Review

CellPress

Purifying biopharmaceuticals: knowledge-based chromatographic process development

Alexander T. Hanke and Marcel Ottens

Department of Biotechnology, Delft University of Technology, Delft, The Netherlands

The purification of biopharmaceuticals is commonly considered the bottleneck of the manufacturing process. Increasing product diversity, along with growing regulatory and economic constraints raise the need to adopt new rational, systematic, and generally applicable process development strategies. Liquid chromatography is the key step in most purification processes and a well-understood unit operation, yet this understanding is still rarely effectively utilized during process development. Knowledge of the composition of the mixture, the molecular properties of the solutes and how they interact with the resins are required to rationalise the design choices. Here, we provide an overview of the advances in the determination and measurement of these properties and interactions, and outline their use throughout the different stages of downstream process development.

Chromatography in bioprocess development

Biopharmaceuticals have been a major driving force for growth in the pharmaceutical industry in recent years [1]. Over 40% of drugs granted FDA approval in 2012 were biopharmaceuticals, of which therapeutic proteins, including monoclonal antibodies (mAbs), constituted the largest group next to therapeutic peptides [2]. From a manufacturing perspective, the increased product titres achieved over the past decade have long shifted the attention towards the downstream process [3]. Despite increasing competition from nonchromatographic techniques [4], and pressure to reduce costs and increase throughput, packed-bed chromatography is still the dominant technique in biopharmaceutical purification [5]. This prevalence is mainly due to the high-resolution purification that can be achieved even for similar components. Advances in resin chemistry have alleviated some of the concerns that packed-bed chromatography could handle the throughput and production needs in coming years [6]. If not during early process stages, then during product polishing where very high purities are required for therapeutics, it seems unlikely that chromatography will lose its place in biopharmaceutical manufacturing in the close future.

Besides having to handle increasing production volumes, downstream scientists and engineers face a

Corresponding author: Ottens, M. (m.ottens@tudelft.nl).

Keywords: process development; chromatography; high-throughput; host cell proteins; mathematical modelling.

0167-7799/$ – see front matter

 http://dx.doi.org/10.1016/j.tibtech.2014.02.001

Glossary

Biosimilars: follow-on biopharmaceuticals produced by a different manufacturer after patent expiration. The higher molecular complexity of biopharmaceutical drugs requires more rigorous regulatory pathways compared to follow-on small molecular drugs.

Column breakthrough experiments: continuous loading of a chromatographic column with sample under constant conditions to determine its dynamic capacity under the specified conditions.

Column scouting: systematic testing of identical process conditions on a small number of chromatographic columns for the purpose of performance comparison.

Critical process parameters (CPPs): process parameters that have been identified to have a significant impact on at least one CQA.

Critical quality attributes (CQAs): a measureable product property that must lie within defined constraints for the product to comply to the quality requirements.

Design of experiments (DoE): parameter combinations to be tested for the optimization of multiparameter problems chosen by statistically principles in order to maximise the information gained per experiment.

Design space: the combination of ranges of process parameters and material quality attributes that have been demonstrated to result in a product compliant to the quality requirements.

Equilibrium dispersive model (EDM): a model for dynamic chromatography lumping dispersion effects into a single parameter.

General rate model (GRM): a more complex chromatography model with dedicated parameters for all mass-transfer effects.

High-throughput process development (HTPD): the systematic application of HTS in combination with statistical tools such as DoE and RSA in the context of process development.

High-throughput screening (HTS): the systematic testing of a large number of process parameters typically with the aid of robotic liquid-handling systems.

Peak parking: a controlled pause in mobile phase flow during a pulse elution experiment to estimate mass-transfer parameters from the resulting peak shape.

Process Analytical Technology (PAT): the use of process understanding, monitoring of raw material quality, and CPPs throughout the process to adjust process parameters in real-time with the goal of producing products with consistent quality.

Response surface analysis (RSA): a tool to describe and analyse interparameter dependencies for which no mechanistic relation is known.

Separation selection coefficient (SSC): an estimated parameter characterising the ability of an operation to separate two proteins.

Shrinking core model: a model describing the solute uptake kinetics across the radius of a spherical chromatography resin bead.

Static batch adsorption experiments: common experiment to determine adsorption equilibria by mixing known amounts of resin and sample and measuring the concentration of the supernatant once equilibrium has been reached.

Quality by Design (QbD): a framework to promote the systematic use of science, process understanding, and risk management to design the production process to consistently deliver the predefined quality objectives.

Box 1. The Purification Challenge

The purification of a biopharmaceutical is not a simple task. Besides the cell debris, host cell proteins, DNA, endotoxins, and viruses that need to be removed, product-related impurities such as product moieties that are misfolded, aggregated, carry the wrong post-translational modifications, or are otherwise chemically degraded may complicate the purification, due to their similarity to the target molecule. Achieving the high levels of product purity required for use as an active pharmaceutical ingredient requires a complex cascade of unit operations (Figure I). The complexity of such multistage processes poses two major challenges to the downstream process developer. The effects that small variations in the upstream process and the quality of the chemicals and auxiliary materials can have on the performance of unit operations further downstream must be accounted for in the process-control strategy. Similarly, the dependencies of the single unit operations need to be considered during the earlier stages of process development, when the choice for specific unit operations and their position in the process are decided.

Despite the importance of understanding these dependencies, most process-development approaches only consider the optimisation of individual unit operations outside of the context of the process in its entirety. In most cases the unit operations are chosen and optimised sequentially in the downstream direction, as the changes in feed composition due to the prior unit operation affect the choice of the subsequent unit operation [38]. In many cases this simplification is necessary, because the exponential increase in possible unit operations sequences with increasing number of unit operations to be considered quickly becomes unmanageable within the timeframe available for process development when time-consuming experimentation is involved. The downside of such a sequential approach is that it poses the risk of missing the global optima by excluding options choosing a worse-performing early step followed by a more efficient subsequent step might lead to a more economical process. With increasing availability of powerful supercomputers the time and resource limitation no longer holds for *in silico* process optimisation. Given enough computational power all, or a large subset of, possible flowsheet options can be optimised and evaluated in parallel.

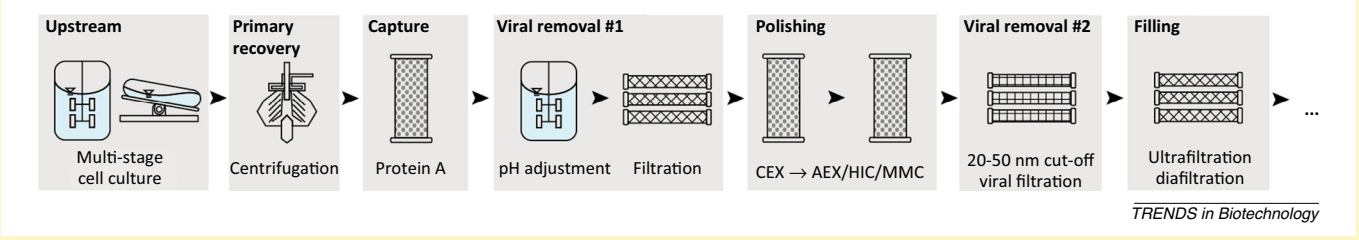

Figure I. Simplified platform process for the production of monoclonal antibodies in bulk form [7]. In reality, the cells undergo a series of inoculum steps and seed reactors before reaching the production reactor. The downstream process, usually considered to start after primary recovery, mostly consists of at least two chromatographic separations to reach the desired purity. Regulations require at least two orthogonal methods of virus removal, commonly low pH inactivation and viral filtration. The greatest process diversity lies in the polishing steps where the choice for the combination of cation exchange (CEX), anion exchange (AEX), hydrophobic interaction (HIC), and mixed-mode chromatography (MMC) is based on the characteristics of the impurities to be removed. After rebuffering by diafiltration further processing steps follow for the drug to reach its final formulation.

plethora of technical, economic and regulatory challenges. Although in the past the dominance of mAbs as a product class allowed us to establish platform processes that required relatively minor adaptations from product to product [7], recent trends towards more diverse therapeutic proteins require more generally applicable process-development approaches (Box 1). [8]. Increased competition through biosimilars (see Glossary) catalysed by abbreviated regulatory pathways have increased the economic pressure on the manufacturing of biotherapeutics [9]. From the regulatory side, the Quality by Design (QbD) and Process Analytical Technology (PAT) initiatives call for increased process and product understanding to ensure each process consistently meets precisely defined quality attributes [10]. Definition of the critical quality attributes (CQAs) and linking them to the underlying critical process parameters (CPPs) requires a thorough and systematic characterisation of the process parameter space.

From a process-development perspective this requirement has rendered trial-and-error-based process development and univariate optimisation largely obsolete. This has led to the widespread adoption of high-throughput screening (HTS) technologies [11]. In this context the relationship between the CPP and CQA is usually of a statistical nature derived from a response surface analysis of a design of experiments (DoE) or multivariate data analysis [12,13]. Genetic algorithms are being increasingly used to identify optima in the design space [14,15]. The combination of these experimental and data-processing techniques has been coined high-throughput process development (HTPD). The statistical relationship allows the ranking of CPP by significance of impact on the CQA but lacks the ability to predict process performance, limiting their use for process optimisation.

The degree to which CPP and CQA can be causally linked reflects the level of process understanding achieved [16,17]. Throughout the biopharmaceutical manufacturing process, both upstream [18], downstream [19], and during formulation [20], there is a trend to replace gradually statistical and empirical correlations with mechanistic models. Mechanistic models typically allow more accurate extrapolation, making them useful as tools for fast and cheap process optimisation. They are derived from fundamental principles, therefore, mechanistic models reflect a higher level of process understanding. Most mechanistic models describing chromatographic separations consist of two parts: equations describing the fluid flow and mass transfer in the column, and a model to describe the interactions between the sample and the resin in the form of adsorption isotherms. Experimental approaches to determine both mass-transfer [21] and resin-interaction parameters [22] have been extensively reviewed, but require large numbers of experiments to gain the parameters needed to model even simple systems (Figure 1). The availability of these parameters often restricts the use these modelling tools to optimisation of specific separation problems during late stages of process development, when