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CHROM^2—A method to enhance the dynamic binding capacity, yield and productivity of a chromatographic column

Mayuratheepan Puthirasigamany*, Peter van Beijeren, Peter Kreis

TU Dortmund University, Emil-Figge-Str. 70, 44227 Dortmund, Germany

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ABSTRACT

Therapeutic proteins are biotechnological products with a fast-growing market. Despite the rapid development of available process technologies, a bottleneck in production capacities is still present due to limitations in the associated downstream process, particularly within chromatographic purification steps. Membrane chromatography has been introduced as a promising alternative for conventional chromatography because it allows for higher throughputs but it does not deliver comparable dynamic binding capacities. To combine the strengths of the two technologies, the so-called "CHROM^2 concepts" are introduced, which merge conventional chromatography with membrane adsorption. The serial connection of a large conventional chromatographic column followed by a small membrane chromatography unit enables to combine the strength of both the individual technologies. The larger column delivers the required high binding capacity, whereas the rapid binding kinetics of membrane chromatography sharpens the breakthrough curve. Furthermore applied higher velocities do not result in poor breakthrough performance since the membrane chromatography is able to compensate for the poor column breakthrough performance. In comparison to column chromatography, the CHROM^2 setup exploits the full column capacity and delivers higher productivities and yields.

1. Introduction

Improving the cell culture productivity has been the main focus over the last few decades in the biomanufacturing industry, with the basic aim to avoid deficiencies in manufacturing capacity due to the increasingly high demand for therapeutic proteins. Improvements in the upstream process have been successfully accomplished by advances in molecular biology that enhance cell line productivity by means of process control, bioreactor design and optimised media [1]. For example, the titre of monoclonal antibodies in cell culture has experienced a 100-fold increase in production from milligrams per litre in the mid-1980s to grams per litre currently [2,3]. Despite satisfying the expanding marketplace for biotechnologically produced therapeutic proteins, the increased titre also has an impact on the cost of goods sold [1]. While innovations in upstream processes have been effectively adopted, the technological advances in downstream processing have failed to keep up with the upstream process development. The deficits in downstream process development are seen as major reasons for existing technological and economic bottlenecks [4] to the method of choice in purifying active pharmaceutical ingredients (column chromatography) [5]. Despite the limitations of high expenses, batch operation, low throughput and complex scale-up, column chromatography remains popular due to the advantage of high resolution in just one selective purification step for bioseparations. Several alternatives to chromatographic separations (classified under bulk, field-based and adsorptive separation techniques) have been reported, which aim to increase the throughput and overcome the aforementioned drawbacks of column chromatography [6].

Among the bulk separation processes (which are characterised by phase changes), precipitation and crystallisation have been utilised in several industrial applications. While only crystallisation exhibits the resolution potential of column chromatography, there are difficulties at larger scales of operation [7,8]. Another bulk separation process that is suitable for high throughput is aqueous two-phase extraction, which has been utilised in selected industrial applications, even though the resolution is not comparable with that of column chromatography. A broad industrial application of aqueous two-phase extraction has been hindered due to the high complexity and number of factors that often govern protein partitioning [9–11].

Continuous chromatographic separations that interconnect several conventional columns, such as the simulated moving bed and the multicolumn counter-current solvent gradient purification

* Corresponding author. Tel.: +49 2317553034; fax: +49 2317553035.
E-mail address: puthirasigamany@bci.tu-dortmund.de (M. Puthirasigamany).

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(MCSGP) [12] are promising alternatives to batch chromatography to enhance separation efficiency, yield and productivity, but these methods presuppose the same performance for all columns.

Adsorptive separations based on sorbent–sorbate interaction involving monoliths and membrane chromatography exhibit a potential to deliver the desired resolution that is comparable with that of the current industry standard (column chromatography). Monoliths are easy to produce in lab-scale but the drawback is the difficulty of producing big monoliths for large scale production.

The more promising separation technology among the adsorptive separation processes is membrane chromatography [13,14]. Basically, the membrane chromatography bed is identical to normal flow filtration membranes in that the membrane pore surface is functionalised with a ligand. This functionalisation gives this unit operation the capability to bind the product of interest from a process solution that is pumped through the membrane [9]. The immobilised ligands, which allow for the selective interaction of target molecules with the membrane, are the same ligands utilised in column chromatography, thereby allowing the operation of both technologies in similar ways using similar equipment. Because of the high porosity, large cross sectional area and minimal membrane thickness, the membrane chromatography process can be operated at low pressure drops. The open pore structure of the membrane predisposes convective solute transport to the binding sites within the pores, which eliminates the rate-limiting pore diffusion associated with column chromatography and leads to the reduction of both process time and recovery liquid volume. Furthermore, high flow rates can be applied because the binding efficiency is independent from the feed flow rate, which enhances the productivity of the overall process. The advantages of membrane chromatography over column chromatography have been demonstrated in previous publications, especially for the purification of large biomolecules [10,14]. Membrane chromatography has therefore been utilised in various applications, not only for the removal of large biomolecules in flow-through mode for polishing purposes (where the process solution is passed through the bed to retain one or more of the contaminants in the bed) but also for capturing molecules in bind and elute mode [15]. In summary, the main drawbacks of column chromatography (such as the mass transfer limitations, the high pressure drop within the column, the low capacity and the time consumption) can therefore be resolved by applying membrane chromatography [7,16,17]. In fact, coupling the functionality of a chromatography resin with the hydrodynamic characteristics of normal flow filtration helps to overcome these drawbacks of column chromatography. Membrane chromatography modules are easy to scale up and inexpensive to mass produce, which enables them to be used as disposables, thereby eliminating the requirement for cleaning and equipment revalidation [18].

To conclude, membrane chromatography allows for larger volumetric throughputs than column chromatography, but it has a significantly lower binding capacity for smaller proteins. Therefore, the development of separation concepts employing membrane chromatography is required to exploit the benefits of membrane chromatography and overcome the bottlenecks in downstream processing. Recently, a novel concept referred to as "CHROM^2" has been patented in cooperation with the laboratory of fluid separations from TU Dortmund and Sartorius Stedim-Biotech GmbH [19]. CHROM^2 has the potential to increase the performance of existing conventional chromatographic columns; thus, it presents a solution for debottlenecking existing downstream processes. Furthermore, it extends the application for membrane chromatography to polishing, capturing dilute products from process solutions and capturing at high product concentrations.

2. CHROM^2—principles and concepts

An ideal unit operation for protein adsorption should combine the high binding capacities of column chromatography with the rapid binding kinetics of membrane chromatography. At first glance, the combination of both technologies into a single unit operation appears impossible due to the large differences in operating windows. However, the operating windows overlap for large chromatographic columns and small membrane chromatography units. The serial connection of a large conventional chromatographic column followed by a small membrane chromatography unit creates a novel unit operation in which the strength of both individual technologies is merged to modernise bioseparations. The larger column delivers the required high binding capacity, whereas the rapid binding kinetics of membrane chromatography sharpens the breakthrough curve. Furthermore, the utilisation of the unexploited capacity of the column is enabled, which allows for enhancing the efficiency of chromatography-based separation processes. To exploit the maximum potential of this unit operation, three CHROM^2 concepts have been developed that aim to improve the three main process characteristics of a chromatography process. These are CHROM^{2}CAP, which aims to increase the binding capacity of the column; CHROM^{2}SPEED, which aims to increase the column productivity; and CHROM^{2}YIELD, which aims to increase the column yield. Generally, these three effects are always present simultaneously, and the dominant effect depends on the stationary phase properties, column dimensions, membrane dimensions, operating conditions and target molecules.

The relevant mechanism underlying these concepts is shown in Fig. 1. It shows the migration of the concentration front along the packed bed at various time events. Fig. 1a depicts a longitudinal cut of an equilibrated column that is ready to be loaded, where the dark and grey dots represent the unsaturated and saturated beads, respectively. At this time, the resins are unsaturated and can be loaded with the product molecule. After equilibration, the next step is loading, and the process solution containing the molecule of interest is pumped through the bed. During loading, the resins become saturated, such that the concentration front moves along the packed bed towards the end of the column (Fig. 1b). Due to internal mass transfer limitations and dispersion, not all beads are saturated equally; thus, the adsorption front is flattened. To avoid product loss, the loading step in industrial processes is usually performed until 10% breakthrough is attained, while not exceeding an outlet concentration of 10% of the inlet concentration. In addition, during this time, many resins are unloaded, and the majority of the column capacity is not utilised (Fig. 1c) if the loading step is interrupted. The shallow breakthrough curve is also noteworthy during this time.

Applying a small membrane chromatography unit after the column results in a steeper breakthrough curve, which helps to use the full column capacity and improve the yield and process productivity (Fig. 1d). Because the breakthrough performance of membrane chromatography is independent from the flow rate, one may apply higher flow rates, and the reduced column performance at the high flow rate can be compensated by the membrane chromatography module to a certain degree. Furthermore, due to the additional capacity obtained through the membrane chromatography module, one may extend the loading interval and therefore utilise more of the existing column capacity (Fig. 1e), which improves the overall process capacity. In Sequential Multicolumn Chromatography (SMCC) a complete utilisation of the column capacity is also achieved but with CHROM^2 large columns instead of smaller ones can be used. Furthermore with CHROM^2 increasing the productivity by increasing the flow rate is possible since the poor column performance at higher flow rates can be compensated by the small membrane module.

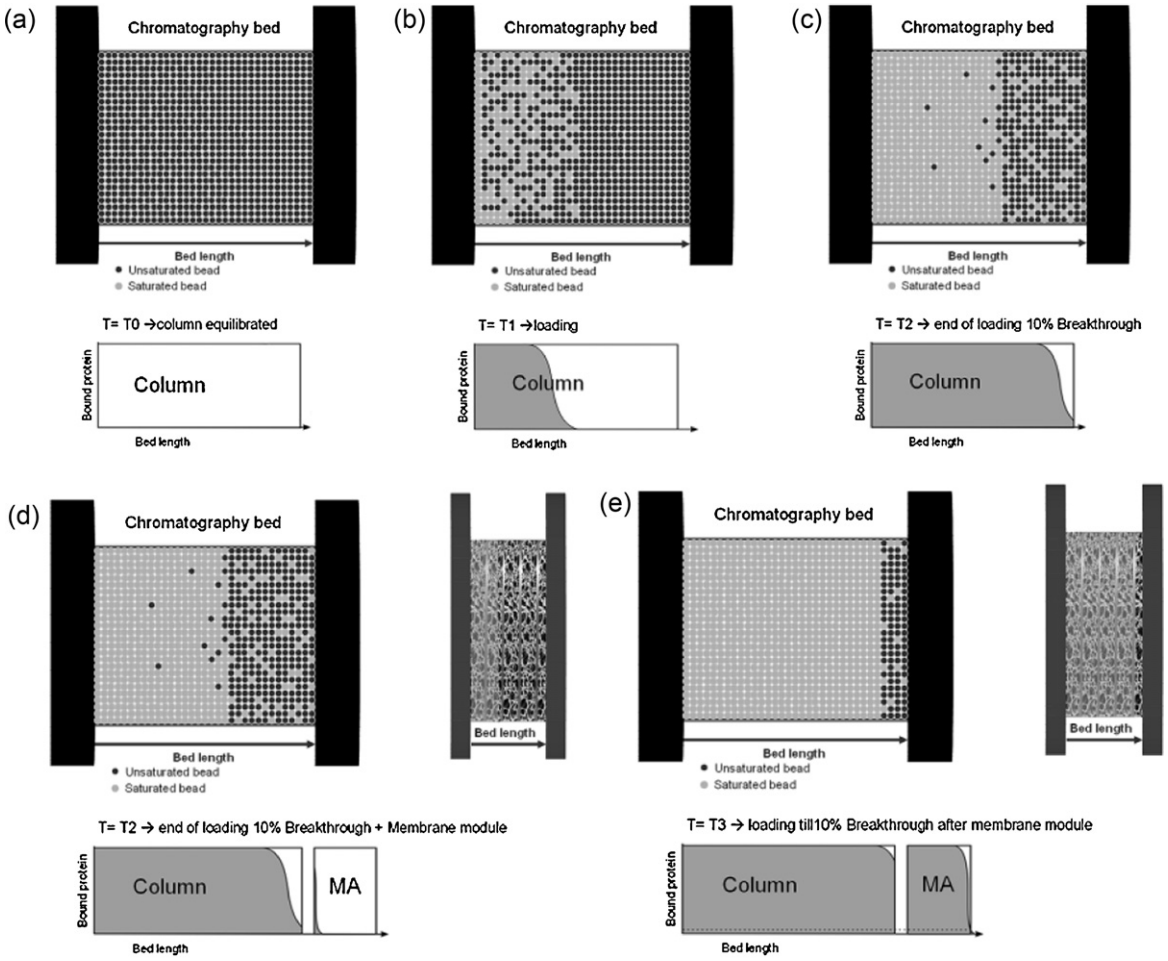

Fig. 1. Schematic representation of CHROM^2—principle.

3. Experimental validation of CHROM^2

The concept of CHROM^2 was investigated experimentally, using commercially available stationary phases, to illustrate the potential of increasing the performance of chromatographic columns.

3.1. Materials and methods

The adsorption of bovine serum albumin on strong anion exchangers was selected as a test system for CHROM^2. The chromatographic medium Q Sepharose FF was obtained from GE Healthcare in the pre-packed HiTrap format. The inner diameter of the column is 1.6 cm and the length is 2.5 cm, yielding a frontal area of 2.0 cm^2 and a column volume of 5 ml. The HiTrap column can withstand a maximum pressure of 3 bars. The CHROM^2 concept was experimentally investigated by connecting the Q Sepharose FF HiTrap column to either a Sartobind Q Nano 1 ml module or a Sartobind Q Nano 3 ml module in series. The module characteristics can be found in Table 1.

Table 1
Characteristic of the membrane modules.

Technical data	Nano 1 ml	Nano 3 ml
Bed volume (ml)	1	3
Bed height (mm)	4	12
Maximum pressure (bar)	4	4
Surface area (cm^2)	36.4	110

For the process steps involved in an ion exchange separation (equilibration, loading and washing), a 10 mM potassium phosphate buffer with a conductivity of 2 mS/cm and a pH of 7 was used. Elution was performed with a 10 mM potassium phosphate buffer solution containing 2 M sodium chloride. Flow rates in the experiments varied from 62.5 cm/h to 600 cm/h, and a BSA feed concentration of approximately 2 mg/ml was applied. Fractions of the elution peaks and of the feed solution were collected, and the BSA concentration was determined using an external UV spectrophotometer at a wavelength of 280 nm. Additionally, non-binding tracer experiments were performed with 0.5 vol.% acetone to determine the dead volumes of the experimental setup and modules. Furthermore, experiments were performed to explore the maximum applicable flow rate that did not violate the pressure drop limitations. All experiments were performed on the ÄKTA Purifier 100 liquid chromatography system (GE Healthcare, Uppsala, Sweden).

3.2. Experimental results and discussion

3.2.1. System dispersion

The system dispersion curves for the ÄKTA Purifier 100 (including the membrane chromatography modules), the HiTrap column and the membrane chromatography module with the HiTrap column were determined at different flow rates, using 0.5% acetone as the tracer in a buffer solution. The corresponding dead volumes are reported in Table 2.

Table 2
Dead volumes for the different configurations.

Configuration	Dead volume (ml)
ÄKTA + HiTrap	4.84
ÄKTA + Nano 1 ml	5.00
ÄKTA + Nano 3 ml	3.99
ÄKTA + CHROM^2 1 ml Nano	9.42
ÄKTA + CHROM^2 3 ml Nano	8.30

It was found that the dead volumes for the different setups were independent from the applied flow rate; therefore, for all flowing calculations, the dead volumes reported in Table 2 were used.

3.2.2. Pressure drops

One of the methods to increase the productivity is to increase the flow rate. Therefore, to determine the maximum applicable flow rate for the CHROM^2 setups, the pressure drop for the HiTrap column and the membrane chromatography modules was measured at different flow rates, as depicted in Fig. 2. The pressure drops were measured for the sample application pumps as well as for the system pumps that were used during the washing and eluting steps.

The pressure drop limitation on the resin bed results in a maximum applicable flow rate of 600 cm/h for the column, but because the serial connection of the column with the membrane chromatography module exhibits a higher resistance, the maximum applied flow rate was reduced to 450 cm/h.

3.2.3. Dynamic breakthrough experiments

Breakthrough curves were measured for the HiTrap column, Sartobind Q Nano 1 ml membrane chromatography module and Sartobind Q Nano 3 ml membrane chromatography module at flow rates ranging from 62.5 cm/h to 600 cm/h. Fig. 3 shows the breakthrough curves for Sartobind Q Nano 1 ml, and the dynamic binding capacities at different flow rates are depicted in Fig. 4.

As expected, no significant influence of the flow rate on the BSA breakthrough curve for Sartobind Q was observed. The total amount of protein bound at 10% breakthrough, $M_{ads,10\%}$, at all applied flow rates was calculated using Eqs. (1) and (2). This calculation resulted in a value of approximately 32 mg, which yielded a dynamic binding capacity of 32 mg/ml (Eq. (3)). $M_{ads,10\%}$ (Eq. (1)) was less than the mass loaded to the unit at 10% ($M_{BTC,10\%}$); the difference was the amount of protein present in the dead volume of the system, which was calculated by multiplying the loading protein concentration (c_{Load}) by the dead volume of the system (V_{dead}).

$$M_{ads,10\%} = M_{BTC,10\%} - c_{load} \cdot V_{dead} \quad (1)$$

The loaded amount of protein, $M_{BTC,10\%}$, was calculated using Eq. (2), which represents the area above the breakthrough curves.

$$M_{BTC,10\%} = c_{load} \cdot V_{BTC,10\%} - \int_0^{V_{BTC\,10\%}} c_{out}(V)dV \quad (2)$$

The dynamic binding capacity at 10% breakthrough ($DBC_{10\%}$) is defined by the ratio of the bound protein at 10% breakthrough to the bed volume ($V_{adsorbents}$) (Eq. (4)).

$$DBC_{10\%} = \frac{M_{ads,10\%}}{V_{adsorbents}} \quad (3)$$

The breakthrough curves of Sartobind Q Nano 3 ml exhibited nearly the same behaviour as that of Sartobind Q Nano 1 ml but

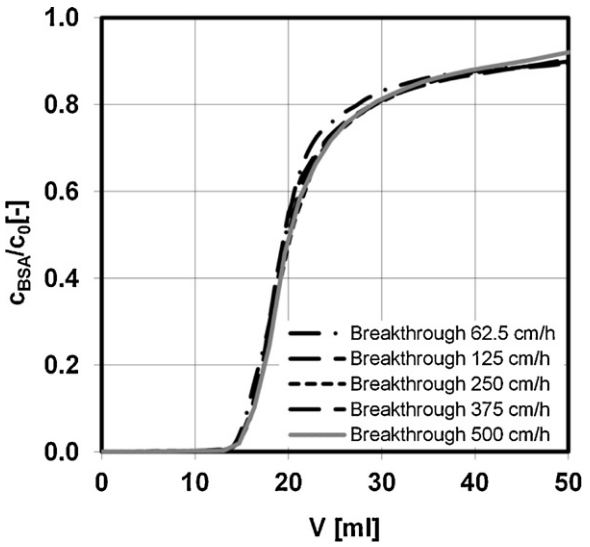

Fig. 3. Breakthrough curves Q Nano 1 ml.

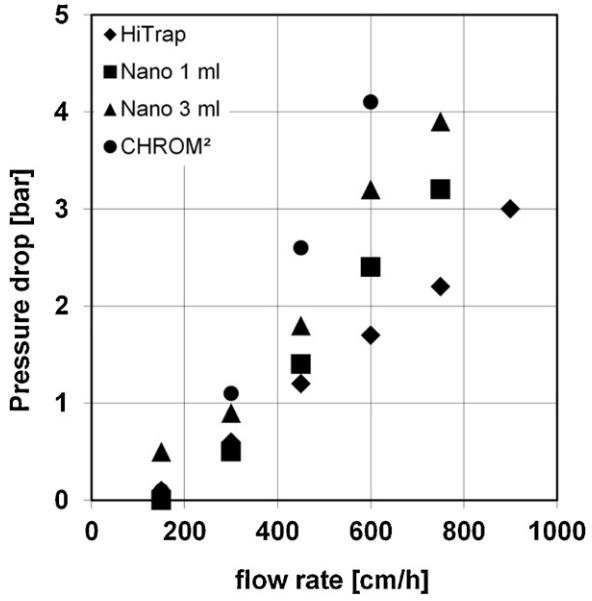

Fig. 2. Pressure drop sample pump.

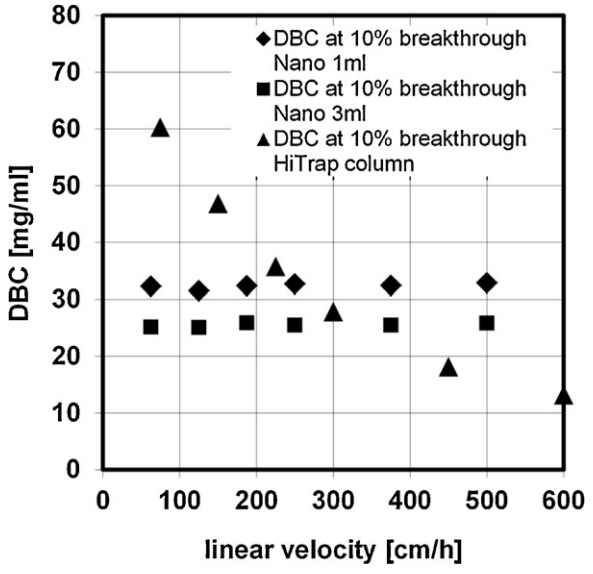

Fig. 4. Dynamic binding capacities (DBC) Q Nano 1 ml, Q Nano 3 ml and HiTrap column.

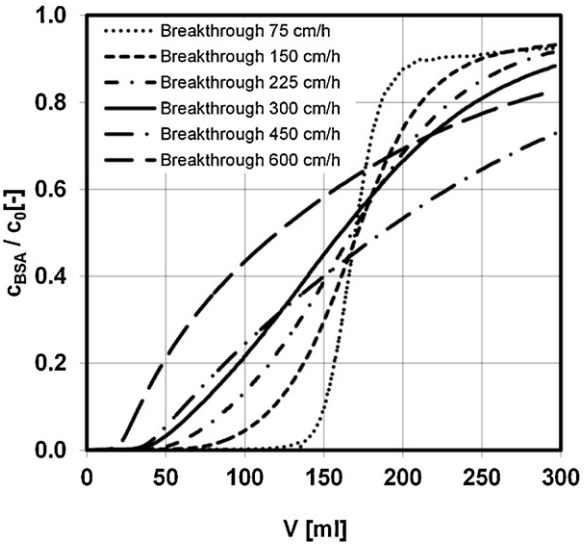

Fig. 5. Breakthrough curves for HiTrap column at different flow rate.

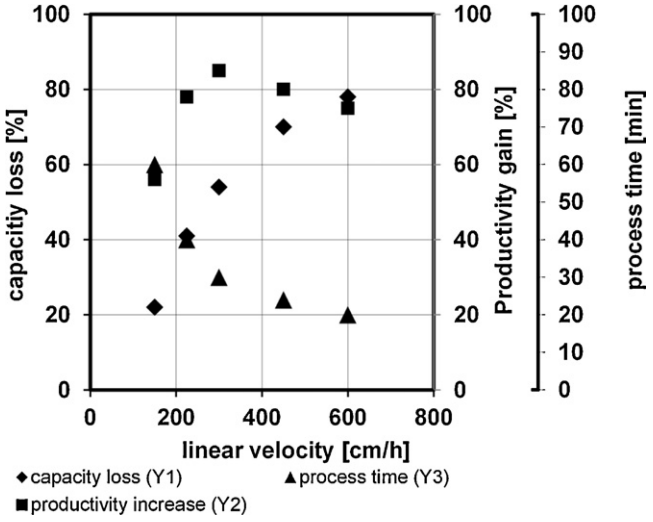

Fig. 6. Capacity loss and productivity increase in relation to linear velocity.

were delayed due to the higher binding capacities. Due to the larger bed volume, the breakthrough was delayed and all breakthrough curves exhibited the same trend. Nevertheless, the total dynamic binding capacity at 10% breakthrough was unaffected by this behaviour. A total amount of approximately 78 mg of protein was bound on the 3 ml module, which yielded a dynamic binding capacity of 26 mg/ml (Fig. 4).

The measured capacities at 10% breakthrough for the HiTrap column are reported in Fig. 4 and the observed breakthrough curves of BSA from the experiments performed at different velocities are depicted in Fig. 5. The breakthrough performance degraded with increasing flow rates, which was due to the rate-limiting pore diffusion of column chromatography. At 600 cm/h, the maximum allowable pressure drop for the HiTrap column was reached; therefore, experiments with higher flow rates were not conducted. As a consequence of the bad breakthrough performance, a significant loss of the dynamic binding capacity (DBC) was observed at increased flow rates. The pressure drop during elution was considerably higher than during loading and washing due to the high viscosity of the concentrated protein solution. To ensure that the pressure drop limitation would not be exceeded, the flow rate was reduced to 75 cm/h during elution.

The loss in the column dynamic binding capacity when the flow rate was increased eight-fold from 75 cm/h to 600 cm/h in order to obtain higher productivity was up to 80%. An increased flow rate should result in higher productivity, but this was not the case for column chromatography. The productivity (P) of the loading phase was calculated as the ratio of the amount of adsorbed protein to the loading time (t_{load}) and the volume of the adsorbents ($V_{adsorbents}$) (Eq. (4)).

$$P = \frac{\Delta M_{ads,10\%}}{V_{adsorbents} \cdot t_{load}} \quad (4)$$

The time required to reach 10% breakthrough is referred to as the loading time and is calculated from the ratio of loaded volume ($V_{load,10\%}$) and loading velocity (F_{load}) following equation:

$$t_{load} = \frac{V_{load,10\%}}{F_{load}} \quad (5)$$

As seen in Fig. 6, an eight-fold increase in flow rate for the HiTrap column did not result in a 800% productivity enhancement; only a productivity increase of 75% was achieved. By increasing the flow rate by a factor of four, a maximum productivity increase of approximately 86% was achieved. Due to the aforementioned poor performance of the column at higher flow rates and the resulting loss in the binding capacity, the productivity cannot be increased economically just by applying a higher volumetric throughput.

3.2.4. CHROM²CAP

The purpose of CHROM²CAP is to enhance the dynamic binding capacity of an existing chromatographic column at its optimal operating point. The HiTrap column and the membrane chromatography modules were subsequently connected in a serial configuration and were operated at 75 cm/h with an inlet BSA concentration of 2 mg/ml. At this flow rate, the column was optimally operated, and therefore, the maximum benefit of applying CHROM² can be stressed accordingly. The principle of the process concept is illustrated in Fig. 7. The black line indicates the breakthrough curve of the column, and the grey line indicates the breakthrough curve of the CHROM² setup. It is assumed that breakthrough is continued until the outlet concentration reaches 10% of the inlet concentration. At 10% breakthrough, the used column capacity is equal to the area A1. Upon the addition of the membrane chromatography unit, not only was the breakthrough delayed, but the breakthrough curve was also sharpened. The membrane capacity is graphically

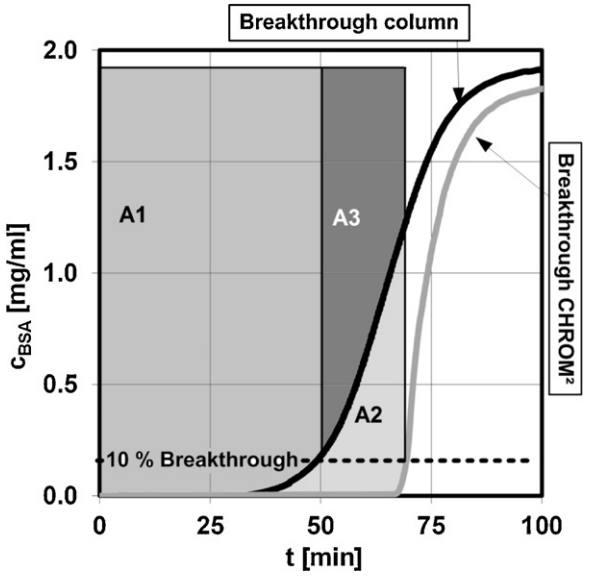

Fig. 7. Principles of CHROM²CAP.

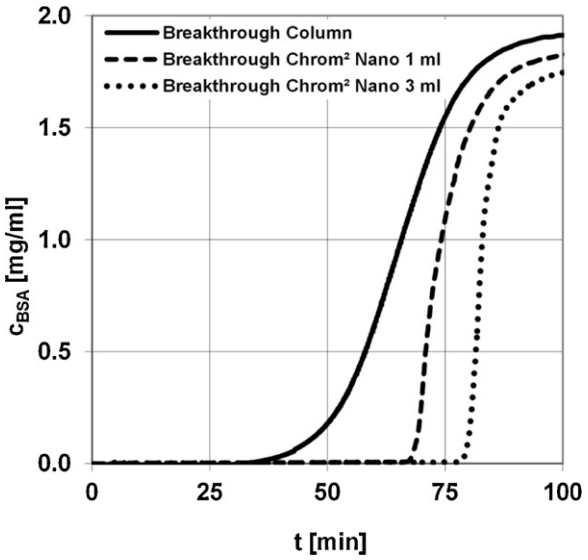

Fig. 8. Breakthrough curves of HiTrap, CHROM² 1 ml Nano and CHROM² 3 ml Nano at a flow rate of 75 cm/h.

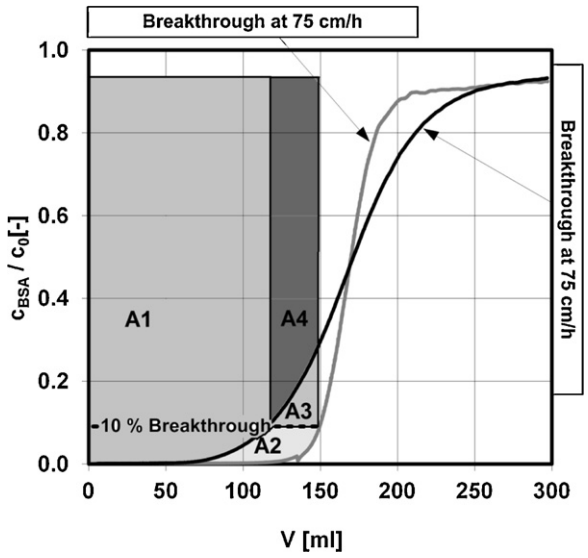

Fig. 9. Principles of CHROM²SPEED.

represented by the area A2. The used column capacity for the serial connection is the sum of A1 and A3. Due to the addition of the membrane module, the used column capacity was therefore increased with A3.

The observed breakthrough curves for the HiTrap column, CHROM² with 1 ml Nano and CHROM² with 3 ml Nano at 75 cm/h are compared in Fig. 8. Upon the addition of the membrane chromatography module, not only was the breakthrough delayed, but the breakthrough curve was also sharpened, which provided higher capacities. All the calculations for determining the capacity were performed till the outlet concentration reached 10% of the inlet concentration.

The increase in the amount of adsorbed BSA ($\Delta M_{ads,10\%}$) due to the addition of the membrane chromatography at constant flow rate was calculated by Eq. (6) as the amount of adsorbed protein in CHROM² setup ($M_{ads,CHROM^2,10\%}$) subtracted by the amount of adsorbed protein in the column ($M_{ads,HiTrap,10\%}$).

$$\Delta M_{ads,10\%} = M_{ads,CHROM^2,10\%} - M_{ads,HiTrap,10\%} \quad (6)$$

The total column capacity before adding the membrane chromatography was 301 mg. In the Chrom² 1 ml Nano setup, after the addition of a membrane capacity of 32 mg (which represented only 10% of the used column capacity), a considerable boost in the used column capacity was observed (to 26%). Further enhancing the membrane capacity (25% of the column capacity, CHROM² 3 ml Nano) also led to an increase in the used column capacity to 31%, but the relative improvement compared to the higher capacity with CHROM² 1 ml Nano was lower due to the limited overall column capacity. A column capacity increase of only 3% was obtained by adding 250% in membrane capacity, which indicated that a very small membrane chromatography module is sufficient to enhance the column capacity.

The increase in the used column capacity can be calculated as follows:

$$E = \frac{\Delta M_{ads,10\%} - M_{ads,10\%,Mem}}{M_{ads,HiTrap,10\%}} \times 100\% \quad (7)$$

The large increase in the used column capacity in the case of CHROM²CAP was caused by the sharp breakthrough curve of the membrane chromatography module. Such a large increase could not have been realised by increasing the column length because, in this case, the breakthrough curves would remain parallel, and the increase in the used column capacity would be nearly proportional with the column length. The overall gain in binding capacity for CHROM²CAP exceeded the added membrane capacity by 400% for CHROM² 1 ml Nano and by 218% for CHROM² 3 ml Nano.

The membrane chromatography efficiency (E_{Mem}) was calculated using equation four, which is the ratio of increase in the amount of BSA upon the addition of the membrane chromatography unit to the amount of bound BSA on the membrane chromatography unit.

$$E_{Mem} = \frac{\Delta M_{ads,10\%}}{M_{ads,mem,10\%}} \times 100\% \quad (8)$$

The potential of CHROM²CAP to increase the column capacity depends strongly on the sharpness of the breakthrough curve of the column and the maximum percentage of breakthrough. Tables 3 and 4 summarise the impact of CHROM² on the binding capacity of the column at different linear velocities and at 5% and 10% breakthroughs, respectively.

From the experiments performed, it is obvious that CHROM² processes operated at high linear velocities and low breakthrough percentages offered the maximum benefit for the CHROM²CAP concept.

3.2.5. CHROM²SPEED

The mass transfer limitations in column chromatography inhibit a productivity enhancement by simply applying higher volumetric throughputs. With the process concept CHROM²SPEED, the productivity of an existing column can be enhanced by increasing the flow rate because the poor performance of the column at higher flow rates and the resulting loss in the binding capacity can be compensated to a certain extent by the membrane chromatography module. As shown in Fig. 5, the column performance (and therefore, the dynamic binding capacity of the column) decreased significantly when the flow rate was increased.

Fig. 9 shows the breakthrough curves of the column operated at linear velocities of 75 (grey line) and 150 cm/h (black line). The used column capacities at 10% breakthrough for the linear velocity of 150 cm/h are represented by the area A1; for 75 cm/h, the used column capacity can be determined from the sum of areas A1 to A4. At 150 cm/h, the used column capacity was 94 mg less than that corresponding to 75 cm/h, but the productivity was approximately twice as high. The required membrane capacity to compensate the loss in the used column capacity can be estimated from the sum

Table 3
Overview of DBC at 5% breakthrough.

Linear velocity (cm/h)	150	225	300	375	450
DBC 5% column (mg/ml)	22.5	16.1	12.3	10.1	8.7
DBC 5% CHROM² 3 ml Nano (mg/ml)	55.6	48.7	42.9	38.1	34.7
Increase in used column capacity (%)	147	202	248	277	298

Table 4
Overview of DBC at 10% breakthrough.

Linear velocity (cm/h]	150	225	300	375	450
DBC 10% column (mg/ml)	28.6	20.4	15.6	12.7	10.8
DBC 10% CHROM² 3 ml Nano (mg/ml)	56.1	49.4	43.8	38.9	35.5
Increase in used column capacity (%)	96	142	180	206	228

of areas A2 and A3. Fig. 10 shows the potential of CHROM²SPEED to sharpen the breakthrough curves obtained from the chromatographic columns. The resulting unit operation is able to deliver high dynamic binding capacities at increased linear velocities.

The column and the CHROM² setup were operated at a velocity of 150 cm/h. The results were compared with a column operated at 75 cm/h (Fig. 11). The addition of a 1 ml membrane chromatography module allowed the volumetric throughput of the column to double without any significant loss in the overall binding capacity. Having a slightly larger module allowed for an increase in the used column capacity by simultaneously doubling the column productivity.

Even higher productivity was achieved by applying higher throughputs (Fig. 12). All experiments were performed with the CHROM² 3 ml Nano setup. As depicted in Fig. 12, the membrane chromatography module (which possessed 25% of the column capacity) was able to compensate for the loss in dynamic binding capacity of the column till the flow rate was four times higher than the initial flow rate. Increasing the flow rate further resulted in higher productivity, but it reduced the column efficiency because the membrane capacity was not sufficient to counteract the poor column performance.

The potential of CHROM²SPEED mainly depends on the impact of flow rate on the sharpness of the breakthrough curves of the chromatographic columns. Therefore, it is most favourable for columns operated at low linear velocities and low breakthrough percentages, which yields sharp breakthrough curves and high dynamic binding capacities.

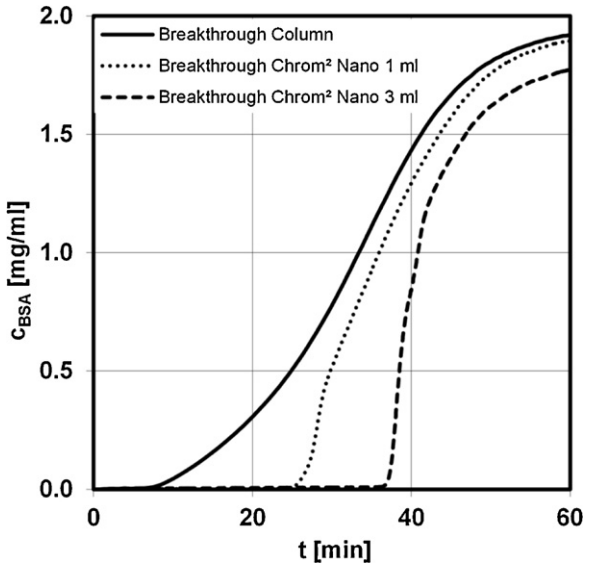

Fig. 10. Breakthrough curves of HiTrap, CHROM² 1 ml Nano and CHROM² 3 ml Nano at a flow rate of 150 cm/h.

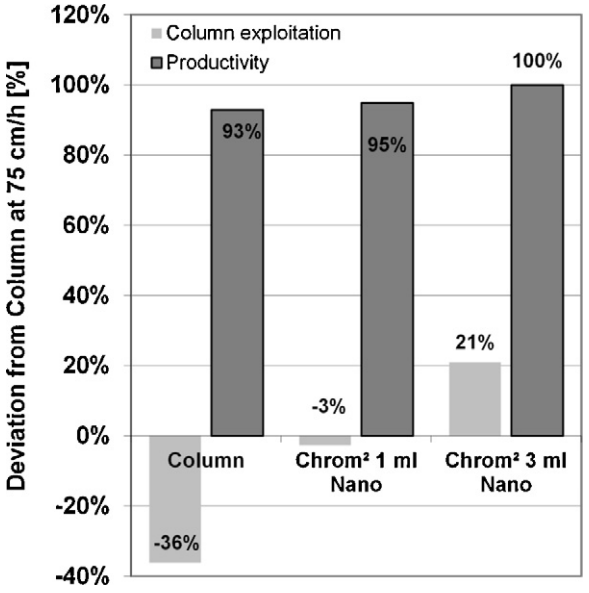

Fig. 11. Capacity gain and productivity for the column in different CHROM² setups compared to the column operated at 75 cm/h.

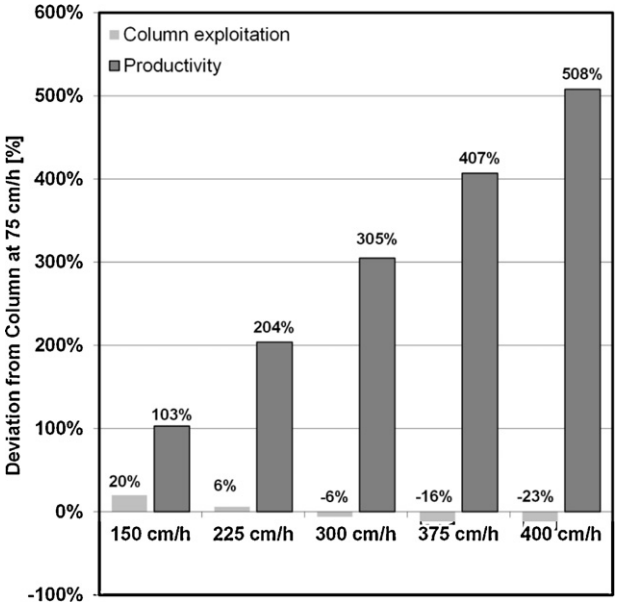

Fig. 12. Performance of CHROM² 3 ml Nano at different flow rates.

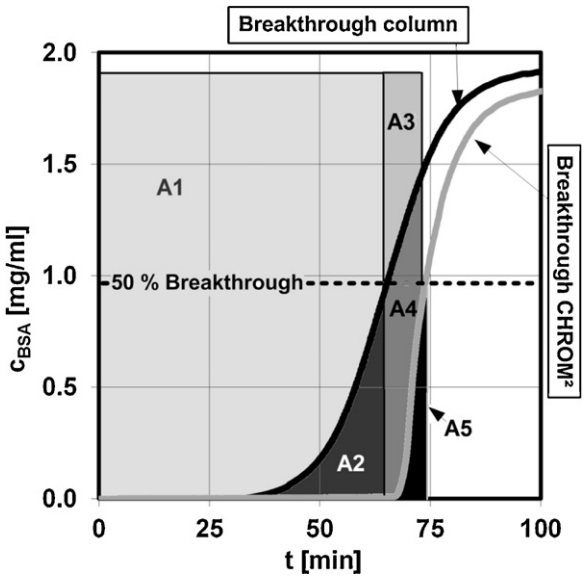

Fig. 13. Principles of CHROM²YIELD.

3.2.6. CHROM²YIELD

The last process concept is CHROM²YIELD, which, in contrast to CHROM²CAP and CHROM²SPEED, is most favourable for columns operated at high breakthrough percentages. Fig. 13 reveals the potential of CHROM²YIELD to reduce the number of cycles, which results in a faster processing time for a batch and the potential to increase the binding yield during protein loading.

The advantage of operating at a high breakthrough percentage is that a smaller number of cycles are required to purify a certain amount of product and that the dynamic binding capacity is higher than that associated with operations at low breakthrough percentages. The main disadvantage is a low binding yield during protein loading. By comparing the breakthrough curves of the chromatographic column with that of the CHROM² setup, it is obvious that the protein loss during loading at 50% breakthrough is equal to A2 for the column and A5 for CHROM². Due to the sharper breakthrough performance of the CHROM² setup, the protein loss is much smaller. The benefit of CHROM²YIELD was demonstrated at a flow rate of 75 cm/h, at which the column delivered the optimal performance. Experiments performed on the HiTrap column and the CHROM² 3 ml Nano setup at different breakthrough percentages are compared in Fig. 14. The yield was calculated as follows:

$$Y = \frac{M_{ads}}{c_{feed} \cdot V_{load}} \quad (9)$$

If the column was operated at 50% breakthrough rather than at 10% breakthrough, this resulted in a yield loss of nearly 10%. By adding a membrane chromatography unit, the yield of CHROM² at 50% breakthrough was nearly the same as the column yield at 10% breakthrough. The protein loss during the breakthrough at the higher breakthrough percentages can therefore be reduced effectively upon adding a membrane chromatography unit behind the column.

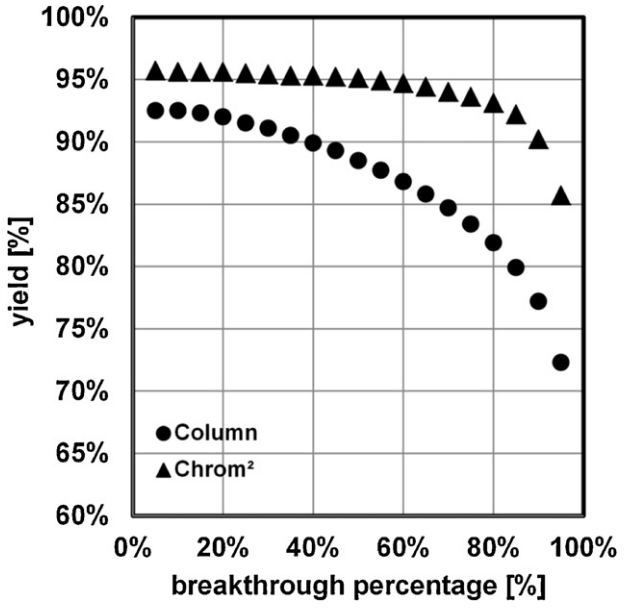

Fig. 14. Yield at different breakthrough percentages for column and CHROM² at 75 cm/h.

4. Conclusion

A novel method for debottlenecking the downstream processing of the biomanufacturing industry has been presented in this work. This novel concept incorporates the benefit of the method of choice for protein purification, column chromatography, with the advantages of membrane chromatography. The possibility of enhancing the performance of conventional column chromatography by a serial connection with membrane chromatography is promising, which eliminates the drawbacks of column chromatography and extends the application area for membrane chromatography. Three process concepts for CHROM² (CHROM²CAP, which aims to increase the dynamic binding capacity; CHROM²SPEED, which aims to deliver higher productivity; and CHROM²YIELD, which aims to increase the binding yield) were evaluated. It was found that the addition of a membrane chromatography unit behind the conventional chromatographic column can be used to enhance the used column capacity, column productivity and the binding yield of the column. A capacity increase of up to 31%, a productivity increase of up to 300% and breakthrough percentages of up to 50% can be achieved with CHROM² without significant losses in the initial column performance. Generally, these three effects are always present simultaneously, and the dominant effect depends on the properties of the stationary phase properties, the column dimensions and the operating conditions.

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