Differential Scanning Calorimetry in the Optimisation of Antibody Manufacturing

Differential scanning calorimetry (DSC) represents a powerful tool for assessing stability parameters, such as pH, ionic strength and excipients, that are critical in the design and optimisation of manufacturing processes.

The development of manufacturing processes for biotherapeutics, such as antibodies, has many challenges, which need to be overcome; these include the constraints of project time-lines as well as limited initial availability of material. As a result, there is a requirement for rapid, robust and sensitive process development tools. Process development and manufacturing steps are routinely evaluated and optimised to improve protein yield, reduce production time and increase long-term stability of the final drug product. Any improvements in final yield and stability are critical to both process and manufacturing economics. Conversely, any purification or production step with a decreased yield and/or stability will result in a financial loss.

Thermodynamic analysis has proven to be an invaluable approach to aid in the characterisation of physical protein instabilities. Understanding the factors that affect stability – such as pH, ionic strength and excipients – is critical in the design and optimisation of manufacturing processes. Differential scanning calorimetry (DSC) is a powerful analytical tool to assess these stability parameters. The technology is utilised throughout the biotherapeutic development pipeline, since it quickly and easily facilitates the selection of process conditions, as well as storage conditions during manufacturing. DSC generates thermodynamic parameters, such as transition temperature ($T_m$), which are indispensable for rapidly evaluating protein stability. Improved thermal stability results in a higher $T_m$ value.

This article describes the use of $T_m$ data obtained from DSC to screen conditions for viral inactivation of an antibody (1). This information provides a quick and easy stability assay during the manufacturing process. For the antibody studied here, the apparent $T_m$ data from DSC was key to the recommendation to increase storage pH immediately after viral inactivation, resulting in increased structural stability. This example illustrates the crucial role of DSC and thermodynamic analysis during therapeutic protein development.

**PROTEIN STABILITY**

The ultimate goal of the development of proteins as therapeutics is the design of dosage forms with sufficient stability to withstand shipping, processing and storage conditions. Biopharmaceutical development is complex and requires expertise and input from multiple groups – such as process development, manufacturing and formulation development. Each biotherapeutic protein is different, requiring its own optimal solution conditions. The timelines for these assessments are often compressed, making 'real-time' shelf-life determinations impractical. Therefore, bioanalytical scientists must rely on a combination of accelerated and forced degradation studies to probe the likely instabilities that might be encountered for a given protein. Methods that help provide an early assessment of the solution behaviour of a protein are very valuable, so scientists are able to eliminate the 'riskier' solution conditions early in the process, and can focus on the processes and formulations, which result in more stable protein. Nevertheless, the amount of material available for analysis during early stage evaluations is often a limiting factor. Procedures that employ small quantities of protein and provide information regarding structure and stability are essential.

An initial thermodynamic evaluation of a protein resulting from these procedures can provide insight into its solution behaviour under a variety of conditions. A preferred tool for such analysis is differential scanning calorimetry (DSC). Although information could also be obtained by alternative methods, DSC has several key advantages: high-throughput capabilities with the automated VP-Capillary DSC system, reproducibility, unlimited buffer compatibility and – most importantly – it is a first-principle technique where the thermal stability of a protein can be directly measured (2-4). Instabilities most commonly observed during the
development of biotherapeutic proteins fall into two categories: biochemical and structural. Some of the most common chemical instabilities include oxidation, deamidation and isomerisation (5-7). Altering protein chemical stability is accomplished by changing solution conditions based on knowledge of the chemistry underlying the instability of interest. Additional degradation pathways observed include: secondary and tertiary structure changes, fragmentation and aggregation/self-association (6,8,9).

THERMODYNAMIC ANALYSIS

The application of thermodynamics to manufacturing development is typically governed by three key stabilisation mechanisms:

- Prevention of partially unfolded intermediates prone to aggregation
- Stabilisation of the folded state, leading to aggregation prevention, through the preferential exclusion mechanism
- Structural stabilisation by maximising protein-ligand or protein-excipient binding interactions

Maximising thermodynamic stability can also have an indirect impact on biochemical stability by changing the solvent exposure of susceptible residues.

There are several challenges that may be encountered when applying thermodynamics to process development and manufacturing issues. First, project timelines often demand the ability to perform high-throughput screening characterisation. Second, the thermodynamic parameters obtained are apparent parameters, useful for relative comparisons of manufacturing conditions. The inability to calculate absolute thermodynamic parameters is a consequence of the irreversible unfolding behaviour typically observed in large, multi-domain proteins, which often contain disulfide bonds, glycosylation sites and other post-translational modifications. Finally, thermodynamics provides no direct link to kinetics of reversible and irreversible protein denaturation. During the development of a drug with an intended shelf-life, it is critical to understand when and how fast a given reaction will take place. Given those basic precautions, DSC analysis provides a researcher with valuable information about the relative differences in stability of a given set of solution conditions. Below, we report on the use of thermal stability data obtained from DSC to screen conditions for viral inactivation of an IgG.

VIRAL INACTIVATION OF AN ANTIBODY

Differential scanning calorimetry was performed using a VP-Capillary-DSC (see Figure 1). Thermograms for each protein (350µL at 1mg/mL) were obtained from 25°C to 100°C using a scan rate of 200°C hr⁻¹, unless otherwise noted. Thermograms of the buffer alone were subtracted from each protein prior to analysis using Origin 7.0 data analysis software equipped with the MicroCal VP-Capillary DSC.

pH-mediated viral inactivation is a standard industry practice in which the protein is exposed to a decreased pH (10). In this antibody example (antibody-C), increased aggregation rates after viral inactivation were reported by the purification group (data not shown). This observation prompted the use of DSC to characterise the behaviour of antibody-C during this processing step, and to subsequently identify an appropriate post-viral inactivation storage condition. Samples were exposed to a simulated viral inactivation step, after which the pH was increased in a step-wise manner. The behaviour of all three transitions as a function of pH was found to trend consistently. Representative data from a single Tm shows increased thermal stability with increasing pH (see Figure 2, page 60). Based on the apparent Tm data, it was recommended to increase storage pH immediately after viral inactivation to maximise structural stability.

DSC was used not only to improve the process, but also to identify the underlying cause of this instability. Significant destabilisation of the first and loss of the third transition is seen when the pH 3.8 thermogram in Figure 3A is compared with the pH 4.5 pre-viral inactivation thermogram. Partial loss of antibody-C structure was confirmed by circular dichroism (data not shown), supporting the observation of Fc domain unfolding at low pH. Antibody-C showed decreased thermal stability after viral inactivation (see Figure 3B),

Figure 1:
VP-Capillary DSC system

(Photograph courtesy of MicroCal)
providing a potential explanation for the increased aggregation rates reported. An analysis such as this can yield a significant amount of valuable information about a protein early in development. It allows for the intelligent design of a viral inactivation strategy, and the associated in-process hold steps that minimise protein instability.

Additionally, preliminary information about pH and unfolding behaviour is collected, which is valuable later in formulation development.

CONCLUSION

The case study described above provides one example of the role that thermodynamic characterisation and DSC specifically should play in the biopharmaceutical development process. While it is far from an exhaustive list, some other key applications of DSC include: identifying appropriate storage temperatures, characterising stability as a function of pH and buffer species, enhancing process development activities, and the characterisation of stabilizing excipients and ligands during late stage formulation development.

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