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Pareto-optimal reversed-phase chromatography separation of three insulin variants with a solubility constraint

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ABSTRACT

With the shift of focus of the regulatory bodies, from fixed process conditions towards flexible ones based on process understanding, model-based optimization is becoming an important tool for process development within the biopharmaceutical industry. In this paper, a multi-objective optimization study of separation of three insulin variants by reversed-phase chromatography (RPC) is presented. The decision variables were the load factor, the concentrations of ethanol and KCl in the eluent, and the cut points for the product pooling. In addition to the purity constraints, a solubility constraint on the total insulin concentration was applied. The insulin solubility is a function of the ethanol concentration in the mobile phase, and the main aim was to investigate the effect of this constraint on the maximal productivity.

Multi-objective optimization was performed with and without the solubility constraint, and visualized as Pareto fronts, showing the optimal combinations of the two objectives productivity and yield for each case. Comparison of the constrained and unconstrained Pareto fronts showed that the former diverges when the constraint becomes active, because the increase in productivity with decreasing yield is almost halted. Consequently, we suggest the operating point at which the total outlet concentration of insulin reaches the solubility limit as the most suitable one.

According to the results from the constrained optimizations, the maximal productivity on the C_4 adsorbent (0.41 kg/(m^3 column h)) is less than half of that on the C_{18} adsorbent (0.87 kg/(m^3 column h)). This is partly caused by the higher selectivity between the insulin variants on the C_{18} adsorbent, but the main reason is the difference in how the solubility constraint affects the processes. Since the optimal ethanol concentration for elution on the C_{18} adsorbent is higher than for the C_4 one, the insulin solubility is also higher, allowing a higher pool concentration. An alternative method of finding the suggested operating point was also evaluated, and it was shown to give very satisfactory results for well-mapped Pareto fronts.

1. Introduction

The downstream processing of biopharmaceuticals based on peptides or small proteins, such as insulin, generally includes one or more steps based on preparative reversed-phase chromatography (RPC) [1–4]. Design and optimization of these chromatographic processes can be performed using either mainly experimental or mainly computational methods. The latter is based on mechanistic modeling and simulation of the process. Despite the advances in mechanistic modeling and reductions in computational time during the last decade, the pharmaceutical industry has traditionally been skeptical towards model-aided process development and analysis. However, the changes made during the last decade in the regulatory framework for pharmaceutical development, manufacturing and quality assurance have, to some extent, served as an incentive for industry to explore modeling and simulation for increased process understanding and controllability [5,6]. Inclusion of models in the filing documentation might allow a more flexible process, for which changes are not confined to a narrow design space, but instead can be approved based on simulations showing that the critical quality attributes are still fulfilled [7].

With increasing competition on the market for biopharmaceuticals, e.g. from the emerging generic drugs [8]; and a need for drastic reductions in production costs for therapeutic proteins going from subcutaneous to oral administration, due to the required large increase of the dose [9]; process optimization within the production

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of biopharmaceuticals is more important than ever. As studies have shown that model-aided optimization is often superior to optimization based on experiments, e.g. design of experiments (DoE) together with a statistical approach, both regarding accuracy of the result and resource utilization [10,11], there are strong incentives to further develop and implement the use of mechanistic models within the pharmaceutical industry.

Considering the high value of the active ingredient, it is not obvious that maximal productivity, constrained by a minimum purity, gives the desired optimal process conditions. The yield of the target component might be equally or more important than the productivity. It is, however, generally not desirable to optimize for maximal yield, since this theoretically corresponds to analytical conditions with column loads within the linear range. A suitable trade-off between productivity and yield can be found by multi-objective optimization in which the objective is a weighted combination of these two quantities [12–14]. Optimization of a chromatographic process is a bi-level optimization with a lower level, at which the optimal cut-points for product pooling are found, and an upper level, at which the values of the chosen decision variables are tuned to maximize the objective function value [15]. In order to find a true optimum for the chosen weight, it is important that the same objective is used on both levels [16]. The results from a multi-objective optimization can be visualized with Pareto fronts, i.e. plots with the different objective values at different weights on one axis each, facilitating a combination of qualitative and quantitative evaluation, as well as comparison of different process set-ups [13] and constraints [14] . Usually, only the purity constraint is varied, and other constraints, e.g. regarding the solubility, are kept constant.

The study presented in this paper is a continuation of a series of papers on investigation and modeling of the effects of the mobile phase composition on the retention of three insulin variants in RPC [17,18]. In the present study, the final model is applied for an optimization of this separation process. As in the previous studies, a dual modulator system, comprised by ethanol and potassium chloride (KCl), was used; and two different adsorbents, with C_{18} and C_4 ligands, respectively, were evaluated. The optimal load factor and modulator concentrations for each adsorbent were determined, using a weighted combination of yield and productivity of the intermediately eluted insulin as the objective. The method presented by Knutson et al. [16], using the same objective function on both optimization levels, i.e. for both product pooling and tuning of process conditions, was applied. In addition to the purity and impurity constraints, a constraint on the total outlet concentration of insulin was applied. The maximum concentration was set to 90% of the solubility, according to the correlation presented in our previous work [18]. The aim of this study was to compare the performance of the two adsorbents, and to investigate the effect of the solubility constraint on the shape of the Pareto fronts. Additionally, an alternative to constrained optimization was evaluated.

2. Modeling, simulation and optimization

2.1. Column description

The kinetic dispersive model [19] applied in this study is given by Eq. (1). Inherent assumptions are mono-disperse adsorbent particles and radial homogeneity of the column packing. The numerator in front of the adsorption term corresponds to the pore volume of adsorbent j accessible to adsorbate i, which was chosen as a basis for the adsorption capacity.

$$\frac{\partial c_{i,j}}{\partial t} = D_{app,j}\frac{\partial^2 c_{i,j}}{\partial z^2} - \frac{v_{sup}}{\varepsilon_{t,i,j}}\frac{\partial c_{i,j}}{\partial z} - \frac{\left(1-\varepsilon_{c,j}\right)\varepsilon_{p,j}k_{D,i,j}}{\varepsilon_{t,i,j}}\frac{\partial q_{i,j}}{\partial t} \quad (1)$$

$c_{i,j}$ and $q_{i,j}$ are the concentrations of solute i in the mobile phase and in the stationary phase, respectively, at the time t and at position z counting from the column inlet. For $c_{i,j}$, index i also includes the modulators. The adsorption model described in Section 2.2 gives the time derivative of $q_{i,j}$. $D_{app,j}$ is the apparent axial dispersion coefficient and v_{sup} is the superficial linear velocity of the mobile phase. $\varepsilon_{t,i,j}$ is the apparent total porosity for solute i (Eq. (2)) in adsorbent j which comprises both the interstitial column porosity ($\varepsilon_{c,j}$) and the part of the void inside the particle pores which is accessible to the solutes. $\varepsilon_{p,j}$ is the particle porosity and $k_{D,i,j}$ is the exclusion factor for solute i, i.e. the fraction of the pore volume of adsorbent j which can be accessed by this solute. The exclusion factor for the modulators is assumed to be unity.

$$\varepsilon_{t,i,j} = \varepsilon_{c,j} + \left(1-\varepsilon_{c,j}\right)\varepsilon_{p,j}k_{D,i,j} \quad (2)$$

Eq. (3) can be used to estimate the apparent axial dispersion coefficient from the Péclet number (Pe_d) based on the particle diameter d_p [20].

$$Pe_{d,j} = \frac{v_{sup}d_{p,j}}{D_{app,j}\varepsilon_{c,j}} \quad (3)$$

A Danckwert boundary condition (Eq. (4a)) was applied for the column inlet while the column outlet was described by a homogeneous Neumann condition (Eq. (4b)).

$$c_t\left(t,z_0\right)\frac{v_{sup}}{\varepsilon_c} - D_{app,i}\frac{\partial_{c_i}}{\partial_z}\left(t,z_0\right) = \begin{cases} c_{load,i}\dfrac{v_{sup}}{\varepsilon_c} & \text{if } i = I_1, I_2, I_3 \text{ and } t \le t_0 + \Delta t_{load} \\ 0 & \text{if } i = I_1, I_2, I_3 \text{ and } t > t_0 + \Delta t_{load} \\ c_{mix,i}\dfrac{v_{sup}}{\varepsilon_c} & \text{if } i = S, M \end{cases} \quad (4a)$$

$$\frac{\partial c_i}{\partial z}\left(t,z_f\right) = 0 \quad (4b)$$

z_0 and z_f are the axial coordinates at the inlet and outlet, respectively, of the column. I_1, I_2, and I_3 refer to the three insulin variants; while S and M represent the salt and the organic modulator, respectively. t_0 is the time at which the injection of the feed starts, and Δt_{load} is the load time.

To account for the changes in flow and density caused by mixing two buffers with different ethanol and KCl concentrations, a mass-based mixing chamber model was used, together with a density correlation from Galleguillos et al. [21], also applied in our previous studies [17,18].

2.2. Adsorption model

The dynamic adsorption of the insulin variants can be described by Eq. (5a), where $k_{kin,i,j}$ is the kinetic constant for the adsorption reaction, Λ_j is the ligand density for adsorbent j, and $\sigma_{i,j}$ is the shielding factor for adsorbate i on adsorbent j. KCl is assumed not to adsorb on the stationary phase, i.e. $q_{S,j} = 0$ and $\partial q_{S,j}/\partial t = 0$ at all times. Similar to the salt in the steric mass-action (SMA) model [22], the ethanol is assumed to occupy all ligands that are neither occupied nor shielded by adsorbate molecules. Consequently, the concentration of ethanol in the stationary phase ($q_{M,j}$) is given by Eq. (5b).

$$\frac{\partial q_{i,j}}{\partial t} = k_{kin,i,j}A'_{0,i,j}\gamma_{i,S}\gamma_{i,M}c_{i,j}\left(1-\sum_{k=1}^{N}\frac{\left(\nu_{k,j}+\sigma_{k,j}\right)q_{k,j}}{\Lambda_j}\right)^{\nu_{i,j}} - k_{kin,i,j}q_{i,j}\left(\gamma_M x_M\right)^{\nu_{i,j}\xi_j} \quad (5a)$$

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