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Optimization of ion exchange sigmoidal gradients using hybrid models: Implementation of Quality by Design in analytical method development

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ABSTRACT

Thorough product understanding is one of the basic tenets for successful implementation of Quality by Design (QbD). Complexity encountered in analytical characterization of biotech therapeutics such as monoclonal antibodies (mAbs) requires novel, simpler, and generic approaches towards product characterization. This paper presents a methodology for implementation of QbD for analytical method development. Optimization of an analytical cation exchange high performance liquid chromatography (CEX-HPLC) method utilizing a sigmoidal gradient has been performed using a hybrid mechanistic model that is based on Design of experiment (DOE) based studies. Since sigmodal gradients are much more complex than the traditional linear gradients and have a large number of input parameters (five) for optimization, the number of DOE experiments required for a full factorial design to estimate all the main effects as well as the interactions would be too large (243). To address this problem, a mechanistic model was used to simulate the analytical separation for the DOE and then the results were used to build an empirical model. The mechanistic model used in this work is a more versatile general rate model in combination of modified Langmuir binding kinetics. The modified Langmuir model is capable of modelling the impact of nonlinear changes in the concentration of the salt modifier. Further, to get the input and output profiles of mAb and salts/buffers, the HPLC system, consisting of the mixer, detectors, and tubing was modelled as a sequence of dispersed plug flow reactors and continuous stirred tank reactors (CSTR). The experimental work was limited to calibration of the HPLC system and finding the model parameters through three linear gradients. To simplify the optimization process, only three peaks in the centre of the profile (main product and the adjacent acidic and basic variants) were chosen to determine the final operating condition. The regression model made from the DoE data yielded a $R^2 > 0.97$ which made it possible to predict and choose the design space where the optimal resolution between the acidic/main peaks and the basic/main peaks could be achieved (>1.2 and >2.5, respectively). The optimal operating condition was validated using experimental runs and was found to give the same resolution as what was predicted by the simulation. The proposed approach aims to significantly reduce the time required for method optimization as well as the extent of experimentation.

1. Introduction

Monoclonal antibodies (mAbs) are today the fastest growing category of recombinant protein therapeutics [1]. These products are complex and this makes it challenging to monitor heterogeneities associated with them so as to ensure product quality and consistency [2]. Some of the heterogeneities result in a change in the surface charge of the antibody, either directly as a change in charged residues, or indirectly as a chemical or physical alteration that changes the surface charge distribution [3]. Hence, charge heterogeneity is a topic of great interest amongst the biopharmaceutical industry because it is known to influence the stability and biological activity of the product [4]. These species may be present at the time of manufacture or they may originate during processing or storage. Thus, monitoring the levels of these variants during processing and also during stability studies is considered essential by the regulatory agencies [5]. Ion-exchange chromatography (IEC)

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is a versatile bioseparation technique in which protein mixtures can be fractionated by either salt-gradient or pH-gradient elution. Cation exchange chromatography is presently the gold standard for analysis of charged heterogeneities in mAbs [6].

Development of a robust IEC method requires optimization of many parameters including the analytical column, pH, mobile phase composition, temperature, and gradients [7]. Since the biotech industry has adopted the Quality by Design (QbD) approach for development of biotherapeutics [8,9], researchers have also applied these principles towards analytical method development [10–13]. The ever-increasing stringency of the regulatory agencies towards product characterization combined with the high cost of the HPLC columns and reagents that are required for method optimization and the large quantity of variables that are known to affect the analytical separation ensure that analytical method development stays non-trivial [14]. Traditional approaches towards analytical method development involve use of a one factor at a time (univariate) philosophy under which a single independent variable (controlled factor) is altered while maintaining all others constant [15,16]. If a large number of variables need to be evaluated, this approach is extremely time-consuming, laborious and expensive [17]. Moreover, this approach can result in suboptimal solutions, since possible interactions between factors are not taken into account [15,17]. Identifying global optima using this approach would require a significantly large number of experiments, time and resources [18].

To identify optimal conditions efficiently, a variety of approaches have been proposed by the researchers including the use of Design of Experiments (DOE) [19–21]. The popularity of DOE arises from the fact that it takes significantly less time, effort and resources for optimization than the univariate procedures [14,22]. In addition, using a minimal number of experiments, one can also measure the extent to which the several variables interact with each other, thus giving an insight about the sensitivity of the analytical method towards the various variables [18]. In the last decade, several researchers have demonstrated the usefulness of DOE based experimentation for implementing QbD towards analytical method development [10,13,23–27].

Another significant development of the past decade in the field of process development is that of process modelling and simulation. There are many software packages available for analysis of HPLC data. Some of them are based on the linear solvent strength (LSS) theory [28,29] including DryLab (Molnár Institute, Berlin, Germany), ACD/LC, and GC Simulator (ACD/Labs, Toronto, Canada), ChromSword (ChromSword Group, Riga, Latvia), and Osiris (Datalys, Grenoble, France). Recently, researchers have introduced an innovative strategy, also based on LSS, for creation of a new method development algorithm that searches the chromatographic parameter space by systematically shifting and stretching the elution window over different parts of the time-axis [30]. More recently, researchers have reported the use of real-time knowledge based approach to automate chromatographic method development [31]. Software strategies have gained more widespread use in evaluation of the results and suggesting the next condition to run so as to maximize separation. These software products enable efficient prediction of the optimum conditions based on two or more initial runs using semi-empirical models. Other software products such as FusionChrom use DOE to generate appropriate retention models that are then used to predict the most favourable analytical conditions [32].

A mechanistic model, if suitably developed, can be capable of simulating analytical separation and predicting the outcomes for a wide range of process perturbations. Once the model parameters have been estimated and the model is validated, it can be used to predict analytical separation under a variety of analytical conditions. Computer assisted analytical method development and optimization in HPLC has been studied by other researchers [33,34]. However, analytical method development of ion exchange (IEX) chromatography has not been widely studied. The reason for this might be the presence of intrinsic non-linearity in IEX systems and the challenges in estimation of model parameters [35]. Various estimation methods based on batch experimentation, frontal analysis, plateau method, and inverse method have been reported in the literature [13,23,36]. Parameter estimation solely by experimentation is time-consuming and prone to experimental errors. The inverse method is most useful when the target is to reduce the number of experiments as parameters are directly obtained from fitting a set of experimental output profile [19].

Optimization of the gradient is likely to be the most important task when creating a rapid analytical method. If the sample contains many heterogeneous components, optimum separation is unlikely in isocratic elution. Method development of a gradient method requires optimization of its steepness, range and shape [5]. In this paper, the ion-exchange separation of charge variants has been demonstrated using cation exchange chromatography (CEX-HPC) with sigmoidal gradient. An extended Langmuir model established previously for ion exchange chromatography has been used to model the HPLC system. The proposed model has been demonstrated to accurately predict elution of the charge variants under different types of gradient. The model has been used to determine the optimal molarity of the binding and elution buffers and the steepness factor of the gradient so as to maximize the resolution between the main product and the adjacent variants. A hybrid approach, wherein general rate model along with modified Langmuir binding kinetics, has been used to successfully optimize an HPLC method.

2. Materials and methods

2.1. mAb samples

Human IgG1 mAb, expressed in Chinese hamster ovary (CHO) cells, was donated by a major domestic producer of biotherapeutics. The mAb has a pI of ~8.2 and a molecular weight of 149 kDa. The input feed for cation exchange chromatography step material consisted of neutralized Protein A elute. CEX-HPLC chromatogram of the mAb resulted in formation of 10 distinct peaks, namely, *A1*, *A2*, *M*, *B1*, *B2*, *B3*, *B4*, *B5*, *B6* and *B7* (Fig. 1).

2.2. Mobile phase preparation

Sodium di-hydrogen phosphate and di-sodium hydrogen phosphate were of HPLC grade and procured from Merck, India. Buffers were prepared by appropriate mixing of sodium di-hydrogen phosphate and di-sodium hydrogen phosphate and filtered with 0.22 μm nylon membrane filter and degassed before usage. The buffer combinations consisted of 15 mM sodium phosphate at pH 6.2 (mobile phase A) and 150 mM sodium phosphate pH 6.2 (mobile phase B). The flow rate used was 1 mL/min.

2.3. Instrumentation and columns

CEX-HPLC was performed on a Dionex® HPLC system equipped with an Ultimate 3000 pump, VWD detector and an autosampler maintained at 4 °C. Instrument control, data acquisition, and data compilation for HPLC were performed on a Chromeleon software version 6.8. The ion-exchange column used in this study was MAbPac SCX-10, RS, 5 μm, 150 mm × 4.6 mm, purchased from Dionex (Sunnyvale, CA).

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