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Model-based high-throughput design of ion exchange protein chromatography

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ABSTRACT

This work describes the development of a model-based high-throughput design (MHD) tool for the operating space determination of a chromatographic cation-exchange protein purification process. Based on a previously developed thermodynamic mechanistic model, the MHD tool generates a large amount of system knowledge and thereby permits minimizing the required experimental workload. In particular, each new experiment is designed to generate information needed to help refine and improve the model. Unnecessary experiments that do not increase system knowledge are avoided. Instead of aspiring to a perfectly parameterized model, the goal of this design tool is to use early model parameter estimates to find interesting experimental spaces, and to refine the model parameter estimates with each new experiment until a satisfactory set of process parameters is found.

The MHD tool is split into four sections: (1) prediction, high throughput experimentation using experiments in (2) diluted conditions and (3) robotic automated liquid handling workstations (robotic workstation), and (4) operating space determination and validation. (1) Protein and resin information, in conjunction with the thermodynamic model, is used to predict protein resin capacity. (2) The predicted model parameters are refined based on gradient experiments in diluted conditions. (3) Experiments on the robotic workstation are used to further refine the model parameters. (4) The refined model is used to determine operating parameter space that allows for satisfactory purification of the protein of interest on the HPLC scale. Each section of the MHD tool is used to define the adequate experimental procedures for the next section, thus avoiding any unnecessary experimental work.

We used the MHD tool to design a polishing step for two proteins, a monoclonal antibody and a fusion protein, on two chromatographic resins, in order to demonstrate it has the ability to strongly accelerate the early phases of process development.

1. Introduction

Downstream processing of biopharmaceuticals relies on chromatographic techniques. In particular, the polishing steps role is to ensure the high purities required are reached. This step is usually based on ion exchange (IEC) or hydrophobic interaction chromatographic principles [1].

Today, the development of this step requires sequential design decisions (trial and error, heuristic design) involving statistical design tools, and often results in suboptimal performance [2]. Furthermore, large and time-consuming experimental sets are often needed to define the process parameter set that yields satisfactory results (i.e. final product quality and production cost). In the era of quality by design (QbD), detailed process knowledge is becoming an indispensable and integral part of any chromatographic step design strategy [3–11]. The goal is no longer to solely find the optimal parameter set, but to define the operating space using comprehensive system understanding. Since this has to be achieved with the same time and material resources (same "time-to-market") [12], traditional chromatography design tools are no longer sufficient. More effective tools are needed; tools that can generate enough process knowledge in less time and with less resources. New tools need to integrate new types of experiments, methods, ideas and theories that increase process understanding. Two potential solutions have emerged: high throughput experiments (HTPE) [3–7] and model-based design [8–11].

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In this work, both HTPE and model-based design approaches are combined into a new tool, called MHD (model-based high-throughput design), with four sections: prediction, HTPE in diluted conditions, HTPE using robotic automated liquid handling workstations (robotic workstation), and operating space determination and validation. Each of the four contributes different types and amounts of information, and combining results from all sections leads to the definition of the operating space.

The MHD tool was applied to the design of a cation exchange polishing step of two industrially relevant proteins. First, the elution behavior of the protein was predicted using available protein and resin information combined with a thermodynamic model [13]. Then, the predictions were refined through a series of HTPE experiments (both diluted HPLC and robotic workstation runs) and finally, a minimum number of HPLC scale experiments were used to validate the model results.

2. Materials and instruments

2.1. Proteins

Two commercially relevant proteins were tested: a monoclonal antibody (mAb) and a fusion protein (FP), both provided by Merck (Fenil-sur-Corsier, Switzerland). They were provided in the form of post-capture material and were directly recovered from the production process at a concentration of ~10 g/L (mAb) and ~2 g/L (FP), respectively. The mAb has a pI of ~8.6 and the FP has a pI of ~5.8. Both proteins have a molecular weight of ~150 kDa.

For both proteins, three components are considered: low molecular weight species (LMW), high molecular weight species (HMW) and the monomer (product of interest). All three are in fact pseudo components: the LMW contains all impurities smaller than the monomer (clipped forms, fragments), the HMW contains all impurities bigger than the monomer (aggregates), and the monomer includes all glycan and charge variants. To consider the differences in each component more explicitly, the proposed methodology can be easily adapted (by differentiating each group into more than one pseudo component).

The materials had the following composition (mass percent; LMW/HMW/monomer): 3/4/93 (mAb) and 29/8/63 (FP). The feed solutions were prepared by dilution of the post-capture material into the appropriate feed buffers and buffer exchange in Vivaspin 3 kDa centrifugal concentrators (Sartorius Stedim Biotech GmbH, Goettingen, Germany) to reach a final protein concentration of 5 g/L in the feed.

2.2. Stationary phases

Two chromatographic resins were used in this study: Eshmuno CPX (CPX) and Fractogel EMD SO_3^- (M) (FGSO3) by Merck Millipore (Darmstadt, Germany) in pre-packed columns by Atoll (Weingarten, Germany). MediaScout RoboColumns (5 × 30 mm) were used for robotic workstation experiments; MediaScout MiniChrom columns (5 × 50 mm) were used for all HPLC experiments.

2.3. Mobile phases

The mobile phases were buffered with 25 mM of the buffering agent (phosphate, pH 5.5–7.0 for mAb, acetate, pH 4.5–5.0 for FP). Mobile phases of different ionic strengths were used. Compounds were purchased from the following sources: Sodium chloride: Fisher Chemical (Pittsburgh, PA, USA); Sodium acetate: Merck KGaA (Darmstadt, Germany); Acetic acid: Sigma Aldrich (Buchs, Switzerland); Sodium phosphate mono and di-basic: Fluka (Buchs, Switzerland). All chemicals were used without further purification. De-ionized water was further purified with a simpak2 unit (Synergy Millipore, MA, USA).

2.4. Analytical techniques

The concentration of all fractions was measured per spectrophotometry using the Labchip DS (PerkinElmer). The LMW mass percent was measured using a LabChip GXII unit in reducing conditions (Caliper Life Sciences, Hopkinton, MA, USA). The HMW mass percent was measured using size exclusion chromatography on a TSK-GEL SuperSW3000 column (4.6 × 300 mm) equipped with a Super SW Guard pre-column (both Tosoh Bioscience).

2.5. Liquid handling workstation

The JANUS BioTX workstation by PerkinElmer (Waltham, MA, USA) was used for all the liquid handling experiments. It was equipped with an 8-channel pipetting arm with eight Varispan tips, allowing the simultaneous injection of eight parallel RoboColumns.

2.6. HPLC

All HPLC experiments were carried out on an Agilent 1100 series HPLC (Santa Clara, CA, USA), equipped with an auto-sampler, a column thermostat, a variable wavelength detector, an online degasser, a quaternary gradient pump, and a. 35900E dual channel interface. A CDD −10AVP conductivity detector (Shimadzu Corporation, Kyoto, Japan) and a TX100 pH/mV 2-Wire Transmitter (Sensorex, Garden Grove, California) were connected to the dual channel interface in order to measure conductivity and pH online. Whenever necessary, A Gilson FC 203 B fraction collector (Middleton, WI, USA) was connected to the HPLC outlet to collect output fractions.

3. Column simulation model and adsorption isotherms

The application of any type of model-based tool needs to be accompanied by detailed knowledge of the system at hand. This is not only true for the actual model describing the physical interactions present in the considered system, but also for the values and dependencies of the model parameters. In this section, we discuss the models used and some of the underlying assumptions.

3.1. Column simulation model

We use the lumped kinetic model to describe the mass balance for a protein i over a chromatographic column:

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

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

where ε_i is the porosity accessible to i [–], c_i and q_i are the mobile and stationary phase concentrations of i [g L^{-1}], t [min] and x [cm] are the time and distance along the chromatographic column, u_{sf} is the superficial mobile phase velocity [cm m^{-1}], $d_{ax,i}$ is the axial dispersion coefficient [cm], $k_{m,i}$ is the lumped mass transfer coefficient [min^{-1}], and q_i^{eq} is the equilibrium stationary phase concentration [g L^{-1}]. The axial dispersion and lumped kinetic coefficients $d_{ax,i}$ and $k_{m,i}$ can be estimated from the Van Deemter curve [9,14–16] or from semi-empirical correlations [17]. The mass balance Eqs. ((1) and (2)) were discretized using the finite-difference method along the column length. The resulting ordinary differential equations were solved using ODEPACK from Netlib (http://www.netlib.org/odepack/).