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Using Differential Scanning Calorimetry to Make Downstream Purification Processing More Economically Viable: A Case Study

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Application Note

Efficient process development must deliver a predictable, stable and reliable process in a short period of time. What makes this task particularly challenging is that increasing purity of the protein product removes it from a stable environment. In the development of protein purification processes, Differential Scanning Calorimetry (DSC) can rapidly, in days compared to weeks, identify stabilizing buffer conditions for chromatography loading and elution. Described here is a DSC investigation demonstrating that the buffering conditions of a therapeutic antibody can be optimized during the development of a Protein A capture step. The results indicate that it is possible to use DSC during process development, which may result in substantial financial savings in downstream biopharmaceutical process development and ultimately manufacturing. In particular, this application note focuses on how DSC was used to save a particular Diosynth Biotechnology client hundreds of thousands of dollars by increasing the efficiency of the antibody capture step during the development of the purification process.

Introduction

Biopharmaceutical process development can be costly and problematic. The ultimate goal is to maximize purified product by the most cost effective, reproducible and robust route. Since batch failures can have major economic consequences, a thorough understanding of protein stability throughout the development pathway, from research through clinical development to commercial manufacturing is fundamental to biopharmaceutical drug commercialization.

Maintaining stability and preserving the active structure of a protein biopharmaceutical until administration is essential. This can be accomplished in part, through stability studies designed at understanding the physical behavior of biotherapeutics. Proteins typically have inherent stability issues due to their complexity and delicate structure. Stability studies to determine the effect of various environmental factors such as pH, temperature and ionic strength on the conformational integrity of a protein, which directly influences biological activity, are indispensable during process development. These “stress” studies help identify features that are critical by revealing the “weak points” in the molecule and provide a rational approach in identifying denaturation mechanisms and in developing effective counter measures, through selection of appropriate buffer, pH, ionic strength and excipients.

Crude Protein Extract

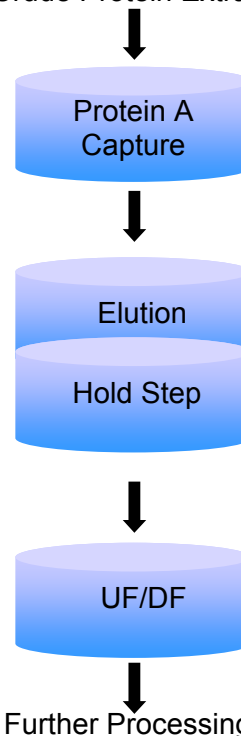


Fig 1: Typical antibody purification scheme

Conducting stability studies in parallel with purification and method development allows scientists to design more effective methods. During development of purification processes, stability studies can:

- Identify appropriate buffers for process steps, including chromatography and ultrafiltration/diafiltration (UF/DF) to increase stability and minimize degradation
- Identify hold steps
- Ensure that processing conditions can be effectively controlled
- Help in maximizing yields

Stability studies also aid in the development of robust and stability-indicating assays. Finally, these data enable accelerated development of formulations for drug product by minimizing the range of conditions that need to be explored during typical formulation development studies. This in turn translates into savings of resources, cost and time.

This application note focuses on the utility of Differential Scanning Calorimetry (DSC), a stability-indicating technique, to guide the development of antibody purification processes. In particular, this note describes how DSC data was used to identify optimal elution conditions during Protein A capture step development that resulted in cost savings at the early clinical manufacturing scale. DSC provides a way to monitor protein stability throughout processing and handling which can lead to improved conditions that can protect the molecule, when atypical conditions or protein behavior occur during processing or assay development.

Materials and Methods

All proteins were prepared in the indicated buffer by dialysis or buffer exchange with a PD-10 desalting column (GE Healthcare, Piscataway, New Jersey). Final protein concentrations were approximately 1 mg/ml. Proteins were analyzed with the VP-DSC (MicroCal, Northampton, Massachusetts) using a temperature range of 5 to 90 °C, at a scan rate of 1 °C per minute. Analyses of thermograms were performed using MicroCal Origin software using either a two-state or a non-two-state model.

Development and Optimization of Protein A Capture in Antibody Purification

Protein A affinity chromatography is commonly used as the initial capture step for monoclonal antibody purification. Protein A binds to the Fc region of many immunoglobulin (Ig) antibody molecules. This binding specificity and selectivity can result in nearly pure product in a single step. Antibody molecules are produced by cell culture, and need to be purified from host cell proteins, nucleic acids and cell culture components. Cell free conditioned cell culture media is applied to the Protein A

affinity resin. The antibody binds to the Protein A ligand at a neutral pH (around pH 7) and is eluted from the resin with a low pH buffer (for example, citrate buffer at pH 3.5). After elution from the column, the antibody solution is neutralized with a solution having a high buffering capacity (such as Tris base at high concentration) or low concentration of base.

There are Protein A affinity resins that are optimized for use as a capture step in a purification process, with high binding capacity and fast flow rates to process thousands of liters of cell culture. However, Protein A chromatography resins are very expensive (typical cost \$7,000 to \$12,000 per liter) and production scale columns can be over 100 liters in size, depending on the bioreactor volume and titer of antibody produced by the cells.

One issue with the use of Protein A affinity chromatography is that many antibodies, as well as other proteins, are unstable at low pHs required for elution. If the protein is unstable at low pH, it can precipitate during or after elution. Precipitation, if it does occur, is a major obstacle in the development and optimization of a Protein A chromatography capture step. Precipitation is usually dependent on protein concentration: high concentration favors aggregation and precipitation. Thus, although most resins can bind at least 20 g of antibody per liter of resin, the stability of the protein at low pH post elution becomes the limiting factor for the loading density of the resin. Unfortunately, most Protein A alternatives also require low pH elution. Although many antibodies are pH-sensitive, the mechanism of denaturation and aggregation varies with structure, and will require different buffers for optimal stability.

To improve the loading capacity and economics of the Protein A chromatography process, the protein needs to be stabilized in the elution buffer. DSC can be used to characterize the stability of the antibody as a function of pH, and determine which additives can improve the protein stability at low pH. In DSC, an increase in the transition temperature (the T_m) suggests an increase in protein stability. The Protein A chromatography step can be made more economically viable by stabilizing the antibody in the low pH elution buffer, thus increasing the loading capacity of the column, and reducing the amount of Protein A chromatography resin needed for the process.

Case Study - Antibody X purification

In this particular case study, a client wanted Diosynth Biotechnology to improve the purification process of their antibody (antibody X). Process information transferred from the client indicated that the initial binding capacity was limited to 2 g of antibody X per liter of Protein A resin due to precipitation of the antibody during elution at higher loading density. To understand the effect of pH on the stability of antibody X, the protein was prepared in citrate buffer adjusted with Tris to pH 7.0, phosphate

buffer pH 7.3, citrate buffer pH 3.0, and citrate buffer pH 5.0. From DSC data (Table 1) antibody X at pH 7.0 and 7.3 showed a higher T_m than antibody at pH 3.5, indicating that the protein was more stable at higher pH. The unfolding onset temperatures were also higher for pH 7.0 and 7.3 compared to pH 3.5. Antibody X at pH 5.0 had a T_m comparable to pH 7.0, and the unfolding onset temperature was lower, suggesting that pH plays a major role in determining unfolding of this antibody.

Antibody X was also prepared in low pH buffers with different additives to determine if any of these additives could stabilize the antibody at low pH. DSC experiments were performed to see which conditions would increase the T_m of the antibody (Figure 2).

	T_m of major transition	Onset of unfolding ($^{\circ}\text{C}$)
Citrate-Tris buffer pH 7.0	68.7	60.1
PBS pH 7.3	69.5	58.5
Citrate pH 5.0	71.5	48.2
Citrate pH 3.5	59.3	34.1
Citrate, Mannitol, pH 3.5	64.7	41.0

Table 1: T_m and onset of antibody X unfolding in various buffers obtained by DSC

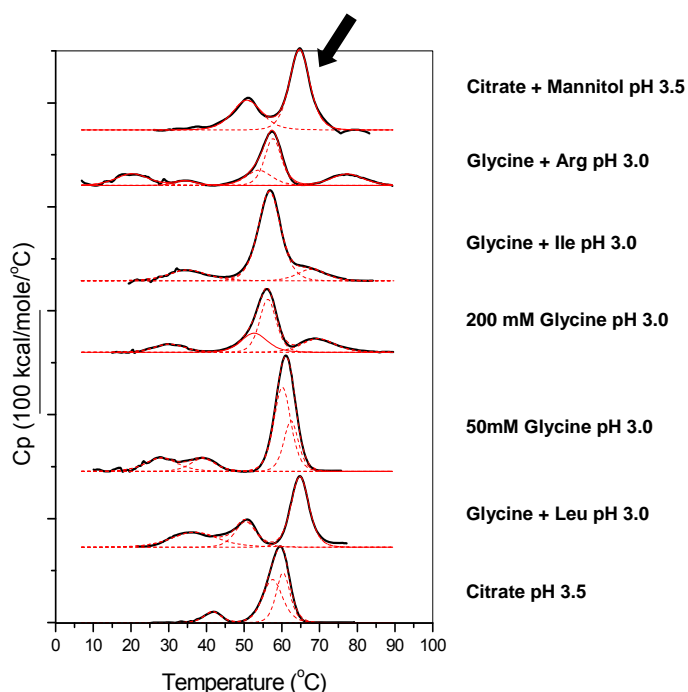


Fig 2: DSC for antibody X with the 7 different elution buffers. Dark lines are DSC data normalized for protein concentration. Broken red lines represent analysis of data fit to either a two-state or non-two-state model. Arrow indicates highest T_m which was for Citrate + Mannitol pH 3.5.

For antibody X, citrate plus mannitol at pH 3.5 resulted in the largest T_m shift, indicating the most favorable stability. The addition of mannitol increased both the T_m and the unfolding onset temperature relative to citrate at pH 3.5. (Figure 3).

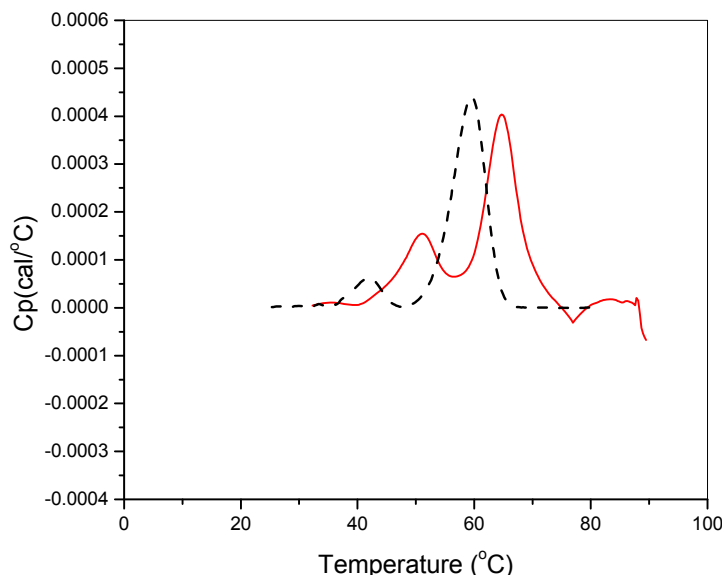


Fig 3: DSC of antibody X in citrate and citrate + mannitol as comparison. The broken line represents the scan of the protein in presence of citrate and red line represents the scan of the protein in

The DSC results suggested that the addition of mannitol to the elution buffer of the Protein A affinity column would improve the stability of antibody X. Improved antibody stability during elution can increase the loading density of the antibody onto Protein A resin, thus reducing the cost of raw materials.

Process development efforts using the protein stability information determined from DSC did result in an increase in Antibody X loading density for the Protein A capture step. Use of citrate plus mannitol as the Protein A elution buffer resulted in at least a 7.5-fold increase in capacity to ≥ 15 g of antibody X per liter of Protein A resin, as compared to 2 g/L with citrate buffer only.

This increased loading capacity directly translates to reduction in the cost of goods related to the purification process. The initial process from the client used a 19.5 liter Protein A column that would have cost $\sim \$175,500$. Using the purification process optimized with DSC data, the capture required a 2.6 liter Protein A column that cost only $\$23,400$. Antibody X after the Protein A capture step was also more concentrated than it was in the earlier process, eliminating an ultrafiltration/diafiltration step, resulting in further savings in materials and processing time. At the 100 liter cell culture scale, assuming an antibody titer of 0.5 g/L and four cycles per run on the Protein A column, the cost reduction would be $\$38,000$ for the resin alone, not including reduced raw materials for the process buffers. At the 1,500 liter cell culture scale the savings would be $\$570,000$ for the resin.

Conclusion

In this application note, we used DSC data to identify the most stabilizing elution conditions for antibody capture during process development. DSC provides valuable information for choosing protein stabilizing buffering conditions before any chromatographic development is undertaken. The ability to improve performance in this critical step in the development of purification processes may result in substantial financial savings. In the case study presented here, optimization of antibody stability during the capture step resulted in reduced processing time and savings well over \$100,000 in raw materials alone.

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