient shape, the resin load and the flow rate. Currently, this problem is widely tackled by the statistical approach of design of experiments (DOE) [4]. Based on a set of experiments at designed conditions, the effects of parameter (factor) changes on one or multiple response variables are quantified.

* Corresponding author. Tel.: +41 61 324 08 76.

E-mail address: bertrand.guelat@novartis.com (B. Guélat).

¹ Current address: Institute of Process Engineering in Life Sciences, Section IV: Biomolecular Separation Engineering, Karlsruhe Institute of Technology, Engler-Bunte-Ring 3, 76131 Karlsruhe, Germany.

Despite only producing a limited insight into the underlying physicochemical processes, the DOE approach is well-established in the industry as it provides a high flexibility, requires a pre-defined number of experiments and a wide variety of software packages are available.

Mechanistic modeling and numerical simulations provide an alternative method to support the optimization and characterization of chromatographic processes. In contrast to DOE, mechanistic modeling is based on a fundamental understanding of chromatography. The benefits of such models for process development include a reduced amount of experimental work compared to the DOE approach, the generation of in-depth process understanding and the possibility to extrapolate behaviors beyond the experimental ranges [5–8].

The underlying equations of chromatographic models describe the local mass balance within the column [1,9] that includes a thermodynamic part (adsorption equilibrium) and a kinetic part (mass transfer). Mass transfer can be simulated at different levels of complexity. Lumped kinetic models (LKM) are among the simplest and have been widely used for chromatographic simulations [5,7,10]. Pore diffusion models, such as the general rate model, include more detailed mass transfer terms, but are also computationally more demanding to solve [11].

For the thermodynamic part, one of the most popular isotherms for IEC is the stoichiometric displacement model (SDM) [12]. The model accounts for the salt dependency of the adsorption equilibrium and is applicable in linear, non-competitive conditions. It is based on the stoichiometric exchange of salt (counterions) and proteins between the liquid phase and the resin. Furthermore, the SDM has been extended to additionally incorporate a pH dependency by empirical [13] and mechanistic approaches [7,14,15]. The SDM also builds the basis for a popular extension to non-linear isotherm conditions, the steric mass-action (SMA) model introduced by Brooks and Cramer [16].

SDM parameters are generally estimated by a method based on linear gradient elutions (LGE), proposed by Yamamoto et al. [17,18]. The simplicity of this experimental method is a major driving force for the popularity of the SDM itself. Still, model calibration remains a significant barrier for the implementation of model-based development approaches in biopharmaceutical industry. A reduction of protein and time demand for parameter estimation would thus enable an easier integration of model-based approaches in process development workflows.

In this work, a combined approach was developed for the estimation of the kinetic parameters (Van Deemter plot) and the thermodynamic parameters (Yamamoto plot) for a chromatography simulation model (LKM). The proposed method is based on a set of LGE experiments with a simultaneous variation of the residence time and of the gradient slope. It aims at reducing the number of runs required for model calibration and simplifying the experimental work.

2. Theory

2.1. Chromatography model and adsorption isotherm

The dynamics of the chromatography column was described by the LKM [9]. It accounts for the convection and axial dispersion in the column (Eq. (1)), as well as the transport to the stationary phase by a solid film linear driving force model (Eq. (2)).

$$\varepsilon_{t,i} \frac{\partial c_i}{\partial t} + \frac{\partial q_i}{\partial t} + u_{sf} \frac{\partial c_i}{\partial z} = u_{sf} d_{ax,i} \frac{\partial^2 c_i}{\partial z^2} \quad (1)$$

$$\frac{\partial q_i}{\partial t} = k_{m,i} (q_i^{eq} - q_i) \quad (2)$$

where $\varepsilon_{t,i}$ is the total porosity accessible for component i , c_i the mobile phase concentration of component i (g/L or mol/L), q_i the stationary phase concentration of component i (g/L column or mol/L column), u_{sf} the superficial velocity (cm/min), $d_{ax,i}$ the axial dispersion of component i (cm), $k_{m,i}$ the lumped mass transfer coefficient of component i (1/min) and q_i^{eq} is the equilibrium stationary phase concentration of component i (g/L column or mol/L column). t and z are the coordinates of time (min) and column axis (cm), respectively. Note that in this work, the stationary phase concentration q_i was defined per unit of column volume V , instead of the usual definition based on unit of solid volume $(1 - \varepsilon_{t,i})V$. The former is a more conventional unit in process development in industry because it is used as scale-up constant to define column loading.

The adsorption isotherm used for ion-exchange chromatography in diluted conditions, i.e. in the linear range of the isotherm, was the SDM [1,12]. This model provides an explicit salt dependence for the equilibrium stationary phase concentrations of the proteins q_i^{eq} , given in Eq. (3).

$$q_i^{eq} = K_i \left(\frac{\Lambda}{c_s} \right)^{v_i} c_i \quad (3)$$

where K_i is the SDM equilibrium constant of component i , v_i the SDM characteristic charge of component i , Λ the ionic capacity of the stationary phase (mol/L column) and c_s the salt concentration in the mobile phase (mol/L).

For the salt, the equilibrium stationary phase concentration q_s^{eq} (mol/L column) was assumed constant and equal to the ionic capacity of the stationary phase (Eq. (4)). This was justified as the isotherm model was used only for diluted conditions with low concentrations of adsorbed proteins.

$$q_s^{eq} = \Lambda \quad (4)$$

2.2. Parameter estimation by combined Yamamoto methods

The combined Yamamoto methods aim at determining both the SDM parameters and the kinetic parameters of the chromatography model (LKM) with a single set of experiments. Therefore, the gradient slope g and the superficial velocity u_{sf} were varied simultaneously within a set of LGE experiments. The parameter estimation method is detailed in the following.

2.2.1. Porosity and phase ratio

The total porosity accessible $\varepsilon_{t,i}$ was determined experimentally for each component i following Eq. (5).

$$\varepsilon_{t,i} = \frac{V_{0,i}}{V} \quad (5)$$

where $V_{0,i}$ is the elution volume (L) of component i in non-binding conditions, for example in elution buffer, and V is the column volume (L).

Due to the definition of the stationary phase concentrations per unit of column volume, the phase ratio ϕ_i was calculated with Eq. (6), which was adapted from the usual definition [9] by replacing the term $(1 - \varepsilon_{t,i})$ by 1.

$$\phi_i = \frac{1}{\varepsilon_{t,i}} \quad (6)$$

2.2.2. Yamamoto method

The adsorption isotherm parameters for the SDM were estimated by the Yamamoto method [1,19]. It requires a set of LGE experiments at various gradient slopes to determine the salt dependence of the model. The usual equations found in the literature [19] were adapted in order to be consistent with the phase ratio definitions used in the chromatography model (stationary phase concentrations per column volume instead of solid phase volume).

Download English Version:

<https://daneshyari.com/en/article/1201173>

Download Persian Version:

<https://daneshyari.com/article/1201173>

[Daneshyari.com](https://daneshyari.com)