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Protein separation in carousel multicolumn setup. Performance analysis and experimental validation

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ABSTRACT

To overcome limitations of periodic separations of proteins in batch chromatographic columns Carousel Multi-Column Setup (CMS) has been recently suggested and theoretically analyzed in a previous study (R. Bochenek, W. Marek, W. Piątkowski, D. Antos, J. Chromatogr. A, 1301 (2013) 60–72). In this system, feed and mobile phase streams are subsequently delivered through parallel columns to mimic their countercurrent movement with respect to the fluid flow. All fluxes in the system are synchronized to ensure continuous feed delivery, which however causes reduction in the size of the operating window compared to batchwise-operating systems.

In this study to improve the performance of CMS, additional process variables have been considered, such as the flow rate gradient and feed concentration. Though altering both variables allowed improving the separation selectivity and extending the operating window, the feed concentration appeared to be the most influential parameter affecting the process performance. Moreover, a procedure for practical realization of protein separations in CMS has been developed, including hints about the process design, configuration of columns and detectors, and use of pumps. As the case study, the separation of a ternary mixture of proteins, *i.e.*, cytochrome C, lysozyme and immunoglobulin G, on hydrophobic interaction columns was used. A target product was a protein with intermediate adsorption strength that was isolated out of a more and less strongly adsorbed compound.

1. Introduction

Proteins manufactured by biotechnological processes are usually obtained in the form of complex multicomponent mixtures; therefore, to isolate a target compound out of a number of contaminants advanced chromatographic techniques are required. Since industrial upstream bioprocesses are often realized in continuous mode, there is growing interest in development of continuous chromatographic separation of proteins [1].

A well-established technique of continuous chromatography is simulated moving bed (SMB) [2]. The conventional 4-zone SMB system is only suitable for processing binary mixtures, where the mixture components are split between a less and more retained fraction. Therefore, to accommodate the process to the separation of multicomponent mixtures several continuous and semi-continuous SMB-based processes have been developed, including, *e.g.* [3–13]: side-stream SMB, 5-zone or 8-zone SMB, three-fraction SMB, intermittent-SMB (I-SMB), which however were intended for separation of small-molecules under isocratic conditions.

Due to the complexity of post-fermentation mixtures and retention behavior of proteins on chromatographic media, those techniques cannot straightforwardly be adopted for separation of protein mixtures. Typical process of chromatographic purification of proteins consists of a few stages including: column loading aimed to deliver the feed mixture, column wash – to remove unretained impurities, selective elution – to separate the mixture components, regeneration – to desorb strongly retained impurities, and re-equilibration – to restore initial conditions. The realization of each stage requires different elution strength of the mobile phase, therefore, the separation is performed in solvent gradient mode, where gradual or stepwise changes of the mobile phase composition are imposed to optimize the separation selectivity.

Several techniques have been suggested for the separation of proteins under solvent gradient conditions, *e.g.*, Multi-column Countercurrent Solvent Gradient Purification (MCSGP) [14–18]. The initial MCSGP setup consisted of six columns and was fully continuous. It was able to separate three fractions, it incorporated linear or step solvent gradients, wash and regeneration steps. The process was further modified by reducing the column number

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to three or four, and eventually to two columns. The latter system, termed as twin-column SMB, assembles the chromatographic columns working sequentially in series and in parallel. The system is based on periodical feed delivery and product withdrawal. Recently, twin-column CaptureSMB has been adapted for the affinity capture of a monoclonal antibody [19].

To realize separation of proteins under solvent gradient conditions, a concept of solvent gradient with steady state recycle (GSSR) has also been developed, where a multicolumn open-loop system was used to perform solvent gradient separations [20]. The system consists of three columns combined in series and in parallel. The feed is injected periodically into the same column and the product is periodically collected from the same column.

The internal recycling of the solid phase in SMB-based systems allows improvement in the utilization of chromatographic resins, separation efficiency and solvent consumption. Nevertheless, it causes undesirable effect of internal solvent mixing [21]. The columns in each zone of such systems are equilibrated with the eluent adequate to the function fulfilled by the zone, *i.e.*, protein binding, separation, resin regeneration, *etc.* After the switch in the position of inlet ports, the columns along with the contained liquid are transported into subsequent zones to realize other stages of the process, which requires eluent exchange. This causes internal distortions of gradient shape, which may be detrimental for the separation efficiency [21].

In our previous study, we have theoretically analyzed separation of multicomponent protein mixtures in carousel multicolumn setup (CMS) under solvent gradient conditions, and compared its efficiency to other continuous systems such as MCSGP and SMB [21]. In CMS systems, the columns are assembled in parallel, which is aimed to increase throughput of the separation process. When the operating cycles of the columns are not synchronized, the system operates batchwise with periodical feed delivery and product withdrawal (batchwise-operating multicolumn system – BMS). In a synchronized CMS, the columns are subsequently supplied with feed and eluents to mimic countercurrent movement of the solid and liquid phase [22–26]. The movement is realized by switching feed and eluent ports in equal time intervals. The switching time and eluent fluxes are properly adjusted to ensure continuous feed delivery, whereas the product withdrawal is realized periodically, analogically as in BMS. The system is simple and easy for handling, which facilitates the realization of the separation process.

Continuous feed delivery may benefit in improvement of the process performance compared to BMS if the chromatographic separation is preceded by a continuous upstream bioprocess. The upstream output incoming into BMS has to be stored between cycles of feed loading, which delays the start-up of the operation and reduces the process economics. On the other hand, because of necessary adjustments of the switching interval and eluent fluxes, the size of the operating window of the CMS processes is smaller compared to BMS [21]. That difference can be reduced by an increase in the number of columns in CMS [21], which however creates higher operating and investment costs.

The goal of this study is to provide an efficient procedure for the design and practical realization of chromatographic separations in CMS. We present a modification of the algorithm for the process optimization developed in our previous study [21]. The modification is based on a combination of solvent gradient and flow rate gradient, which is aimed to improve the separation performance by widening the operation window. The algorithm also accounts for the feed concentration as an additional adjustable parameter. The concentration overload, which results from isotherm nonlinearity, is a crucial process parameter that can influence dramatically on the performance of the separation realized in both CMS and BMS.

Furthermore, we demonstrate how the separation process can be designed and accomplished based on a mathematical model and optimization algorithm, and how to assemble CMS using columns, pumps and multiposition valves. We also describe the manner of efficient scheduling chromatographic pumps in CMS to reduce the investment costs of the whole unit.

The separation system under consideration consists of a ternary protein mixture that is subjected to the so-called center-cut separation, where the target compound is intermediately eluted between weakly and strongly adsorbed impurities. The mixtures contain lysozyme, (intermediately adsorbing compound), which is a target product of the separation, cytochrome C (weakly adsorbing compound) and immunoglobulin G (the most strongly adsorbing compound), which are product contaminants. The separation is performed on hydrophobic interaction chromatography (HIC) columns. The system works under nonlinear isotherm conditions in regards to the target component.

2. Theory

2.1. Concept of the CMS process

The concept of CMS has been described in detail in our previous study [21]. For the sake of brevity, here we quote only main principles of the process. CMS consists of parallel columns, which are supplied with streams of feed and eluents with a stepwise changed composition, to realize all stages of the process: loading, wash, separation, regeneration and re-equilibration. The continuity of feed delivery is ensured by shifting the operating cycles of subsequent columns in time, so that in each time interval the same number of columns could be loaded with the feed mixture. Fig. 1 illustrates a four-column setup, in which two columns are in loading position in the same time interval, whereas two remaining ones perform separation, regeneration and re-equilibration. If the operating periods of pumps are properly scheduled, the same pump can be re-used in the same operating cycle to accomplish different functions, *e.g.*, pump 3, in Fig. 1 can serve twice to perform two stages: wash and regeneration.

The operating cycle of a single column, t_c, is a sum of durations of all process stages [21]:

$$t_c = t_l + t_{wash} + t_{sep} + t_{reg} + t_{re\text{-}eq} = t_l + t_e \quad (1)$$

where t_l, denotes duration of feed loading, which is a termed as the productive period; t_{wash}, t_{sep}, t_{reg}, $t_{re\text{-}eq}$ denote duration of four remaining stages of wash, separation, regeneration and re-equilibration, which make up the nonproductive period, t_e, where eluents are supplied into the systems.

The operating cycle of columns is divided into time units. The number of the productive time units, N_p, is calculated as follows:

$$N_p = \mathrm{INT}\left(\frac{t_l}{t_c} N_{col}\right) \quad (2)$$

where INT is an integer function rounding down; N_{col} denotes the number of columns in CMS.

The number of productive and nonproductive units is equal to the number of columns in CMS. Hence, the number of the nonproductive units is calculated as:

$$N_{np} = N_{col} - N_p \quad (3)$$

The length of the time unit is defined as follows:

$$t_u = \frac{t_l}{N_p} \quad (4)$$

The durations of the productive and nonproductive periods may be different, therefore, to guarantee the continuity of feed delivery some of the columns have to wait for the use in work stoppages.