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Model-based design of peptide chromatographic purification processes

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ABSTRACT

In this work we present a general procedure for the model-based optimization of a polypeptide crude mixture purification process through its application to a case of industrial relevance. This is done to show how much modeling can be beneficial to optimize complex chromatographic processes in the industrial environment. The target peptide elution profile was modeled with a two sites adsorption equilibrium isotherm exhibiting two inflection points. The variation of the isotherm parameters with the modifier concentration was accounted for. The adsorption isotherm parameters of the target peptide were obtained by the inverse method. The elution of the impurities was approximated by lumping them into pseudo-impurities and by regressing their adsorption isotherm parameters directly as a function of the corresponding parameters of the target peptide. After model calibration and validation by comparison with suitable experimental data, Pareto optimizations of the process were carried out so as to select the optimal batch process.

1. Introduction

With increasing production needs of therapeutic polypeptides, there is a significant interest in developing more efficient production processes. Since the purification step is often the bottleneck in the synthetic peptide production, it is important to develop procedures for its optimization. Among the various possible purification techniques, reversed-phase chromatography is the method of choice for peptide purification.

Usually purification processes development is done under strong time limitation and with small amount of target product. Those constraints usually prevent the use of detailed process modeling due to the time and product consuming model calibration (e.g. frontal analysis) and to the lack of methodology to build an appropriate model. However, in the recent years, strong efforts have been made to incorporate process modeling in the optimization and validation of chromatographic processes [1–9].

The chromatographic models found in the literature to describe the analyte elution can incorporate various levels of complexity. However, they all contain the same basic building blocks: a chromatographic mass balance and a multi-component adsorption isotherm. Several forms of the chromatographic mass balances can be used to describe the elution of the components (e.g. the equilibrium-dispersive model, the lumped kinetic model, the general rate model, etc.) [10]. The selection of the appropriate model depends on the level of complexity required to describe accurately the elution profiles. On the other hand, the selection of the appropriate adsorption equilibrium isotherm required to describe the distribution of the analyte between the mobile and the stationary phase is strongly dependent on the properties of the system studied (i.e. analyte properties, stationary phase type and mobile phase composition) [10]. Several methods are available for the determination of the adsorption equilibrium isotherm (e.g. frontal analysis, perturbation method, inverse method, etc.). The selection of the method used to determine the adsorption equilibrium isotherm mainly depends on the amount of product available for the model calibration and on the accuracy required to describe the analyte elution.

Typically, once the chromatographic model has been selected and calibrated, an optimization procedure is used to determine the optimal batch process based on a Pareto optimization [1–5,7]. In addition, a sensitivity analysis can be used to characterize the sensitivity of the process and to determine the critical process parameters [6–8]. These results can then be used to validate the

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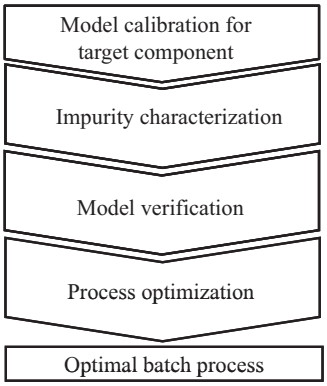

Fig. 1. Schematic of the model-based process development.

process and to ensure the process and product quality according to FDA regulations.

This paper presents the model-based optimization of a peptide purification process. The model calibration and the optimization study were done in a systematic manner following the step sequence in Fig. 1. It will be shown that by carefully following these steps, it is possible to develop a very robust and predictive chromatographic model, based on which reliable process optimization can be carried out. In addition, such a model could also be very useful for process sensitivity analysis and Quality-by-design.

The general procedure presented in this paper can be applied to any peptide chromatographic purification process. Of course, the choice of the adsorption isotherm will depend on the system studied. However, the methodology and the simplifications presented in this paper are valid for most of the chromatographic purification processes. In fact, the procedure was successfully applied by our research group to several peptide purification processes.

2. The framework

Often, the optimization of chromatographic processes is done empirically on the basis of heuristics and experience. This empirical approach leads in general to suboptimal process performances. In this work, an attempt was made to rationalize the process design by developing a general procedure that can be followed to design peptide purification processes using a model based approach. The procedure is sketched in Fig. 1 and it contains four main steps:

2.1. Model calibration for target component

The first step in a model based process optimization is to select the appropriate model for the system and to calibrate the model parameters. To do this, it is important to first characterize the adsorption characteristics of the pure target component and then to develop a model able to predict its elution behavior.

2.2. Impurity characterization

Once the target component adsorption has been characterized, the impurities elution has to be determined. The impurities having similar elution behavior are usually lumped into pseudo-impurities. Their selectivity is measured in diluted conditions and the remaining adsorption isotherm parameters are regressed from the target peptide isotherm parameters. The justification for doing this is that the most critical impurities are the ones eluting closer to the target component, which therefore, exhibit a chromatographic behavior very similar to that of the target component.

Table 1
Mobile phase composition.

Buffer	Composition
A1	AcOH/H_2O/AcN: 0.5/97.5/2.0 (v/v/v)
B1	AcOH/H_2O/AcN: 0.5/49.5/50.0 (v/v/v)
A2	200 mM KH_2PO_4 pH 2/AcN: 93.5/6.5 (v/v)
B2	200 mM KH_2PO_4 pH 2/AcN: 55/45 (v/v)

2.3. Model verification

Before using the model, it is important to test its prediction validity against a suitable set of experimental data.

2.4. Process optimization

Finally the model can be used to perform a multi-objective optimization. The results are represented as productivity–yield Pareto curves at constant product purity and the optimal batch process can be selected based on the optimization results and on additional economical constraints.

3. Definition of the purification process

3.1. The preparative purification

The purification of a long peptide with more than 30 amino acids was optimized using a model-based approach. The crude mixture was directly obtained from the Lonza production process, so that a real industrial multi-component mixture could be investigated. The polypeptide was synthesized by solid phase synthesis [11]. The structures of the major impurities are therefore very closely related to the target peptide structure. The starting material had a purity of about 60%. This crude mixture was purified using a 2 steps purification process. The first purification step was carried out to increase the purity of the starting material up to 94.5%. This product pool was then further purified to reach the final purity specification of 98%. This second purification step will be used in the sequel as a case study to illustrate the model-based process development procedure presented in this work.

The second purification step was performed on a Kromasil 100A 10 μm C8 4.6 mm × 250 mm column obtained from EKA chemicals AB (Bohus, Sweden). The preparative buffer composition is summarized in Table 1 (i.e. buffer A1 and B1). The temperature was set to 25 °C and the flowrate was 0.5 mL/min.

The feed for the second purification step had a concentration of 3.5 g/L and the purity was 94.5%. An analytical chromatogram of the feed is shown in Fig. 2. It can be seen that the feed is a complex mixture containing more than 20 impurities. To simplify the feed characterization, the impurities were classified in 3 groups, based

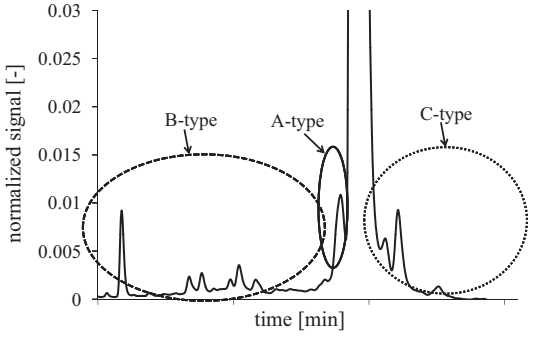

Fig. 2. Analytical chromatogram of the feed. *Note*: Three different types of impurities have been defined.