TECHNICAL NOTE

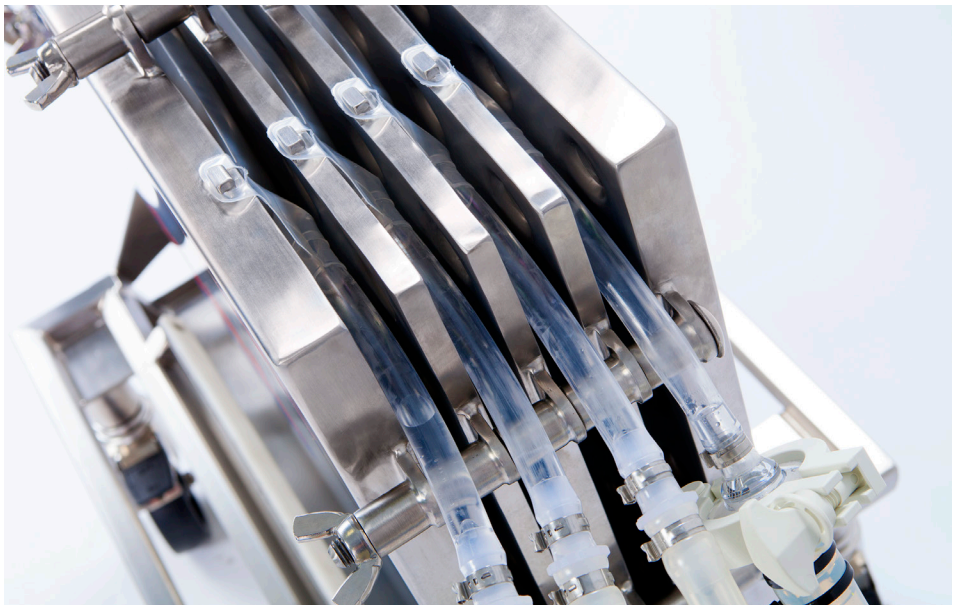
COL01676

Downstream Protein Purification

Integrate sterile and efficient temperature control with the Thermo Scientific DHX Heat Exchanger

Scope: The purpose of this technical note is to present the benefits of the Thermo Scientific™ DHX™ Heat Exchanger and its applications:

- Downstream protein purification equipment configuration
- Benefits of using the DHX in harvest clarification, chromatography, ultrafiltration/diafiltration, viral filtration and bulk drug substance
- Heating/cooling efficiencies in a single pass



Introduction

Bio-therapeutic manufacturing requires precise control of process parameters throughout production to ensure high yield and high quality products that are safe for patient use. The pressure on manufacturing facilities to increase yields, decrease turnaround time and reduce cost has never been greater than it is today, and presents a challenge that users must deal with on a day-to-day basis.

Downstream processing, specifically downstream protein purification is the final stage in bio-therapeutic manufacturing; the point when the target protein is harvested and purified.

Single-use technologies have found their place during downstream protein purification providing a sterile processing environment without the cost and time of clean in place or steam in place sterilization (CIP/SIP). although users are typically most concerned about bioburden control rather than sterility until the viral filtration step, the FDA still mandates sanitization standards that must be met. Single-use technologies are a practical solution to meet these standards, decrease costs, reduce contamination risk, increase process speed and turnover between processes.

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Unfortunately it is still challenging for a user to create a 100% single-use process in downstream protein purification. Several components of the manufacturing process including heat exchangers have not previously been available in a single-use format, requiring that users either insert stainless steel equipment into an otherwise single-use process flow, or use other single-use equipment such as a jacketed mixing tote in place of a heat exchanger, a purpose for which temperature control is not optimized. The ideal solution to this problem is to include a single-use heat exchanger at key points in the process to gain precise temperature control of the process solution without losing the benefits of a single-use process (see figure 1 to the right).

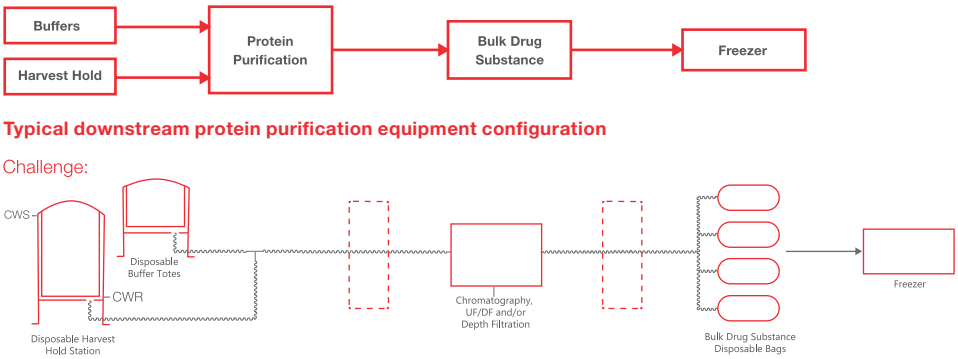


Figure 1. Typical downstream processing configuration. Dashed boxes indicate where technical challenges exist.

Temperature control during downstream protein purification

Protein purification is technically challenging; proteins are heat labile, and most purification steps expose the process solution to unwanted heat. In order to maximize quality and quantity of the final product, it is often desirable to cool the process solution after each step in order to prevent unwanted binding or folding.

Downstream processing is a multi-step process that typically includes the following steps:

1. Harvest clarification to separate the cells from media and cell debris
2. Chromatographic separation to isolate and purify
3. Ultrafiltration/diafiltration to further purify and concentrate
4. Viral filtration to leave a pure, clean, pharmaceutical grade product
5. Bulk drug substance freezing prior to shipment to Fill/Finish facility

While it is true that every step in the process generates heat that must be controlled, there are critical points where temperature control has a significant impact on the yield and quality of final protein.

Harvest Clarification

Typically performed by means of centrifugation or filtration. Processing large batch volumes through harvest clarification can take a significant period of time, which can lead to increased temperature. It is common practice to use a jacketed mixing tote or hold station after clarification and prior to column load to control temperature. The DHX heat exchanger provides an alternative solution with increased heat transfer rates, enabling more rapid cooling of the process solution, while maintaining the benefits of single-use.

Chromatography

Chromatography is one of the most important steps for determining final yields. Proper optimization of the chromatographic separation steps can have a significant impact on recovery rates. This is compounded by the fact that reaching the desired level of purity often necessitates multiple rounds of chromatography. while there are always losses during chromatography (recovery is never 100%), loading conditions are a critical factor in determining the percent recovery from each pass. The process solution and loading buffer must be at the same temperature during mixing and loading onto the column. Failure to properly control the temperature of either solution will lead to non-specific binding and a reduced recovery.

Ultrafiltration/Diafiltration

Polishing via ultrafiltration/diafiltration (UF/DF) is another critical step in determining final yield. Recovery can be significantly improved through proper temperature control of buffers and intermediates prior to loading, and through rapid cooling of the retentate or permeate immediately after filtration.

Viral filtration and bulk drug substance

Final steps in downstream processing call for viral filtration and cooling of the bulk drug substance prior to freezing. Once viral filtration is complete, sterility of the bulk drug substance is critical and single-use options for cooling can expedite the freezing process. The DHX single-use heat exchanger provides an alternative; enabling sterile cooling in a single pass (depending on temperature and flow rate of the bulk), with heat transfer rates comparable to a stainless steel heat exchanger, while maintaining all the advantages of single-use.

Given all the time and expense that has gone into getting the protein to this final stage it is critical to maximize the final yield. Proper set up of clarification, purification, and polishing techniques, and control of key parameters such as temperature, are all- important in the determination of final yield. The DHX single-use heat exchanger enables temperature control at critical points during downstream processing; increasing yields, driving down cost, reducing clean up and improving turnaround times of plant and equipment. (See figure 2 to the right).

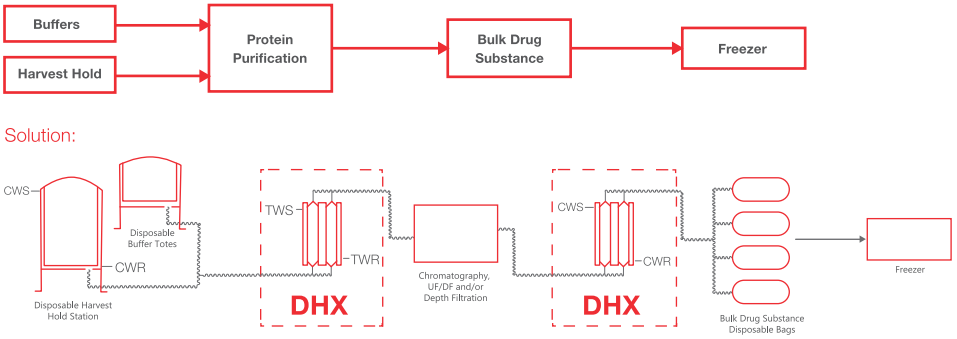


Figure 2. Utilizing the DHX Heat Exchanger in downstream protein purification.

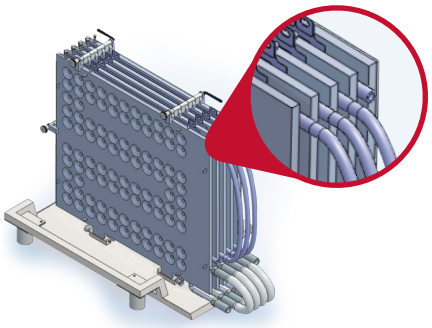
Benefits

- **Increased yields**
  The DHX enables the user to accurately control the temperature of their process solution before and after critical filtration and chromatography steps without the need to incorporate stainless steel equipment into the process flow. Precise temperature control is a key determinant of recovery and yield, especially during chromatography and polishing steps.

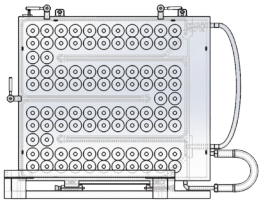
- **Reduced infrastructure requirements**
  The DHX Heat Exchanger enables the user to cool or warm the process fluid and buffers with the benefits of single-use technology. The DHX eliminates the need for separate cold or warm storage rooms, which greatly reduces the complexity and cost of the manufacturing facility requirements.

- **Reduced bioburden**
  The DHX heat exchanger creates a sterile, temperature controlled fluid path for process solution, buffers and any other process components. The single-use benefits of the DHX addresses any weak points in the CIP or sanitization processes that typically arise when stainless steel equipment is used.

- **Rapid turnaround of process equipment**
  anytime stainless steel equipment is used within a process, it creates a requirement for time consuming CIP and sometimes SIP operations. These cleaning and sterilization steps can increase the turnaround time from hours to days, locking up expensive capital equipment that could otherwise be put back into service. The DHX Heat Exchanger addresses one critical point in the process where previously only multi-use options were available.



A plate and frame design promotes efficient heat transfer. Single-use BioProcess Containers (BPCs) fit tightly between the stainless steel plates.



Serpentine flow paths of process fluid and heating/cooling fluid maximize heat transfer.

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Heat transfer rates in both heating and cooling mode are comparable with stainless steel heat exchangers

Table 1. Cooling efficiencies

DHX cooling efficiencies measured by temperature vs. time.

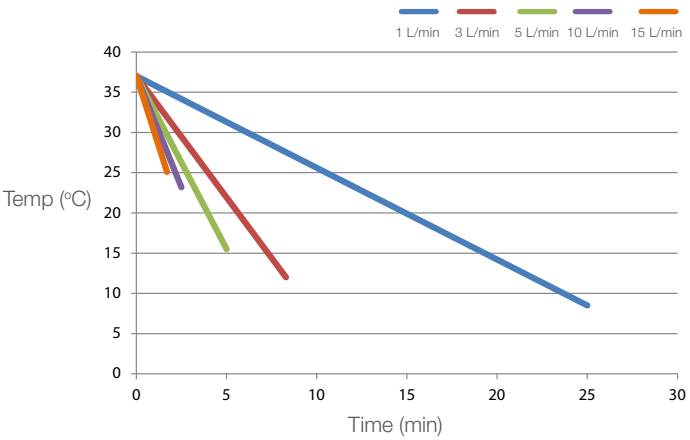
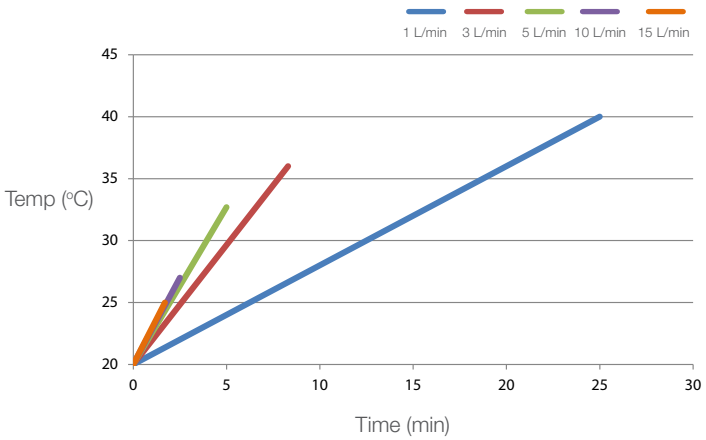


Table 2. Heating efficiencies

DHX heating efficiencies measured by temperature vs. time.



Conclusion

There are several reasons to choose the DHX Heat Exchanger, but the most compelling argument is the increased quality and improved yields that can be achieved by controlling the temperature of the process solution before and after critical steps such as filtration and chromatography. The cost and time required to take a modern bio-therapeutic all the way through upstream and downstream processing is immense. Any tool or technique that can increase the final yield will positively impact the process; reducing cost and increasing profit.

References

1. State-of-the-art in downstream processing of monoclonal antibodies; process trends in design and validation. Marichal-gallardo, P.A., and Alvarez, M.M. Biotechnology Progress. Volume 28, Issue 4. 899-916, June 2012
2. Single-use disposable technologies for biopharmaceutical manufacturing. Shukla A, gottschalk U. Trends in Biotechnology, Volume 31, Issue 3, 147-154, 26 November 2012

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