Economic Benefits of Single-Use Membrane Chromatography in Polishing

A Cost of Goods Model

Janice Ai Chye Lim, Andrew Sinclair, Doug Sang Kim, and Uwe Gottschalk

Applications of single-use chromatography techniques present attractive alternatives to conventional column chromatography for purifying monoclonal antibodies (MAbs) (**1**). Over the past few years, disposable membrane chromatography technologies are increasingly being used in purification processes to separate proteins of interest from process-related impurities (**2–4**). The benefits of such technology include reductions in capital requirements, greater process flexibility, and elimination of cleaning validation costs. In addition, disposable-based engineering enables facilities to manufacture multiple products in a single plant without risk of cross-contamination between batches.

Product Focus: Monoclonal antibodies (MAbs) and other proteins

Process Focus: Downstream processing

Who Should Read: Process development and manufacturing, separation scientists, project managers

Keywords: Purification, Single-use (disposable) technologies, chromatography, anion-exchange (AEX), ion-exchange, membrane absorbers, membrane chromatography

Level: Intermediate

Disposable membrane chromatography is used in the flow-through mode (the matrix binds the contaminants and not the product) for removal of a variety of impurities and viruses during late-stage purification. The flow-through anion-exchange (AEX) chromatography unit operation is an effective polishing step for trace-contaminant removal and virus clearance during large-scale production of a typical MAb (Figure 1). Most endogenous and adventitious viruses, DNA, dyes, endotoxins, and many host-cell proteins are negatively charged and will bind to positively charged ligands while the basic, positively charged antibody-based product flows through the media. The ligands are either strong anion exchangers such as quaternary ammonium (Q) or weak anion exchangers such as diethylamine (D).

Traditional column chromatography depends on diffusion of molecules into the pores of beads to the binding sites. Conventional column chromatography for the flow-through AEX polishing step operates at a linear flow rate between 100 and 150 cm/hr, thereby requiring columns with large diameters (100–160 cm) to enable greater flow rates. At such diameters, column volumes can reach up to 400 L. Therefore, packed-bed columns are dramatically underused in relation to contaminant loads. In addition, current conventional column chromatography technologies has limitations in purifying large molecules: Host-cell DNA, viruses, endotoxins, and large proteins are often too large to diffuse into the pore structures of resins.

A purification operator handles a single-use 30-inch membrane-based chromatography device.
(WWW.SARTORIUS.COM)

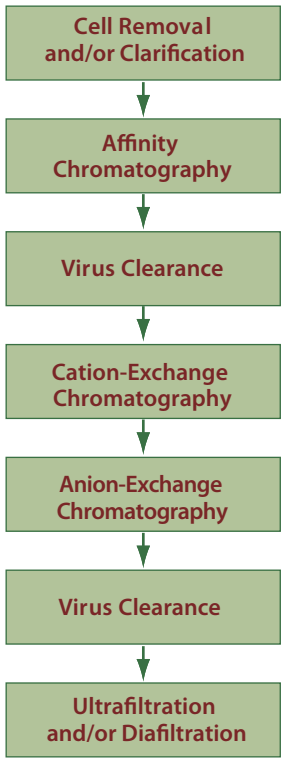
The disadvantages of AEX columns have resulted in development of membrane adsorber chromatography for trace-contaminant removal. The technique relies on convective flow to bind contaminants directly to ligands in the membrane adsorber, requiring no pore diffusion; hence, faster processing times can be achieved. The large pore size associated with membranes provides available channels for all molecules, thereby permitting faster flow rates

and high binding capacities. High linear flow rates reduce the membrane volume of disposable chromatography devices. The antibody yield for membrane chromatography is comparable with that of conventional column chromatography. Table 1 summarizes key differences between traditional resin-based chromatography and disposable membrane chromatography.

Here we describe an approach to designing an economic analysis comparing disposable membrane adsorber technology with equivalent, conventional stainless steel column chromatography. It uses a spreadsheet-based cost of goods (CoG) model developed by BioPharm Services Ltd. Our objective was to gain insights into the cost structures of the two chromatographic formats. The representative disposable membrane-based chromatography devices used were Sartobind SingleSep Q ion-exchange capsules (**5**, **6**). A scale-up concept based on devices with a 15-layer spiral wound membrane was implemented with membrane adsorber formats from 1 mL (validation element) to 1.6 L (large process element) (Table 2) and validated (**7**). The conversion factor between membrane surface and chromatography volume was about 36 cm^2/mL (3.6 m^2/L).

Here we describe the methodology, assumptions, and key results of the cost model. Our analysis considers operational issues such as maintenance and validation, and it quantifies the economic benefits of disposable membrane chromatography technology in terms of capital, labor, materials, and cost of goods.

Figure 1: Downstream purification steps for a typical MAb production process



Method

We based the feed mass into this AEX chromatography unit operation on the throughput from a 10,000-L bioreactor (Table 3 provides details). The AEX chromatography is operated in a flow-through mode with a step yield of 95%. Stainless steel vessels are used to hold the product and to prepare and hold the buffer solutions required for the unit operation. The bed diameter and height of the column are specified as 63 cm and 15 cm, respectively. A typical linear velocity is 150 cm/hr. The column is limited to 100 cycles.

For the comparison, we selected the 10-inch Sartobind Q SingleSep capsule (a volume of 180 mL and a flowrate of 300 L/hr; www.sartorius.com/sartobind). Our cost model determines the number of capsules required by the unit operation. We did not consider an economy-of-scale effect with the use of larger devices and introduction of bulk amounts such as in resin chromatography.

The flow-through capacity of our device depends on the level of contaminants, and hence on the position of the step during manufacturing. For the base case, we selected 10 kg of MAbs per liter of membrane (3.6 m^2). This is a typical value for a relatively pure feed stream after the second chromatography step (cation exchange), but it is not the upper limit (**3**). To challenge the model, we simulated a flow-through load of 2 kg of MAbs — representing a less pure feed stream after the first chromatography column (protein A).

The comparison is based on the steps used to define the chromatography operation: column packing, HETP (height equivalent to the theoretical plate) testing, equilibration, loading, washing, and regeneration. Buffers used for the disposable membrane operation are similar to those used in the conventional resin-based chromatography operation. Tables 4 and 5 show the subunit operations and the buffer and utility requirements for the reusable column chromatography and the disposable membrane chromatography, respectively.

Capital Requirements

The unit operation for the single-use method requires a peristaltic pump — a setup that is more typical for a

Table 1: Key factors between the two chromatography methods

Description	Column Chromatography	Membrane Chromatography
Hardware (investment)	High	Low
Pore size	~15–30 nm	>3,000 nm
Speed	100–150 cm/hr	450–600 cm/hr
Handling	Column packing	Plug and play
Space requirement	High	Low
Cleaning validation	Yes	No

Table 2: Data of Sartobind SingleSep standard capsules

Capsule Size	Membrane Volume (mL)	Flowrate (L/hr)	Membrane Surface (cm^2)
Nano	1	1.3	36
Mini	3.5	4.5	126
Mini	7	9.0	250
Midi	70	90.0	2,500
Maxi 10-inch	180	300.0	6,550
Maxi 20-inch	360	600.0	13,100
Maxi 30-inch	540	900.0	19,600
Mega	1,620	2,700.0	59,000

Table 3: Mass balance calculations

Typical Values for Unit Operation	Yield	Product		
		Concentration (g/L)	Volume (L)	Mass (g)
Production bioreactor		1.00	10,000	10,000
Cell removal/clarification	80%	1.00	8,000	8,000
Protein A	90%	2.30	3,130	7,200
Virus clearance	100%	2.06	3,500	7,200
Cation exchange	95%	5.00	1,368	6,840
Anion exchange	95%	10.00	650	6,498
Virus clearance	100%	8.12	800	6,498
Ultrafiltration	98%	25.00	255	6,368
DSP Overall Yield	64%			

Figure 2: Structure of the CoG model

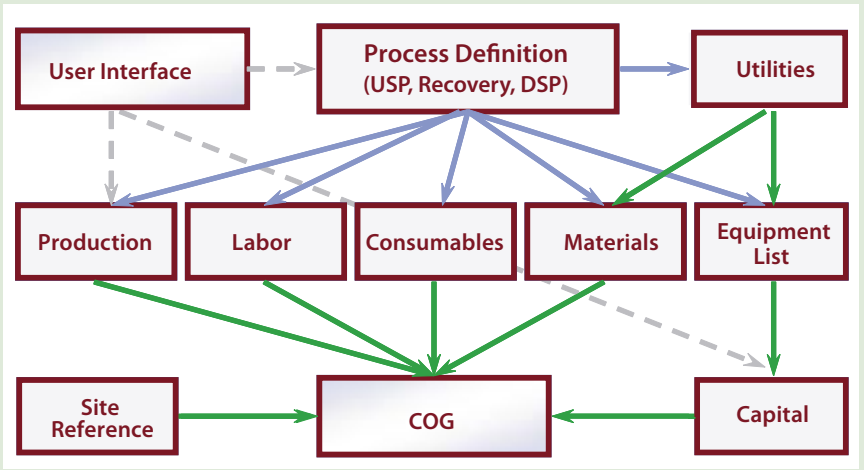


Table 4: Subunit operations for conventional resin-based chromatography (CVs = 3.0)

	Step	Buffer/Utility
1	Setup	
2	Column packing	
3	HETP test	0.2 M NaCl
4	Flush filters	WFI[a]
5	Prewash	10 mM Tris pH 7.0
6	Equilibration	25 mM NaCl, 20 mM Tris pH 7.2
7	Flow through	
8	Wash	25 mM NaCl, 20 mM Tris pH 7.2
9	Regeneration	1 M NaCl
10	Column repack	
11	HETP test	0.2 M NaCl
12	Postuse integrity test	WFI
13	Storage	1 M NaOH

[a] WFI = water for injection

filtration step. The equipment required for column chromatography is more complex and includes a stainless steel chromatography column, a chromatography skid, and a packing skid. In both cases, stainless steel vessels are used for preparing and holding process solutions and for holding the product.

Process equipment costs are typical sales-to-market prices. Additional cost data were drawn from BioPharm Services' internal cost database built from benchmarking analyses containing information from biomanufacturing operations in the United States and Europe. In cases where the cost of equipment was unknown, we used cost-estimation factors based on the known cost for that type of equipment (**6**). Other engineering costs (e.g., building and civils, instrumentation and control, validation) for both methods were estimated by applying Lang factors based on major equipment cost estimates (**8**).

Cost of Goods

The cost of goods (CoG) model provides a complete economic evaluation of the two chromatographic formats because it considers indirect (fixed) costs, direct (variable) costs, and plant productivity. Indirect costs consist of capital charges, and direct costs include consumables, materials, labor, and utilities. A spreadsheet-based approach is proposed for development and implementation of the CoG model. It is configured as modules (e.g., capital, materials, and consumables) using Excel spreadsheets (Figure 2).

Each module is configured as a user-defined entry worksheet, a calculation worksheet, or a combination of the two. Operating costs are determined by process information, which is captured in the cost model. A brief description of the key modules follows, including details of user inputs that form the basis of the model, the calculation method, and the key results.

Process Definition: The model captures a detailed breakdown of the subunit operations in the chromatography step. For each such operation, model parameters include the type of solutions and/or utilities used, the feed quantity per cycle, the total feed quantity, the number of column volumes (CVs) required, the number of capsules required, the operating time, and the number of personnel needed to carry out the operation. This worksheet computes the total operating time and manual hours required per subunit operation.

Capital Charge: The total fixed capital investment is included in this cost model as an annual annuity charge, termed the *capital charge*. The annuity capital charge is the payment for a loan based on constant payments and a constant interest rate. In the model, that charge is calculated based on an eight-year period (the total number of repayments for the loan) (**9**), a value of 12% for the cost of capital (the interest rate for the capital investment) (**8**), and a future value of 10% (the future value you want to attain after the last payment is made, expressed here as a percentage of the

Table 5: Subunit operations for disposable membrane chromatography (flush volume 5.0 L)

	Step	Buffer/Utility
1	Setup	
2	Flush filters	WFI[a]
3	Prewash	10 mM Tris pH 7.0
4	Equilibration	25 mM NaCl, 20 mM Tris pH 7.2
5	Flow through	

[a] WFI = water for injection

capital investment, which are typical values used in the industry).

Labor: The number of manual hours required per batch is calculated for the following categories: production, quality, maintenance, materials, and consumables.

Production covers direct production operators and supervisors required in the unit operation and supporting activities such as buffer preparation and CIP. This figure is used to determine the manual hours required for supervisors.

Quality describes staff required in validation, quality assurance (QA) and quality control (QC). These are estimated using a function of the direction production labor.

Maintenance personnel are required to maintain the chromatography skid and vessels. Different categories of personnel, annual salaries, and overheads are user input parameters. The wage per hour for each personnel type is calculated using the annual salary, operating weeks per year, operator hours per week, and overheads. The number of manual hours and the hourly wage are then used to determine the total labor costs per batch.

Materials: Process materials include buffer solutions and concentrated cleaning chemicals (caustic and acid), which are made up from solid salts using purified water (PW) or water for injection (WFI). Process equipment for cleaning includes vessels to prepare and hold the buffers, a vessel to hold the product, and a chromatography skid. The molecular weight, pack size, pack unit, and cost per pack for raw chemicals are user-defined variables, which are required to determine the unit cost/L for the solutions. Those costs can be obtained from vendors.

Table 6: Buffer and utility requirements for the base case

	L/Batch	
Buffer and Utility	**Resin (reusable)**	**Membrane (disposable)**
0.2 M NaCl	147	0
1 M NaOH	281	0
10 mM Tris pH 7.0	140	20
25 mM NaCl, 20 mM Tris pH 7.2	281	20
Total	**849**	**40**
Water for injection	1,129	60
Purified water	0	0
Total	**1,129**	**60**

The compositions of each solution are indicated by specifying the molarity of each chemical contained in the solution. The total volume per batch is reported for each type of buffer and cleaning chemical. That figure is used to determine the quantity of utilities (PW and WFI) needed per batch. The amount used is multiplied by the unit cost to calculate the total cost per batch.

Consumables: User-defined consumable costs include the chromatography resin, the single-use membrane, and the sterile filters for the stainless steel vessels. The total consumption per batch for the consumables is determined. For disposable consumables, the costs per batch are calculated by multiplying the number of each type of consumables used and the unit cost. (We did not consider the economy-of-scale effect for consumables coming from larger devices and higher unit numbers.) In the case of reusable consumables, the cycle limit and cycles per batch are used to calculate the cost per batch.

Results and Discussion

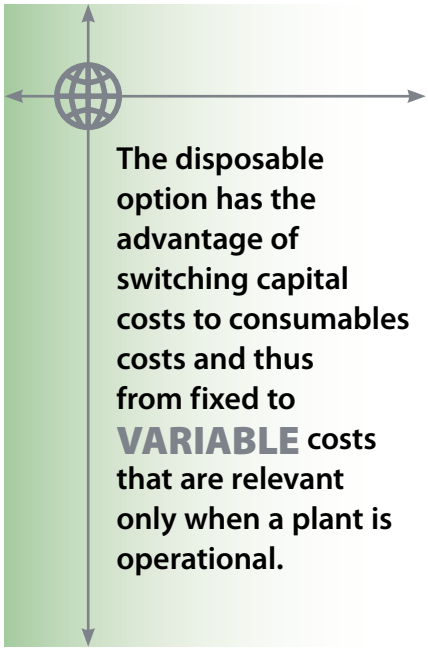
Materials Requirements: Table 6 shows the buffer and utility use per batch for the two chromatographic formats in the base case (polishing after intermediate purification with the cation-exchange chromatography). To highlight differences in material use, the figures indicate amounts required for the main process unit operation and do not include the use for the cleaning operations. In comparison with the conventional resin-based chromatography, the disposable membrane chromatography showed a significant reduction (>95%) in both buffer and utility requirements. The single-use technology eliminates the need for several steps such as wash, elution, regeneration, and storage, thereby reducing the amount of buffers and number of utilities required.

Cost of Goods (CoG) Comparison: Figure 3 illustrates the breakdown of each cost element for the column chromatography and the disposable membrane chromatography. The values of the membrane savings are relative to the conventional resin-based chromatography. The maximum antibody load is limited by contaminant levels in the feed. Figure 3A indicates the CoG comparison for the base case: polishing after intermediate purification with cation-exchange chromatography at a loading capacity of 10 kg of antibody per liter of membrane. The model was challenged with a flow-through load of 2 kg of MAbs per liter of membrane for polishing after initial capturing with protein A chromatography (Figure 3B).

The graphs indicate that the ratio of cost categories differs between the two chromatography formats. In the case of resin-based chromatography, the capital charge is the major cost contributor, suggesting that process equipment has a significant impact on the operating costs. Conversely, for single-use membrane-based chromatography, the consumables costs contribute substantially to total operating costs.

For the base case, CoG is broken down into more details to provide insight into those areas that are affected by the single-use technology. Table 7 indicates the cost comparison for both types of chromatographic techniques, taking into account the capital charge and the running costs. The values of the membrane savings are relative to the resin-based chromatography.

The main impact of the single-use membrane technology is reflected in the amount of equipment required for the unit operation. The cost model indicates significant savings in the capital charge (57%). Reduction in



process equipment minimizes the extent of installation and design efforts, which significantly reduces capital requirements. Reduction in materials consumption (7% savings) is a direct result of the single-use nature of the technology and the smaller volumes of membrane chromatography devices. In this study, the column volume is 46.8 L, whereas the total membrane volume is 0.72 L (requiring four capsules), which is >60 times smaller. The disposable technique eliminates a substantial number of processing steps such as wash, elution and regeneration, thereby reducing the amount of materials. In addition, disposability removes the need for column packing, HETP test, and repacking. Labor costs represent a 12% savings, attributed to the reduced number of staff needed to prepare buffer solutions.

The disposable option has the advantage of switching capital costs to consumables costs and thus from fixed costs to variable costs that are relevant only when the plant is operational. The cost model predicts an 11% increase in the consumable category. This is largely because a new membrane capsule is used for each chromatography step. On the other hand, a column is reusable (up to 100 times), thereby distributing the cost of resin evenly over each chromatography cycle.

Cost benefits provided by reduced materials consumption, process equipment, and labor have more than compensated for the increased cost of membrane chromatography media, translating to a significant total CoG savings of about 66% relative to the resin-based chromatography. Further potential savings comes from the fact that the use of bulk resin amounts were compared with current list prices for membrane chromatography devices.

Figure 4: Cost savings as a function of MAb loading capacity

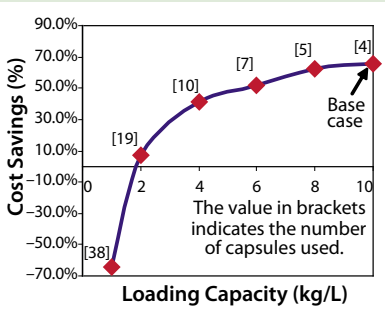


Figure 5: Cost savings as a function of WFI cost

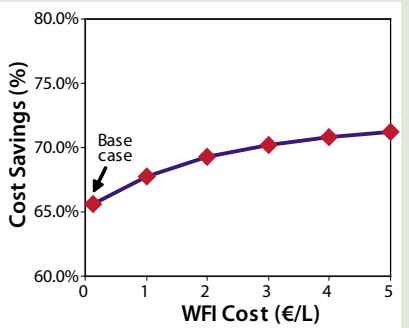


Figure 4 illustrates cost savings as a function of loading capacity for disposable membrane technology relative to conventional resin-based chromatography. The value in the bracket indicates the number of capsules required to process the feed volume. Examining the curve in Figure 4 reveals that operating costs break even when membranes are loaded to about 2.0 kg MAbs/L. Figure 3B shows the breakdown of the cost elements for both chromatographic formats. Consumable costs have increased considerably, resulting in a significant increase in the CoG. Even beyond this economic break point, it should be considered because of other driving factors such as product yield increase, protection of consecutive column step, and risk avoidance.

The cost of WFI can vary greatly depending on whether it is prepared in-house or brought directly from vendors. The curve in Figure 5 indicates that the cost of WFI has a relatively significant impact on the cost savings. At €5/L — a value that some companies calculate with — savings increase to about 71%. High use of WFI in conventional column chromatography accentuates difference between the two methods. All calculations have been used, however, with the conservative assumption of €0.2/L as in the base case.

A Strong Case

We have focused on an economic comparison of using single-use membrane technology and a conventional stainless steel column for the flow-through AEX chromatography unit operation to separate active proteins from contaminants. The cost model considers all aspects of operation, including capital equipment, materials, consumables, and labor. Overall operating costs for disposable membrane chromatography are reduced despite an increase in consumable costs. The financial benefits are derived from a significant reduction in the amount of equipment to support the unit operation. We also see a considerable reduction in requirements for personnel and materials (chemicals and utilities) associated with processing steps.

The use of disposable membrane chromatography technology is a potential approach toward lower capital investment and operating costs. Other benefits that can be gained from single-use technology such as elimination of cross contamination between batches and simplification of material and people flow in the facility cannot be calculated easily, but they may provide an even stronger driving force than the benefits discussed in this article.

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Figure 3: Breakdown of cost categories for the reusable resin-based chromatography and the disposable membrane chromatography: The two typical scenarios were (A) polishing after intermediate purification with cation-exchange chromatography and (B) polishing after initial capturing with Protein A.

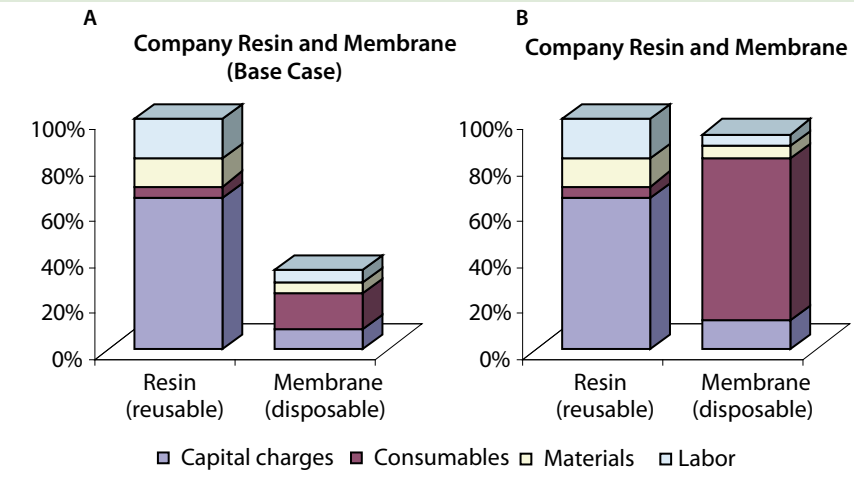

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Table 7: CoG comparison (including capital) for the base case

Category	Description	Cost per Batch (€) Resin (reusable)	Membrane (disposable)	Membrane Savings (%)
Capital charges	**Subtotal**	**18, 901**	**2,340**	**57**
Consumables	Resin/MA capsules	267	4,000	
	Sterile filters	630	120	
	Maintenance spares	453	207	
	Subtotal	**1,350**	**4,327**	**–11**
Materials	Chemicals	1,344	649	
	Utilities	2,001	678	
	Subtotal	3,345	1,328	7
Labor	Direct production labor	1,872	582	
	Quality	2,642	821	
	Maintenance	192	86	
	Subtotal	**4,706**	**1,490**	**12**
Total		**27,493**	**9,484**	**66**

The financial benefits for disposable membrane chromatography are derived from a significant REDUCTION in the amount of equipment to support the unit operation — as well as a reduction in requirements for personnel and materials associated with processing steps.