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Review

Scale-up of monoclonal antibody purification processes☆

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Abstract

Mammalian cell culture technology has improved so rapidly over the last few years that it is now commonplace to produce multi-kilogram quantities of therapeutic monoclonal antibodies in a single batch. Purification processes need to be scaled-up to match the improved upstream productivity. In this chapter key practical issues and approaches to the scale-up of monoclonal antibody purification processes are discussed. Specific purification operations are addressed including buffer preparation, chromatography column sizing, aggregate removal, filtration and volume handling with examples given.

Keywords: Monoclonal antibodies; Antibody purification; Scale-up; GMP antibody production

Contents

1. Introduction

Monoclonal antibodies have proved to be a highly successful, if expensive, class of therapeutic product. One factor contributing to their high cost is the fact that therapeutic doses of most antibodies are much higher than the doses of other therapeutic proteins or small molecules. One dose of a hormone, e.g. erythropoietin or human growth hormone, is typically a few micrograms of protein, but one dose of a therapeutic antibody may be a million-fold higher with doses of a gram or more quite common. Consequently, very large scale production facilities are required for monoclonal antibodies that are clinically successful and scaling-up an antibody purification process can cover a wide range of operational scales.

The industrial manufacture of pharmaceutical antibodies is a complex task that requires considerable effort in both process and analytical development [1]. The manufacturing processes for such antibodies are likely to require scale-up at several

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stages of product development. Processes are typically developed in the laboratory with milligram quantities of product. Eventually, if the product is a clinical success, a cGMP manufacturing process will need to be developed to produce many tens of kilograms per batch. This represents a scale-up factor of approximately 10^6, a considerable technical challenge. Scale-up is further complicated by the fact that other changes to the manufacturing process are likely to be made in parallel with a simple increase in scale [2]. For example, a more productive cell line or new cell culture conditions may be introduced in parallel with an increase in scale. Also, as the project passes through the different stages of pre-clinical and clinical development, the process will be operated by different people in different departments using different equipment, e.g. process development, pilot plant, manufacturing, etc. Furthermore, when scale-up difficulties arise, there is limited time available to investigate the problem and often robust but pragmatic solutions are put in place to circumvent the problem without developing a thorough understanding of the mechanisms involved. Therefore, the implementation and scale-up of a cGMP manufacturing process for a therapeutic antibody is not a straightforward exercise [3]. This is even more challenging for multi-product facilities where processes can be much more variable and one-off batches form the bulk of the manufacturing capability. Processes developed several years ago, employing older technology will be operated alongside newer processes developed with totally different types of chromatography resins and will require different modes of operation.

There are however two features of monoclonal antibodies (MAbs) that make the scale-up task significantly easier for this class of protein than for other proteins. Firstly, MAbs are generally very stable molecules that tolerate relatively harsh treatments, e.g. extremes of pH, shear, etc. Secondly, a highly specific affinity ligand, Protein A, is available that binds to most classes of therapeutically relevant human antibodies [4–7]. Antibody purification processes that avoid the use of Protein A affinity chromatography have been investigated but it is difficult to develop an alternative step that can achieve the same degree of purification as Protein A chromatography [8,9]. There are now commercially available affinity chromatography resins based on Protein A where the Staphyloccal Protein A molecule has been specifically tailored by amino acid deletion and substitution for the industrial scale manufacture of monoclonal antibodies [10]. The availability of chromatography resins specifically designed for large scale antibody manufacture, coupled to the stability of most monoclonal antibodies, has greatly aided the scale-up of manufacturing processes, but there are still many scale-up considerations and many pitfalls that need to be avoided.

Strategies for the development and scale-up of purification processes are constantly changing due to the rapid improvement in the productivity of antibody-producing cell lines in the last few years [11]. The downstream processing areas of most large scale cGMP manufacturing facilities were not designed to handle the quantity of product that the new cell lines can produce, and this is driving the need to maximise product loading onto chromatography columns and optimise throughput whilst limiting product aggregation. In addition, concerns over the safety of therapeutic proteins have driven the development of completely chemically defined fermentation media free of any animal components. This has aided the purification of monoclonal antibodies to the extent that it is often possible to purify these molecules to the required standard using just two chromatography steps instead of the three steps that are generally used for most therapeutic antibodies.

2. Scale-up considerations for the large scale manufacture of therapeutic antibodies

The scale-up of manufacturing processes can be divided into a number of stages. In some organisations, the transition from one stage to another is formalised into “Manufacturability Reviews” which may correspond to the project moving from one department to the next, e.g. from research to process development, from process development to pilot plant, etc. Ideally, large scale manufacturing considerations should be taken into account even before a candidate antibody molecule is first purified for research purposes, as this can save a great deal of time and effort later in the development of the therapeutic product.

Some of these considerations are straightforward, for example the use of chromatographic resins that are chemically and physically robust and easy to clean. However, there are many other considerations that may not be obvious and may be ignored or at best are considered a low priority when making research grade antibodies. Many of these requirements are specified in cGMP rules and guidance documents. For example, the use of fully traceable cell lines; the avoidance of any animal-derived raw materials in the production process; the use of endotoxin-free components supplied with Certificates of Analysis, and the incorporation of virus clearance steps into the manufacturing process are all key aspects of the manufacture of proteins for therapeutic use. Neglecting any of these aspects can be an expensive mistake as it can result in a requirement to make process changes once clinical trials are underway, which in turn can lead to time-consuming comparability studies and potentially even additional clinical trials if significant process changes are introduced.

There are other considerations that apply to any protein that will be manufactured at a large scale. We have used the term ‘large scale’ to describe processes that produce multi-kilogram quantities of drug substance per batch, roughly equivalent to manufacturing facilities that can produce close to tonne quantities per year. At this scale, a fully optimised process is essential to maximise the throughput of the production plant, as measured in the number of kilograms produced per week, and to control manufacturing costs [12], often measured in dollars per gram [13]. cGMP regulations specify that the critical parameters of a manufacturing process are identified and controlled, but it is possible to have a cGMP-compliant process that has not been optimised. For example, the use of large volumes of buffer at a slow flow rate to wash a chromatography column, or the use of chemicals that cannot be piped into a wastewater treatment plant, are cGMP-compliant operations, but are not an efficient use of time and resources. Thus, process optimisation is essential for efficient, cost-effective processes and often goes hand-in-hand with process scale-up. Later sections of this chapter will illus-

trate the impact of process optimisation on the final full-scale manufacturing operation.

3. Typical stages of the scale-up of antibody purification processes

The first stage of process scale-up is usually a paper exercise where calculations are performed to determine what the small scale process would look like if scaled-up linearly. For example, when scaling-up a process 100-fold from a 20 L fermenter to a 2000 L fermenter, is a 100-fold increase in the volumes of the chromatography columns feasible? For ultrafiltration operations, is a 100-fold scale-up of membrane area feasible? Key questions at this stage are (1) how does the linearly scaled process compare to the capabilities of the manufacturing plant? (2) what would the throughput of the plant be? and (3) what would the manufacturing costs be? Often a linear increase in chromatography column volume and membrane area is either not practical or is prohibitively expensive and some optimisation work is required. The output of this first stage of scale-up is a list of unit operations that need optimisation with performance targets, e.g. a requirement to filter 500 L of product in less than 2 h.

The second stage is the experimental stage where laboratory studies are performed to optimise the process. Key parameters for chromatography operations are the dynamic binding capacity and the cycle time. Other parameters that are important for large scale operations include the number of different buffers required and their volume, and the volume of the product collection tanks. For ultrafiltration (UF), it is advantageous to operate at as high a protein concentration as possible as this has a direct impact on the volumes of buffers required and the size of the product tanks required. Therefore, studies are often performed to test the maximum protein concentration achievable with different UF membranes without causing protein aggregation or other changes to the product. For a cGMP manufacturing operation, it is useful to have experimental data on the stability of the product at different stages of the purification process so that possible hold points can be defined. Even if such hold points are not planned in the purification process, unforeseen events can hold up processing and stability data can be very useful to determine where and how to hold part-purified product. All this optimisation data is generally captured in one or more documents sometimes called a "Purification Process Description" that details all the relevant flow diagrams, unit operations, operating parameters, in-process assays required, etc.

The third stage of scale-up is often a trial of the optimised process at an intermediate scale, i.e. one or more pilot runs. Traditionally, pilot runs are one-tenth of the final manufacturing scale, but limiting scale-up to 10-fold jumps in scale is not necessary. However, more than one pilot run can be particularly useful as they will provide information on: (a) how well the process has scaled up, (b) information on the reproducibility of the process, and (c) useful data on the re-use of purification components, such as chromatography resins and filtration membranes. Ideally these pilot runs would be scheduled to allow a full set of analytical data to be collated on each completed run before the next run is commenced but this might not always be possible within the time constraints of a development programme. However, the risk is reduced if the process parameters and their limits have been well defined and characterised at laboratory scale.

Assuming that the results of the pilot runs indicate that the process has scaled-up successfully, the next stage is a transition into the final scale of operation. This may be in the form of one or more "engineering runs", which are designed to test the equipment and the documentation at full scale. These runs also serve to train the operators and the runs may be the final chance to make any minor process modifications, e.g. to the chromatographic peak collection criteria, which are often based on the output of a UV detector. Again assuming that the engineering runs are successful, the process is considered scaled-up and ready for full-scale manufacture. For therapeutic products that require approval from Regulatory bodies, the engineering runs may be followed by a series of production runs termed process validation or consistency runs. These are a set of consecutive runs that are analysed in detail to demonstrate that the process can operate reliably at the manufacturing scale and that all the unit operations can consistently perform their intended functions. These runs are a requirement from a regulatory perspective but are not an inherent part of scaling up a process and therefore they are not considered further here.

Subsequent sections of this chapter illustrate aspects of the scale-up of purification processes in the form of case studies or specific examples. In this short chapter, we have not been able to cover all aspects of process scale-up and therefore we have used four examples to illustrate some of the specific processing aspects: buffer preparation, column dimensions/packing on scale-up, aggregate removal and filtration operations.

4. Scale-up of buffer preparation

Several buffers and various cleaning and storage solutions are required to operate a purification process and the total number of buffers/solutions is typically between 10 and 20. As scale increases, cost of goods becomes increasingly important and buffers commonly used at laboratory scale may not be cost effective at larger scale. For example, when a formulated HEPES buffer is used to equilibrate an affinity column, the chemicals required cost the equivalent of approximately £3 per litre. The chemicals required for a formulated phosphate buffer used for the same purpose on the same resin costs the equivalent of approximately £0.4 per litre. Therefore, apart from assessing the suitability of a buffer in the process, the cost of raw materials is a significant factor for scaling up.

On determining the suitability of buffers for large scale operation, the buffers should be monitored routinely during process development to check their pH and conductivity. Preparation of each buffer at different scales by different operators in different laboratories, with routine checking of pH and conductivity, will result in pH and conductivity specifications which are more likely to be achieved during cGMP operation. The specifications set should include an acceptable processing range based on process limits studies which include an assessment of the effect on processing when the specifications are exceeded. For example,

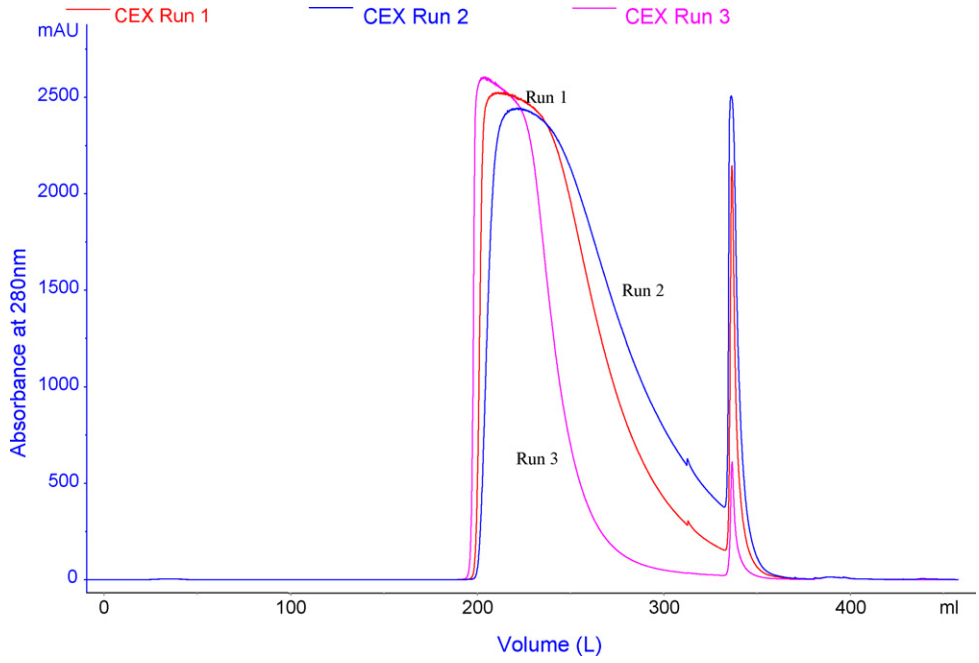

Fig. 1. Overlay of three cation exchange chromatograms recorded using elution buffers with different conductivities.

the pH specification for a buffer may be the target pH value ±0.1 pH units. For conductivity and osmolality, a suitable range may be a target value ±10%. All specifications should be based on a particular temperature range, this is especially important for conductivity.

Buffers and solutions used in cleaning processes or in column regeneration may not need tight specifications; pH and conductivity values may be acceptable as 'greater than' or 'less than' values ensuring that a defined limit is achieved. Buffers containing high concentrations of salts may require a review to ascertain their compatibility with the materials of construction of the process equipment. Where such buffers are used, their conductivity can only be measured approximately given the non-linearity of conductivity at high salt concentrations.

Other buffers which have a specific purpose in the purification process (such as product elution buffers on ion exchange resins) may need a much tighter conductivity range. This is illustrated in Fig. 1, where three runs of antibody purification on a cation exchange chromatography column are overlaid. The cation exchange column was equilibrated with the same equilibration buffer and loaded with the same amount of product in each of the three runs. The only variable was the conductivity of the elution buffer which was adjusted by the addition of sodium chloride. This buffer had a specification of 15.00 ± 0.75 mS/cm, i.e. a midpoint of 15 mS/cm with a relatively narrow range of ±5%.

Run 1 used an elution buffer at the target or midpoint conductivity; for Run 2 the elution buffer was at the lower limit of the conductivity specification, i.e. 5% below the target; for Run 3 the buffer was at the upper limit of the specification, i.e. 5% above the target. The elution buffer at the highest conductivity resulted in a reduced eluate volume and increased level of aggregated product (see Table 1), whilst the elution buffer with the lowest conductivity resulted in an increased elution volume and a reduced aggregate level. In this example, additional measures were put in place to minimise buffer variability and process limits studies were conducted to demonstrate that the purification process could meet all specifications irrespective of whether the buffer was at the high or low end of its conductivity range. Therefore, for particularly sensitive processing steps, careful control of buffer make-up is essential for robust and reproducible results.

Differences in the procedures used for buffer preparation should also be taken into account. Buffer preparation at laboratory scales tends to be based on volume. At increasing scales, buffer preparation is usually based on weight and for dilute buffers a default value of 1 l = 1 kg can be used. However, when preparing certain buffers by weight, e.g. buffers used for hydrophobic interaction chromatography, which have high

Table 1
Comparison of the performance of a cation exchange column using elution buffers with different conductivities

Run	Elution buffer conductivity (mS/cm)	Eluate volume (CV)[a]	% Product recovery in eluate fraction	% Aggregated product in eluate fraction
1	15.00 (midpoint)	3.6	88.3	0.42
2	14.25 (lower limit)	3.8	79.2	0.26
3	15.75 (higher limit)	2.2	89.1	0.72

[a] CV, column volumes.

concentrations of sodium chloride or ammonium sulphate, or preparing buffers with high concentrations of sucrose or similar chemicals used in final formulations, buffer density must be taken into account.

To maintain consistency throughout process development and scale-up, buffers should be prepared using the same grade of chemicals. If chemicals from different suppliers are used, conductivity specifications in particular may vary. For example, a 100 mM citric acid buffer prepared with citric acid monohydrate supplied by Merck has a conductivity specification of 3.30 ± 0.50 mS/cm (when measured at a temperature of 23.0 ± 1.0 °C). When the same buffer is prepared using citric acid of the same chemical composition supplied by JT Baker, the conductivity specification is 2.80 ± 0.50 mS/cm measured at the same temperature.

Ideally all buffers should be formulated and not titrated to the required pH using acid or base even during initial process development. This allows the formulations to be evaluated as the scale of buffer preparation increases. The use of formulated buffers simplifies buffer make-up operations at large scale and also reduces the risk of overshooting a pH target with a non-formulated buffer.

5. Column packing and column dimensions on scale-up

The scale-up of chromatography operations is usually achieved by increasing the column diameter whilst maintaining the resin bed height and linear flow rate. This ensures that the residence time is the same at all scales of operation. Generally, resins are easy to pack at laboratory scale although columns with diameters of 1 cm and less can be problematical and prone to drying out. Pre-packed laboratory scale chromatography columns are available from suppliers, but these are more useful for evaluating operating conditions rather than a basis for scale-up. As scale increases, column packing becomes more troublesome [14,15], and certain operating ranges may not be achievable, particularly with non-rigid chromatographic media [16,17]. For example, when using a Sepharose 4 Fast Flow chromatography resin with a bed height of 20 cm at a temperature of 6 °C, production scale columns (diameter ≥45 cm) could not be operated at a linear flow rate of 100 cm/h [18]. To achieve this flow rate either the bed height had to be reduced to 15 cm or the temperature increased to 22 °C.

With large diameter columns (> approximately 30 cm) equipment is available that can automate column packing by pumping a resin slurry into the column. This type of equipment is now commonplace in large manufacturing facilities, but it should be noted that each type of resin behaves differently in terms of the conditions required to achieve optimum packing. Therefore, extensive trials are required to develop robust and optimal packing and unpacking procedures for such pack-in-place columns.

Table 2 below shows an example of a scale-up scenario for a monoclonal antibody process comprising three chromatography steps. This scale-up scenario was the result of both technical considerations and also pragmatic commercial ones. Common technical considerations include the rigidity of the resins, their validated lifetime and how well they withstand packing and unpacking (some ceramic chromatography matrices are almost impossible to unpack without damaging them). Commercial considerations may include the length of the manufacturing campaigns, the resin costs, and the relative economics of using a small column to purify one batch of product with a large number of sequential cycles of operation, versus using a large column without multi-cycling. Often shift patterns, buffer volume constraints, tank limitations as well as raw material costs and product stability issues need to be considered. These commercial and technical considerations are often complex and may be com-

Table 2
Column dimensions used at different scales of production – example for a monoclonal antibody purification process

	Lab scale	Pilot scale	Small scale manufacture	Large scale manufacture
Fermenter volume	1–10 L	130 L	2,000 L	20,000 L
Grams per batch	<10 g	156 g	2.4 kg	24 kg
Purpose of purification	Process development/troubleshooting	Pilot runs	Manufacturing for clinical trials	In-market supply
Affinity chromatography				
Column volume	119 mL	1.63 L	14.8 L	323 L
Column diameter	2.6 cm	10 cm	30 cm	140 cm
Column bed height	22.4 cm	20.8 cm	21 cm	21 cm
No. of cycles per batch	n/a	5	9	4
Anion exchange				
Column volume	48.2 mL	2.0 L	13.1 L	385 L
Column diameter	1.6 cm	10 cm	25 cm	140 cm
Column bed height	24 cm	25.6 cm	26.7 cm	25 cm
No. of cycles per batch	n/a	2	4	2
Cation exchange				
Column volume	28.1 mL	4.71 L	39.6 L	471 L
Column diameter	1.6 cm	20 cm	60 cm	200 cm
Column bed height	14 cm	15.0 cm	14.0 cm	15 cm
No. of cycles per batch	n/a	2	4	3
Column packing technology	Manual	Manual	Manual or automated pack-in-place	Automated pack-in-place

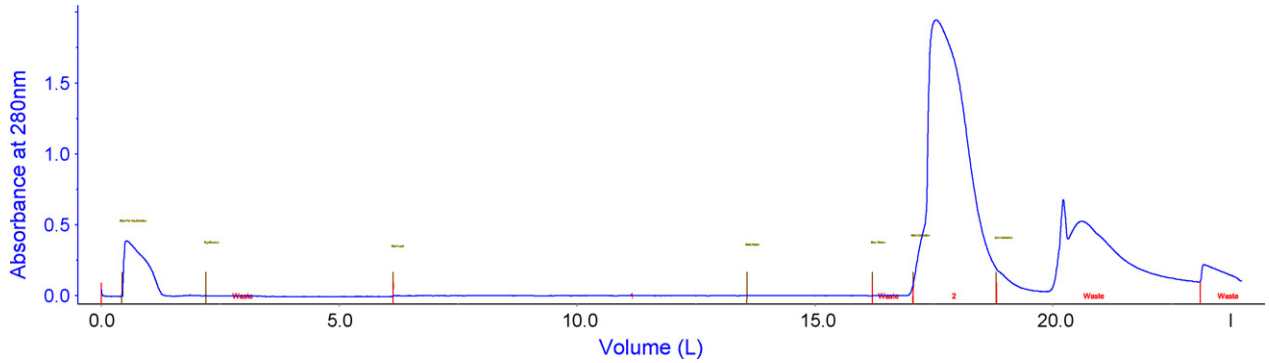

Fig. 2. Chromatogram recorded for a 7 cm diameter, 22 cm bed height HIC column.

pletely different from one project or one manufacturing facility to another.

Often the technical considerations are more straightforward than the commercial ones, but nonetheless difficulties can arise. For example, when scaling up chromatography operations, increasing pressure and bed instability can become problems. The latter can sometimes be corrected by making adjustments to the column packing procedure [18]. This has been observed with a hydrophobic interaction chromatography (HIC) step.

At small scale, using a 7 cm diameter column packed to a bed height of 22 ± 2 cm using a linear flow rate of 300 cm/h, and operated at 200 cm/h, there were no operational issues observed (Fig. 2). The process was scaled-up to a 35 cm diameter column and a similar chromatogram was obtained (Fig. 3).

However, when the column diameter was increased further to 44 cm, the bed became unstable and channelling was observed during elution of the product from the resin. An indication that there was a problem was observed during column loading, when the absorbance at 280 nm increased slightly above the baseline indicated product breakthrough, and also during product elution when a split peak was observed (Fig. 4).

This problem was resolved by a change to the packing method and the operating conditions. In this case, an increase in the packing flow rate to 450 cm/h and a reduction in the operating flow rate resolved the problem (Fig. 5).

6. Aggregate removal at different scales of operation

Protein aggregation is a common difficulty encountered during protein drug development but the mechanism of aggregate formation is poorly understood [19]. The level of aggregates in the product often determines the requirement for a third chromatography step in an antibody purification process. Aggregate removal at laboratory scale can be achieved readily using

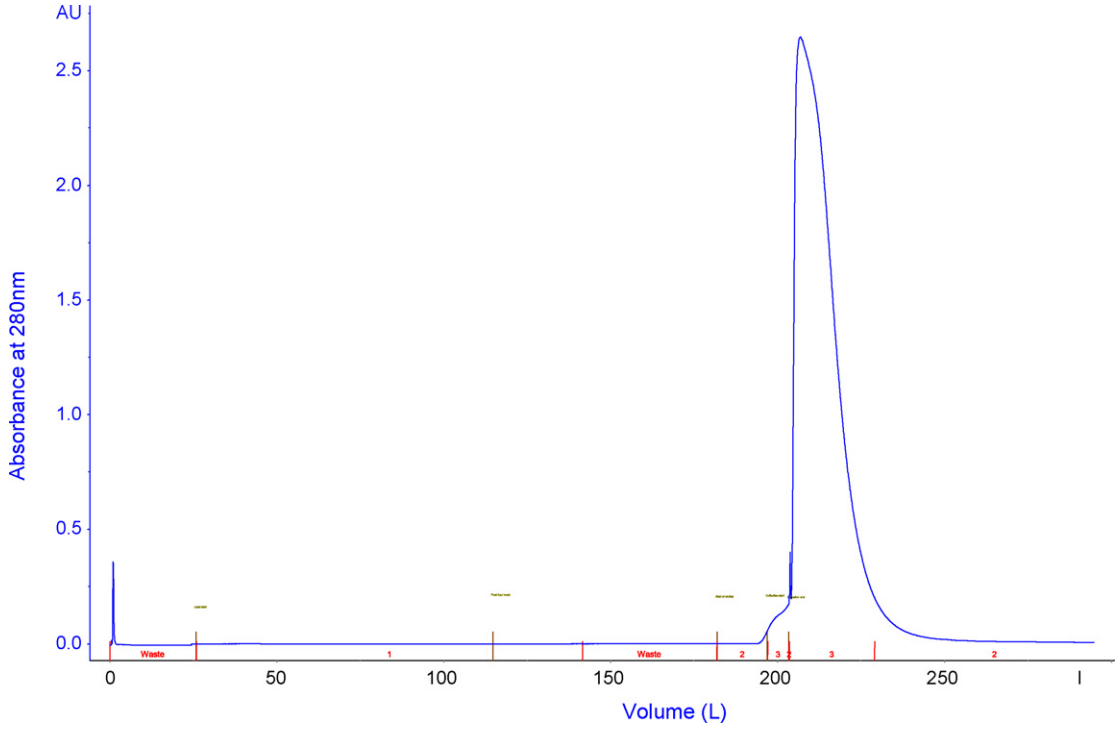

Fig. 3. Chromatogram recorded for a 35 cm diameter, 22 cm bed height HIC column.

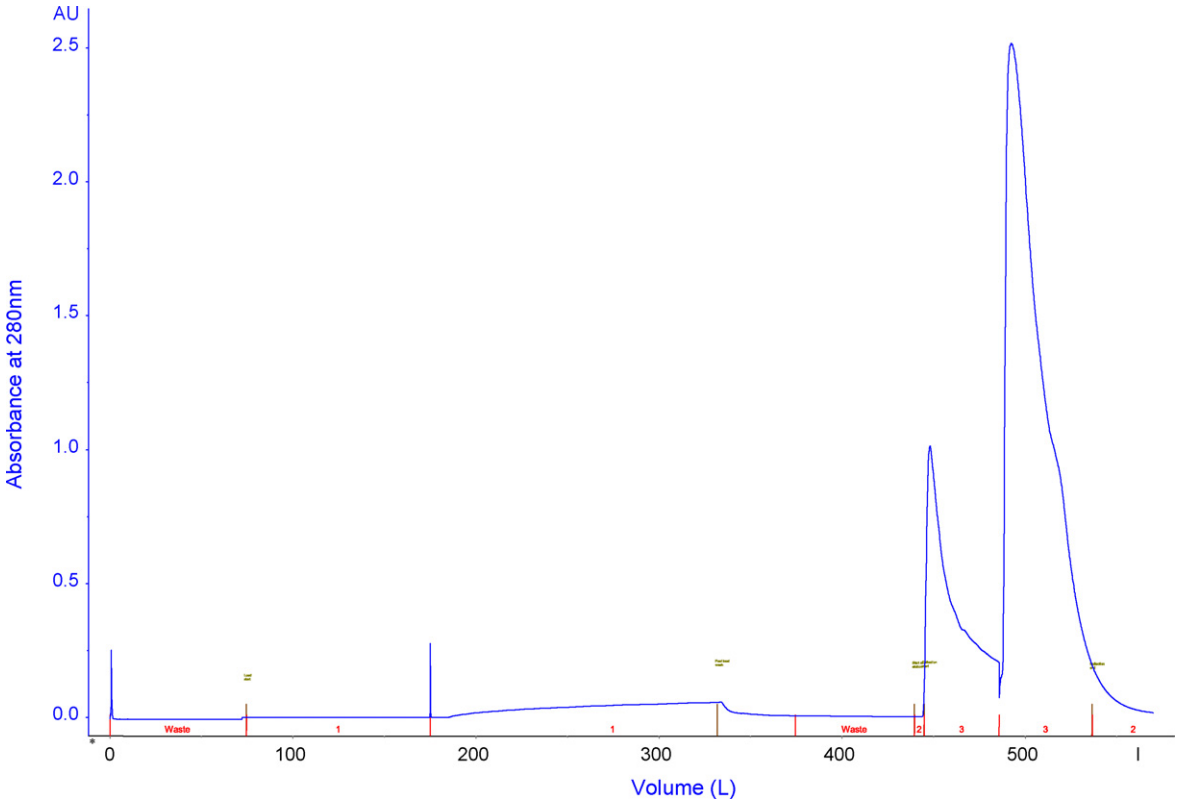

Fig. 4. Chromatogram recorded for a 44 cm diameter, 22 cm bed height HIC column.

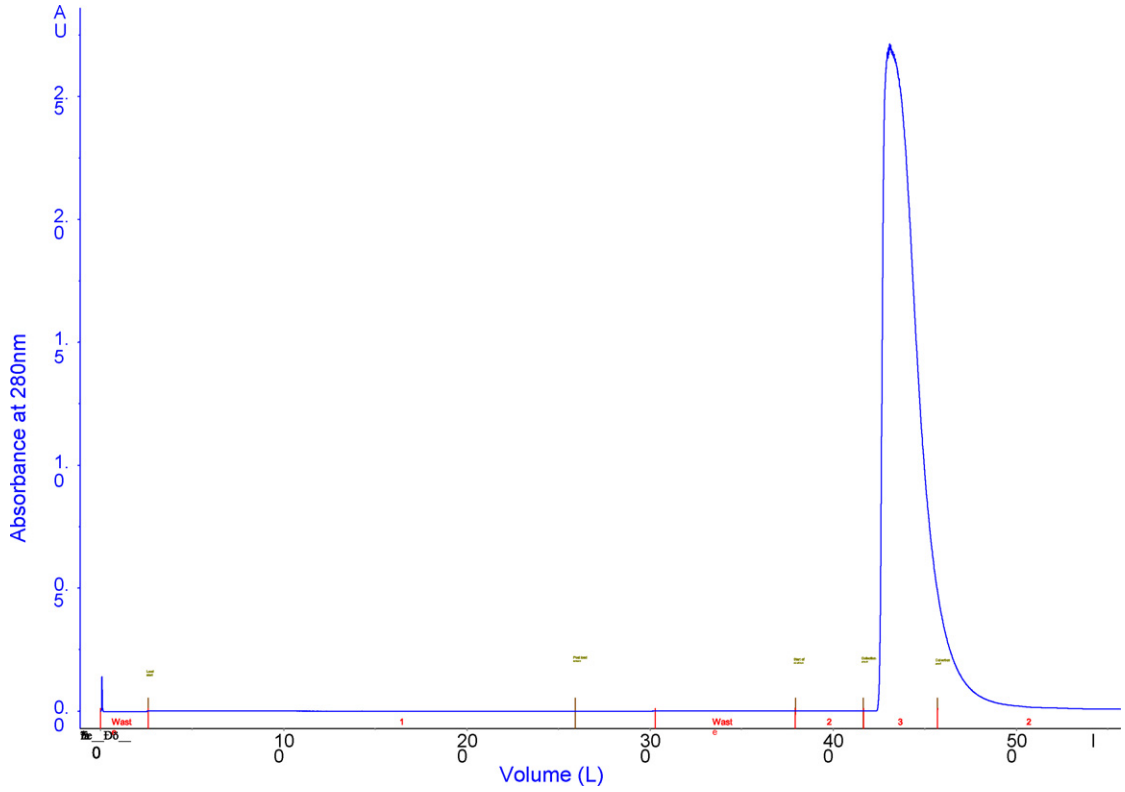

Fig. 5. Chromatogram recorded for a 44 cm diameter, 22 cm bed height HIC column packed with a modified packing procedure.

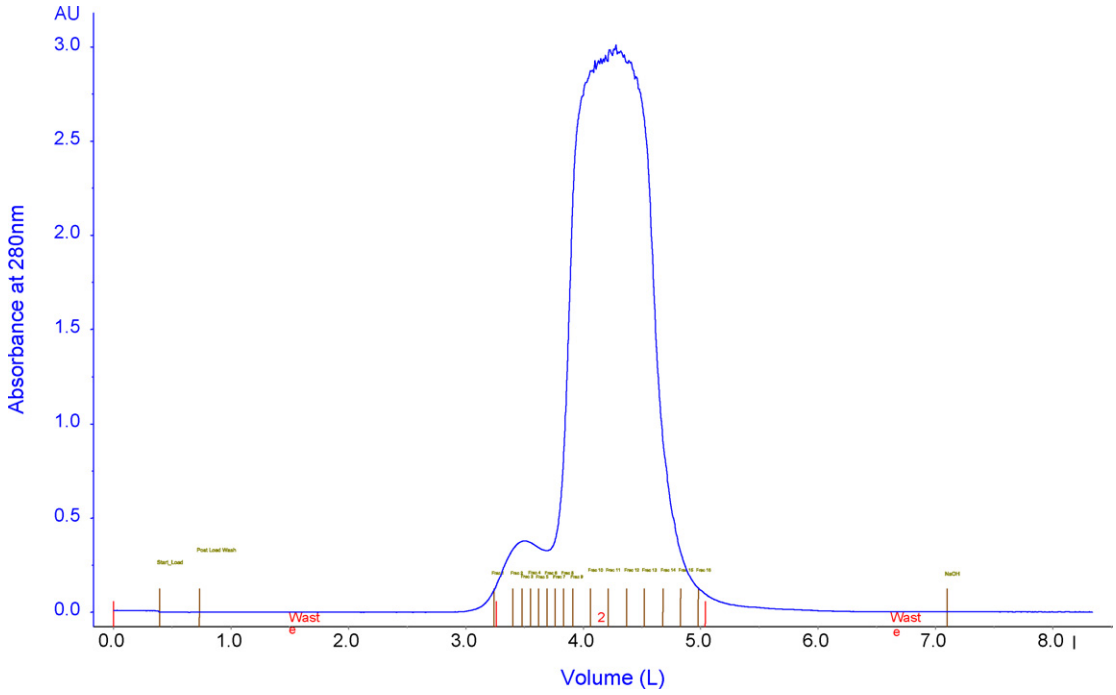

Fig. 6. Pilot scale chromatogram recorded for two 10 cm diameter Superdex 200 size exclusion columns connected in series with a combined bed height of 85 cm.

size exclusion (gel permeation) chromatography. This method employs much slower flow rates than those routinely used for affinity or ion exchange resins. Whilst size exclusion chromatography can be scaled-up to an extent, time constraints, product hold times, column sizes and the possible requirement for fraction collection all become major factors in the use of such resins outside the laboratory.

An example of a pilot scale size exclusion chromatography step is shown in Fig. 6. The process was initially operated at laboratory scale with a 5 cm diameter, 85 cm bed height column with the eluate collected as fractions (Fig. 7). The chromatogram shows separation of the aggregate peak from the monomer peak. This step was scaled-up to a 10 cm diameter column and found to operate comparably. The resin used (Superdex 200) allowed for a faster flow rate than the older Sephacryl resins (approximately 25 cm/h compared to 10 cm/h). By loading product for the next cycle as product from the previous cycle had eluted, throughput was increased so that three cycles could be completed in 1 day.

Analysis of the fractions from the 10 cm diameter columns using a size exclusion HPLC (SE-HPLC) assay showed significant aggregate removal from fraction 10 onwards (Table 3). Fractions 9 to 14 were pooled and the product recovery was 81.7%. Collection of the elution peak for subsequent cycles on the same column were set to be triggered at 2.2 AU on the upslope and collection stopped at 0.3 AU on the down slope.

To provide cGMP material for a clinical trial, two 20 cm diameter columns were used in series. For further scale-up, size exclusion columns with diameters up to 100 cm could be used although the bed height needs to be restricted to approximately 30 cm or less to provide support to the chromatographic bed. By connecting several of these columns in series, total bed volumes of many hundreds of litres can be achieved. However, the operation of such large size exclusion chromatography columns is often problematic and is always a major bottleneck in any manufacturing plant [2]. With the slow flow rates and long processing times, the product can also be put at risk due to increasing levels of bioburden. Therefore, alternative methods for the removal of aggregated product are often employed and there are several potential methods available. Hydrophobic interaction chromatography, ceramic hydroxyapatite and cation exchange resins have all been used for aggregate removal but none are ideal.

Hydrophobic interaction chromatography resins require large amounts of salts that are expensive, can be difficult to dispose of, and may not be compatible with the materials of construction of buffer and product holding tanks. Furthermore, the density difference between the buffers used for a HIC step can cause bed stability problems. Ceramic hydroxyapatite can also be used for the separation of aggregate from monomer, but the ceramic resin can be very difficult to unpack with-

Table 3
Analysis of fractions collected during the elution of two 10 cm diameter size exclusion chromatography columns connected in series

Sample	% Aggregated product	% Monomeric product
Fraction 8	15.98	84.02
Fraction 9	2.64	97.36
Fraction 10	0.41	99.59
Fraction 11	0.1	99.9
Fraction 12	0.04	99.96
Fraction 13	0.04	99.96
Fraction 14	0.02	99.98
Fraction 15	0.02	99.98
Pool 9-14	0.41	99.59

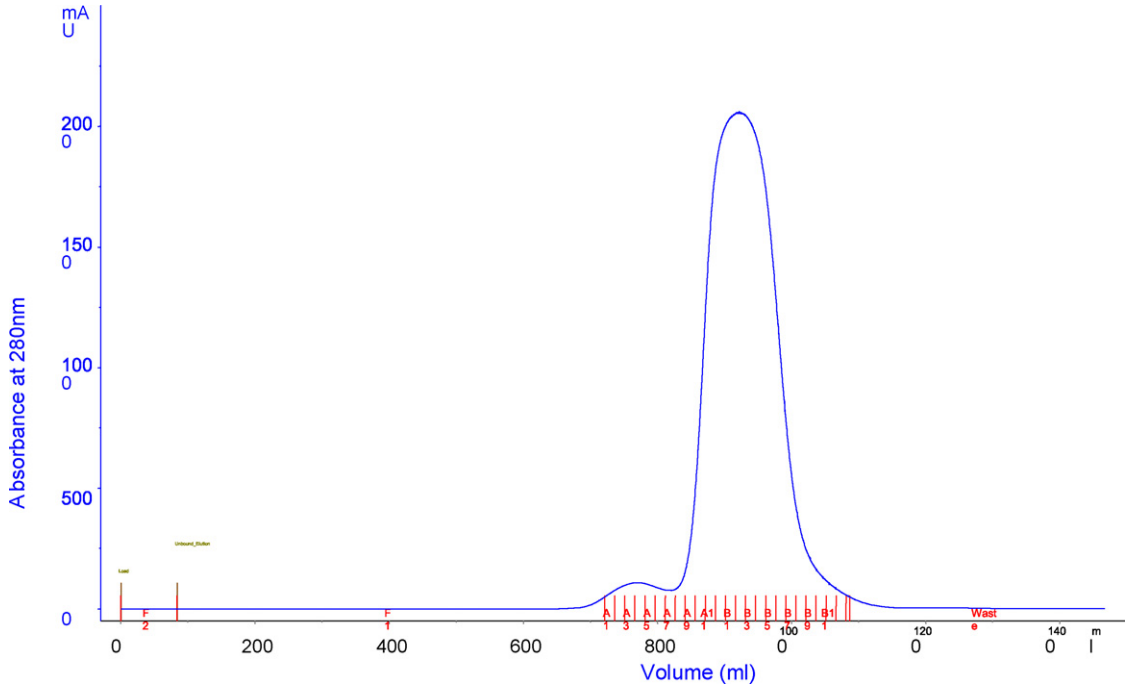

Fig. 7. Laboratory scale chromatogram recorded for a single 5 cm diameter, 85 cm bed height Superdex 200 size exclusion column.

out damaging the resin. Therefore, storing the resin outside the column for re-use in a subsequent manufacturing campaign may not be possible. Cation exchange chromatography can be a useful way to separate aggregate and monomer but it can be difficult to develop a high yielding step with a high capacity.

Fig. 8 shows one example of the removal of aggregate product and other impurities by a HIC step. In this example, aggregated product was reduced from >20% to below 1% (as measured by size exclusion HPLC). This can be seen by SDS-PAGE analysis (lanes 14, 15 and 16) which shows the removal of a high molecular weight band at >200 kDa.

Ion exchange chromatography steps can also be used to reduce the level of antibody aggregates. Fig. 9 shows a process characterisation run on a cation exchange column used for aggregate removal. The eluate was collected as fractions and each fraction was analysed for aggregate content by SE-HPLC (see Fig. 10).

Fig. 10 shows that the first few fractions are free of aggregated antibody but the percentage of monomer decreases with increasing fraction number. Therefore, a trade-off is required between aggregate removal and product recovery.

The removal of aggregated antibody from monomer is of key concern due to the possible immunogenicity of antibody aggregates. Targets of $\leq$1% aggregates are desirable and new technology is directed towards achieving this goal. The traditional approach is to remove aggregates by chromatography steps and new resins, such as mixed mode resins, are coming onto the market. These do not necessarily offer the desired operating conditions (high dynamic binding capacity, high flow rates) to achieve adequate aggregate removal. Consequently, alternative methods for aggregate removal are being evaluated, for example using solubilisation (see [19] for references) or ultrafiltration to retain aggregates [20].

7. Filtration steps

Although the chromatography steps play the major role in the purification of antibodies, filtration steps are integral to any process. These can be in the form of ultrafiltration steps in TFF (Tangential Flow Filtration) mode, 0.2 μm membrane filtration steps for particle reduction/bioburden control, or virus reduction filtration steps to reduce the level of viruses in the process stream.

7.1. In-process filtration

Downstream processing operations are generally not considered sterile operations and although many processes incorporate 0.2 μm filters after each step, particularly if operated at ambient temperature, their function is to control bioburden to a low level rather than to eliminate bioburden completely and ensure sterility.

Filtration issues in antibody purification processes are most likely to occur after pH adjustments. Retroviral inactivation at low pH is routinely incorporated into Protein A affinity chromatography steps as the product is eluted from the resin by an acidic buffer. After incubation of the product at low pH, the pH is usually adjusted to match that of the next processing step which is typically in the range pH 5.0 to 8.0. During this pH adjustment step, precipitation is frequently observed.

Very few filtration difficulties are observed at laboratory scale and 0.2 μm filters, such as syringe filters may be sufficient for the small product volumes. However, filtration difficulties are enhanced as processes are scaled-up. Filters should either be

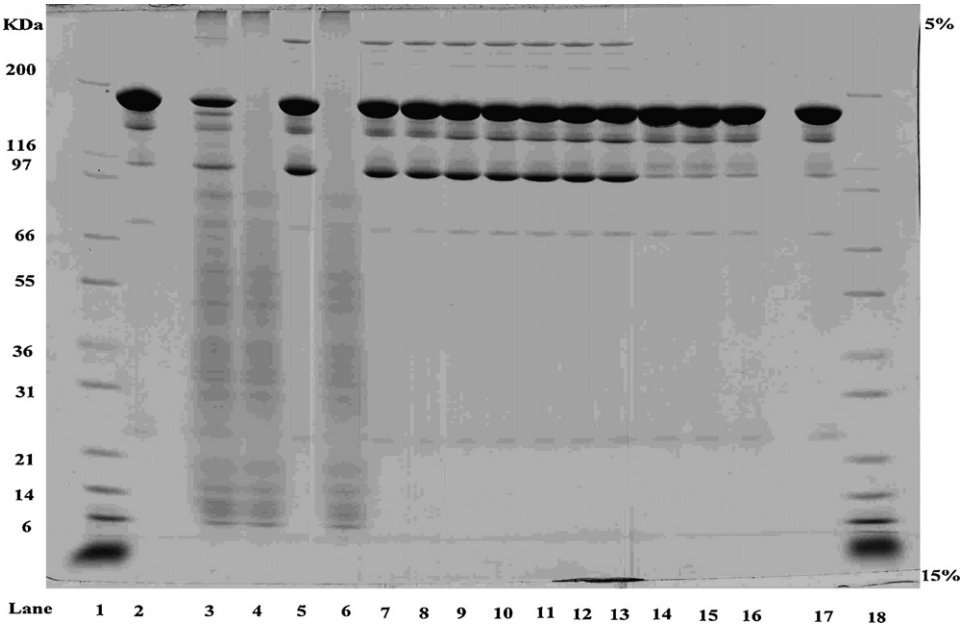

Fig. 8. SDS PAGE analysis of monoclonal antibody in-process samples showing aggregate reduction by hydrophobic interaction chromatography (5 to 15% (w/v) gradient SDS PAGE, non-reduced samples visualised with Coomassie Blue Staining).

Lane	Sample
1	Molecular weight markers
2	Reference standard
3	Protein A load
4	Protein A unbound fraction cycle 1
5	Protein A eluate cycle 1 (post pH adjustment)
6	Protein A unbound fraction cycle 2
7	Protein A eluate cycle 2 (post pH adjustment)
8	Protein A eluate pooled
9	Cation exchange eluate cycle 1
10	Cation exchange eluate cycle 2
11	Cation exchange eluate pooled
12	Virus reduction filtrate
13	Anion exchange membrane filtrate
14	HIC eluate cycle 1
15	HIC eluate cycle 2
16	HIC eluate pooled
17	Bulk purified product
18	Molecular weight markers

sized appropriately for a particular application or in the absence of data, a generic approach may be taken whereby a train of pre-filters of decreasing pore size may be used prior to the final 0.2 μm filter. This is particularly suitable for a multi-product facility to avoid storage of a large variety of filters specific to the filtration of a particular product.

For filter sizing, an adequate volume of product is required so that ideally the filtration operation can be continued until the filter becomes blocked. Hence, the process may need to be partially scaled-up before this data can be obtained. One pilot process operated at 130 L fermentation scale was observed to have filtration issues in 2 out of 4 batches. During a further pilot batch, filter sizing was performed at two stages in the purification process which had proved to be difficult to filter previously. These were after neutralisation of the affinity chromatography purified product and after the subsequent concentration/diafiltration step.

The eluate from a Protein A chromatography column was filtered under pressure through a dual-layer 300 cm^2 Sartopore 2 (0.45/0.2 μm) sterile filter capsule until flow terminated. The 300 cm^2 Sartopore 2 filter blocked after 6.05 L had passed through the filter (the data are shown in Fig. 11). Based on these findings, the estimation was made that one 20″ filter would be required for the in-process filtration of the eluate of the Protein A affinity column at the 2000 L fermenter scale.

Filter sizing was also performed on the same filter type with 18.7 L of product after it had been concentrated and diafiltered into a defined buffer. All the concentrated/diafiltered product passed through the capsule without flow terminating. These data are presented in Fig. 12. Based on these findings, the in-process

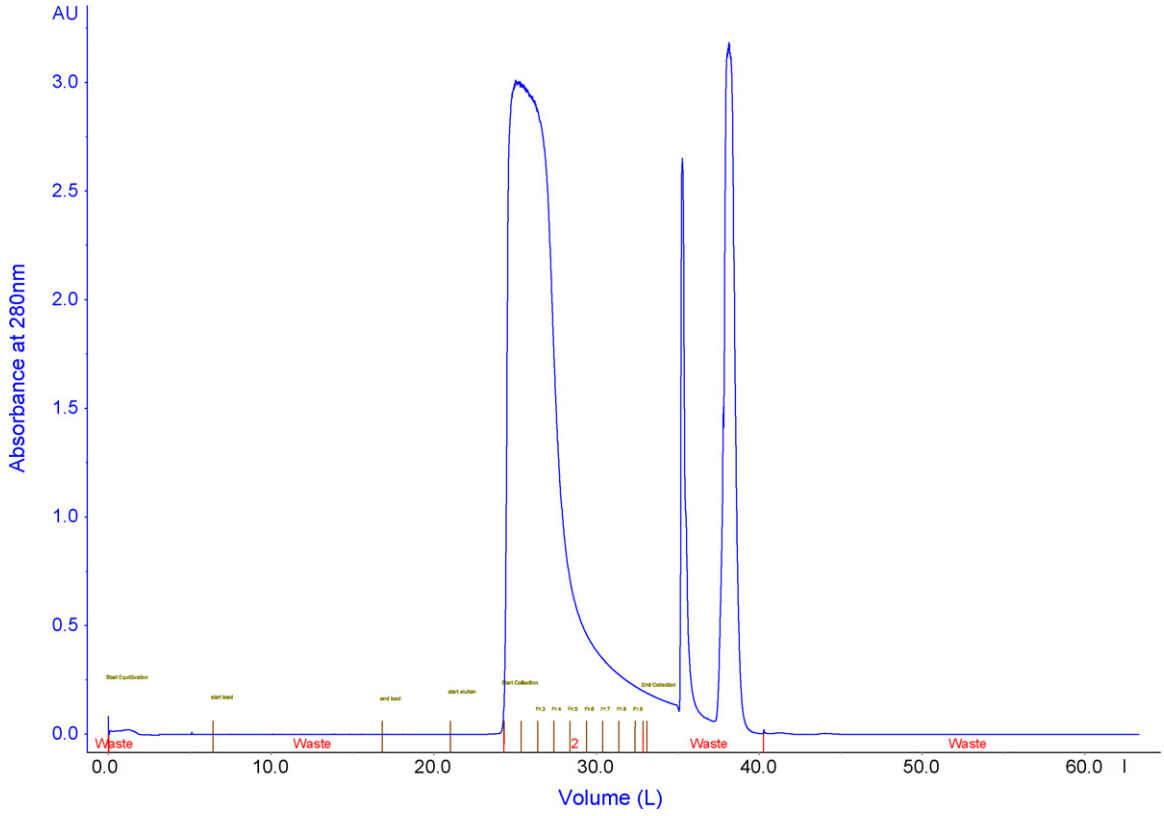

Fig. 9. Cation exchange chromatogram recorded for a monoclonal antibody showing the eluate peak collected as fractions and the subsequent strip and sanitisation peaks.

filtration operation at this stage of the process was scaled-up linearly to the 2000 L fermentation scale. No filtration problems were expected and this proved to be the case.

The use of larger pore size pre-filters can also be effective at reducing filter area and costs. For example, filter sizing data for the neutralised eluate from a Protein A chromatography column showed the requirement for three 30″ 0.45/0.2 μm Sartopore 2 filters or alternatively one 30″ 0.8/0.45 μm pre-filter in line with one 10″ 0.45/0.2 μm Sartopore 2 filter. The second option resulted in a cost reduction of >£1000 per cycle. With the larger

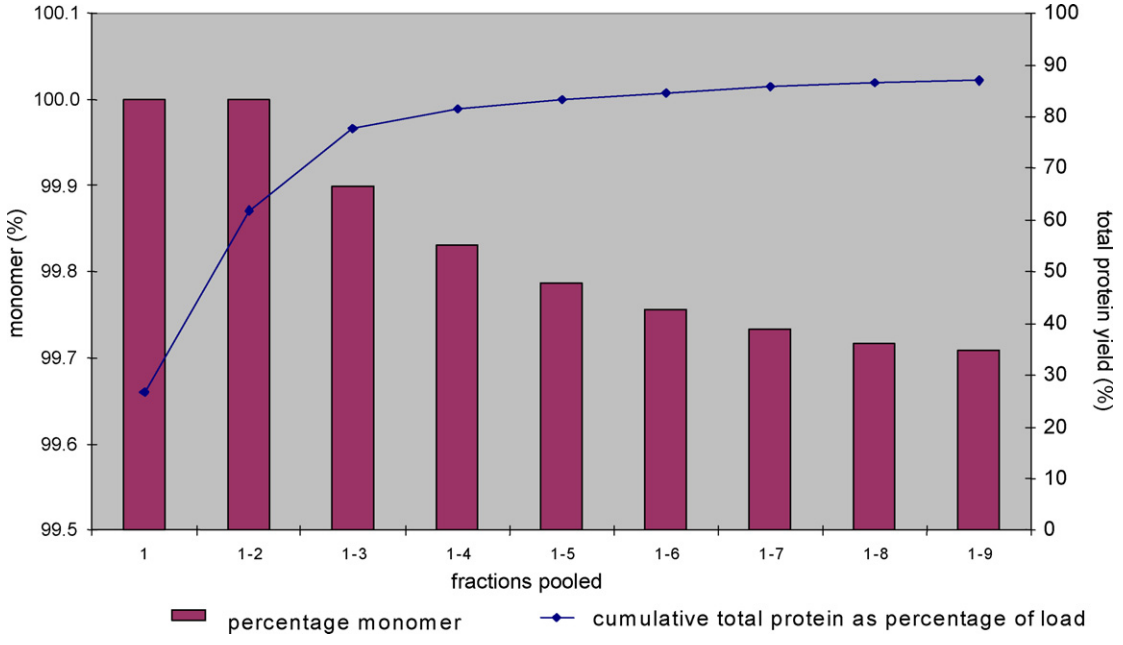

Fig. 10. Cumulative total protein yield and percentage monomer for theoretical pools of fractions on cation exchange chromatography column.

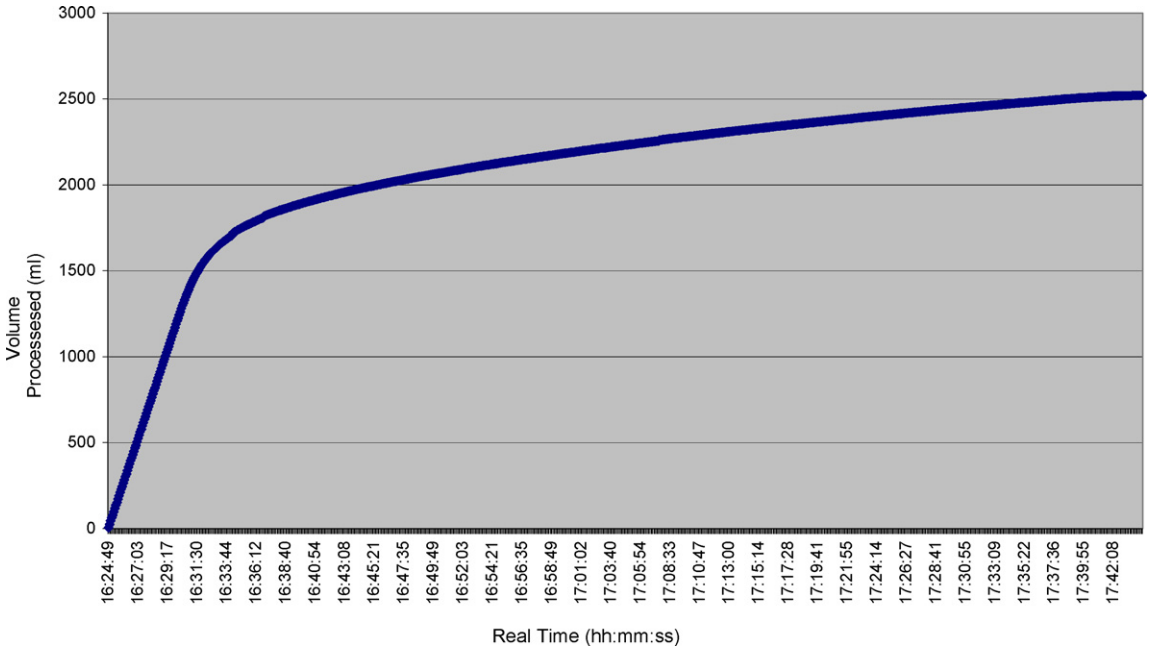

Fig. 11. Throughput of Protein A eluate vs. time through a Sartopore 300 cm^2 capsule filter.

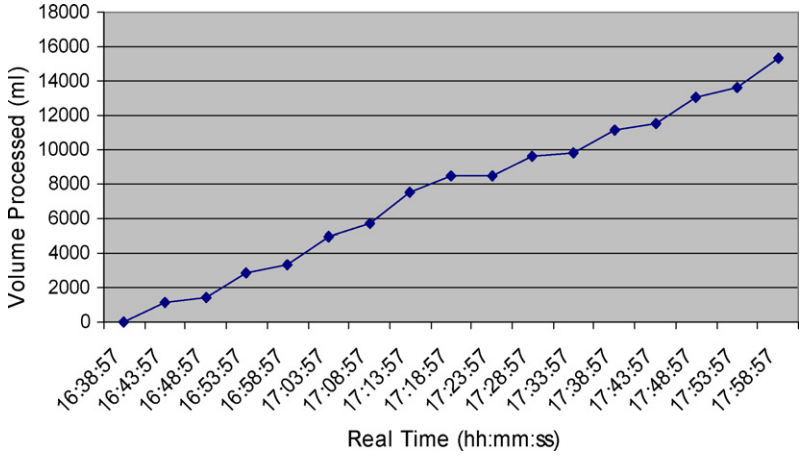

Fig. 12. Throughput of a Protein A-purified antibody after concentration and diafiltration plotted vs. time through a Sartopore 300 cm^2 capsule filter.

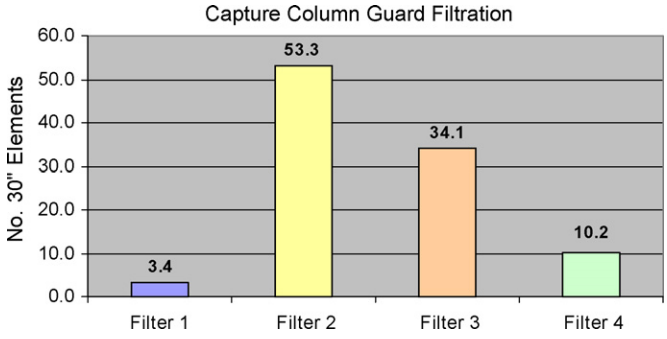

Fig. 13. Number of 0.2 μm filter cartridges required to filter 20,000 L of cell culture supernatant – results of a filter selection study.

volumes anticipated with titre increases, filter sizing should be a key consideration of any scale-up process.

Filter performance can vary depending on supplier and material of construction as shown in Fig. 13, emphasising the advantages of evaluating several filter types prior to scaling up. This study resulted in filter 1 being selected to minimise filter surface area.

7.2. *Virus reduction filtration*

The inclusion of a virus reduction filter is now virtually a prerequisite for any monoclonal antibody manufacturing process using mammalian cell culture [21]. In recent years, the availability of small pore size filters specifically designed for virus removal has driven an expectation that these filters will be incorporated into manufacturing processes for improved removal of small viruses, e.g. Minute Virus of Mice (MVM) [22]. However,

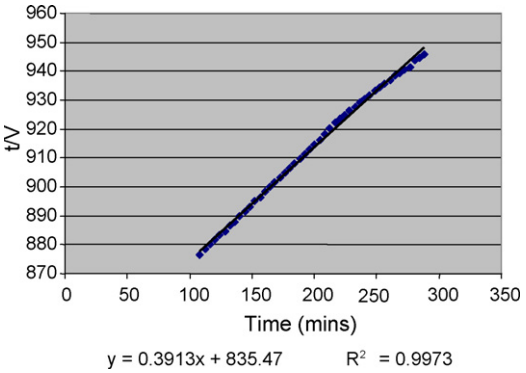

Fig. 14. V_{max} – graph of time/volume vs. time for an Asahi Planova 20N filtration of a partially purified antibody product.

these filters are expensive and the greater the volume of product filtered per unit membrane area, the lower the level of virus reduction.

For filter sizing studies, as with 0.2 μm in-process filtration, a relatively large volume of product is required and therefore filter sizing is often performed during the operation of a partially scaled-up process. Typically, 300 mL of material is required for loading onto the scaled down filter or filter disc.

Some typical data is shown in Fig. 14 where a 300 mL aliquot of a partially purified antibody product was filtered under pressure through an Asahi Planova 20N virus reduction filter (0.001 m^2 membrane area). The time versus volume data were logged throughout the filtration using a data logging balance. The data collected was then used to calculate the throughput of material through the filter based on an operation time of 4 h. This was estimated as 206 L/m^2. The estimated filter area and number of filters required for a typical 2000 L scale fermentation batch is presented in Table 4. Filters with 15 nm pore size are now available which may prove more effective at removal of the very small viruses, but at the cost of a reduced flux rate.

Table 4
Determination of the filter area requirement for a 2000 L fermentation batch

Parameter	Result
Protein concentration of filter load	4.51 g/L
V_{max}	2556 Lm^2
Ji	72 $L/m^2/h$
Estimated volume to be processed at 2000 L scale	500 L
Filter area required (4 h processing time)	2.42 m^2
Number of 1.0 m^2 filters required (4 h processing time)	3

8. Effect of scale-up on product volume and buffer volume

With increasing process size, both the volume of product to be handled and the volumes of the different buffers and solutions required to operate purification steps become a major concern with regards to handling and storage. This becomes an even bigger consideration with increasing titres and purification processes will either need to use larger columns or employ greater re-use of chromatography resins.

During process development, there are no concerns with volume handling whether product elutes from a chromatography column in two column volumes or ten column volumes. However, as the process scales up, this volume and cost become increasingly significant and equipment constraints at larger scale may then determine how the process can be operated. Using the same column sizes as quoted in Table 2 for the affinity chromatography column, the effect of increasing elution volume per cycle at various scales is shown in Table 5. Product volume is further increased by the number of cycles performed. When

Table 5
Impact of the elution volume from an affinity chromatography column on the size of product tanks required

	Lab scale	Pilot scale	Small scale manufacture	Large scale manufacture
Fermenter volume	1–10 L	130 L	2000 L	20000 L
PA column volume	119 mL	1.63 L	14.8 L	323 L
PA eluate volume per cycle if product elutes in 2 CVs	238 mL	3.3 L	29.6 L	646 L
PA eluate volume per cycle if product elutes in 4 CVs	476 mL	6.5 L	59.2 L	1292 L
PA eluate volume per cycle if product elutes in 8 CVs	952 mL	13.1 L	118 L	2584 L
Volume for multiple cycles (2 CV elution volume)	n/a	16.5 L	267 L	2584 L
Volume for multiple cycles (8 CV elution volume)	n/a	65.5 L	1062 L	10336 L

The cycle numbers are based on data in Table 2.

Table 6
Comparison of elution conditions on Protein A chromatography

	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7
Post load wash buffer	pH 8.0	pH 4.5	pH 8.0	pH 5.5	pH 5.5	pH 8.0	pH 8.0
Elution buffer	Acetate pH 3.7	Formate pH 3.3	Formate pH 4.0	Formate pH 4.0	Formate pH 3.3	Acetate pH 3.7	Citrate pH 3.5
% Product recovery[a]	96	107	110	108	74	119	124
Eluate volume	2 CVs	9 CVs	5 CVs	6 CVs	4 CVs	2 CVs	6 CVs
Absorbance at 340 nm pre-neutralisation	0.26	N/A	0.05	0.03	0.05	0.39	0.53
% Aggregated product in eluate	2.01	N/A	1.13	1.24	2.5	2.33	2.12

[a] Recovery determined using A280 analysis for the column eluate sample and PA-HPLC analysis for the column load sample.

considering process scale-up, the number of chromatography cycles should be predicted based on the highest titre expected from the fermenter so that the maximum anticipated volume can be estimated to ensure appropriately sized tanks are available.

Some examples of elution buffer volumes required with different composition buffers are shown in Table 6. For choosing a suitable elution buffer for scaling up, elution volume was one of several factors to be taken into account. Product recovery and effect on product precipitation and aggregation were also considered.

The elution volume from each chromatography column should be monitored during scale-up. Product collection conditions can be selected to obtain an acceptable balance between maximising product recovery and minimising product volumes to allow the collection of column eluates into existing tanks in the manufacturing facility.

As an example of this balance, an anion exchange chromatography step was operated in flow-through mode on a 2 L column. The resulting chromatogram showed significant tailing on the downslope which was not apparent at laboratory scale (it is not known why there was a difference between laboratory scale and pilot scale chromatograms. However, tailing profiles appear to be a feature of this particular resin and have been noted at laboratory scale during purification of other products). When product collection was maximised, the elution product volume was 20 L (equivalent to the load volume plus 9 column volumes of wash buffer) and the recovery was 91.5%. The collection conditions were adjusted subsequently, which resulted in the collection of a reduced product volume of 15.6 L (equivalent to load volume plus 6 column volumes) with a recovery of 89.4%. When scaling up to a 13 L column using the latter collection conditions, product collection from a potential eight cycles would have exceeded the tank volume available for product pooling. Therefore, collection conditions were further adjusted to collect the load volume plus 3 column volumes. Product recovery was reduced by approximately 6%. Although this approach was not ideal due to the reduction in product recovery, the product volume was manageable in the purification facility.

If the product requires conditioning prior to the next process step, e.g. pH or conductivity adjustment, ideally the product from multiple cycles should be pooled and treated as one volume. At small scale, such adjustments can be achieved by addition of a suitable titrant or dilution buffer. For pH adjustment, it is often difficult to give an accurate prediction of the amount of titrant that will be required at an increased scale. An example of titrant volumes required with increasing scale of processing is shown in Table 7 below. On scale-up, the volume of titrant required to adjust the pH had decreased, which could result in overshooting the target pH if the guidelines for titrant addition had been based on the small scale trials.

In pilot facilities with chromatography column volumes up to a few tens of litres, mobile and disposable tanks and bags are often used for holding buffers and product. With increasing scale, the equipment in the purification facility is less flexible and tanks are static and of a defined volume, whether in multi-product or single product facilities. Hence, certain product conditioning steps may not be suitable and the inclusion of a volume reduction step, e.g. ultrafiltration, may be necessary to make a process fit into a facility. Ultrafiltration operations are readily scaled-up [23] but they can result in increased levels of product aggregation. This is particularly critical when scaling up high titre processes and may require challenging concentration conditions. For example, concentrating 10 kg of product to a volume of 100 L requires a product concentration of 100 mg/ml, whereas 2 kg product would only need to be concentrated to 20 mg/ml to achieve the same volume. If the antibody is prone to increased aggregation at higher concentrations, then the introduction of such a concentration step could result in the requirement for process modifications to improve aggregate removal. Ideally UF process steps should be optimised to minimise the buffer requirements and the amount of time the product is in the UF system [24]. Therefore, equipment restrictions in the large scale manufacturing facility should always be considered when a process is scaled-up and optimised or process modifications and additional product comparability studies may be required.

Table 7
Effect of scale-up on the titrant volume required after the affinity chromatography step

	Lab scale	Pilot scale	Small scale manufacture
Fermenter volume	1–10 L	130 L	2000 L
Column volume	120 mL	1.63 L	14.8 L
Eluate volume	0.40 L	3.31 L	27.3 L
Volume of titrant required per litre of eluate	62.5 mL	43.8 mL	24.9 mL

8.1. Buffer volumes

Increasing process scale also affects the volume of buffers required. During process development, the volumes of different buffers should be defined and ideally minimised. Fig. 15 shows the volumes that would be required when scaling up a process from 2000 to 20,000 L fermenter scale for a product with a titre of approximately 3 g/L. The volume required even at 2000 L fermentation scale is approximately 15,000 L with 2500 L of this being the equilibration buffer for the affinity chromatography step. Approximately 40% of the total buffer requirement is for the affinity chromatography step.

One option is to use buffer concentrates especially for equilibration buffers. To be able to implement in-line dilution, the buffer must be readily soluble so that 5-fold or 10-fold concentrates can be prepared. The temperature of the water and concentrate need to be controlled because temperature influences conductivity. Appropriate equipment is required to implement in-line dilution [25]. Equilibration of a chromatography column using a 10-fold concentrate of the equilibration buffer at flow rates of 20 and 120 L/h was compared with improved conductivity control being demonstrated with the faster flow rate. In-line dilution gives the added benefit of a process being more reproducible between batches as with this approach, a conductivity set point is determined which is monitored by the probes within the system. Smith showed that a stable

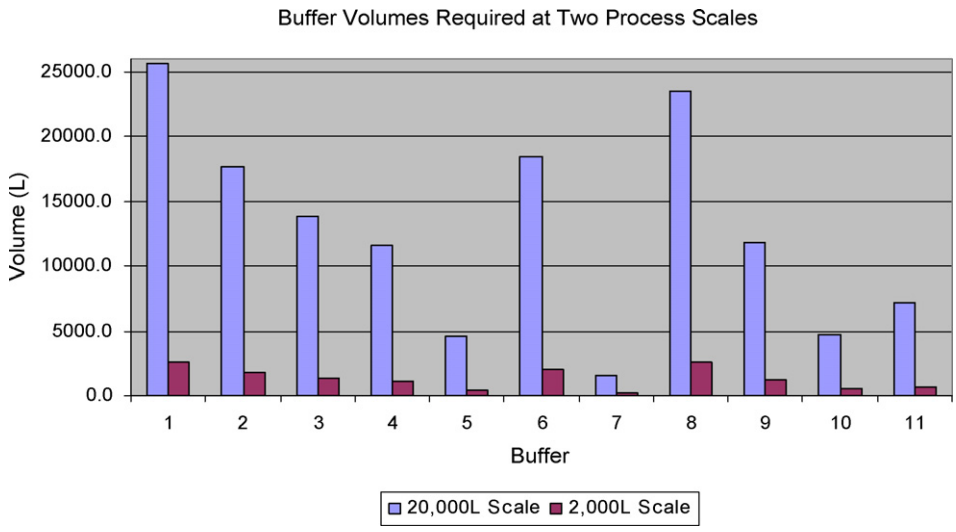

Fig. 15. Comparison of buffer volumes at 2000 and 20,000 L fermentation scale.

conductivity profile can be achieved within a minute of enabling the system at the higher flow rate. He calculated that a 30–40% reduction in buffer volume was possible using in-line dilution.

Using membrane adsorbers in place of polishing chromatography steps may be one way of reducing some of the buffer volumes required. A new process capacity equivalent at both small and large scale was successfully achieved recently [26].

9. Conclusions

Improvements in the productivity of antibody-producing cell culture processes have resulted in the need to purify very large quantities of monoclonal antibodies as a single batch. The current technologies used for the purification of therapeutic monoclonal antibodies rely on chromatography and various types of filtration operations. While it is technically feasible to scale-up these operations to purify up to 100 kg quantities of product per batch, very large volumes of chromatography resins, buffers and large filter areas are required, which are often incompatible with current manufacturing facilities. Additional ultrafiltration steps may be required at large scale to reduce volumes but product concentration steps can affect aggregate content. Improvements in chromatography resins have resulted in faster throughput due to enhanced flow rates and binding capacities which is of particular importance in purification of high titre products. However, one of the major bottlenecks in antibody purification processes is aggregate removal by current chromatography methods. Research into alternative, high throughput aggregate removal methods in particular, and high capacity, cost-effective purification methods in general, is required to ensure that downstream operations keep pace with upstream ones.

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