

Mini-review

Challenges and trends in bioseparations

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Abstract: In this paper the issues and challenges presented 15 years ago for performing efficient separation processes for recombinant proteins are revised and discussed. Competitive advantage in production was seen as not only dependent on innovations in molecular biology and other areas of basic biological sciences but also on innovation of separations and downstream processes. The trend to develop techniques that exploit fundamental physicochemical principles more efficiently was emphasized, including analysis of the physicochemical properties of proteins and its relation to efficiency in bioseparation. 15 years ago the main thrust was also focused on the development of novel techniques. Clearly the challenges faced today, where highly optimized and efficient production processes exist, are dramatically different. The use of mathematical models for optimizing chromatographic separations and simplifying validation of such operations is extremely advantageous. Their use constitutes an example of how the challenges that bioseparations are facing and will be facing within the next few years can be met. Such models should be extended to a larger number of proteins, chromatographic procedures and experimental conditions.

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INTRODUCTION

Some 14 years ago we addressed the issue of the separation methods needed for biotechnology given the challenges that the 'new' recombinant proteins were posing to the biotechnology industry at that time.^{1,2} These challenges included the extremely high purity required for proteins for use in human therapeutics (99.9% or even higher) and that these had to be separated from a very large number of contaminants, other proteins, nucleic acids, polysaccharides and many other components present in the cell culture or cell lysate used to manufacture these proteins. Competitive advantage in production was seen as not only dependent on innovations in molecular biology and other areas of basic biological sciences but also on innovation and optimization of separations and downstream processes. The main issues that were considered then as important for the development of novel separation techniques to give improved resolution, simplicity, speed, ease of scale-up and possibly continuous operation were presented, discussed and evaluated. The trend to develop techniques that exploit fundamental physicochemical principles more efficiently was emphasized, including analysis of the physicochemical properties of proteins such as pI, charge as a function of pH, biological affinity, hydrophobicity and size and its relation to efficiency in a bioseparation. It had already been pointed out that some properties of proteins can show extremely high resolution in purification

operations (e.g., charge and affinity), whereas others show much less resolution (e.g., molecular weight). The main thrust was focused on the development of novel techniques such as cross-flow electrofiltration,^{3,4} reversed micelles,^{5–7} centrifugal partitioning,⁸ membrane chromatography,^{9,10} new affinity ligands,^{11,12} aqueous two-phase partitioning^{13–16} and continuous processing (e.g., CARE).^{17,18}

It was also stressed that the development of new and efficient separation processes had to be based on more efficiently exploiting differences in the actual physicochemical properties of the protein product (surface charge/titration curve, surface hydrophobicity, molecular weight, biospecificity, pI, stability) compared to those of contaminant components in the crude broth. In addition, the main physicochemical factors involved in the development of standard and novel separation processes were listed and described in some detail. The effect of process kinetics and mass transfer in the development of novel separation processes was also analysed in detail.

In addition to evaluating novel processes such as liquid–liquid extraction (e.g., aqueous two-phase systems (ATPS), reverse micelles) and solid-based systems (e.g. perfusion, membrane adsorption), the important potential of continuous processing was evaluated (e.g., continuous adsorption recycle extraction (CARE) and ATPS). Much hope was expressed in those days on continuous processing for

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protein purification and many protein products have been very successfully purified by such processes (e.g., monoclonal antibodies (MAbs), virus-like particles (VLPs), α -amylase). However, the fact that most biotech proteins produced today and required as high-purity products are still high-cost therapeutics has not made necessary the use of continuous or liquid–liquid purification systems until now.

TODAY'S CHALLENGES

The challenges the biotechnology industry faces today are very different. The reason for this is mainly because the production of recombinant proteins, in the large majority of cases, has been optimized and has become very efficient; product protein yields and concentrations are virtually hundreds of times higher than they were 15 years ago. Another reason is that, partly due to this, but also to the increase in experience gained in finding optimized 'purification trains' or purification sequences, most recombinant proteins can be purified to the level of therapeutic-grade injectables, in most cases by one or two ion-exchange chromatography (IEC) steps followed by a hydrophobic interaction chromatography (HIC) step. This was already predicted by the Expert System, developed by our group more than 10 years ago.^{19–22} This Expert System included a 'proteomic' characterization of the contaminants present in the culture supernatant well before the term 'proteomics' was even used.

A typical example of the 'change of paradigm' is shown by the purification of monoclonal antibodies using protein A. The use of protein A, which shows great affinity for monoclonal antibodies (MAbs), was justified when this separation constituted a 'protein capture' step due to the very low concentration of MAbs and the large number of contaminants present in the culture supernatant. However, the much higher relative concentration of MAbs *vis-à-vis* contaminants, that are obtained in present-day processes, does not justify the use of an affinity process. Furthermore, the generic use of affinity capture was well justified when low protein product concentrations were obtained in production cultures, but affinity does have an important limitation. This is usually a consequence of the very high affinity of the ligand for the protein product, which can often result in leaching of the ligand. This has important cost consequences as it becomes necessary to test the protein product for the presence of ligand, which in many cases can be highly expensive. Conversely MAbs are highly hydrophobic and hence can be very efficiently separated by ion-exchange chromatography and HIC.

MATHEMATICAL MODELS

Two recent papers have shown how mathematical models developed in an academic environment can

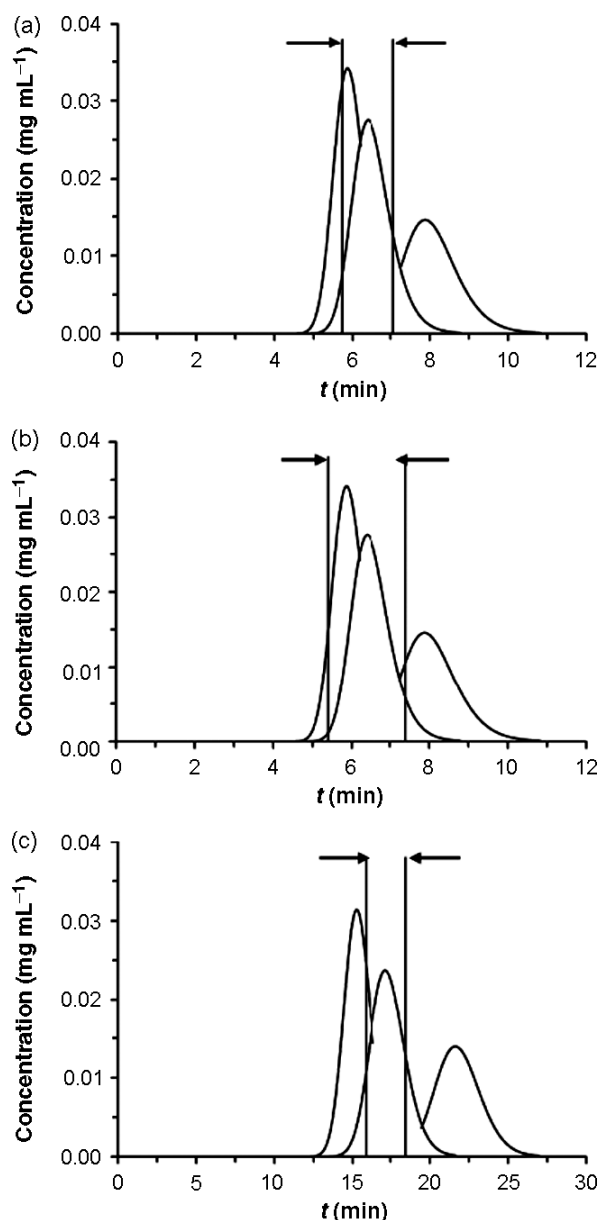


Figure 1. Elution curves of a mixture of three proteins in a chromatography separation for the minimum production cost given in Shene *et al.*²³ Between the arrows the fraction of protein product collected for three different cases is shown ('peak cutting'): (a) case 1; (b) case 2; (c) case 3. The three cases correspond to products that require different levels of purity.

be put to efficient use to optimize chromatographic separation processes in an industrial environment.

The first one shows two mathematical models – the plate model and the more fundamentally based rate model – which have been used to satisfactorily simulate the behaviour of elution curves in ion exchange chromatography of a mixture of proteins.²³ Simulated elution curves were compared with experimental data not used for parameter identification. Deviation between experimental data and simulated curves using the plate model was less than 0.0189 (absorbance units); a slightly higher deviation (0.0252 (absorbance units)) was obtained when the rate model was used. A cost function was built that included the effect of the different production stages, namely fermentation,

purification and concentration. These considered the effect on performance of IEC: yield, purity, concentration and the time needed to accomplish separation. Operational conditions in the IEC such as flow rate, ionic strength gradient and operational time can be selected using this model in order to find the minimum cost for the protein production process depending on the characteristics of the final product desired, such as purity and yield. This cost function was successfully used for the selection of operational conditions as well as the fraction of the product to be collected (peak cutting) in IEC. It can be used for protein products with different characteristics and qualities, such as purity and yield, by choosing the appropriate parameters. Figure 1 shows the result given by the model for 'peak cutting'. Cases 1 and 2 correspond to an enzyme needed in high yield and relatively low purity, whereas case 3 corresponds to a protein that is

required at much higher purity and where yield is not that relevant.

More recently, a second paper shows how the plate model has been used for an industrial practical application of chromatographic theory for process characterization towards validation of an ion exchange operation.²⁴ When a chromatographic operation utilized to purify a human therapeutic protein is prepared for validation before commercial production, numerous tests have to be performed to establish the relative importance of each operating parameter to define its future role and importance in the framework on in-process controls. This prioritization process is usually performed using an entirely empirical approach. The process flow chart from a decision to commercialize a biologic to product launch is shown schematically as a process flow chart in Fig. 2. This paper demonstrates the application of a

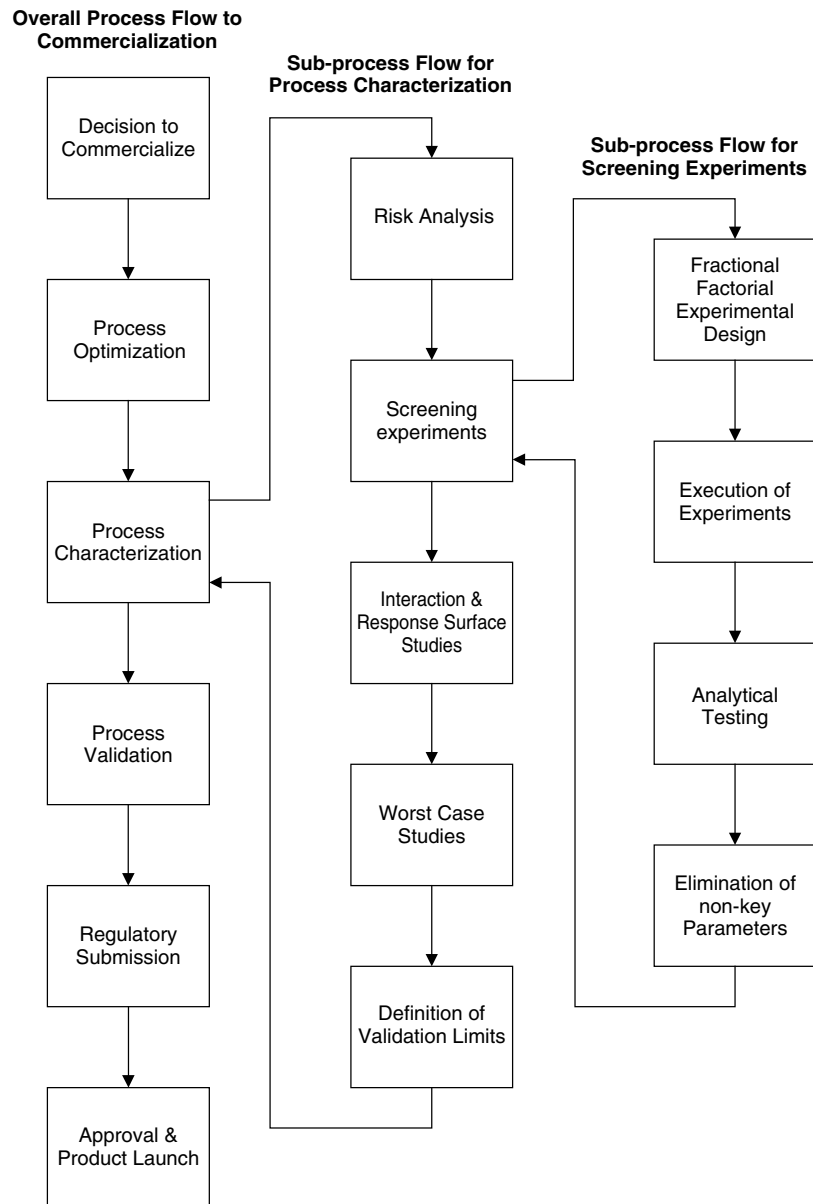


Figure 2. Process flow chart from a decision to commercialize a biologic to product launch. The sub-processes of process characterization and screening experiments are outlined.²⁴

rational approach based on chromatographic theory to prioritize operating parameters. Both methodologies, empirical and rational, were performed to evaluate a specific IEC operation for the preparative separation of closely related protein species. The paper shows that the application of the rational approach has the potential to accelerate the evaluation and significantly reduce the amount of analytical testing needed.

These two examples that show how relatively complex and sophisticated mathematical models can be successfully applied to solve and optimize problems of a very practical and use-oriented nature, and illustrate the challenges that bioseparations are facing and will be facing within the next few years.

CONCLUDING REMARKS

The challenges and trends in bioseparations faced by biotechnology today and in the near future are different from those that existed 15 years ago. Two such examples correspond to complex mathematical models that have recently been used to simulate accurately and optimize chromatographic procedures and to largely simplify validation of such operations. Such models should be extended to a larger number of proteins, chromatographic procedures and experimental conditions. An important challenge includes modelling and simulation that realistically mimic the behaviour of 'real' situations and chromatography in bioseparations.

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