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Design of two-column batch-to-batch recirculation to enhance performance in ion-exchange chromatography

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ABSTRACT

Preparative liquid chromatography is a separation technique widely used in the manufacturing of fine chemicals and pharmaceuticals. A major drawback of traditional single-column batch chromatography step is the trade-off between product purity and process performance. Recirculation of impure product can be utilized to make the trade-off more favorable. The aim of the present study was to investigate the usage of a two-column batch-to-batch recirculation process step to increase the performance compared to single-column batch chromatography at a high purity requirement. The separation of a ternary protein mixture on ion-exchange chromatography columns was used to evaluate the proposed process. The investigation used modelling and simulation of the process step, experimental validation and optimization of the simulated process. In the presented case the yield increases from 45.4% to 93.6% and the productivity increases 3.4 times compared to the performance of a batch run for a nominal case. A rapid concentration build-up product can be seen during the first cycles, before the process reaches a cyclic steady-state with reoccurring concentration profiles. The optimization of the simulation model predicts that the recirculated salt can be used as a flying start of the elution, which would enhance the process performance. The proposed process is more complex than a batch process, but may improve the separation performance, especially while operating at cyclic steady-state. The recirculation of impure fractions reduces the product losses and ensures separation of product to a high degree of purity.

1. Introduction

Preparative liquid chromatography is a separation technique widely used in the manufacturing of fine chemicals, pharmaceuticals and other biotechnical products [1]. A promising approach to enhance the performance of preparative chromatography is the usage of process integration, where two or more process steps are combined [2]. By combining different chromatographic columns an integrated column sequence (ICS) is constructed [3]. When linked in an optimal design, these integrated columns can improve downstream processing of target components. For facilities with limited storage capacity the improvement may be achieved by reducing hold times between steps and performing in-line adjustment of intermediate products [2]. Straight through processing with high recovery yield and minimal time from expression to formulation is desirable. However, the overall process performance of the ICS may be limited by one or several separation steps where separation is more difficult to achieve. For such performance limiting steps, it may be favorable to introduce local recycle streams, enhancing the purity and/or the recovery yield of the target component.

A major drawback of traditional single-column batch chromatography step is the trade-off between product purity and process performance. A high purity product comes at the cost of reduced recovery yield of product, increased solvent consumption and/or decreased productivity [4]. Hence, various chromatographic processes that utilize recirculation have been introduced to make the trade-off more favorable. These processes include the closed-loop recycling (CLR) [5] chromatography, the steady-state recycling (SSR) [6,7] chromatography, the simulated moving bed (SMB) [8,9] chromatography and the multicolumn countercurrent solvent gradient purification (MCSGP) [10–12] process. A comparison of all but the latter is given in [13]. A simplified two-column SMB concept has also been demonstrated [14,15].

Modelling and simulation is a widely used tool to predict the behavior of chromatography systems. Ion-exchange chromatography has been widely used in modelling and simulation studies, such as the separation of monoclonal antibodies [16]. A separation of the same ternary protein mixture on the same stationary phase has also been modelled and simulated before [17]. An overview

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of modelling of ion-exchange chromatography can be found in for example [18,19].

The aim of the present study was to investigate the usage of a two-column batch-to-batch recirculation process step to increase the performance of a part in an integrated column sequence. The investigation started with modelling and simulation of the process step, which then was constructed and validated in lab. Finally, an optimization study was performed on the simulated process. The separation of a ternary protein mixture (ribonuclease A, cytochrome c and lysozyme) on ion-exchange chromatography (IEC) columns was used to evaluate the proposed process [17].

2. Theory

2.1. Two-column batch-to-batch recirculation process step

The proposed two-column batch-to-batch recirculation process step consists of a main column and a twin column, see Fig. 1. The first batch, coming from the previous step in the ICS, is run on the main column. Impure product fractions from the main column are recirculated to the twin column. During the second batch the twin column is used as main column, while the original main column is used for recirculation of impure product. The columns keep altering their roles to form a batch-to-batch recirculation process step. Odd cycles are run on the main column, while even cycles are run on the twin column. The injection of new batch in every loop prevents concentration decrease from cycle to cycle, as for one-column SSR [13]. In the present setup, the recirculated fluid enters the columns before fresh fluid enters. The absence of mixing of recirculated and fresh fluid results in that the profiles of recirculated fractions are maintained. The usage of a two-column process enables the ability to perform simultaneous actions on the columns, e.g. clean one column while separation is performed on the other.

Fig. 2 shows a chromatogram where three proteins are insufficiently separated. In the two-column batch-to-batch recirculation process step the following time points and intervals are defined:

- $t_{\alpha 1} \leq t < t_{\alpha 2}$: recycling of impure product, fraction I
- $t_{\alpha 2} \leq t \leq t_{\beta 1}$: extraction of pure product
- $t_{\beta 1} < t \leq t_{\beta 2}$: recycling of impure product, fraction II

The pure product that leaves the main column is extracted. Impure product fractions are recirculated. Streams of impurities or pure buffer go to waste. It is important that unwanted components can leave the system to reduce build-up of impurities in the recycle fractions.

The two-column batch-to-batch recirculation process bears some similarity with the MCSGP process even though the MCSGP may be superior in performance the process performance should be comparable. The main benefit of the suggested process is its suitability to run in an integrated column sequence where it can be integrated just as easily as any batch step because the receive phases are connected. In the MCSGP process the receive phases are divided because the process starts with the first receive phase followed by the loading phase and the second receive phase. In the suggested process the second receive phase is moved before the loading phase, enabling the two recirculated fractions to be recirculated to the twin column where it is parked until the next cycle while the ICS continues to the next separation steps in the chain. In the next ICS cycle of the integrated column sequence, the twin column is used instead with fresh feed from upstream.

2.1.1. Dilution of recirculated fractions

In this study the system utilized ion-exchange chromatography (IEC) columns to perform separation of three proteins. By changing the competitive salt ion concentration over time, creating a gradient elution, proteins with different affinity could be displaced at different times [20]. The salt concentrations in recycling fraction I and II were high enough to displace the proteins. Recycling of these fractions would therefore result in instant displacement of recirculated proteins as they enter the following column. Dilution of recirculated fractions was implemented to prevent this behavior. The dilution decreased the concentrations of recirculated proteins and salt, and temporarily increased the flow in the following column.

3. Material and methods

3.1. Proteins and chemicals

Ribonuclease A (bovine pancreas, R5503), cytochrome c (horse heart, C2506), lysozyme (chicken egg white, 62971), monosodium phosphate, disodium phosphate, sodium chloride and blue dextran were purchased from Sigma-Aldrich (Steinheim, Germany). Acetone was purchased from Merck (Darmstadt, Germany). Cytochrome c represented product in this study since it has an isoelectric point in between [21] the other two proteins. Ribonuclease A and lysozyme represented by-products.

A ternary protein mixture (ribonuclease A, cytochrome c and lysozyme) was prepared by solving 1 g of each protein in 20 mM sodium phosphate buffer (pH 7). Buffer solutions were prepared using deionized water. Two mobile phases were used in the protein separation. Buffer A was a 20 mM sodium phosphate buffer. Buffer B was a 20 mM sodium phosphate buffer with 0.5 M NaCl.

3.2. Apparatus and columns

Two coupled ÄKTA explorer 100 chromatography systems from GE Healthcare (Uppsala, Sweden), denoted system 1 and system 2, were used in the experiments. The accessory equipment included two 2 mm UV cells, a 1/16" conductivity cell (system 1), a fraction collector Frac-900 (system 2) and two 10 ml super loops. The protein absorbance was measured using UV light at a wavelength of 280 nm. Two 5 ml HiTrap SP HP columns purchased from GE Healthcare (Uppsala, Sweden) were used in the experiments. The columns were cation exchangers consisting of an agarose matrix with sulfopropyl ligands. The median particle size of the cumulative volume distribution was 34 μm [22] and the column length was 2.5 cm.

The two-column batch-to-batch recirculation process was realized by connecting two identical systems symmetrically as seen in Fig. 3. The buffers and feeds to the system were introduced to the system in the inlet valve and continued to the column and sensors. Depending on the process phase the outlet was separated to product, recycle or waste. The recycled material was mixed with the inlet of the other system using a three-way junction.

The systems were controlled using the Orbit controller developed in Python [3,23]. The communication to the systems in Orbit used an OPC server using the OpenOPC package. Orbit allows scripting to set flow rates and valve positions of chromatography systems offering the OPC interface. To run the two-column process, the systems needs to be completely synchronized and was accomplished using two computers that communicated over the network using the *zmq* package. This enabled simultaneous control of two systems and in a simple way iterate over several cycles of the process. The systems were represented by two instances s1 and s2 in orbit that had references to each valve on the system that could be changed using commands like s1.valve1.set(position).