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Immobilized metal affinity chromatography in open-loop simulated moving bed technology: Purification of a heat stable histidine tagged β-glucosidase

Deepti Sahoo, Jonatan Andersson, Bo Mattiasson*

Department of Biotechnology, Lund University, P.O. Box 124, SE-22100 Lund, Sweden

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ABSTRACT

Open-loop simulated moving bed (SMB) has been used for immobilized metal affinity chromatographic (IMAC) purification of his-tagged β-glucosidase expressed in *E. coli*. A simplified approach based on an optimized single column protocol is used to design the open-loop SMB. A set of columns in the SMB represent one step in the chromatographic cycle i.e. there will be one set each of columns for load, wash, elution etc within the SMB. Only the wash and elution are operated with columns in sequence. The β-glucosidase was purified to almost single band purity with a purification factor of 15 and a recovery of 91%. SMB-performance showed reduced buffer consumption, higher purification fold, a better yield and higher productivity.

1. Introduction

The term 'affinity' plays a very important role in life sciences and beyond it. The affinity of an enzyme to its substrate, antigen to a corresponding antibody are two examples of affinity interactions. Many more such examples display the importance of affinity interactions in the biological world. Major applications of affinity interactions are widely used in the field of bioseparation especially separation of proteins. Affinity based separations are combining affinity interactions with traditional separation techniques namely precipitation, membrane filtration, two phases/three phases extraction [1] and chromatography. Immobilized metal affinity chromatography (IMAC), a form of affinity chromatography, introduced by Porath et al. [2] is an effective method for isolating proteins supplied with an extra peptide tag which can bind to the chelated metal ions. The advent of recombinant DNA technology has facilitated the introduction of affinity tags, thereby simplifying the purification of such tagged target proteins. Affinity membranes and aqueous two phase extractions are other alternatives to chromatography which are gaining selective industrial attention but it is the packed bed chromatography which is considered as an industry golden standard [3]. Still it suffers from its own pitfalls especially high cost and a low through-put operation mode. It is for above mentioned reasons used for separation of high price products such as therapeutic proteins where purity is the ultimate goal.

Due to the resolution of IMAC, it can be used for separation of industrial enzymes if the classical problem of low productivity and high buffer consumption are overcome. In order to broaden the area of applications new process technology must be developed to address the above-mentioned problems.

One way to improve separation economy is to use continuous countercurrent chromatographic systems. Simulated moving bed (SMB) technology is an established chromatographic technique based on simulating a countercurrent contact between the solid and the liquid phases. Its benefits, raised productivity and product concentration as well as reduced buffer consumption have been shown in many studies and the concept has been successfully commercialized [4,5].

Use of SMB in protein purification is very rare although some examples have been reported. Most of the described work is based on binary mixtures of model protein as SMB is better suited for separation of binary mixtures than for resolving a complex protein mixture. Proteins have been separated using SMB by ion exchange [6], affinity [7–9] and size exclusion chromatography [10,11].

Still the use of SMB for protein purification is very sparse especially for affinity separations. Affinity separations of proteins using SMB have been done on binary model mixtures with target proteins which show very high affinity or well characterized interactions to the matrix. Gottschlich and Kasche [9] showed purification of monoclonal antibodies against penicillin amidase from cell culture supernatant on a protein A-Sepharose column using non-isocratic condition by introducing two purge zones to increase purity. Gottschlich et al. [7] optimized the four zone SMB system under non-isocratic conditions using the model system of α-chymotrypsin and immobilized soybean trypsin inhibitor. A non-isocratic process was established by determining switch time and flow ratio between zones by using the adsorption equilibrium parameters and mass transfer coefficient.

* Corresponding author. Tel.: +46 46 2228264; fax: +46 46 2224713.
E-mail address: Bo.Mattiasson@biotek.lu.se (B. Mattiasson).

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Gueorguieva and co-workers [8,12] have reported purification of human bone morphogenetic protein-2 (BMP-2) dimer from the monomer forms present in a ratio of 3:1 (monomer: dimer) with 1% of host cell protein as contaminants using heparain columns as a stationary phase. Gueorguieva also used three-zone open-loop step gradient SMB chromatography for purification of model system IgG from lysozyme and purification of BMP.

In a conventional closed-loop SMB for binary separations all the columns are connected in series with two inlets, for the load and eluent streams, and two outlets, for the extract and raffinate steams. Optimization of the process requires modeling in order to determine flow rates and switch time. Different methods for SMB design have been suggested; the triangle theory [13,14], the safety margin method [15,16] or standing wave design [17,18].

In the current work an open-loop non-isocratic SMB technology is used to purify target protein from a crude mixture of proteins from cell fermentation. Each set of columns in the SMB represent a step in the chromatographic cycle; i.e. there will be one set of columns for load, wash, elution and regeneration respectively within the SMB. The columns in wash and elution are coupled sequentially while the loading takes place in columns operated in a parallel configuration. A simplified approach based on an optimized single column protocol is used to design the SMB [19].

The objective with this study was to set-up an efficient SMB purification process based on results from a single column IMAC protocol for the separation of a cloned heat stable His-tagged β-glucosidase from *E. coli*.

2. Materials and methods

2.1. Materials

Sepharose–CL 4B was obtained from GE Healthcare (Uppsala, Sweden). PageRuler Protein ladder came from Fermentas (St. Leon-Rot, Germany). Acrylamide (40%), ammonium persulfate (APS) and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) were obtained from BioRad (Hercules, CA, USA). Allyl glycidyl ether (AGE, 99%), iminodiacetic acid (IDA), 1,4-butanediol diglycidyl ether, BCA (bicinchoninic acid) protein reagent, copper sulphate were purchased from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade.

2.2. Preparation of IDA-Sepharose

Coupling of IDA to Sepharose was carried out as described by Porath and Olin [20]. Oxirane-activated gel (150 ml settled gel) was transferred to a 500 ml shaking flask containing 5.0 g IDA in 150 ml of 1.0 M sodium bicarbonate, pH 10.0. The gel suspension after overnight incubation was filtered and later washed with NaCl and deionized water.

The resulting IDA-Sepharose was packed in glass columns (I.D. 1.1 cm), height of 10 cm. Cu(II) was loaded to the gel by passing 100 ml of 100 mM copper sulphate solution followed by washing with deionized water to remove any unbound copper. Then the gel was washed with imidazole buffer (20 mM imidazole in 20 mM citric–phosphate, pH 7.0 containing 0.2 M NaCl) to remove loosely bound copper. Cu(II)-IDA-sepharose was then equilibrated with 20 ml of equilibration buffer (20 mM citric–phosphate, pH 7.0 containing 0.2 M NaCl) at a flow rate of 10 ml/min.

2.3. His–tagged β-glucosidase

A gene construct containing β-glucosidase from the thermophilic bacteria *Thermotoga neapolitana* was cloned into plasmid pET22b(+), which is under the control of T7/*lac*- promotor and was transformed into *E. coli* strain BL21(DE3) which was grown in a 3 L fermentor. To start the flask cultivation, 1.0 ml frozen inoculum of recombinant *E. coli* was added to a 1 L baffled shake flask containing 100 ml of NYAT salt solution (a defined medium containing 2 g/L $(NH_4)_2SO_4$, 14.6 g/L K_2HPO_4, 3.2 g/L $NaH_2PO_4{\cdot}H_2O$, 0.5 g/L $(NH_4)_2{\cdot}H{\cdot}$citrate), 10 g/L glucose, 0.2 ml 1 M $MgSO_4$, 0.2 ml trace elements [21], and ampicillin solution (0.1 g/L) and was grown at 30 °C, on a rotary shaker for 18 h. The batch cultivation was performed by adding the 100 ml inoculum to the fermentor containing the same medium stated above with an addition of 0.1 ml antifoam. For the cultivation the temperature was kept at 37 °C, the pH was maintained at 7.0 (using 12.5% NH_3 solution), aeration was 3 L/min and the pO_2 was kept above 40% of saturation by the automatic adjustment of the stirrer speed. The cultivation parameters were controlled by using the computer program Phantom 2000 [22]. When the optical density at 620 nm reached 3, IPTG was added to a final concentration of 1 mM to induce the expression of the $(His)_6$-β-glucosidase gene. After 2 h the cells were harvested by centrifugation at 6000 × *g* for 30 min and stored at −18 °C until use.

2.4. Cell disrupture and clarification

A pellet of 81.4 g wet weight was after thawing suspended in 800 ml of buffer and sonicated in 200 ml portions. The crude cell homogenate was further diluted to a final volume of 4 L which corresponds to an initial cell density of 20.3 g/L. The cell homogenate was heat treated at 70 °C for 30 min in order to denature the *E. coli* host cell proteins thereby facilitating further downstream work. The heat treated cell homogenate was then centrifuged at 7000 × *g*.

Heat-treated cell homogenate showed better recovery of enzyme activity (74%) than not heat-treated (63%) after centrifugation. The heat treatment itself did not have any noticeable impact on the enzyme activity. There is a loss of 25% in the centrifugation step that can be traced to the pellet. Efforts were made to enhance recovery by re-suspension of the pellet in buffer followed by a second centrifugation but a major part of the enzyme activity could still only be found in the new pellet (results not shown). This clarified heat-treated sample was used as a feed for the rest of the chromatographic steps.

2.5. Single column chromatography: optimization for separation of β-glucosidase

Single column experiments were performed using Biologic DuoFlow Chromatography System (BioRad, Hercules, USA). Chromatography columns (Ace Glass, USA), 10 ml column volume, 11 mm I.D. were used for both the single column runs and the SMB-runs.

The chromatographic procedure included: (1) conditioning of the column with 20 mM citric–phosphate buffer, pH 7.5, 0.2 M NaCl and 10 mM imidazole; (2) loading with clarified *E. coli* homogenate; (3) washing with conditioning buffer; (4) elution of histidine tagged glucosidase with 20 mM citric–phosphate buffer, pH 7.5, 0.2 M imidazole. Experiments were performed at room temperature.

Two single-column experiments were run in order to find suitable loading volumes and washing conditions for the SMB configuration. In the first experiment 100 ml of clarified homogenate was loaded onto the column and imidazole up to 2 mM was added to the washing buffer in order to wash off non-specifically bound proteins. In experiment no. 2, 200 ml of clarified homogenate was loaded onto the column and imidazole up to 10 mM was added to the washing buffer.

Both of the single column batch experiments showed very high recovery but the chromatographic profile for the first experiment (feed volume of 100 ml and 2 mM imidiazole added in washing buffer) showed high absorbance at UV_{280} by the eluted protein fractions However, in the second experiment (feed volume of 200 ml

and 10 mM imidiazole added in washing buffer) a substantial amount of non-target protein was removed in the washing step. The specific activity and the purification fold of the recovered protein from the second experiment was much higher (almost three times) than that from the first experiment since the concentration of added imidazole in the second case is high enough to wash off the nonspecifically bound host cell proteins (data not shown).

The efficiency in binding of glucosidase to the IMAC-column was also determined as the loss of glucosidase activity in the flow-through. From these studies it was possible to find the suitable volume of clarified supernatant to be loaded on the column. There is a small leakage corresponding to 4% loss at 100 ml load and around 5% loss at 200 ml load.

Based on the results obtained the optimum conditions to operate the purification of β-glucosidase was load volume of 100 ml and a washing buffer containing 10 mM imidiazole

2.6. Open-loop simulated moving bed chromatography

The pilot scale SMB unit, C920, with columns and fittings came from Calgon Carbon Corporation (Pittsburgh, USA). The heart of the equipment is a central multi-port valve which coordinates the flow of liquid into and out of the columns. The valve has an upper stationary head and below a rotating head which rotates along with the columns on a turntable. A time based controller regulates the column switching. The maximum columns that can be used to design the SMB run are 20. Five single-piston pumps (model RHV) from Fluid Metering (Syosset, NY, USA) were used to control flow rates.

The simplified approach for designing the open-loop SMB is based on an optimized single column experiment. Each step (loading, washing, elution, regeneration and conditioning) in the chromatographic cycle is translated into a set of columns in the SMB. In this case it was chosen to run a 10 column SMB.

In order to design the SMB, there are choices to be made based on the characteristics of the single column protocol. The first consideration is to determine the design of the loading zone. If it is worthwhile to pump the flow through from the loading step over one more column connected in series or if parallel loading is desired. It was decided not to pump the flow-through over one more column as long as the volume loaded onto a single column is below 100 ml (10 cv), since the leakage is small (4%). Three columns were loaded in parallel since the other steps (wash, elution and conditioning) require less volume (2–3 cv) and when parallel loading is used different steps can be run at desirable flow rates thereby improving the productivity. A parallel three column loading zone would then lead to a load of 33.3 ml (≈3 column volumes) in each column position in the loading zone.

A serial three column elution zone was chosen with a buffer load of 20 ml/switch and in total 60 ml (six column volumes) will be used to elute the target. Two columns each were chosen for washing and conditioning respectively and in total 10 columns were used for the SMB run (Fig. 1).

The parallel loading had the highest loading (ml/column) 33.3 ml/column and was run at maximum flow rate 10 ml/min (compression effects were seen on the resin over 10 ml/min (631 cm/h) which limits the flow rate), thus the switch time will be 33.3/10 = 3.33 min. Flow rates of the other steps are then determined as volume/switch time (Table 1).

A high product concentration was desired and therefore elution was carried out through three columns in sequence instead of putting a forth column in the loading zone and elute over two columns. The SMB configuration can be seen in Fig. 1 with its characteristics summarized in Table 1.

An increased number of columns in a zone will decrease the buffer consumption (until a certain point) and thereby increase product concentration. Still, enough liquid must be pumped during each cycle in order to move the elution front forward to the next column. Otherwise the front will follow in the direction of the column when switching. However, there will also for each zone be a trade-off between number of columns used in the zone and the productivity, since the productivity is scaled by the total column volume.

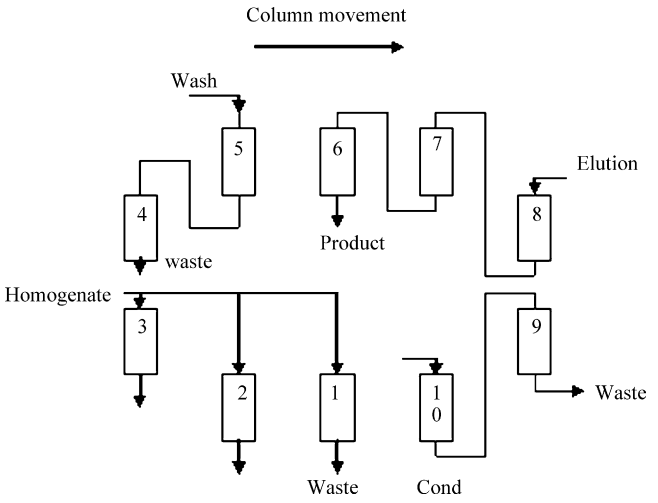

Fig. 1. Schematic design of SMB configuration, a 10-column set-up for β-glucosidase purification on IDA-Sepharose. Clarified *E. coli* homogenate is loaded in parallel over three columns (column positions 1–3). Saturated columns are washed in column positions 4–5. The glucosidase is eluted in positions 6–8. Conditioning of the columns is done in column position 9–10.

It was chosen not to regenerate the columns between each cycle in this process set-up. Just to strip the copper ions off the column and to replace them with a fresh loading requires five steps (Cu-stripping by EDTA, washing, Cu-loading, washing of loosely bound Cu and conditioning). To this further CIP procedures might be necessary. The strategy was instead to run the separation cycle as many rounds as possible before the capacity decreased to levels when regeneration was necessary and at that point perform Cu-reloading.

2.7. Analytical determinations

β-Glucosidase activity was determined spectrophotometrically with the formation of *p*-nitrophenol at 405 nm from the substrate *para*-nitrophenyl β-D-glucopyranoside. The substrate was dissolved in 20 mM citrate–phosphate buffer pH 5.6 to give the reaction mixture (2.8 mM final concentration). The prepared reaction mixture (960 μl) was taken and placed in a heating block set at 80 °C. To this 40 μl of enzyme was added and incubated for 10 min. The tubes were cooled on ice for 5 min and adjusted to room temperature before the absorption was recorded.

Total protein concentration was determined using BCA (bicinchoninic acid) reagents [23]. For eluted samples total protein was determined by UV_{280} nm using an extinction coefficient of 1.8 cm^{-1} mg^{-1} ml [24] since there was major interference from the elution buffer when using the BCA method.

Table 1
SMB process characteristics.

Step	No. of columns	Flow
Loading	3	10 ml/min/column
Washing	2	6.3 ml/min/column
Elution	3	6.3 ml/min/column
Conditioning	2	6.3 ml/min/column

Ten-column set-up for SMB and distribution of columns for each step of chromatography.

Fractions from the chromatographic runs were analyzed by SDS–PAGE according to the method of Laemmli [25]. It was performed on a 12% resolving gel. Reduction of protein was accomplished with 10% β-mercaptoethanol (v/v) in the sample buffer. Gels were stained with 0.1% coomassie blue. The molecular weights of the bands were determined by using Fermentas PageRuler Protein ladder as reference.

2.8. Productivity and buffer consumption

Productivity for this system can be calculated as amount of enzymatic activity collected in the eluent stream per unit time per unit volume of the adsorbent.

Productivity of SMB process is calculated as:

$$\mathrm{SMB_{prod}} = \frac{(\text{enzyme activity})}{(\text{switch time})} \times \frac{(\text{eluted volume})}{(\text{column volume})}$$

Productivity of conventional chromatographic process is calculated as 10 single columns operated in parallel mode:

$$\mathrm{Batch_{prod}} = 10 \times \frac{(\text{enzyme activity})}{(t_{\mathrm{cycle}})} \times \frac{(\text{eluted volume})}{(\text{column volume})}$$

$$t_{\mathrm{cycle}} = \sum (V_{\mathrm{step}}/\nu_{\mathrm{step}})$$

Abbreviations: t_{cycle} (cycle time), V_{step} (step volume), ν_{step} (step flow rate).

Buffer consumption was expressed as the volume of buffer in relation to the volume of feed. Buffer consumption of a system is calculated as below:

$$(\text{buffer consumption}) = \frac{(\text{washing volume}) + (\text{elution volume}) + (\text{conditioning volume})}{(\text{loaded feed volume})}$$

3. Results and discussion

3.1. Simulated moving bed chromatography

3.1.1. Results from the SMB

When using affinity separation a high purification factor can be expected. In this experiment the target protein is purified by a factor of 15. Recovery from the SMB experiment reached 91%. The outcome of the experiment is shown in Table 2. The SDS–PAGE (Fig. 2) shows a very distinct band at 81 kDa concluding that the purification was successful. Although there are still some contaminants observed as minor bands in the electrophoresis gel. They might be removed by further increasing the imidazole concentration in the application solution or if a higher loading was applied they might be displaced by the His-tagged enzyme. This might however reduce the yield of the process. The elution data can be seen in Fig. 3 together with the elution and washing profiles in Figs. 4 and 5. The elution data show that column to column variation is small. Also, the appearances of the washing and elution profiles show uniform column performance. There are two peaks corresponding to each interval in the elution step (Fig. 4). There was no enzymatic activity observed in the fraction relating to the first big peak (peak a) while most of the target enzymatic activity corresponded to small peak b. Thus, there is a fraction of impurities that is not removed in the washing zone but comes as a separate peak during the elution.

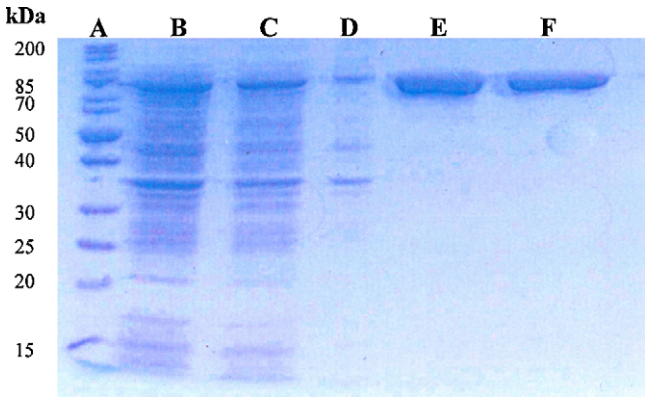

Fig. 2. SDS–PAGE showing the purification of β-glucosidase from the heat treated supernatant. A-molecular marker, B-Crude homogenate, and C-Supernatant after heat treatment and centrifugation, D-redissolved pellet, E-elution sample 1, and F-elution sample 2. This later showed an 81 kDa band of β-glucosidase.

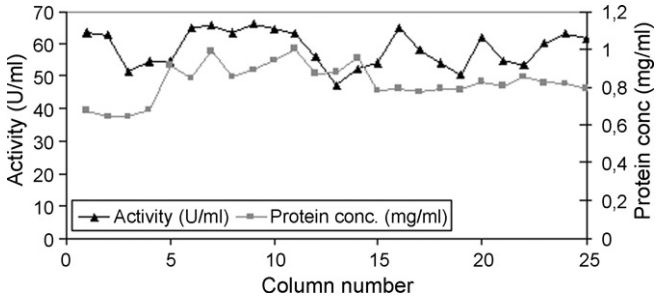

Fig. 3. Elution data for 26 sequential (2.6 cycles) β-glucosidase elutions. Black triangles indicates β-glucosidase activity in the effluent (U/ml). Grey squares indicates protein content (mg/ml).

Table 2
SMB–IMAC separation of His–tagged β-glucosidae from *E. coli*.

Steps	Volume (ml)	Protein (mg/ml)	Activity (U/ml)	Specific activity (U/mg protein)	Fold purification	Yield (%)
Raw material	100	1.5	7.0	4.7	1	100
Breakthrough	100	0.88	0.17	0.2	0.04	2.4
Wash	20	0.76	0.17	0.2	0.04	0.5
Peak a	8	1.2	0.27	0.2	0.04	0.3
Peak b	12	0.78 ± 0.10	53 ± 7	68	15	91
Conditioning	20	0.10	0.9	9	1.9	2.8

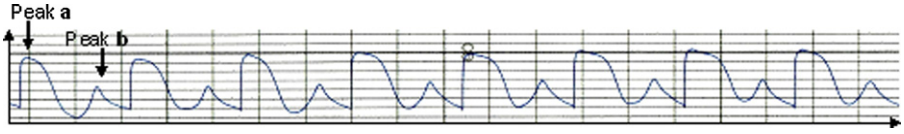

Fig. 4. Absorbance of the effluent at UV_{280} for eight sequential column switches. Peak a does not contain any β-glucosidase activity. Peak b contains β-glucosidase activity.

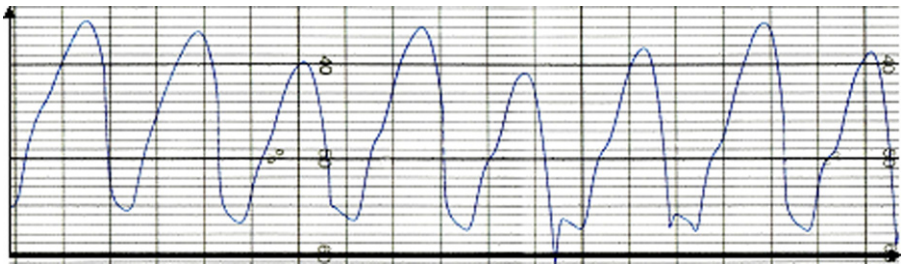

Fig. 5. Profile of absorbance at UV_{280} of the washing stream for eight sequential column switches.

Table 3
Comparison between SMB and batch chromatography.

Parameters	Batch (100 ml load)	SMB	SMB/batch
Productivity (U/min/ml)	1.4	1.9	1.4
Buffer consumption (ml/ml load)	1.5	0.6	0.4
Purification fold	5.7	15	2.6
Specific activity (U/mg)	42	68	1.6
Yield (%)	67	91	1.4
Cycle time (min)	31.25	3.3	0.1

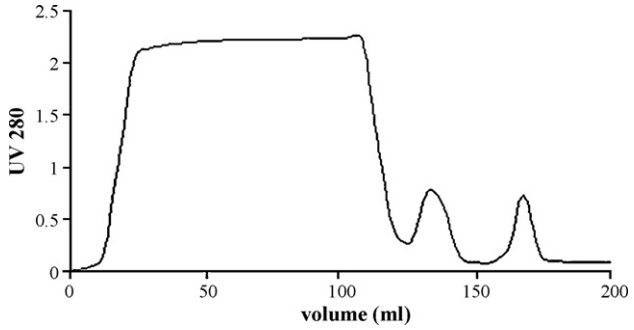

Fig. 6. Chromatographic profile of β-glucosidase on IDA sepharose using 10 mM imidazole in the washing step. Chromatographic steps performed—0–100 ml loading step, 100–150 ml washing step with buffer containing 10 mM imidiazole, 150–200 ml elution step. Experiments performed at room temperature and the flow rate used is 8 ml/min. Details concerning buffers used are presented in Section 2.

3.1.2. Process comparison

In open literature, SMB processes have shown higher productivity and lower buffer consumption as compared to those of the conventional chromatographic processes. A comparative study on SMB chromatography and the single column runs (Table 3) is thus of high interest. Therefore a single column run with the previously optimized parameter (100 ml of clarified homogenate containing 10 mM imidiazole in the washing buffer) was performed in the same lines of SMB to have a fair comparison (Fig. 6 and Table 4). A comparison between results from a SMB process and a conventional chromatographic process cannot be made directly but it still gives an illustrative idea about the advantages of the used process technology. The SMB process used is compared with a conventional chromatographic process involving ten columns connected in parallel.

The eluted fraction collected through the SMB is represented by two peaks (Fig. 4), peak a showing meager activity represents final washed proteins present in dead volume in the tubing whereas almost all the activity was found in peak b containing more pure protein with higher activity.

Productivity of the SMB is higher when columns are connected in series and the flow through from the previous column is loaded onto the next to ensure minimum losses of target protein. This has been shown in an earlier report [26]. However, due to a very small loss in activity in the flow through even by loading 100 ml it was decided to only apply sample over one column length using three parallel columns which is similar to loading on to a single column.

3.2. Discussion

This report with the design of an open-loop SMB process using a new simplified approach shows the target protein is purified by a factor of 15 and recovered to 91%. The SDS–PAGE indicates that the target is purified to almost single band purity, thus the purification was successful. It also demonstrates reduction in buffer volume used by a factor of 2.5 and higher productivity as compared to conventional batch chromatography. A simple single column protocol is used to design the SMB and this is done by translating the different steps in the chromatographic cycle into sets of columns in the SMB.

The possibility of open-loop operation renders application of different buffers which are inevitable in protein separation. This could be one of the reasons which restrain protein separation through conventional SMB (operated at isocratic condition). Much focus has been placed on modeling of the process, and separation of binary mixtures of two model proteins. By the approach that is introduced here, it becomes relatively easy to set-up a SMB-system for separating proteins, also from realistic complex sources.

Another benefit with the open-loop design is that the system is very flexible with possibilities of using parallel loading and recycling. Different process characteristics could easily be traded against each other. For example, the productivity could be raised with a lower eluted target protein concentration and raised buffer consumption by removing columns from the elution zones to be used in the loading zone instead etc. Also, the linear gradient problem in closed-loop SMB can at least partly be circumvented by step changes of the eluting agent in different zones, i.e. introduce a wash step and then do a step gradient elution of the target substance.

Table 4
IMAC separation of His-tagged β-glucosidae from *E. coli*.

Steps	Volume (ml)	Protein concentration (mg/ml)	Activity (U/ml)	Specific activity (U/mg protein)	Fold purification	Yield (%)
Raw material	100	0.94	6.91	7.4	1	100
Breakthrough	100	0.65	0.903	1.4	0.19	13.1
Wash	50	0.38	0.301	0.8	0.11	2.2
Eluate	50	0.22	9.2	42	5.7	67

Purification table for 100 ml clarified load, washing buffer with 10 mM imidiazole on a 10 ml column packed with IDA sepharose.

The major drawback with open-loop SMB in relation to conventional SMB-processing is that buffer volumes will increase and these increased volumes will result in loss in productivity. Another drawback is that it might be hard to work with the same maximum flow rate in the different zones which can be seen in this work where the elution, washing and conditioning are run at a lower flow rate than necessary. This will also lead to lower productivity.

A simple single column run was used to determine the buffer volume for each step and the switch time. Based on these two parameters a simple SMB configuration can be designed and its performance can be checked with the single batch run. The mode of operation in developed SMB with sequential multi column switching is similar to batch chromatography but the effect of countercurrent contact between the solid and the liquid phase and the affinity of the target protein to the solid phase is the main reason which results in a better performance. The benefits with this design could be that: (1) a more flexible system (2) with optimized single column run it becomes easy to set-up a process (3) the gradient problem in closed-loop SMB can be circumvented by step changes of the eluting agent (4) since all the columns are not connected in series it will be more robust for process disturbances (5). The drawback is that a closed-loop SMB will be more efficient with more pronounced reduction in buffer consumption but is harder to design in reality within the area of protein purification and especially when handling complex mixtures.

To combine affinity chromatography with SMB has the advantage that the adsorption of the target is expected to be specific so that elution (after a prior washing step) of a pure target can be performed with a step gradient instead of a linear gradient. On the other hand, the full potential of the SMB might not be utilized due to the same specific adsorption will deplete the feed from target over one column (if not overloaded), making it un-necessary to apply the flow-through into another column. However, due to a very small loss in activity in the flow-through in one-column experiments, it was decided to use the concept of sequential columns only in the washing and elution steps. Loading over parallel columns reduces application time and results in the use of sequential column operations in the subsequent steps leading to a high yield, low buffer consumption and a high concentration of the target protein in the eluate.

Chromatography is the workhorse within protein separation due to its gentle and high resolving power. Still it is an expensive method because of low productivity, diluted product streams, large waste streams and large consumption of high purity water used for buffer and washing solution and expensive support material leading to an overall increase in production cost. By improving productivity and reducing costs, chromatography might become realistic separation technology in situations when it was earlier regarded as too expensive, both with regard to low productivity and to high operating costs. The development might be of interest for isolation of proteins aimed for technical applications and also designed proteins for material science applications.

There is a clear interest to make the technology cheaper to use both for existing pharmaceutical and fine chemical applications and also to open doors to new areas like processing of effluents from the food industry.

To use chromatography to remove impurities from aqueous solutions is an attractive area of applications since then relatively low capacity will be needed and that leads to possibilities to process large volumes with small beds of chromatographic material. Such applications are certainly found in pharmaceutical processing but there the validation problems may still hamper the development. However, there are many other cases when such a treatment would be highly beneficial, e.g. treatment of liquid feedstocks (milk, fruit juice etc.) as well as water. The pollutants to be removed could be pesticide residues or other environmental pollutants.

Since many of such pollutants are present at low concentrations, it seems attractive to use affinity chromatography to enrich the pollutants, thereby purifying the effluent. This will be the topic of a future study.

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References

[1] K. Mondal, M.N. Gupta, Biomol. Eng. 23 (2006) 59.
[2] J. Porath, J. Carlsson, I. Olsson, G. Belfrage, Nature 258 (5536) (1975) 598.
[3] T.M. Przybycien, N.S. Pujar, L.M. Steele, Curr. Opin. Biotech. 15 (5) (2004) 469.
[4] M. Saska, G.J. Rossiter, J. Am. Oil Chem. Soc. 75 (1998) 1421.
[5] R.M. Nicoud, in: S. Ahuja (Ed.), Handbook of Bioseparations (Separation Science and Technology), Academic press, San Diego, CA, 2000, p. 475.
[6] J. Houwing, H.A.H. Billiet, L.A.M. van der Wielen, International Symposium on Preparative and Process Chromatography, Washington, D.C., 2001.
[7] N. Gottschlich, S. Weidgen, V. Kasche, J. Chromatogr. A 719 (2) (1996) 267.
[8] L. Gueorguieva, L.F. Vallejo, U. Rinas, A.S. Morgenstern, J. Chromatogr. A 1135 (2) (2006) 142.
[9] N. Gottschlich, V. Kasche, J. Chromatogr. A 765 (2) (1997) 201.
[10] J. Thommes, A.M. Sonnenfeld, J.P. Pieracci, L. Conley, M. Bisschops, M. Pennings (2007) US Pat. Appl. 11/751.147.
[11] Y. Xie, S.Y. Mun, N.H.L. Wang, Ind. Eng. Chem. Res. 42 (7) (2003) 1414.
[12] L.C. Kessler, L. Gueorguieva, U. Rinas, A.S. Morgenstern, J. Chromatogr. A 1176 (2007) 69.
[13] G. Storti, M. Mazzotti, S. Carra, M. Morbidelli, Chem. Eng. Sci. 44 (1989) 1329.
[14] S. Abel, M. Mazzotti, M. Morbidelli, J. Chromatogr. A 944 (2002) 23.
[15] C.B. Ching, D.M. Ruthven, K. Hidajat, Chem. Eng. Sci. 40 (1985) 1411.
[16] G. Zhong, G. Guiochon, Chem. Eng. Sci. 51 (1996) 4307.
[17] Z. Ma, N.H.L. Wang, AIChE J. 43 (1997) 2488.
[18] Y. Xie, D.J. Wu, Z. Ma, N.H.L. Wang, Ind. Eng. Chem. Res. 39 (2000) 1993.
[19] J. Andersson, B. Mattiasson, J. Chromatogr. A 1107 (2006) 88.
[20] J. Porath, B. Olin, Biochemistry 22 (7) (1983) 1621.
[21] Holme, Proc. Biochem. 5 (1970) 62.
[22] www.belach.se, Belach Bioteknik AB-3L autoclavable reactor (Kent Laboratory series) BIO_Phantom based soft control.
[23] P.K. Smith, R.I. Krohn, G.T. Hermanson, A.K. Mallia, F.H. Gartner, M.D. Provenzano, E.K. Fujimoto, N.M. Goeke, B.J. Olson, D.C. Klenk, Anal. Biochem. 150 (1985) 76.
[24] H.F. Seidle, I. Marten, O. Shoseyov, R.E. Huber, Prot. J. 23 (2004) 11.
[25] U.K. Laemmli, Nature 227 (1970) 680.
[26] J. Andersson, D. Sahoo, B. Mattiasson, Biotech. Bioeng. 101 (6) (2008) 1256.