Pilot-scale process for magnetic bead purification of antibodies directly from non-clarified CHO cell culture

Nils A. Brechmann (ORCID)
AdBIOPRO, VINNOVA Competence Centre for Advanced BioProduction by Continuous Processing, Stockholm, Sweden
Cell Technology Group (CETEG), Dept. of Industrial Biotechnology, School of Engineering Sciences in Chemistry, Biotechnology and Health, KTH Royal Inst. of Technology, Stockholm, Sweden

Per-Olov Eriksson
PE Bioprocess Consulting AB, Strängnäs, Sweden

Kristofer Eriksson
AdBIOPRO, VINNOVA Competence Centre for Advanced BioProduction by Continuous Processing, Stockholm, Sweden
Lab-on-a-Bead AB, Uppsala, Sweden

Sven Oscarsson
Dept. of Organic Chemistry, Stockholm University, Stockholm, Sweden

Jos Buijs
Dept. of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden

Atefeh Shokri
AdBIOPRO, VINNOVA Competence Centre for Advanced BioProduction by Continuous Processing, Stockholm, Sweden
Cell Technology Group (CETEG), Dept. of Industrial Biotechnology, School of Engineering Sciences in Chemistry, Biotechnology and Health, KTH Royal Inst. of Technology, Stockholm, Sweden

Göran Hjälm
AdBIOPRO, VINNOVA Competence Centre for Advanced BioProduction by Continuous Processing, Stockholm, Sweden
Lab-on-a-Bead AB, Uppsala, Sweden

Véronique Chotteau
AdBIOPRO, VINNOVA Competence Centre for Advanced BioProduction by Continuous Processing, Stockholm, Sweden
Cell Technology Group (CETEG), Dept. of Industrial Biotechnology, School of Engineering Sciences in Chemistry, Biotechnology and Health, KTH Royal Inst. of Technology, Stockholm, Sweden

High capacity magnetic protein A agarose beads, LOABeads PrtA, were used in the development of a new process for affinity purification of monoclonal antibodies (mAbs) from non-clarified CHO cell broth using a pilot-scale magnetic separator. The LOABeads had a maximum binding capacity of 65 mg/mL and an adsorption capacity of 25–42 mg IgG/mL bead in suspension for an IgG concentration of 1 to 8 g/L. Pilot-scale separation was initially tested in a mAb capture step from 26 L clarified harvest. Small-scale experiments showed that similar mAb adsorptions were obtained in cell broth containing 40×10^6 cells/mL as in clarified supernatant. Two pilot-scale purification runs were then performed on non-clarified cell broth from fed-batch runs of 16 L, where a rapid mAb adsorption ≥96.6% was observed after 1 h. This process using 1 L of magnetic beads had an overall mAb yield of 86% and 16 times concentration factor. After this single protein A capture step, the mAb purity was similar to the one obtained by column chromatography, while the host cell protein content was very low, <10 ppm. Our results showed that this magnetic bead mAb purification process, using a dedicated pilot-scale separation device, was a highly efficient single step, which directly connected the culture to the downstream process without cell clarification. Purification of mAb directly from non-clarified cell broth without cell separation can provide significant savings in terms of resources, operation time, and equipment, compared to legacy procedure of cell separation followed by column chromatography step. © 2019 American Institute of Chemical Engineers Biotechnol. Prog., 00:000–000, 2019.
Keywords: magnetic beads, purification, monoclonal antibody, pilot-scale, downstream-bioprocess

Introduction

Biomolecules, such as monoclonal antibodies, represents a large and fast expanding class of biopharmaceuticals that are targeting a variety of diseases.[1,2] With an increasing demand of mAbs, a significant burden has been placed on the mAbs capture and downstream process.[2] Protein A affinity chromatography is today's gold standard for industrial mAbs capture due to its high selectivity and yield.[3–5] However, the capture step by protein A column chromatography is a bottleneck for the field, as its performance cannot keep up with the production from upstream bioreactors.[2,6,7] A major limitation of this chromatography is the time needed to adsorb the target antibody product to the protein A separation matrix.[8] Depending on the volume of the applied solution and column size, the adsorption phase in column chromatography-based processes can take hours or even more than a day. In contrast, batch separation completes the adsorption phase in laps of minutes or a few hours.[8] The limitations of a chromatography-based process, regarding throughput, scale-up, and cost, have therefore increased the interest in alternative methods for the capture step.[9] A cell separation step, typically performed by centrifugation and/or filtration, is required prior application of a protein A column chromatography step. This step is costly, can take many hours, and is known to potentially increase host cell protein (HCP) levels and proteolytic activity in the clarified material, due to cell lysis.[10,11] A one-step batch separation product-recovery, directly applied on the cell broth, has thus the potential to provide high savings in terms of time, material, and other resources, while also possibly reducing the HCP level.

Magnetic affinity adsorbents possess interesting characteristics, such as being rapid, gentle, compatible with complex particulate-containing biological suspensions[8,12] and highly selective for a variety of biomolecules,[13–15] particularly mAbs.[16] Magnetic separation is based on functionalized magnetic particles and magnetic filter,[17–21] and consists of three typical phases: (i) target adsorption directly in the sample, e.g., affinity for antibody; (ii) separation of magnetic beads by a temporary applied magnetic field; and (iii) bead washing and recovery of the target molecule.[22] Purification with protein A coupled magnetic beads, combines the high selectivity of this antibody capturing ligand and the benefits of magnetic separation. This provides the possibility to adsorb mAbs directly from a culture by the functionalized bead surface,[8,22–25] while minimizing the capture of unwanted biomolecules of the cell broth.[12,24,26,27] Separation based on magnetic bead has proven its applicability as a one-step purification method from crude suspension using bacitracin functionalized glutaraldehyde particle.[7]

Magnetic separation eliminates pretreatment, such as centrifugation and filtration,[22] and fuses the steps of clarification, purification, and concentration.[12] This decreases process costs, while providing high product purity in a single step.[24,28] Magnetic particles, however are mainly used in milligram quantities for diagnostic and analytical purposes,[8,29,30] whereas only few applications of large-scale purification with magnetic beads have been reported. Holschuh and Schwämmle reported a process with a magnetic separator of up to 100 L. This process had a product yield of 75% and resulted in 2 L elution volumes, i.e., larger than 10 times the magnetic bead volume. This volume is unfavorable for large scale industrial application.[8] Recently, another study with magnetic particles and a magnetic separator based on electromagnetism, reported a capture process of mAb from CHO cell culture.[31]

The aim of the present study, was to develop an efficient mAb capture step applied to a cell culture broth using magnetic beads, showing a proof of concept of suitability for industrial manufacturing with high product yield and elution volumes suitable for large-scale process, comparable to protein A column chromatography process, while reducing the overall operational burden compared to the legacy of cell separation followed by capture step. This work was based on newly developed high-capacity magnetic protein A agarose beads and a proprietary magnetic separator. The study included the characterization of the beads, pilot-scale evaluation with clarified supernatant, small-scale adsorption efficiency experiments, evaluation with non-clarified cell broth at pilot scale, and comparison to commercial affinity column chromatography.

Materials and Methods

Growth of CHO cells

For all the assays, a CHO—M cell line stably expressing a humanized IgG1 antibody was used, kindly provided by Selexis, Switzerland. Growth in fed-batch mode was performed according to the medium manufacturer's basic protocol CHO cells were cultivated for 11 days in run cult_B1 and 14 days in run cult_B2 with BalanCD CHO growth A kindly provided by Irvine Scientific (Santa Ana, California), supplemented with 8.25 mM Glucose and 4 mM Glutamine; called here Base Medium. The cells were inoculated at 0.55 and 0.67×10^6 cells/mL in 9 L of Base Medium (Day 0), in runs cult_B1 and cult_B2, respectively. From Day 1, cultures were supplemented with Feed Medium (10% Feed concentrate BalanCD CHO Feed 4 in Base Medium, Irvine Scientific). Glucose and glutamine were added according to the cells need. At the harvest of run cult_B1, end volume was 15.73 L, total cell density at 14×10^6 cells/mL with a viability of 89.9%, and a mAb titer of 1.31 g/L. At the end of run cult_B2, cell broth volume was 16.25 L, total cell density 11.2×10^6 cells/mL with a viability of 75.9%, and a mAb titer at 1.51 g/L. The cell broths of runs cult_B1 and cult_B2 were harvested at Day 11 and 14 and used for pilot-scale purification in runs B1 and B2, respectively.

Magnetic protein A agarose beads

The experiments using magnetic separation were performed with commercially available LOABeads PrtA (Lab-on-a-Bead AB, Uppsala, Sweden), a superparamagnetic 4% agarose resin with an average diameter of 90 μm and coupled covalently with a standard recombinant protein A. The LOADBeads PrtA provide a magnetic saturation of 40 emu/g beads and they have a loading capacity as high as the MabSelect SuRe. Furthermore, the beads show a high reusability of around 100 purification cycles.

Magnetic protein A bead capacity assays

The adsorption equilibrium data for LOABeads PrtA were collected in a set of batch experiments using purified mAb IgG1 as a model antibody. For each experimental data set, 50 μL beads were mixed with IgG (0.5–5 mg/mL in PBS) in a total volume of 1 mL. After rotation end-over-end for 2 h at room temperature, the unbound IgG concentrations were determined by measuring UV absorbance at 280 nm in the supernatants. The amount of bound IgG, obtained by subtracting the

unbound IgG from the input value, was used to calculate the amount of IgG adsorbed per mL of settled beads.

Dynamic Bead Binding Capacity (DBBC) for LOABeads PrtA is defined as the amount of beads, as a function of IgG concentration, required to bind 90% free IgG within 1 h of adsorption time and was determined performed on a humanized monoclonal IgG antibody spiked in PBS with concentrations of 1, 2, 4, 6, and 8 g/L. For each input concentration, four tubes were set with various amounts of magnetic beads, while maintaining a fixed total volume. Adsorption took place for 1 h with end-over-end mixing. Unbound fractions were measured for IgG content and the adsorbed IgG portion was calculated as described above. Data from the four samples were plotted (not shown) and capacity per mL beads at a specific IgG concentration was determined at 90% adsorption. Final data points at 90% adsorption, for the five different input concentrations, were plotted to obtain a dynamic bead binding capacity graph.

Determination of mAb concentration

The concentration of mAb in cell supernatant samples prior purification was determined using a POROS A 20 μm protein A column (Thermo Fisher Scientific, Waltham) coupled to a 2695 HPLC separation module and a 2996 Photodiode Array Detector (Waters, Milford). The column was equilibrated with 20 mM NaH_2PO_4 pH 7.0 and bound material was eluted with 20 mM NaH_2PO_4 pH 2.7. The detection was performed with UV at 214 nm. Standard curves were generated with total IgG from human serum (#I4506; Sigma-Aldrich, Missouri) and elution peak areas were used for quantification.

After purification the concentration of mAb in eluted fractions was determined using a Libra S12 spectrophotometer (Biochrom, Cambridge, UK) using $A_{280} = 1.40$ at 1 mg/mL.

Micro-scale purification of mAb in the presence or absence of CHO cells

1.5 mL cult_B2 samples, either from non-clarified harvest or harvest clarified by centrifugation, were mixed with 83 μL LOABeads PrtA end-over-end at room temperature for 90 min. The LOABeads resin were magnetically separated using a handheld LOABeads MagSep5 cube magnet. Unbound fractions, with cells separated for the non-clarified samples by centrifugation, were collected for later analysis by SDS-PAGE. The beads were washed five times with 0.9 mL PBS. Bound mAb were released with 1.86 mL 60 mM citrate, pH 2.8.

Pilot-scale purification of mAb from clarified and non-clarified CHO cell culture harvest

The proprietary pilot-scale magnetic separator prototype with a magnetic flux density of 1.0 Tesla in direct proximity to the magnetic rods, developed by Lab-on-a-Bead AB (Uppsala, Sweden), is a system that includes a chamber equipped with retractable magnetic rods, allowing ON/OFF mode, in which the magnetic attraction is applied (ON) or switched OFF, as well as a dedicated compartment for the elution, which enables a concentration of the magnetic beads. An initial pilot-scale experiment of mAb capture was performed on 26 L clarified cell-free harvest, which had been obtained from a perfusion experiment. This was followed by two experiments of mAb purification from 15 to 16 L

non-clarified cell broth, i.e., in the presence of CHO cells, obtained by fed-batch, as described above, conditions for all three purifications are shown in Table 1. The two first pilot-scale purifications were essentially performed in the same way as the third purification (Table 2), described in more detail below.

1 L LOABeads PrtA (volume of settled beads) was batch equilibrated with PBS and then incubated with 16.25 L of fresh non-clarified cell broth, constituting mixture A. Gentle continuous stirring was carried out to keep the beads in suspension. 1 mL samples were taken at 5, 10, 15, 30, 60, and 120 min, after contact of the cell broth with the beads. The cells were removed by centrifugation from these samples and the supernatants were stored for later analysis. At 120 min, the rest of mixture A was transferred at 100 L/h, using a low shear force peristaltic circulation pump, into the magnetic separator, where the retractable magnetic rods were positioned in ON mode for separation of the beads by magnetism. After complete separation, the unbound fraction was displaced using PBS. Subsequent bead washes with PBS were then performed in cycles at a flow of 254 L/h to homogenize the bead suspension; (i) magnetic mode OFF; (ii) magnetic mode ON to capture the magnetic beads; (iii) PBS solution change, followed by (i) and so on. An additional tubing cleaning step was performed (Table 2) to ensure that no magnetic beads were lost in the tubing or connections. After completion of the wash cycles, the beads were transferred into a second compartment where the elution took place. Adsorbed mAbs on the beads were released using 100 mM citrate, pH 2.8, and passed through a Millipak 0.22 μm filter (Merck, Darmstadt, Germany) for sterile filtration. In total, 2.9 L eluate was collected in a container. 48 aliquots of 50 mL volumes were taken at regular interval from the elution line to measure the absorbance at 280 nm, before pooling them. For the virus inactivation step, the eluted mAb was maintained at low pH for 1 h before reconstituting a neutral pH by adding 310 mL of 2 M Tris–HCl, pH 9.0.

Preparative HiTrap protein A column chromatography

Column chromatography was performed on a 5 mL HiTrap MabSelect SuRe coupled to an ÄKTAexplorer chromatography instrument, controlled by Unicorn software (version 5.11; GE Healthcare, Uppsala, Sweden). The column was equilibrated with PBS, whereafter 82 mL clarified cell culture sample were applied at 4 mL/min. Wash was performed with 12 column volumes of PBS and remaining material bound to the protein A-column was released using 100 mM citrate,

Table 1. Comparison of the conditions for the three mAb purifications of clarified cell broth (run CF) and non-clarified cell broth (run B1 and run B2)

	run CF	run B1	run B2
Feed volume [L]	26	15.57	16.25
Calculated amount of magnetic beads [mL][*]	380	680	820
Amount of magnetic beads [mL]	1000	800	1000
mAb titer [g/L]	0.44	1.31	1.51
Total process time (including adsorption)[†]	N/A[‡]	≈ 7.5 h	≈ 5.5 h

[*]According to DBBC 90%.
[†]Process time in a completely manual controlled, process time will most likely decrease with automatization.
[‡]During the test run several additional parallel tests were performed and the real operational time could not be measured.

Table 2. Comparison of process steps, buffers, volumes and durations for all 3 mAb purifications of clarified cell broth (run CF) and non-clarified cell broth (run B1 and run B2)

	run CF*				run B1*			run B2*		
Process Step	Buffer	Volume [L]	Duration [min]	Process step	Buffer	Volume [L]	Duration [min]	Buffer	Volume [L]	Duration [min]
Equilibration	PBS	3	10	Equilibration	PBS	3	10	PBS	3	10
Adsorption	Cell culture supernatant	26	240	Adsorption	Cell Culture Broth	15.73	240	Cell Culture Broth	16.25	120
Sample Application			N/A†	Sample Application			15			15
Wash step 1	PBS	15	N/A†	Wash step 1	PBS	30	30	PBS	30	30
Wash step 2	PBS	15	N/A†	Wash step 2	PBS	5	15	PBS	5	15
				Wash step 3	PBS	7.5	15	PBS	7.5	15
				Wash step 4	PBS	7.5	15	PBS	7.5	15
				Wash step 5	PBS	7.5	15	PBS	7.5	15
				Tubing Cleaning	PBS	5	25	PBS	5	25
Additional Wash	PBS	N/A	N/A†	Additional Wash	PBS	7.5	36	PBS	7.5	36
Elution	0.06 M Citrate	2.5	N/A†	Elution	0.1 M Citrate	3.5	30	0.1 M Citrate	2.9	30

*All processes were performed in manual mode and buffer volume will most likely decrease with the increase of automatization.
†During the test run several additional parallel tests were performed and the real operational time could not be measured.

pH 2.9. 13.5 mL eluted material were collected and neutralized using 2 M Tris–HCl, pH 9.0.

Sodium dodecyl sulfate polyacrylamide gel-electrophoresis

Separation of proteins by SDS-PAGE was performed on precast 4–20% Mini-PROTEAN TGX gels at 200 V, using the Mini-PROTEAN Tetra System and a PowerPac Basic, according to the manufacturer's instruction (Bio-Rad Laboratories, Hercules, CA). The unstained Precision Plus Protein Standards from Bio-Rad were used as a relative size marker and separated proteins were visualized using QC Colloidal Coomassie Stain from Bio-Rad. The samples were heated for 5 min at 95°C prior to loading, either under non-reduced or reduced conditions using 100 mM dithiothreitol (DTT).

Enzyme-linked immunosorbent assay for measurement of CHO host cell proteins

Assessment of HCP concentrations in supernatant and eluted material, were performed using CHO HCP ELISA kit 3G (#F550), according to manufacturer's instructions (Cygnus Technologies, Southport, NC).

Results and Discussion

Magnetic protein A bead capacity assays

The data collected in the adsorption equilibrium experiment were fitted using Langmuir isotherm to display the static binding capacity for the magnetic bead at adsorption saturation (Figure 1A). The maximum binding capacity (Q_{max}) for the beads was calculated by linearizing the Langmuir isotherm (Hanes plot), where 1/slope is equal to Q_{max} (Figure 1B). The static capacity at saturation and Q_{max} were found to be 65 mg mAb IgG/ml of magnetic beads and comparable to the maximum binding capacity measured for humanized IgG1 mAbs in the case of non-magnetic protein A resin rProtein A Fast Flow (64 mg/mL), Mabselect SuRe (66 mg/mL), CaptivA PriMAB (63 mg/mL) and Amsphere Protein A JWT203 (63 mg/mL).[32] The closest magnetic protein A resin Protein A Mag Sepharose, which is a prototype resin, provides a maximum binding capacity at IgG concentrations below 1 g/L of 87 mg/mL.[31]

Additional studies with rabbit IgG provided a Q_{max} of 55 mg IgG/ml magnetic beads (Supporting Information Figure S1).

Static and maximum capacities are usually measured for the resin characterization of column chromatography using non-magnetic protein A resin. However, the dynamic binding capacity (DBC) is recognized to be more useful to determine the operating conditions. DBC is defined as the amount of target that binds to the chromatography resin under specific flow rate conditions.[33–35] Besides the determination of the static and maximum capacities of the LOABeads PrtA, we wanted to obtain a term similar to the DBC as support for the selection of practical operating conditions in magnetic bead-based purification process. Based on our knowledge of the bead binding dynamics, we introduced the concept of dynamic bead binding capacity defined as 90% load of mAb after 1-h residence time $DBBC_{1-h}$, which can be used as a tool to help for estimating the right amount of beads. $DBBC_{1-h}$ provides 90% adsorption of antibody when applying 1 h of adsorption and the exact amount of beads specified by the $DBBC_{1-h}$ value. *Vice versa* this would mean 10% of the target molecule
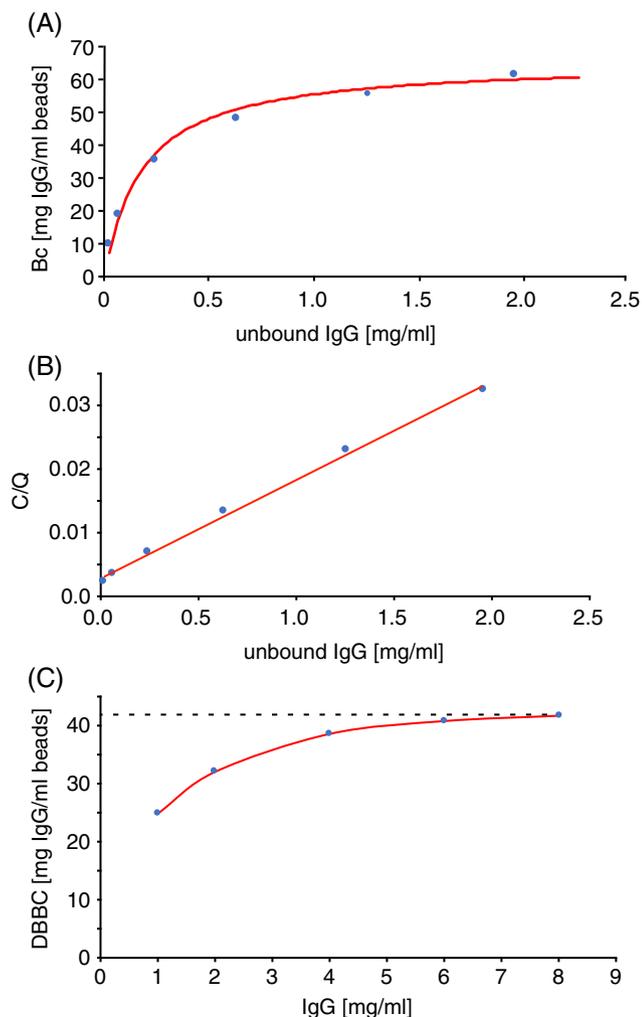
Figure 1. Capacity assays with a humanized mAb IgG1 on the magnetic protein A agarose beads, LOABeads PrtA. (A) Static binding capacity from adsorption equilibrium experiments fitted with Langmuir isotherm. (B) Linearized Langmuir isotherm (Hanes plot), to obtain the maximum binding capacity Q_{max}. $r^2 = 0.9962$. (C) Dynamic bead binding capacity, defined as 90% load of antibody after 1-h residence time, $DBBC_{1-h}$, for different concentrations of monoclonal antibody.

will be lost in the supernatant. In the case of IgG concentration higher than 1 g/L, if a higher adsorption is desired, a 10–20% excess of beads compared to the $DBBC_{1-h}$ value can be used.

In the case of purification using magnetic beads in suspension (of antibodies in present case), some of the main parameters that affect the adsorption and end yield are the amount of accessible protein A-ligands per bead, the concentration of antibodies and the time allowed for the antibody adsorption to the beads. To determine the $DBBC_{1-h}$ of the LOABeads PrtA, IgG1 antibodies were spiked in PBS at different concentrations reflecting a range of typical final antibody titers (1 to 8 g/L) in fed-batch process. The binding load capacity at 90% was measured and represented as function of these antibody concentrations. As shown in Figure 1C, the 90% binding load capacity for LOABeads PrtA increased with higher mAb input concentrations until a plateau was reached at ~7 g/L mAb concentration at a maximum of 42 mg IgG/mL bead resin. This latter value of 42 mg IgG/mL bead resin was the maximum $DBBC_{1-h}$ of the LOABeads PrtA. We used this $DBBC_{1-h}$ value as a first approximation to preliminary guide the bead usage in the first pilot scale experiment in absence of other available information. Notice however that the $DBBC_{1-h}$ is specific to an antibody due to the specific affinity (K_d) of an IgG for the protein A bead. It is therefore a valuable parameter to determine the practical operating conditions of bead concentration and time allowed for the adsorption.

Pilot-scale purification of clarified cell culture supernatant

The magnetic separator prototype process for pilot-scale purification, schematically represented in Figure 2, was initially tested for liquid handling and capability to separate and magnetically hold the high capacity superparamagnetic agarose LOABeads resin at various buffer flows (not shown). Thereafter, a first experiment was performed with 26 L clarified cell-free supernatant (*CF*) with a titer of 0.44 g mAb/L, run *CF*, to preliminary test the mAb capture by magnetic separation. For this first proof-of principle, the amount of 1 L beads was selected for the magnetic separation with operation target of 100 L/h. Although this was somewhat over-dimensioned for the bead loading capacity, this provided a test of the magnetic separator capacity. The adsorption took place in an external vessel, from which analytical samples were withdrawn at selected times up to 4 h. The bead suspension was then pumped into the magnetic chamber, the separated beads were washed, and finally eluted twice in a second chamber, i.e., the elution chamber. In this prolonged adsorption phase, a complete removal of mAb from the clarified cell suspension was obtained. The collected material from the consecutive elution´s was 9.5 and 0.5 mAb grams respectively in a total eluted volume of 2.5 L, corresponding to a high total yield of purified mAb of 87.4% (Figure 3A) and a concentration factor around 10 times. The first elution was monitored in the collected fractions by UV-detection and showed a near symmetrical elution profile (Figure 3B). Overall, this experiment demonstrated a purification of mAbs from a pilot-scale volume of supernatant using novel magnetic separator system and magnetic beads, and resulting in a significant concentration factor, comparable to legacy techniques based on column chromatography.
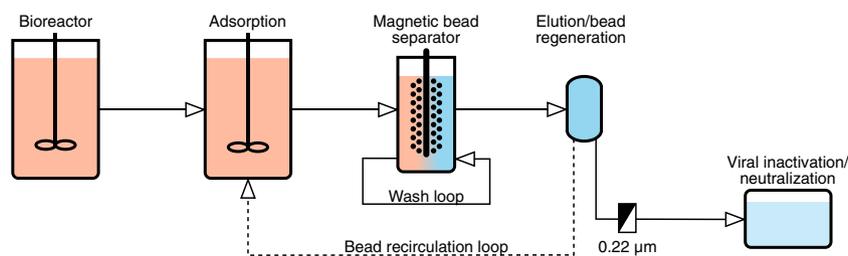

Figure 2. Schematic representation of the pilot-scale magnetic separation system used herein.
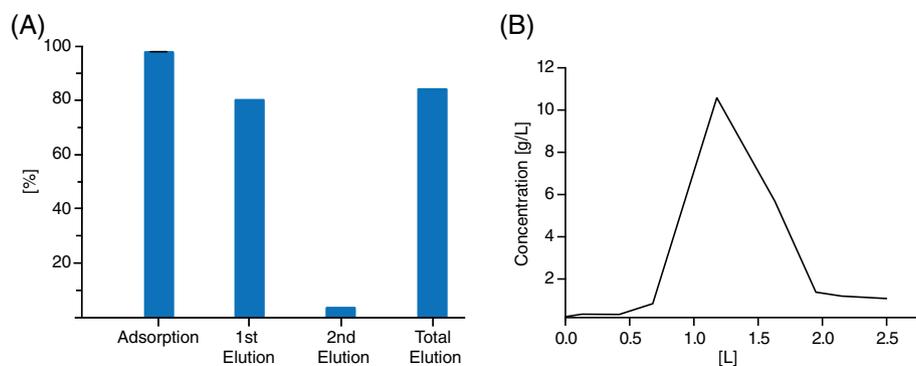
Figure 3. Pilot-scale purification using cell-free harvest, run *CF*. (A) Adsorption efficiency from 26 L clarified harvest with a mAb titer of 0.44 g/L, using 1 L of magnetic LOABeads PrtA, and the yields of the two sequential elution's as well as the total yield of elution. (B) Release profile from the first mAb elution measured by absorbance at 280 nm.

Effect of cell density on magnetic bead mAbs separation

The purpose of the present study was to achieve a one-step purification process of non-clarified cell broth based on magnetic beads. The effect of the cell density on the adsorption efficiency of mAb in cell broth was therefore studied for densities up to 40×10^6 cells/mL. An equal amount of mAb was present in several samples of 20 mL cell broth, where 1.5 mL magnetic protein A agarose beads was added. Cell-free supernatant, *i.e.* absence of cells, was used as reference. As shown in Figure 4, the mAb adsorption in the presence or absence of cells showed an equally high mAb capture, larger than 90%. These results obtained with non-clarified cell broth using magnetic beads, showed great promise for further development, and prompted us to proceed with pilot-scale purification using non-clarified cell broth.

Pilot-scale purification of non-clarified cell broth

The demonstrated functionality of the magnetic prototype separator in *CF* run and the high mAb adsorption in presence of cells, showed in previous sections, built the premise to perform pilot-scale purifications using non-clarified cell broth. Two experiments, run B1 and run B2, were performed essentially in the same way as run *CF*, from a technical point of view. The amount of magnetic beads was based on the mAb titer
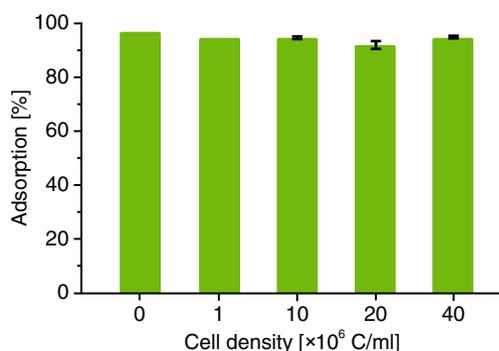

Figure 4. Study of mAb adsorption in cell broth at various cell densities up to 40×10^6 cells/mL. Culture samples of 20 mL volume containing a fixed amount of 20 mg mAb were tested for adsorption efficiency to magnetic LOABeads PrtA resin, with cell free sample as reference. Study performed in duplicate (indicated by error bars) except for the cell-free and 1×10^6 cells/mL samples.

determined the day before harvest. The input IgG concentrations, determined by HPLC the day before harvest, was expected to be between 1 and 2 g/L at harvest. Based on the guidance of the $DBBC_{1-h}$ chart (Figure 1C), and a bead capacity usage of 80%, 0.8 and 1 L beads were used for the 15.73 and 16.25 L of non-clarified cell broth of runs B1 and B2. For these pioneer experiments, we decided to opt for a conservative approach and used 20% more magnetic beads instead of the bead amount given by the $DBBC_{1-h}$ value from Figure 1C.

Learning from the experience of run *CF*, the total adsorption time was reduced from 4 h to 2 h. The adsorption curves of run B1 and run B2 (Figure 5A), showed a fast binding of the mAb to the beads with 99.5% and 95.5% mAb captured after 1 h as seen in Figure 5A, the adsorption time of 2 h could potentially be reduced, since an efficient mAb capture was already achieved after 1 h, and the adsorption rate was significantly lower after this time. After adsorption, the bead capture by the magnetic rods in the separator chamber was performed at 100 L/h. The remaining cell broth in the separation chamber was removed by buffer displacement at 100 L/h flow rate. The subsequent washing steps were carried out at the same flow rate. For the elution, the beads were transferred into the elution chamber connected to the magnetic separator by a liquid flow designed to obtain the beads in a concentrated form. The bottom of the elution chamber contained a nanofilter to retain the beads. Therefore, the elution profile resembled a chromatography elution and generated a highly concentrated product (Figure 5B).

In run B1, the elution was low with a total yield of 52% of the mAb input. An investigation of the possible causes of this low yield, revealed that the 100 mM citrate buffer used for run B1 elution had been erroneously prepared at pH of 3.56 instead of pH 3.0. To confirm that the pH was the source of the low yield of run B1, a test for elution efficiency at different pH was performed. As suspected, the elution efficiency was significantly affected at pH ≥ 3.5 with a sharp yield decrease at higher pH (Supporting Information Figure S2). For further experiments, fresh elution buffer was prepared and adjusted at pH 2.8. The elution profile for run B2 is given in Figure 5B, where the antibody was finally eluted in a volume of 1.0 L, generating a 16.25 times concentration factor, and a total mAb yield of 86%.

An advantageous feature of magnetic bead affinity is that the product capture can take place simultaneously throughout the whole input volume. In the present setup, over half of the mAb amount was already captured after 5 min, and an average of 88% and 97% was bound after 30 min and 1 h
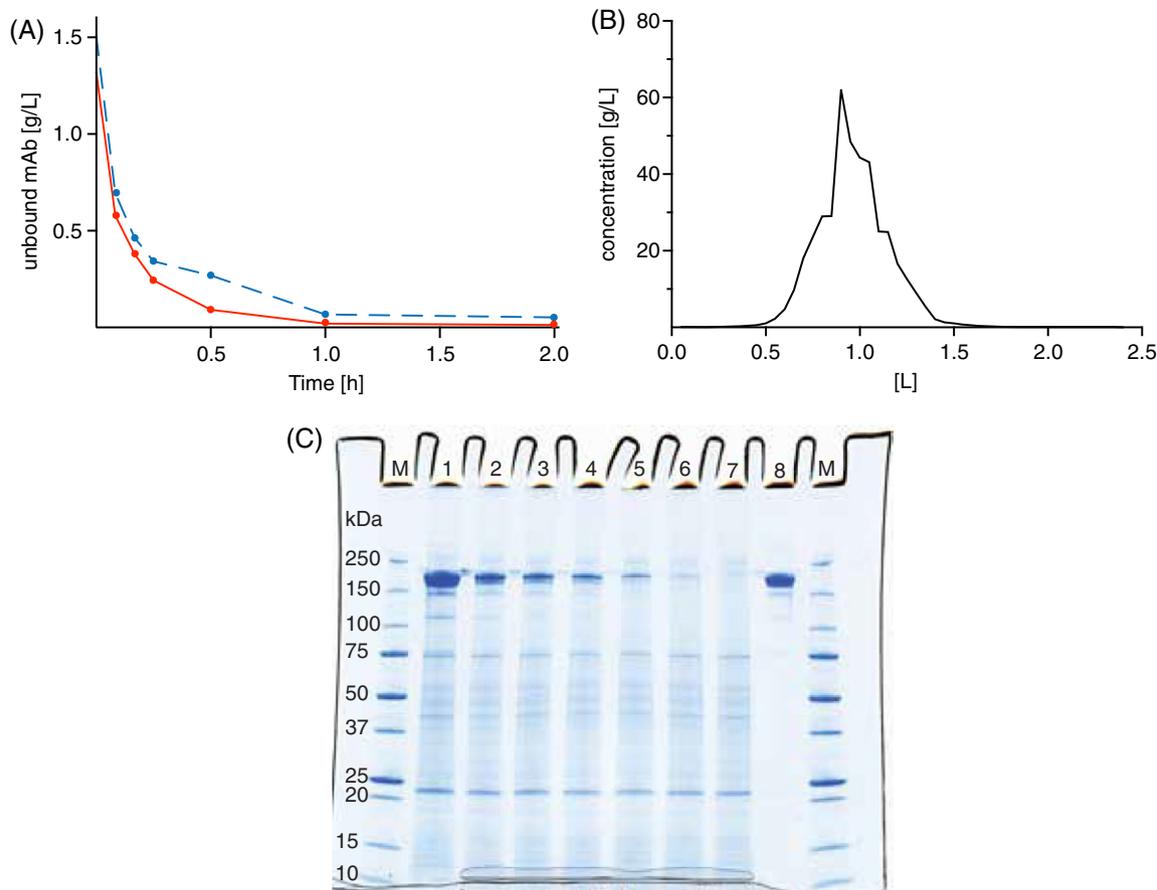
Figure 5. Pilot scale purification using non-clarified cell broth. **(A)** Adsorption efficiency from run B1, 15.73 L (red/solid), and run B2, 16.25 L (blue/dashed), with mAb concentrations of 1.31 and 1.51 g/L, respectively, using 0.8 and 1 L magnetic beads. **(B)** Elution profile from run B2, as measured at A_{280}. **(C)** Cell broth from run B2 at time points 0, 5, 10, 15, 30, 60, and 120 min, were centrifuged and analyzed by nonreduced SDS-PAGE (lanes 1 to 7), together with the purified and eluted mAb (lane 8).

(Figure 5A). The SDS-PAGE carried out on fractions collected at different time points during the adsorption visually confirmed the fast rate of mAb adsorption to the beads (Figure 5C). Importantly, the presence of cells in runs B1 and B2, with viabilities 89.9% and 75.9%, respectively, had no influence on the process. To support this, samples from run B2 cell broth, either as such or clarified by centrifugation, were purified in small-scale using magnetic beads. Highly similar outcomes were observed in this experiment where comparable total yields of 93 and 95%, respectively, were obtained and identical purity of eluted material as shown in the SDS-PAGE of these purifications (Figure 6). Overall, the purification at pilot-scale, performed here in a non-automated mode, took a total of 5.5 h, from the harvest of the cell broth to obtaining the purified mAb. Such a process performed in an automatized way and in optimized conditions would represent a substantial reduction of operation time and resources compared to column chromatography-based methods.

Concerning the elution step, the traditional way to work with magnetic beads batch-wise requires up to 20 bead volumes of final eluate to reach a high total yield.[8,31] This is due to the fact that a significant proportion of the mAb remains in the void volume of the separated beads and inside the stationary phase of the porous agarose bead; both factors requiring a large amount of elution buffer to extract the mAbs from the beads. On the contrary, elution from agarose beads packed in a chromatography column is efficiently achieved in 2 to 3 bead volumes of elution buffer. The elution step of the present

process was performed in a dedicated chamber in a semi-packed form. Compared to other separator systems introduced before, we were able to achieve a highly concentrated eluted product with only 1 bead volume of eluted material, which both systems reported by Hohlschuh and Schwämmle (20 bead volume eluted material)[8] and Ebeler (15 bead volume eluted material)[31] were not able to reach.

The magnetic beads and the separator are key elements to develop a purification process based on this kind of technology. The magnetic ProteinA Sepharose, a variant of agarose, used by Ebeler et al.[31] and the LOABeads PrtA, used in the present report, present similarities in a way that both are porous, made of agarose and have a diameter of comparable range. The magnetic separation performance is also important for a purification process and both studies used dedicated equipment with rods based on electromagnetism in Ebeler's report while we used retractable magnetic rods. Ebeler's and our report demonstrate that protein A purification process of CHO cell broth based on magnetic beads is feasible at scale by far larger than analytical purpose. The facts that these developments occurred separately in different academic groups and that the beads are issued from different manufacturers show that this type of technology has very good potential for the biopharmaceutical field. Importantly magnetic based purification can also be advantageous from an economical point-of-view. In the present study, both cell broth purification runs (B1 and B2) provided total process times of 7.5 h and 5.5 h (Table 1). These operations were performed in
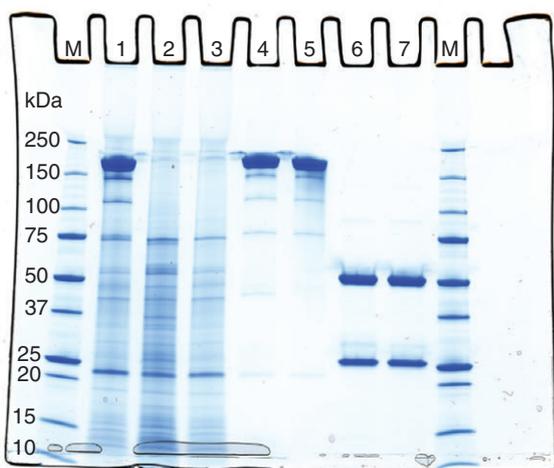
1. Input CB
2. Unbound fraction CB
3. Unbound fraction CF
4. Eluted mAb CB
5. Eluted mAb CF
6. Eluted mAb CB (+DTT)
7. Eluted mAb CF (+DTT)
M. Size marker

Figure 6. **Comparison of small-scale mAb purification (1.5 mL) by LOABeads PrtA carried out either on non-clarified cell broth (CB) or on cell-free harvest (CF) clarified by centrifugation, with efficiency and purity assessed by SDS-PAGE. The experiment was performed on the cell culture harvest of run cult_B2 in triplicate giving similar results (and of which one gel is presented here). Loaded amount of material: 4 μL of input CB and unbound fractions (the equivalent of flow-through in column chromatography), and 4 μg of the eluted antibody containing fractions, with the latter nonreduced or reduced (+DTT).**

manual mode. The automation of the system will allow reducing significantly this time so that two cycles will be feasible during a working day. Using magnetic beads will remove the cell separation step, and the mAb capture will require roughly one working day for an operator performing two cycles in a raw on the automatized magnetic separator. The amount of magnetic beads needed for 15 L cell culture with various mAb concentrations (Table 3) is within the range of the usage of traditional chromatography resins.

Assessment of HCP

The concentration of HCP during the process steps and in the final mAb product is a critical quality attribute.[36–40] The HCP of the input and the final eluted materials from runs B1 and B2 was investigated by ELISA. The input HCP concentrations of the supernatant gently clarified by low-speed centrifugation from runs B1 and B2, were 3758 and 5978 ppm/mg mAb, respectively. After the one-step process purification, described in Section Pilot-scale purification of non-clarified cell broth, the

Table 3. Amount of beads as a function of mAb titer. Display of different bead amounts calculated for various mAb titer for a 15 L cell culture

mAb concentration [g/L]	Needed amount of beads [L]*
1.5	0.8
3	1.2
5	1.9

*For a 15 L cell culture.
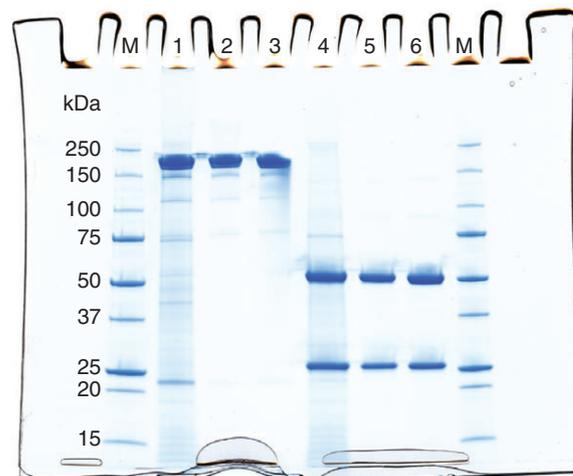
1. Input CB
2. Eluate LOABeads
3. Eluate HiTrap
4. Input CB (+DTT)
5. Eluate LOABeads (+DTT)
6. Eluate HiTrap (+DTT)
M. Size marker

Figure 7. **Comparison of mAb purification of non-clarified cell broth (CB) from run cult_B2 with magnetic LOA-Beads PrtA resin and purification of clarified cell-free harvest (CF) with a 5 mL HiTrap MabSelect SuRe column coupled to an ÄKTA instrument. Four microliter input CB material of run cult_B2 were loaded, as was 5 μg of purified and eluted products, either as nonreduced or reduced (+DTT) form.**

HCP levels of the eluted product were 4.2 and 7.5 ppm/mg mAb, respectively. This represents a significant decrease in HCP concentration compared to legacy steps including cell clarification and mAb capture (300 ppm/mg).[37,38,41] Furthermore, the magnetic protein A bead process system used herein, showed comparable or higher HCP removal, with a log removal of 2.9 for run B2 and 2.95 for run B1, compared to Borildo´s report using boronic acid magnetic resin for antibody purification.[42] The observed HCP levels achieved in a single step in runs B1 and B2 were < 10 ppm/mg mAb, a concentration at the lower end of the regulatory requirements (<1–100 ppm) for therapeutic biopharmaceuticals.[10,36,43–45] Our data suggest that a purification process based on magnetic bead has a strong advantage for the industry. It is highly plausible that the adsorption directly applied to the cell broth keeps the integrity of the cells, and therefore releases a significant smaller HCP amount compared to a process including a clarification step, and that likewise the host genome DNA level might be also significantly reduced. Low HCP and DNA-contents in the early phase of downstream process are attractive, since these might simplify the final polishing step(s) and represent as well an advantage for the patient safety.[36,39]

Comparison of magnetic beads purification and chromatography column purification

Column chromatography is the legacy for affinity-based purification. The yield and purity of the LOABeads-based process of run B2 was compared to affinity chromatography. For this, a sample of 82 mL harvest of cult_B2 run clarified by centrifugation was purified using a pre-packed 5 mL HiTrap MabSelect SuRe column coupled to an ÄKTA chromatography instrument and compared to run B2 performed on cell broth using magnetic beads. The HiTrap-column process had a yield of 88%, while run B2 yield was 86%. No difference in purity of the eluted

mAb in run B2 and in the column chromatography process was observed as visualized by SDS-PAGE (Figure 7). Significant differences in scales of operation, *e.g.*, bench-, pilot- and manufacturing-scale, can influence the process performances. The two different processes compared here differed not only in their size and resin volume, presence or absence of cells, but also in the technique. Regardless of these differences, the total yield and purity of both methods were the same. Furthermore, the HCP level of the magnetic protein A-bead purification of the antibody was very low as presented above.

Conclusions

An efficient pilot-scale process for affinity magnetic bead purification of antibodies was developed and evaluated using cell broth from fed-batch cultures. The performances obtained with this novel magnetic separator system show an interesting alternative method to the legacy protein A column chromatography used in the industry for the production of monoclonal antibodies. The use of magnetic beads added directly in the cell broth lift out the cell/debris/particle-removal step, and provides very low HCP levels in a one-step process. In fact, the HCP levels were so low after this single step, that the concentration required by the Authorities for human use of recombinant biotherapeutics was reached, representing a vast improvement over the classical chromatography method. Furthermore, the high increase in cell densities ($>100 \times 10^6$ cells/ml), driven by industrial intensification introduces more severe issues for the clarification step.

Acknowledgments

Conflicts of Interest

POE, KE, SO, and GH, have direct or indirect ties with the Swedish company Lab-on-a-Bead AB, whom owns intellectual property for the LOABeads magnetic protein A agarose resin and magnetic separator system used herein. NB, JB, AS, and VC, declare no conflict of interest.

Literature Cited

1. Beck A, Wurch T, Bailly C, Corvaia N. Strategies and challenges for the next generation of therapeutic antibodies. *Nat Rev Immunol*. 2010;10:345.
2. Swartz AR, Xu X, Traylor SJ, Li ZJ, Chen W. One-step affinity capture and precipitation for improved purification of an industrial monoclonal antibody using Z-ELP functionalized nanocages. *Biotechnol Bioeng*. 2018;115(2):423–432.
3. Ghose S, Allen M, Hubbard B, Brooks C, Cramer SM. Antibody variable region interactions with protein A: implications for the development of generic purification processes. *Biotechnol Bioeng*. 2005;92(6):665–673.
4. Kelley B. Industrialization of mAb production technology: the bioprocessing industry at a crossroads. *MAbs*. 2009;1(5):443–452.
5. Branco RJF, Dias AMGC, Roque ACA. Understanding the molecular recognition between antibody fragments and protein A biomimetic ligand. *J Chromatogr A*. 2012;1244:106–115.
6. Shukla AA, Thömmes J. Recent advances in large-scale production of monoclonal antibodies and related proteins. *Trends Biotechnol*. 2010;28(5):253–261.
7. Käppler, T.; Posten, C., In-situ Magnetic separation (ISMS): protein fishing in reactor-scale. 2008.
8. Holschuh K, Schwämmle A. Preparative purification of antibodies with protein A—an alternative to conventional chromatography. *J Magn Magn Mater*. 2005;293(1):345–348.
9. Thömmes J, Etzel M. Alternatives to chromatographic separations. *Biotechnol Prog*. 2007;23(1):42–45.
10. Hogwood CEM, Tait AS, Koloteva-Levine N, Bracewell DG, Smales CM. The dynamics of the CHO host cell protein profile during clarification and protein A capture in a platform antibody purification process. *Biotechnol Bioeng*. 2013;110(1):240–251.
11. Pohlscheidt M, Lieser G, Bertermann B-E, Eberhardt H, Herter S, Tebbe H. At scale analysis and optimization of a cell culture harvest process– mitigating risk and optimizing performance. *Am Pharm Rev*. 2013.
12. Hubbuch JJ, Thomas OR. High-gradient magnetic affinity separation of trypsin from porcine pancreatin. *Biotechnol Bioeng*. 2002; 79(3):301–313.
13. Franzreb M, Siemann-Herzberg M, Hobley TJ, Thomas ORT. Protein purification using magnetic adsorbent particles. *Appl Microbiol Biotechnol*. 2006;70(5):505–516.
14. Dunnill P, Lilly MD. Purification of enzymes using magnetic bio-affinity materials. *Biotechnol Bioeng*. 1974;16(7):987–990.
15. Wu W, He Q, Jiang C. Magnetic iron oxide nanoparticles: synthesis and surface functionalization strategies. *Nanoscale Res Lett*. 2008;3(11):397.
16. Morgan PE, Thomas OR, Dunnill P, Sheppard AJ, Slater NK. Polyvinyl alcohol-coated perfluorocarbon supports for metal chelating affinity separation of a monoclonal antibody. *J Mol Recognit*. 1996;9(5–6):394–400.
17. Yavuz CT, Prakash A, Mayo JT, Colvin VL. Magnetic separations: from steel plants to biotechnology. *Chem Eng Sci*. 2009;64 (10):2510–2521.
18. Gañán J, Pérez-Quintanilla D, Morante-Zarcero S, Sierra I. Comparison of different mesoporous silicas for off-line solid phase extraction of 17β-estradiol from waters and its determination by HPLC-DAD. *J Hazard Mater*. 2013;260:609–617.
19. Elmizadeh H, Khanmohammadi M, Ghasemi K, Hassanzadeh G, Nassiri-Asl M, Garmarudi AB. Preparation and optimization of chitosan nanoparticles and magnetic chitosan nanoparticles as delivery systems using box–Behnken statistical design. *J Pharm Biomed Anal*. 2013;80:141–146.
20. Toh PY, Yeap SP, Kong LP, Ng BW, Chan DJC, Ahmad AL, Lim JK. Magnetophoretic removal of microalgae from fishpond water: feasibility of high gradient and low gradient magnetic separation. *Chem Eng J*. 2012;211-212:22–30.
21. He J, Huang M, Wang D, Zhang Z, Li G. Magnetic separation techniques in sample preparation for biological analysis: a review. *J Pharm Biomed Anal*. 2014;101:84–101.
22. Batalha IL, Hussain A, Roque A. Gum Arabic coated magnetic nanoparticles with affinity ligands specific for antibodies. *J Mol Recognit*. 2010;23(5):462–471.
23. Schügerl K, Hubbuch J. Integrated bioprocesses. *Curr Opin Microbiol*. 2005;8(3):294–300.
24. Cerff M, Scholz A, Franzreb M, Batalha IL, Roque ACA, Posten C. In situ magnetic separation of antibody fragments from *Escherichia coli* in complex media. *BMC Biotechnol*. 2013;13(1):44.
25. Roque ACA, Silva CSO, Taipa MÂ. Affinity-based methodologies and ligands for antibody purification: advances and perspectives. *J Chromatogr A*. 2007;1160(1):44–55.
26. Käppler TE, Hickstein B, Peuker UA, Posten C. Characterization of magnetic ion-exchange composites for protein separation from biosuspensions. *J Biosci Bioeng*. 2008;105(6):579–585.
27. Maury TL, Ottow KE, Brask J, Villadsen J, Hobley TJ. Use of high-gradient magnetic fishing for reducing proteolysis during fermentation. *Biotechnol J*. 2012;7(7):909–918.

28. Fish NM, Lilly MD. The interactions between fermentation and protein recovery. *Bio/Technology*. 1984;2:623.

29. Safarik I, Safarikova M. Use of magnetic techniques for the isolation of cells. *J Chromatogr B Biomed Sci Appl*. 1999;722.

30. Safarik I, Safaříková M, Forsythe SJ. The application of magnetic separations in applied microbiology. *J Appl Bacteriol*. 1995;78 (6):575–585.

31. Ebeler M, Lind O, Norrman N, Palmgren R, Franzreb M. One-step integrated clarification and purification of a monoclonal antibody using protein A mag Sepharose beads and a cGMP-compliant high-gradient magnetic separator. *New Biotechnol*. 2018;42:48–55.

32. Zhuo L, Mostafa SS, Shukla AA. A comparison of protein A chromatographic stationary phases: performance characteristics for monoclonal antibody purification. *Biotechnol Appl Biochem*. 2015;62(1):37–47.

33. Do T, Ho F, Heidecker B, Witte K, Chang L, Lerner L. A rapid method for determining dynamic binding capacity of resins for the purification of proteins. *Protein Expr Purif*. 2008;60(2): 147–150.

34. McCue JT, Kemp G, Low D, Quiñones-García I. Evaluation of protein-A chromatography media. *J Chromatogr A*. 2003;989(1): 139–153.

35. Hahn R, Bauerhansl P, Shimahara K, Wizniewski C, Tscheliessnig A, Jungbauer A. Comparison of protein A affinity sorbents: II. Mass transfer properties. *J Chromatogr A*. 2005; 1093(1):98–110.

36. Tait AS, Hogwood CE, Smales CM, Bracewell DG. Host cell protein dynamics in the supernatant of a mAb producing CHO cell line. *Biotechnol Bioeng*. 2012;109(4):971–982.

37. Follman DK, Fahrner RL. Factorial screening of antibody purification processes using three chromatography steps without protein A. *J Chromatogr A*. 2004;1024(1):79–85.

38. Shukla AA, Hubbard B, Tressel T, Guhan S, Low D. Downstream processing of monoclonal antibodies—application of platform approaches. *J Chromatogr B*. 2007;848(1):28–39.

39. Shukla AA, Jiang C, Ma J, Rubacha M, Flansburg L, Lee SS. Demonstration of robust host cell protein clearance in biopharmaceutical downstream processes. *Biotechnol Prog*. 2008;24(3): 615–622.

40. Adith V, Patrick H, Sandro M, Are B, Arne S, Susan S, Georges B. Optimized removal of soluble host cell proteins for the recovery of met-human growth hormone inclusion bodies from *Escherichia coli* cell lysate using Crossflow microfiltration. *Biotechnol Prog*. 2007;23(3):667–672.

41. Ghose S, Hubbard B, Cramer SM. Evaluation and comparison of alternatives to protein A chromatography: mimetic and hydrophobic charge induction chromatographic stationary phases. *J Chromatogr A*. 2006;1122(1):144–152.

42. Borlido L, Azevedo AM, Sousa AG, Oliveira PH, Roque ACA, Aires-Barros MR. Fishing human monoclonal antibodies from a CHO cell supernatant with boronic acid magnetic particles. *J Chromatogr B*. 2012;903:163–170.

43. Hogwood CE, Bracewell DG, Smales CM. Host cell protein dynamics in recombinant CHO cells: impacts from harvest to purification and beyond. *Bioengineered*. 2013;4(5):288–291.

44. Champion K, Madden H, Dougherty J, Shacter E. Defining your product profile and maintaining control over it, part 2. *Bio Process Int*. 2005;3(8):52–57.

45. Chon JH, Zarbis-Papastoitsis G. Advances in the production and downstream processing of antibodies. *New Biotechnol*. 2011;28 (5):458–463.