Rigorous Modelling, Simulation and Evolutionary Optimisation of Generic Protein Downstream Processes

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The aging of society leads to an increasing demand for high value biopharmaceuticals addressing age- and prosperity related diseases such as cancer or Alzheimer. On account of this increasing demand, the growing number of biopharmaceuticals in clinical trials requires that more efficient manufacturing strategies have to be developed [1]. Therefore rapid prototyping of the processes and efficient scale-up methods from lab-scale to an industrial production are crucial for the commercial success of such engineering projects [2]. While in chemical and oil industry the use of computer aided process design tools based on detailed mathematical models is a state of the art method for the efficient design, scale-up and optimisation of integrated process [3], the application of models considering mass and heat transfer phenomena for process analysis and process optimisation purposes, to design biological processes is not common yet. However the potential benefits of computer-aided design methods have led to an increasing acceptance in the biopharmaceutical industry since the downstream process costs often exceed 50% of the overall production costs [4] and reduced time-to-market is critical despite of limited budgets. The application of such tools helps to reduce process development time by allowing different process sequences and operating conditions to be examined inexpensively by model-based design, thus saving time and reducing the required number of pilot–scale experiments [5]. In 2004, the Federal Drug Administration (FDA) published the Process Analytical Technology (PAT) guidelines which emphasize the necessity of process understanding when designing and validating a new process [6]. The PAT guidelines suggest that experimental process development databases could be used to develop bioprocess simulation tools which would contribute to knowledge gain and, ultimately, reduction of scale-up time and costs. The application of computer aided design methods allows for decreasing the number of required experiments hence reducing the development time and the process costs. Such computer aided process analyses require the implementation of rigorous dynamic models of each unit operation when studying an entire downstream process. Compared to empirical modelling, this methodology can account for large variations in process parameters and reveal interactions between the respective unit operations.

In this work, a simulation and optimisation platform for generic protein purification processes has been developed which is based on rigorous dynamic models of ion-exchange membrane adsorbers, ion-exchange and size-exclusion chromatography and ultra-/diafiltration.

To demonstrate the applicability of this methodology for entire process simulation, four process alternatives based on the chromatographic separation of human serum albumin from human plasma were chosen as benchmark processes. All process alternatives consisted of six consecutive purification steps, comprising the sequential connection of an anion-exchange and cation-exchange step for product capturing and purification.

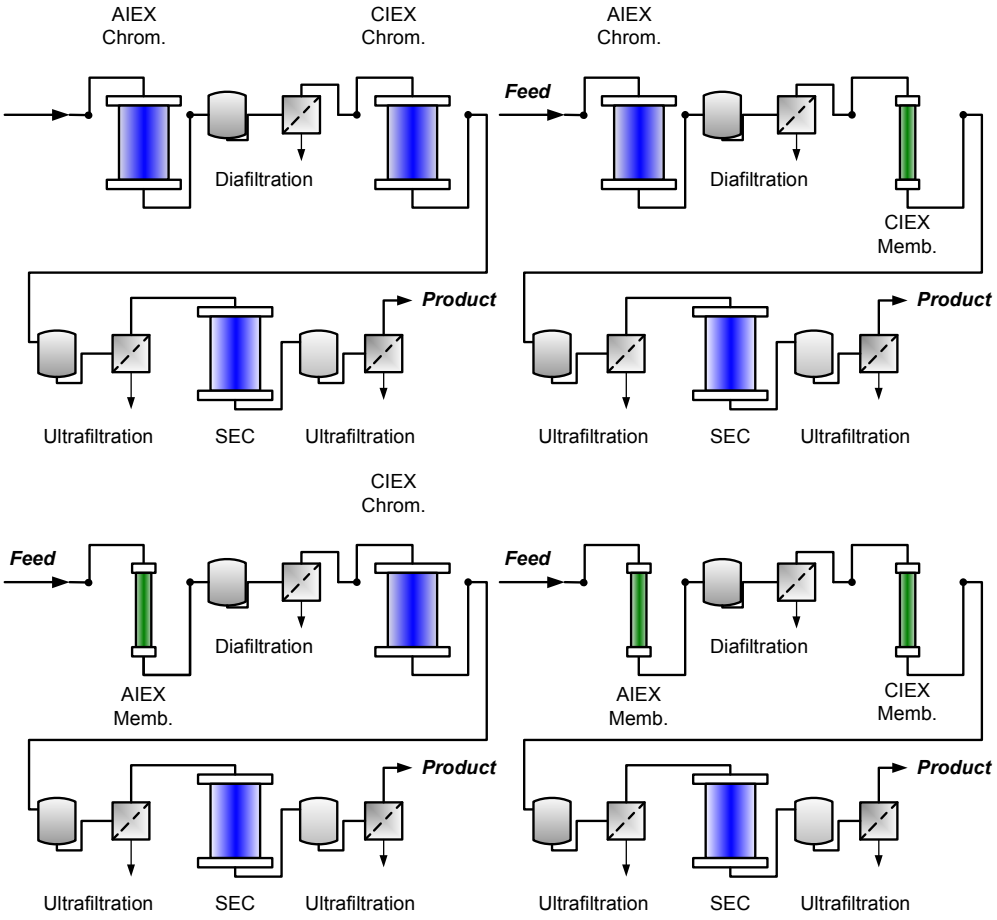

Figure 1: Alternative process sequences for the purification of human serum albumin based on the chromatographic sequence proposed by *Curling* and *Berglöf* [7, 8]

The polishing step was performed by utilizing a size-exclusion column. An artificial protein mixture comprising bovine serum albumin, α-lactalbumin and immunoglobulin G was selected to mimic the separation behaviour of human plasma, due to safety and cost issues in the framework of this thesis.

The developed process model provides a flexible structure incorporating various modelling approaches and different modelling complexities allowing for easy exchange of unit operations (Figure 2) and modelling depths.

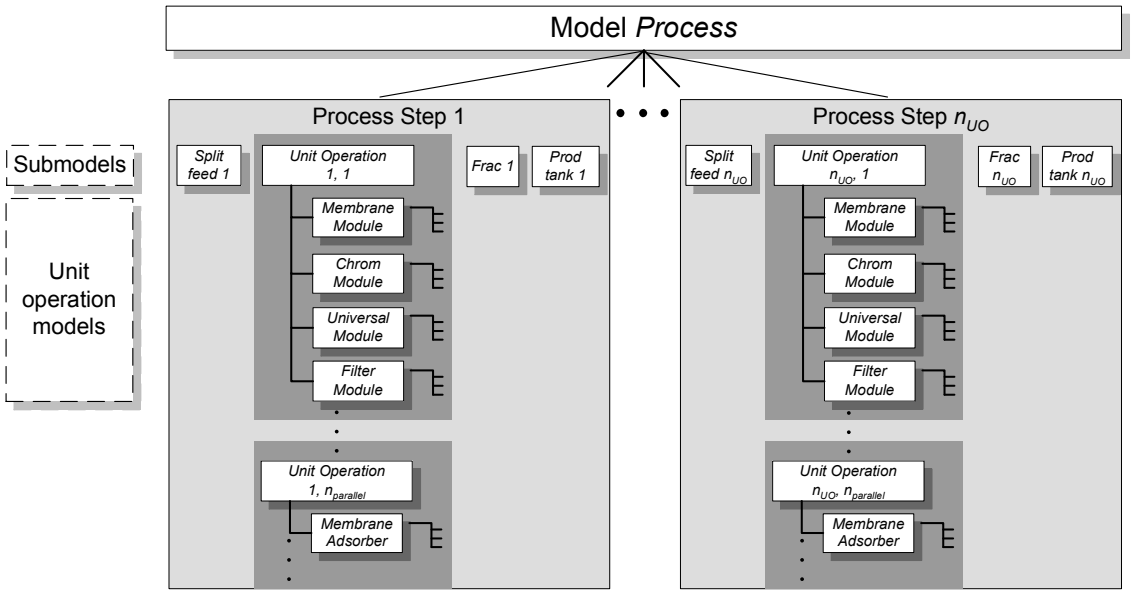

Figure 2: Hierarchical structure of the generic process model

To provide the user the option of selecting the modelling depth suitable for the given modelling task, various modelling depths available for each process step were implemented in the generic process model [40], which are shown in

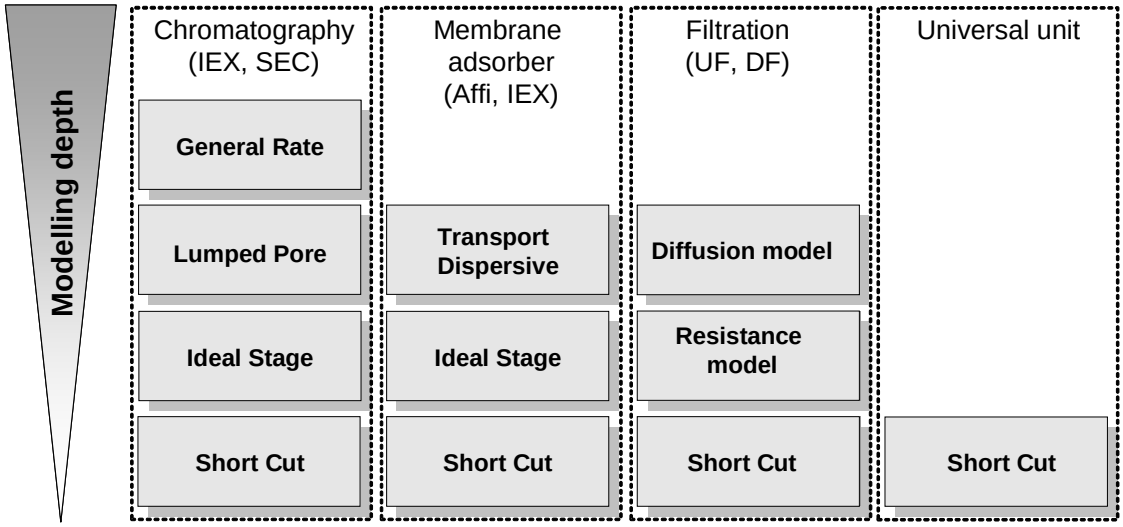

Figure 3: Modeling depth of the implemented unit operations

To account for the discrete events occurring in batch purification processes, a time-discrete event modelling approach was utilized. The resulting model equations have been implemented in the equation-oriented simulator ASPEN CUSTOM MODELER™. Despite the complex transport phenomena, such as convection, axial dispersion, radial diffusion and nonlinear adsorption, considered dynamically in the chromatographic models, the process simulator provides stable convergence properties even for processes consisting of more than seven different unit operations. These properties of the process model allow for simulation and evaluation of arbitrary batch downstream protein process with respect to process performance and process costs. In contrast to commercially available bioprocess simulators

based on short-cut or black-box models [9], the impact of varying process conditions on the process behaviour can be predicted by utilising the developed process model. Furthermore, the dynamic concentration profiles of each component can be utilised for detailed process analysis. In simulation studies for the benchmark processes, it was demonstrated that the replacement of a cation-exchange chromatographic resin by a cation-exchange membrane adsorber had a significant impact on the performance of the subsequent unit operations. These interactions between unit operations would not have been identified by examining the individual unit operations separately.

The basis of modelling the separation of proteins in ion-exchange membrane adsorbers is the selection of an appropriate adsorption isotherm and the determination of the respective model parameters. In this work, the adsorption of the solutes on ion-exchange membrane adsorbers was described by the steric mass action model which was originally derived for modelling the ion-exchange processes in chromatographic resins. A methodology to determine model parameters consistently, proposed by *Epping* [10] for chromatographic separations, was successfully adapted to determine the model parameters required to describe the adsorption of IgG, BSA and α-lactalbumin on a strong anion-exchange membrane adsorber. Comparison of the simulation results with the experimental results showed reasonable agreement, validating the model (see Figure 3).

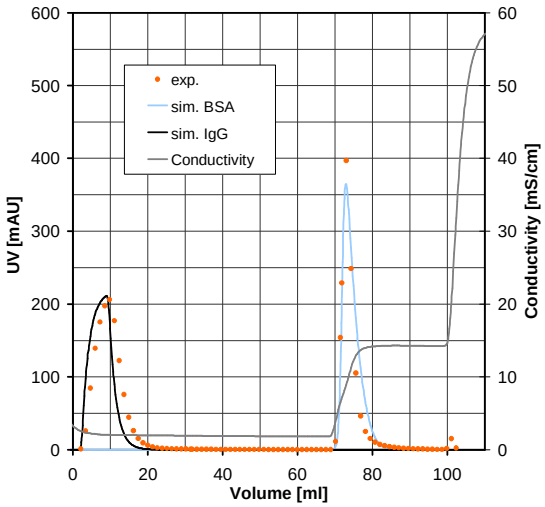

Figure 3: Separation of a BSA/ IgG mixture from a Q75 membrane adsorber module at a flow rate of 16 ml/min and feed composition: 1.8 mg/ml BSA, 0.8 mg/ml IgG, V_{load}= 7 ml

Nevertheless, fundamental research is necessary since the mass-transport phenomena causing band broadening of the eluting protein peaks and flattening of the breakthrough curves at high protein loading close to the saturation capacity of membrane adsorbers are not fully understood.

In a biopharmaceuticals production plant, downstream processing, which consists of a sequence of several unit operations, significantly contributes to the overall production costs. As a result, optimising the design and operation of the downstream process has the potential for significant cost savings. Particularly, designing of processes with new alternative unit operations or reduced number of unit operations can have a significant influence on the process yield and process costs [11]. The application of computer-aided design methods to determine the optimal operating conditions and design of the unit operations typically leads to nonlinear or mixed-integer nonlinear global optimisation problems. To utilise the generic process model for process simulation and process design tasks, it has been linked to an optimisation algorithm based on differential evolution [12]. This approach yields a powerful optimisation platform for downstream processes of protein purification, which allows for the discrete-continuous optimisation of multistage batch purification processes involving detailed dynamic models. The adaptability of the applied evolutionary strategy for global optimisation has been demonstrated by solving benchmark problems previously described in the literature. Because of its flexible interface, the developed evolutionary optimisation algorithm can be connected to any process models implemented in ASPEN CUSTOM MODELER™, allowing for solution of complex optimisation problems involving multistage membrane separation and hybrid distillation processes as demonstrated in several other works. In a case study, a superstructure comprising four alternative purification sequences for the production of bovine serum albumin, each consisting of six consecutive separation steps, was solved globally optimised by simultaneously altering the process structure and the continuous design variables of the respective unit operations. For this case study, the combination of an anion-exchange membrane adsorber module followed by a cation-exchange resin yielded the minimum production costs of all four process alternatives. Since the optimisation was performed on the basis of rigorous dynamic models, the dimensionless outlet concentrations of the different unit operations are plotted versus the time in Figure 4.

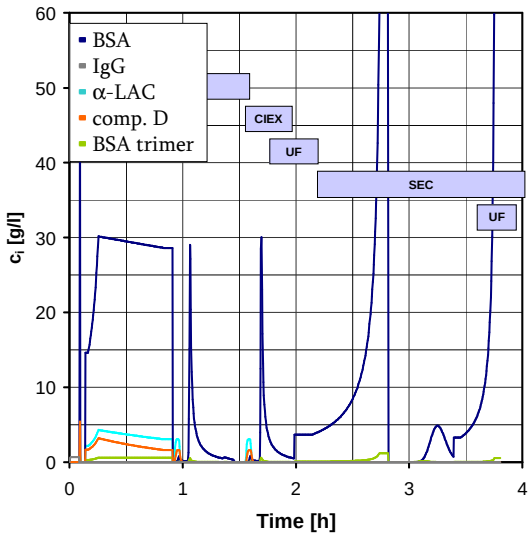

Figure 4: Time dependent outlet concentration profiles of the proteins for the optimal downstream process DSP_3(comprising an AIEX membrane and CIEX column

The simulation results show that the polishing step executed in a size-exclusion column constitutes the bottleneck of the downstream process with respect to process time and process costs.

The impact of several process parameters, such as feed load, salt concentrations in the buffers, etc., on the process performance of the two best process alternatives determined in the optimisation study was investigated. It was shown that the interactions between the unit operations at first glance lead to unexpected separation results when the process parameters are perturbed around the base case. For example, a reduction of the feed volume leads to a decreased yield in the fifth separation step, a size-exclusion column. These simulation results emphasise the necessity of incorporating the entire purification process, not only single unit operations, into the process analysis.

Rigorous modelling of protein separation processes contributes to the fundamental understanding of the entire process performance, revealing interactions between unit operations within the process. The developed simulation and optimisation platform can be utilised to efficiently design the experiments required for scale-up from lab to industrial scale. Furthermore, due to the PAT guidelines initiated by the FDA, an increased understanding of the process allows for faster validation of new downstream processes.

[1] Reis, R.v. and A. Zydney, Bioprocess membrane technology. *Journal of Membrane Science*, 297: 16-50, 2007.

[2] Lightfood, E.N., Speeding the design of Bioseparations: A Heuristic Approach to Engineering Design. *Industrial Chemical Engineering Research*, 38: 3628-364, 1999.

[3] Petrides, D., A. Koulouris, and P.T. Lagonikos, The Role of Process Simulation in Pharmaceutical Process Development and Product Commercialization. *Pharmaceutical Engineering*, 22(1): 1-8, 2002.

[4] Chmiel, H., *Bioprozesstechnik*. 2nd ed, ed. H. Chmiel, Elsevier, München, 2006.

[5] Gadewar, S.B., G. Schembecker, and M.F. Doherty, Rapid Process Design. *Chemical Engineering Progress*, 102(9): 22-32, 2006.

[6] *PAT — A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance*. [cited 2005 06.30]; Available from: http://www.fda.gov/Cder/OPS/PAT.htm, 2004.

[7] Curling, J.M., *Albumin Purification by Ion Exchange Chromatography*. in *Separation of Plasma Proteins*, J.M. Curling, Editor, Academic Press, London, 1980.

[8] J.H Berglöf, S.E.E., *Plasma Fractionation by Chromatography of Albumin and IgG*. in *Biotechnology of Plasma Proteins*, C.R. J.F. Stoltz, Editor, Colloque, 1989.

[9] Petrides, D., BioPro Designer: An Advanced Computing Environment for Modelling and Design of Integrated Biochemical Processes. *Computers & Chemical Engineering*, 18: S621-S625, 1994.

[10] Epping, A., *Modellierung, Auslegung und Optimierung chromatographischer Batch-Trennungen*. Fachbereich für Bio- und Chemieingenieurwesen, Universität Dortmund, Dortmund, 2004.

[11] Lightfood, E.N., Bioseparations. *Biotechnology and Bioengineering*, 87(3): 259-273, 2004.

[12] Angira, R. and B.V. Babu, Optimisation of process synthesis and design problems: A modified differential approach. *Chemical Engineering Science*, 61: 4707-4721, 2006.