Simulation of continuous preparative chromatography: A case study in MCSGP

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Abstract

Purification of proteins in the pharmaceutical industry is often performed with batch chromatography. For increase of productivity and yield there is a need for continuous chromatography. The purpose of this master thesis was to develop a general simulation toolbox for continuous chromatography that could simulate a defined configuration of moving beds. Recently a process for separation of ternary systems called *multicolumn solvent gradient purification process* (MCSGP) was developed. This is a six bed arrangement, where the solid phase move countercurrent to the mobile phase after certain periodical time intervals. Optimization of the time between bed rotations showed that long times favored purity, while short times favored yield and productivity. This result follows the same trend as the batch chromatography cycle time.

Keywords: Continuous chromatography, Simulation, Optimization, MCSGP, MATLAB

Introduction

Production of biopharmaceuticals is an expensive industry, where the main cost is due to separation in downstream processing. A good separation technique, which does not require high temperatures, is chromatography. An important biopharmaceutical is antibodies that usually are purified with solvent gradient batch chromatography. A mobile phase carries the substances that are going to be separated through a column, filled with stationary phase, constituted of particles with adsorbing sites. The fact that the binding strength differs for each substance makes separation possible by gradually changing of the mobile phase conditions with a solvent modifier concentration, such as salt. The purified components are collected by cutting the outlet stream into fractions which is called peak shaving. Batch chromatography is run in discontinuous cycles, starting with a load step, followed by a wash step, elution step, regeneration step. Thereafter follows a re-equilibrium step to prepare the column for a new cycle.

When substances have similar properties higher separation efficiency can be achieved by running the process in a counter-current manner. In large scale productions counter-current chromatography has proved to be more cost-effective concerning yield, solvent consumption and productivity. The fact that it is practically impossible to truly move the stationary phase in a counter-current way can be solved by dividing it into several columns that are moved discretely with certain time intervals. An example is the *simulated moving beds* process (SMB) with at least four beds that separates the substances into two fractions of high purity.

This thesis focuses on the process called *multicolumn countercurrent solvent gradient purification* (MCSGP) that has recently been developed at ETH by Aumann and Morbidelli (1). This six column arrangement makes it possible to separate a ternary component system and to apply a smooth solvent gradient which usually is needed for good separations of complex systems. The MCSGP introduces the concept of *short circuited moving beds*, which means that the inlet and outlet is not connected to other beds and can be treated as batch units (2). They are represented by the three upper beds in Fig. 1, which are also referred to as the batch lane, while the three lower beds are referred to as the interconnected lane. All beds are however in a way connected because of a periodical bed rotation scheme countercurrent to the mobile phase direction. This scheme means that the bed at position 6 rotates to position 5, 5 to 4, 4 to 3, 3 to 2, 2 to 1 and 1 to 6 after each switch cycle time. A complete cycle is when a bed has gone through all positions i.e. after 6 switch cycles.

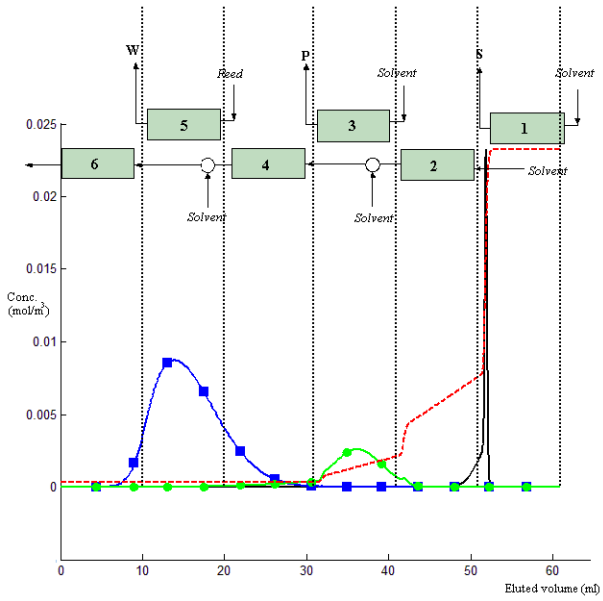

Figure 1. A batch like chromatogram is build up by putting together the outlets of the beds during one switch cycle. This batch diagram is thus looped time after time with the switch cycles. The outlet concentrations of the beds are separated with dotted lines and represent the bed in the schematic process scheme.

The feed mixture, that is going to be separated, is injected to bed 5. In contrast to batch chromatography the

feed can be added continuously. In all beds but the feed bed a solvent is added to get a desired solvent gradient profile. The outlet of the feed bed is used for collecting weakly adsorbed component (W). The product component (P) and the strongly adsorbed component (S) are collected at the outlets of bed 3 and 1, while inert components are found in the outlet of bed 6. The interconnected lane beds are used to recycle the impure part of the batch diagram where two peaks overlap and gives unseparated components another chance. This gives different constraints on the tasks of the beds.

A good design of the MCSGP process is important in order to get high yield, productivity and purity. The simplest way to achieve good behavior is to translate an optimized batch diagram to MCSGP. In Fig. 1 it is illustrated how the outlet concentrations during one switch cycle for each bed is simulated and joined together side by side from bed 6 to bed 1. The appearance of a chromatogram of joined beds is very similar to a batch chromatogram, which can be perceived from the following reasoning. The feed is injected in bed 5 where the weakly adsorbed component is eluted. The bed, containing all components that are strongly bound is now moved to the next position, position 4. By changing the conditions with the solvent concentration, a protein with stronger bindings can be retained. The bed now once again moves to the next position and gradually builds up a complete batch chromatography. For each switch cycle a new batch chromatogram can be produced. In the joined diagram the width of each bed fraction corresponds to the eluted volume of the bed according to the scale of the horizontal axis. The start and end of the switch cycle is shown from the left to the right edge of a bed fraction.

The applied modifier gradient is of central focus to control in which bed the components will elute. The solvent can be considered to follow the convective flow because it is not bound to the adsorption sites in the chromatography column. To get the gradients to follow a predefined behavior, different factors have to be taken into account. The dead volumes in the pipes between the beds are one important matter. When the beds are connected to an inflow bed its concentration must be considered. Furthermore the outlet of a bed is lagged with one column volume, CV, compared to the inlet of a bed.

Multiple bed simulation

To be able to simulate general configurations of continuous units the beds are divided into *monomer units* consisting of a mixer, loop, column, splitter and five different flows, see Fig. 2.

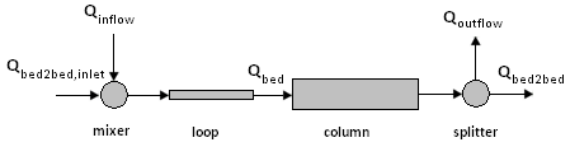

Figure 2. The structure of a single bed, called monomer unit, that builds up a multiple bed process in the simulator.

To not generate or destruct material in the simulator a simple material balance over each monomer unit must hold.

$$Q_{inflow} + Q_{bed2bed,in} = Q_{outflow} + Q_{bed2bed,out}$$

This material balance can be directly derived from a given bed configuration and is easily understood from a look at the inlets and outlets of the monomer unit. The feed and the solvent are added in the inflow stream, while the waste or product is found in the outflow stream. The notation $Q_{bed2bed}$ is the flow to the next connected monomer unit, where it is called $Q_{bed2bed,inlet}$. The simulation of multiple beds is thus built up by connecting monomer unit from a given configuration. The mixer and splitter are modeled by simple material balances while the model of the loop and column are described below.

Mathematical model

The column is described with a homogeneous convective-dispersive model, consisting of three main equations, describing the flow through the column, the diffusion in the particles and the adsorption of components on the particles. The mobile phase is modeled with three terms describing diffusion, convection and sorption as follows:

$$\frac{\partial c_i}{\partial t} = D_{ax}\frac{\partial^2 c_i}{\partial x^2} - v_{int}\frac{\partial c_i}{\partial x} - \frac{(1-\varepsilon_c)}{\varepsilon}\cdot\frac{\partial q}{\partial t}$$

,where c_i is the concentration of a component in the mobile phase (mol/m^3), t is the time (s), D_{ax} is the apparent dispersion coefficient (m^2/s), *x* is the axial coordinate along the column (m), v_{int} is the interstitial velocity (m/s), ε_c is the void fraction in the column (m^3 bulk mobile phase / m^3 column), ε is the total void fraction in the column (m^3 total mobile phase / m^3 column) and q_i is the concentration of component i in the stationary phase (mol / m^3). The loop is described in a similar way by omitting the part describing the sorption.

The boundary conditions for the inlet and the outlet of the mobile phase are described by a Robin condition and by a von Neumann condition.

$$\frac{\partial c}{\partial x} = \frac{v_{int}}{D_{ax}}(c - c_{inlet}) \qquad \text{at } x = 0$$

$$\frac{\partial c}{\partial x} = 0 \qquad \text{at } x = L$$

Here, c_{inlet} is the inlet concentration of a component (mol/m^3) and L is the length of the column (m).

To describe the adsorption rate on the sites, $\partial q/\partial t$ (mol/m^3 sp, s) an expanded Langmuir isotherm is used, by adding modulators for the mobile phase. Thus the model is called Langmuir mobile phase modulators (MPM).

$$\frac{\partial q_i}{\partial t} = c_{p,i}k_{ads,i}q_{max,i}\left(1 - \sum_{j=1}^{N}\frac{q_j}{q_{max,j}}\right) - k_{des,i}\cdot q_i$$

Here, $q_{max,i}$ is the maximal number of sites that the component can be bonded to (mol/m^3), $k_{des,i}$ is the desorption rate coefficient (1 / s), $k_{ads,i}$ is the adsorption rate

coefficient (m^3/mol, s) and N is the number of interacting components (-).

The mobile phase modulators, which describe non-idealities within the column, are accomplished by modifying the desorption and adsorption rate with the salt concentration,

$$k_{des,i} = k_{des\,0,i} \cdot S^{\beta_i}$$

$$k_{ads,i} = k_{ads\,0,i} \cdot e^{\gamma_i \cdot S}$$

,where $k_{des\,0,i}$ is a constant describing the desorption rate ($(m^3/mol)^{\beta} \cdot s$), $k_{ads\,0,i}$ is a constant describing the adsorption rate ($m^3/mol,s$), β_i describes the ion-exchange characteristics (-), γ_i describes the hydrophobic characteristics (m^3/mol) and S is the concentration of the elution component (mol/m^3).

Case study

MCSGP claims to be able to completely separate a ternary system making the case with the proteins *myoglobin, immunoglobulin* (IgG) and *bovine serum albumin* (BSA) suitable to investigate. Here, the binding strength to the stationary phase of the components is in ascending order with IgG considered as the product. The simulated column is an ion-exchange chromatography type.

The optimization of the process is performed with a genetic algorithm, which is a population-based algorithm where the best individuals survive to the next generation (3). The algorithm can optimize multiple objective functions, which often are in conflict with each other, and therefore the optima become a set of points. Optimization with genetic algorithms with large populations requires a lot of computer capacity and consequently a computer cluster was developed and used. The three objective functions used were the purity of the product flow and the yield and productivity of the process as defined by Karlsson (4).

For continuous chromatography processes, the yield is somewhat differently defined compared to batch chromatography because it cannot be directly related to the feed stream until steady-state is reached. The reason is that there is always accumulation in the MCSGP unit. When steady-state is reached, the same amount that is being fed into a MCSGP process must be eluted in the outlet streams at each time unit. When calculating the total amount product lost in the process, only the outlets of the process (bed 1, 3 5 and 6) are considered. To calculate the yield for a cyclic process, a complete cycle has to be considered.

The elution modifier gradient is of great importance for the retention times of the proteins in the batch diagram and is the best way to influence that the right compound elutes in the right bed. In the optimization a linear salt gradient is applied to all beds, with start and stop points at the start and stop of a switch cycle. The gradient points are defined as added amount of high concentration elution modifier buffer. If the switch cycle time and the flows are given the operating conditions are fully supplied. The decision variables for the simulation are chosen as three gradient points, flows for four beds and the switch cycle time.

The way to steady state

It takes time for the feed to propagate through all columns at the startup of a MCSGP because there is just one feed column, bed 5. After one switch cycle the only outlet with protein is bed 5, which is seen in Fig. 3. The protein content that is not eluted is switched to bed 4 that is connected to bed 6. In the next switch the protein content continues to bed 3 which explains the profile after three switch cycles. The profile after six switch cycles, when a total cycle is completed is also shown and will be close to a steady-state profile.

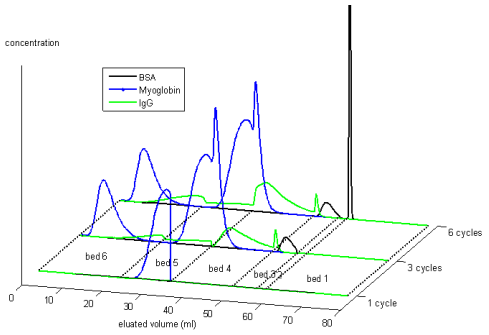

Figure 3. The propagation of the proteins is illustrated after the end of 1, 3 and 6 switch cycles.

How fast the solution will reach a steady state profile is measured by comparing the profile during a switch cycle with the profile for the previous switch cycle for a component. In Fig. 4 a mean value of the absolute value in 10000 points are plotted. It should be interpreted as the mean change between two adjacent switch cycles. The less the difference between the switch cycles, the closer is the steady state.

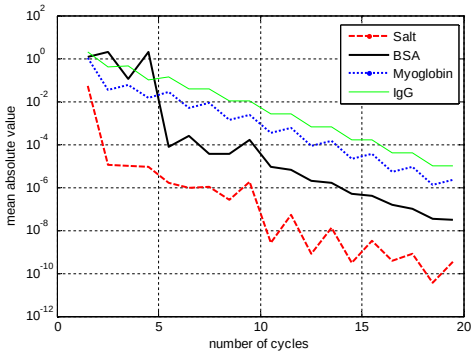

Figure 4. How the solution reaches steady state as a function of the number of switch cycles.

The component that reaches steady state quickest is the modifier. The reason is that the elution modifier is added in all beds, in contrast to the proteins. The biggest difference is between switch cycle 1 and 2 with a mean absolute value of approximately 0.1 mol/m^3. The difference decreases quickly with the next switch cycle. For BSA with the highest peak a big drop is seen with the fifth switch cycle. This is because that peak is first eluted after the fifth switch cycle and after that the profile is not changed much. It is hard to spot any changes with the eye after 2 switch cycles for the elution

modifier and after 6-7 switch cycles for the protein components, which is illustrated in Fig. 5. The convergence is followed after switch cycle 3, 6 and 18. For myoglobin there are just a tiny difference between 6 and 18 switch cycles. All MCSGP optimization calculations are done after a complete cycle when steady state is close.

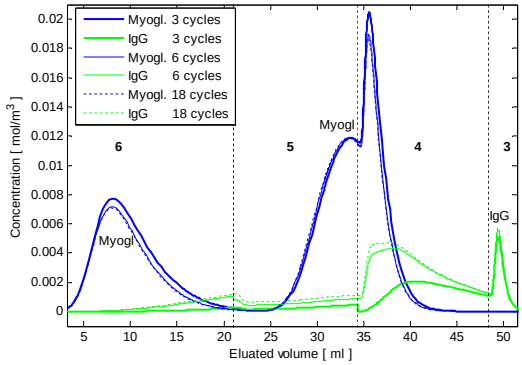

Figure 5. The way to steady state after 3, 6 and 18 cycles for myoglobin and IgG.

Results

For the best yield with a purity constraint of 99 %, see Fig. 6a, the yield is 74 %, which is not as high as hoped. The profile of the best yield with no purity constraint is shown in Fig. 6b. The purity is here decreased to 33 % seen in the fraction representing bed 3 which is contaminated with both W and S. The salt gradient is here lower until the product bed where it is raised to elute all product in the product bed. The eluted volume for the product bed is also larger when yield is optimized, while the eluted volume of the adjacent beds is smaller.

The effect of changing the switch cycle time is shown as a purity-yield pareto front in Fig. 7. A tendency between the switch cycle time can be clearly seen in the figure. Shorter switch cycle times gives higher productivity and yield while longer switch cycle times gains high purity. The switch cycle time cannot be directly compared to the cycle time for a batch process, which feed is just added as a part of that cycle in contrast to the MCSGP process that is fed continuously. However, for MCSGP a batch diagram is repeated for each switch cycle, making a connection between the switch cycle and batch cycle that could explain the behavior. In optimization of yield in batch chromatography the cycle time is maximized, while optimizing the purity will result in minimizing the captured fraction which leads to a short cycle time. The productivity is significantly lowered with long cycle times in both MCSGP and batch chromatography.

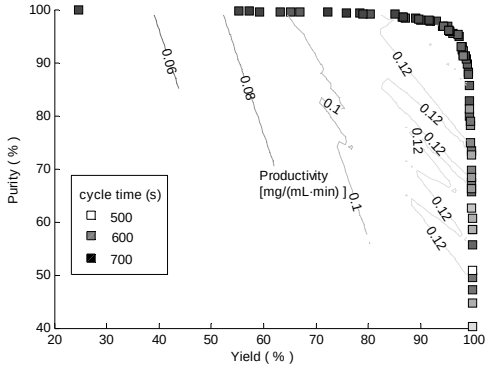

Figure 7. A purity yield pareto front from the optimization of the switch cycle time that is shown as a floating grey scale of filled squares where darker means longer switch cycle time. The productivity is shown as a contour plot.

Conclusions

When simulating MCSGP a steady state profile is reached after approximately 1-2 switch cycles for the elution modifier gradient and 6-7 switch cycles for the components to be separated. High switch cycle times gains high purity, while low switch cycle times gains yield and productivity.

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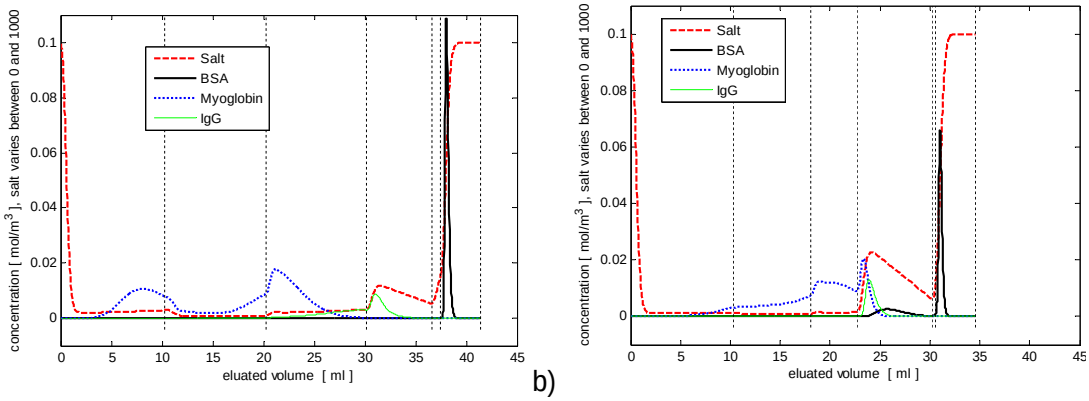

Figure 6. The result from the optimization where the best yield with a purity constraint of 99 % is shown in (a). Purity for this simulation is 76 % and productivity is 0.72 mg/(mL·min). The best yield with a no purity constraint is shown in (b). Purity for this simulation is 33 % and productivity is 0.74 mg/(mL·min).