

In-Line Dilution Capabilities of Mobius[®] Multi Column Capture System for Effective Buffer Management Strategies

Enhancing Intensified Chromatography Performance through Automated Buffer Dilution

The interest in process intensification and continuous bioprocessing in biopharmaceutical manufacturing is growing. By optimizing unit operations through advanced technologies and streamlining processes, productivity can be increased, efficiency improved and costs reduced.

As production capacity grows, buffer preparation becomes more resource-intensive and presents significant challenges. Hence, effective buffer management is crucial to meet these increasing demands of process intensification and continuous manufacturing.

In this context, there is a growing body of comparative studies incorporating specific in-line dilution systems. If such a solution is considered, it usually necessitates high facility utilization to achieve a return on investment.¹ However, integrating in-line dilution capabilities within purification systems introduces the benefits of this approach without additional investment.



In downstream processing (DSP), key targets for process intensification are the chromatography steps, often considered the primary bottlenecks in DSP. Strategies such as multi-column chromatography (MCC) provide solutions to this challenge by increasing the efficiency and throughput of bind and elute (b/e) steps.

Utilizing this approach, the Mobius® Multi Column Capture system is designed to enable three-column operation for any b/e steps, whether for primary capture using affinity resins or intermediate purification with ion exchange resins. It enables continuous loading of a feed stream, with two columns being loaded in series and one set in parallel for non-loading steps (Figure 1). This multi column cycling results in higher resin utilization and a semi-continuous elution stream with minimal surge tank requirements.

As resin utilization increases, the volume of resin, and consequently costs of goods, are reduced. The multi column approach to purification using the Mobius® Multi Column Capture system can be used either to intensify existing fed-batch processes or serve as a key operation within continuous processes. These dual

capabilities of the system complemented by single-use flow paths, provide flexibility for agile multi-product facilities like CDMOs and others producing early clinical phase material.

To address the challenges of buffer preparation, the Mobius® Multi Column Capture system has in-line buffer dilution functionality within the system that enables preparation of up to six buffers to user specifications through automated dilution of buffer concentrates. The in-line buffer dilution feature not only reduces the storage space needed for buffer concentrates but also streamlines the final buffer preparation process, minimizes handling efforts, and contributes to overall operational efficiency. By leveraging these innovations, biopharmaceutical manufacturers can improve efficiency of their processes while addressing the challenges posed by increased production demands.

In this Application Note, we present the economic benefits of incorporating in-line dilution functionality of the Mobius® Multi Column Capture system on Protein A capture step.

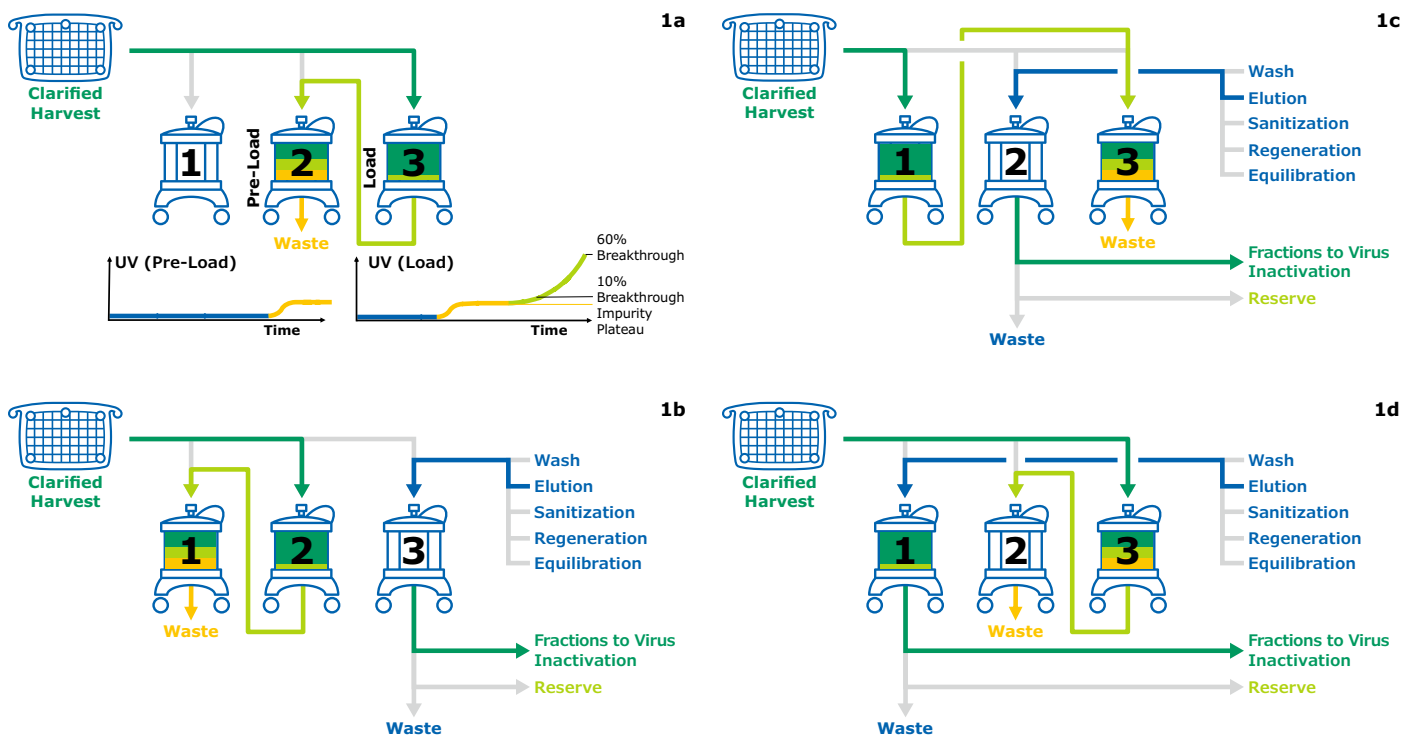


Figure 1.

Multi-Column Chromatography (MCC), three columns are operated simultaneously. Once column 3 is loaded (1a), it is disconnected from column 2 to be washed, eluted, and regenerated (1b). At the same time, column 2 (already partially loaded) is set in series with column 1 which remains unused and ready to accept product load. Once column 2 is loaded, it is disconnected from column 1 for wash, elution, and regeneration (1c). The same procedure is repeated with column 1. This complete sequence is called a cycle and can be repeated as many times as necessary to process all material or until the defined maximum number of cycles is reached.

How Buffers are Diluted In-Line?

The Mobius® Multi Column Capture system offers two types of dilution control: Percentage (volumetric) or Conductivity. Both methods use control loops, with a pre-pump flowmeter regulating volumetric dilution and a pre-column conductivity sensor regulating conductivity controlled dilution (**Figure 2**).

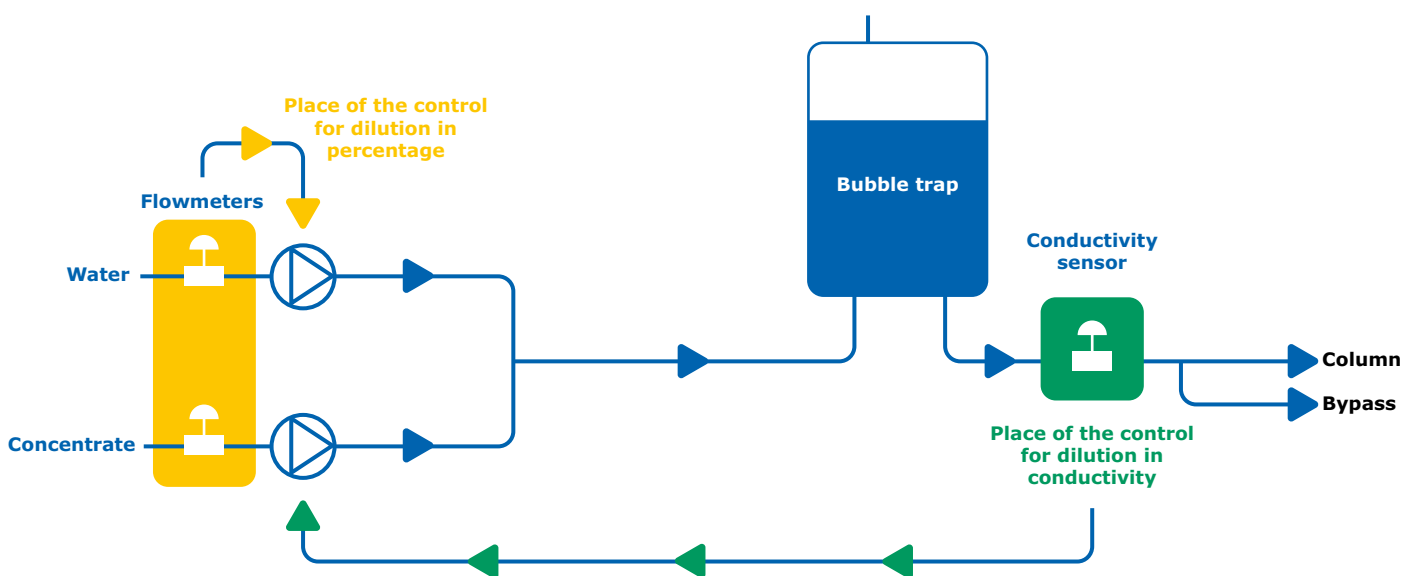


Figure 2. Regulation loop for in-line dilution: Volume dilution control (yellow) and Conductivity control (green).

Volumetric dilution

When using volumetric dilution, the dilution is controlled by setting the buffer concentrate flow rate as a percentage of the total flow rate.

- Once the dilution setpoints are defined, the system incrementally increases pump speeds, maintaining setpoint proportions until reaching the total flow rate setpoint, during which the buffer is directed to pre-column waste.
- Once the target flow rates are attained, the pump flow rates are fine-tuned and stabilized.
- Upon meeting the stability criteria, the valve transitions from the bypass to the column forward flow path.

This method allows for short stabilization times, as they correspond to the pump stabilization times, but requires precise concentrations of buffer concentrates.

Conductivity controlled dilution

When using conductivity controlled dilution, the buffer dilution pump flow rates are controlled in response to the pre-column conductivity sensor.

- The system first increases pump speeds to reach the desired total flow rate. During this flow increase, the flow rate proportion is derived from user-defined concentrate and diluent conductivities, and the liquid is directed to pre-column waste.
- Once the total flow rate is stable, iterative feedback control adjusts the flow rate proportion after each hold-up volume has passed between the pump and pre-column conductivity sensor.
- Upon meeting the stability criteria, the valve transitions from the bypass to the column forward flow path.

With this approach, the stabilization times are longer due to the greater hold-up volume between the conductivity sensors and actuating pumps, and therefore a longer response time of the control loop. However, the benefit of conductivity dilution control is that it allows for greater precision of the buffer dilution. To further reduce stabilization time, the bubble trap, located upstream of the conductivity sensor, can be bypassed, particularly during non-loading phases.

In-line Dilution Performance Studies

Performance of the in-line dilution capabilities of the Mobius® Multi Column Capture system is summarized in **Tables 1** and **2**. The details of these studies are available in the Performance Guide.²

Volumetric dilution

In-line dilution of an acetone concentrate was performed utilizing the volumetric dilution method. UV absorbance was monitored as an indication of the buffer concentration, **Table 1**.

Conductivity controlled dilution

In-line dilution of a 3M NaCl solution was performed utilizing the conductivity controlled dilution method. The conductivity was monitored as an indication of the buffer concentration, **Table 2**.

With conductivity controlled dilution, the time required to achieve stability criteria may decrease from one cycle to the next. In fact, the dilution rate from the same step of the previous cycle is saved and set as the new setpoint. Therefore, if no change is made to the buffer concentrate or diluent between two cycles, flow rate corrections required by the regulation loop are minimized, leading to more rapid attainment of dilution stability.

Table 1.

Performance study results for in-line volumetric dilution, without bubble trap. Results show time required to achieve stability, maximum mixing error (%) and volume of buffer concentrate used before achieving stability criteria. Stability criteria are: a. flow deadband: 10%, b. flow stable Time: 30s.

Total flow rate [L/min]	Dilution rate								
	×10			×20			×40		
	Time [mm:ss]	Mixing error [%]	Volume [L]	Time [mm:ss]	Mixing error [%]	Volume [L]	Time [mm:ss]	Mixing error [%]	Volume [L]
0.17	00:57	4.36	0.0159	00:47	3.80	0.0261			
0.33				00:58	1.21	0.0160	00:43	0.44	0.0595
0.67							00:42	0.39	0.0117
2.00							00:59	0.03	0.0492

Table 2.

Performance study results for in-line conductivity controlled dilution, without bubble trap. Results show time required to achieve stability, maximum mixing error (%) and volume of buffer concentrate used before achieving stability criteria.

Total flow rate [L/min]	Dilution rate [conductivity setpoint – mS/cm]								
	×10 [25.5]			×20 [14.5]			×40 [8.8]		
	Time [mm:ss]	Mixing error [%]	Volume [L]	Time [mm:ss]	Mixing error [%]	Volume [L]	Time [mm:ss]	Mixing error [%]	Volume [L]
0.17 ¹	04:02	2.27	0.067	05:23	0.44	0.045			
0.33 ¹				06:10	1.38	0.103	14:19	0.58	0.119
0.67 ¹							08:02	0.73	0.134
2.00 ²							01:19	0.96	0.066

1. Flow deadband 10%, flow stable time 30s, conductivity deadband 5%, conductivity stable time 30s.

2. Flow deadband 20%, flow stable time 30s, conductivity deadband 35%, conductivity stable time 30s.

Economic Analysis of Buffer Preparation Strategies in a Capture Step using the Mobius® Multi Column Capture system

The in-line dilution performance data of the Mobius® Multi Column Capture system were incorporated into an economic analysis of buffer preparation in a Protein A capture step.

The different scenarios considered a 2000 L facility intended to produce 20 batches of mAb per year with a 5 g/L fed-batch upstream process and three columns with a height of 6 cm and a diameter of 20 cm each. The various buffer preparation strategies differed in the concentration of the buffers and if they were made-in-house (MIH) or purchased externally. All other parameters were set to be identical. Cost components included consumables cost, labor time, wfi, raw materials, warehousing, risk, footprint.

- Base case: all buffers are made-in-house at the right concentration.
- Scenario #1: three out of six buffers are made-in-house at a higher concentration and diluted by the Mobius® Multi Column Capture system.
- Scenario #2: three out of six buffers are purchased externally at a higher concentration and diluted by the Mobius® Multi Column Capture system.

The combined cost profile (**Figure 3**) illustrates the overall savings potential derived from using the in-line dilution capabilities of the Mobius® Multi Column Capture system. **Figure 4** breaks down the profiles by cost components.

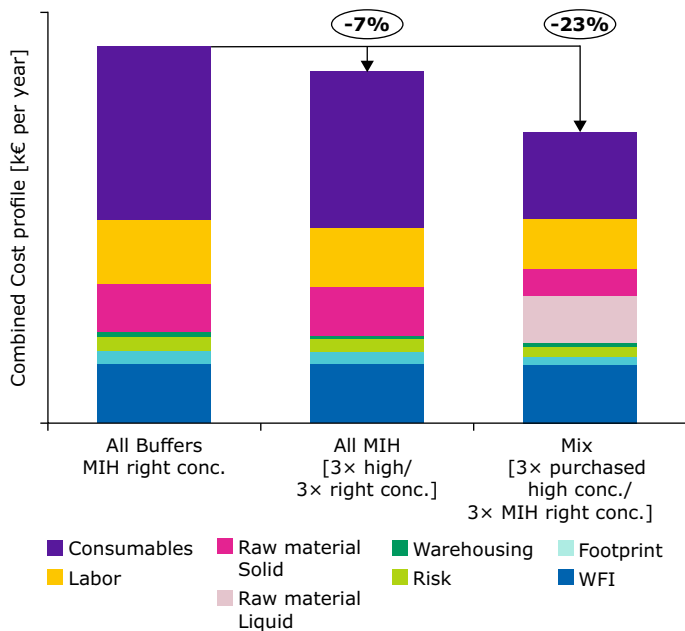


Figure 3.
Combined cost profile.

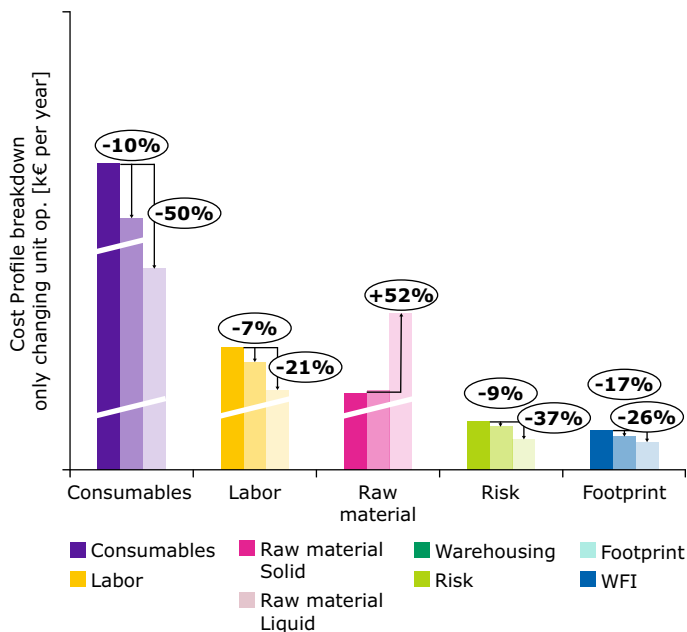


Figure 4.
Cost break-down. For each cost component, the darkest color represents the model base case (all buffers MIH right conc.), the medium color represents scenario #1 (all MIH – 3x high/3x right conc.), and the lightest color represents scenario #2 (Mix – 3x purchased high conc./3x MIH right conc.).

A separate focus was drawn on constraint resources (**Figure 5**) including labor hours and hardware footprint (Mobius® Single-Use Mixing and Storage Systems, Mobius® Multi Column Capture system). The more efficient utilization of constraint resources is not only valuable in a monetary way, but also brings more flexibility for future projects.

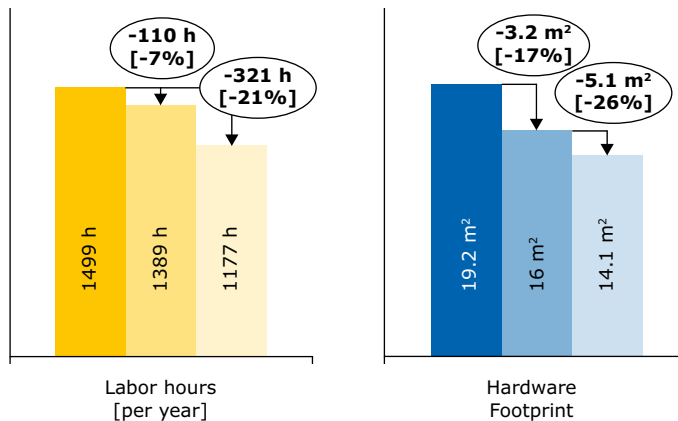


Figure 5.
Constraint resources.

The biggest component of cost in buffer preparation is consumables. Comparing the three buffer preparation scenarios, this is where the in-line dilution capabilities of the Mobius® Multi Column Capture system can capture the biggest savings by using smaller mixing and storage bags. Sourcing concentrated buffers externally further reduces these costs by eliminating the need for large volume mixer and storage bags. Higher raw material prices for concentrated liquid buffers were offset by larger cost reductions in other areas. Other value drivers include reduced labor costs connected to shorter filtration time and easier storage handling, along with less cleaning operations in the event of a leak.

While the Mobius® Multi Column Capture system footprint is identical in each of the three buffer preparation scenarios, depending on manufacturer decisions on how buffers are prepared, there are potentially large savings in consumables, labor and storage space.

Conclusion

The Mobius® Multi Column Capture system demonstrates its potential to enhance the efficiency of the capture step through its integrated in-line buffer dilution capabilities. By enabling both volumetric and conductivity controlled buffer dilution, the system provides manufacturers with the flexibility to optimize for precision or speed, depending on process requirements.

- Conductivity controlled dilution offers high precision but comes with longer stabilization times linked to feedback loop response. These times, which also depend on the stability criteria, can be reduced through optimizations such as bubble trap bypassing or cycle learning between steps.
- Volumetric dilution provides an efficient alternative for most applications, with shorter stabilization times, minimal buffer waste, and straightforward setup when precise buffer concentrates are available.

Economic analysis further underscores the value of in-line buffer dilution and highlights the substantial savings in consumables, labor, and facility footprint. Beyond cost reductions, the potential for the Mobius® Multi Column Capture system to address resource constraints and mitigate operational risks enhances its appeal for agile manufacturing environments. This adaptability, combined with reduced buffer storage needs, streamlined preparation workflows, and labor-saving benefits, positions the system as a key enabler for process intensification.

To determine the best approach for implementing the Mobius® Multi Column Capture system, contact your local Mobius® representative for expert support.

References

1. Economic Analysis of Buffer Preparation Strategy for Single-Use Bioprocessing of Monoclonal Antibodies, 2024.
2. Performance Guide – Mobius® Multi Column Capture system, 2023 PG 11657.



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