

Preformulation and stability studies of biotherapeutics using DSC

It is important for a biotherapeutic to remain stable throughout manufacturing, storage, and delivery to the patient. Differential scanning calorimetry (DSC) allows for the accurate, rapid and easy measurement of T_m , the thermal transition midpoint of a protein, which has been shown to be a good indicator of the relative stability of biotherapeutics in solution.

In this application note, MicroCal VP-Capillary DSC has been used for rapid screening of buffers in preformulation development and for the optimization of storage conditions of antibodies during process development. The results correspond well with those obtained by other more time-consuming methods.

Introduction

The use of biotherapeutics, such as antibodies and other protein molecules, to treat diseases is a rapidly growing field in pharmaceutical industry. Biotherapeutics are often required at high concentrations and multiple doses; hence a manufacturer must produce kilogram quantities or more of the protein drug. The manufacturing process of biotherapeutics involves protein expression in thousands of liters of bioreactor media followed by a purification process using large-scale chromatography columns and filtration systems.

The stability of the protein to process conditions, reversibility of conformational changes, and any propensity to form aggregates depends on factors such as pH and buffer composition. A thorough understanding of these factors is important for the selection of process conditions, formulation, and development of analysis methods. Steps in the antibody purification process that could render a

protein unstable include low pH elution step from protein A column, low pH hold step for viral inactivation and any stage involving pH and/or ionic strength adjustment including final formulation.

DSC provides information on the thermal stability of a protein under different pH and cosolutes, by monitoring thermal transition midpoints, T_m . A higher T_m reflects higher thermal stability, which correlates well with long term stability. This application note describes how Diosynth Biotechnology uses thermal stability data obtained from DSC to characterize the stability of an antibody during initial pH and buffer screening for preformulation development and for optimization of the low pH viral inactivation used in the manufacturing process. Low pH viral inactivation is desirable for protein manufacturing if it does not cause any decrease in protein stability.

Materials and methods

DSC was performed using a MicroCal VP-Capillary DSC system (GE Healthcare). In the preformulation development study, a range of buffers with pH between 3 and 8 were used. The protein (antibody X) was stored in each buffer, and assays were performed immediately ($t = 0$) and after one week storage ($t = 1$ week).

For the optimization of purification conditions, DSC thermograms of antibody Y in a neutral Tris buffer containing NaCl and EDTA, pH 7.4 were studied and compared to a pH 3 citrate buffer and the citrate buffer adjusted to pH 6 with 2 M Tris, pH 9.0.

Thermograms of the buffer alone were subtracted from each protein prior to analysis using Origin™ 7.0 software equipped with MicroCal VP-Capillary DSC analysis software.



Results and discussion

Initial pH/buffer screening during preformulation development

The values of the main T_m peak at $t = 0$ for antibody X in the initial study of 19 buffers for preformulation development are shown in Figure 1, and DSC thermograms for the antibody in two of these buffers are shown in Figure 2. From the T_m values, the most stable buffer conditions are found between pH 5.0 and pH 7.5. At $t = 0$, other analytical methods (UV, size exclusion chromatography (SEC), light scattering, and SDS-PAGE) showed much less discrimination between the buffer conditions as compared to DSC (data not shown).

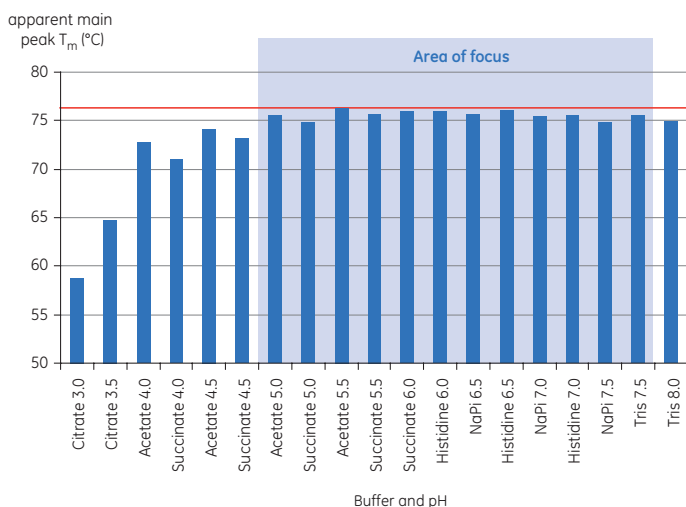


Fig 1. Range of T_m values of antibody X in preformulation buffers. Samples were analyzed in MicroCal VP-Capillary DSC at $t = 0$.

$T_{1/2}$ values were used to further discriminate between conditions, as indicated in Figure 2. $T_{1/2}$ is the peak width at half maximal height for the major transition in the DSC thermogram and typically reflects the cooperativity of the thermal transition. A lower $T_{1/2}$ value can indicate a more compact structure and is therefore preferred for formulations. Here, the lowest $T_{1/2}$ values were found for buffers with pH values between 5.5 and 6.5 (Fig 3).

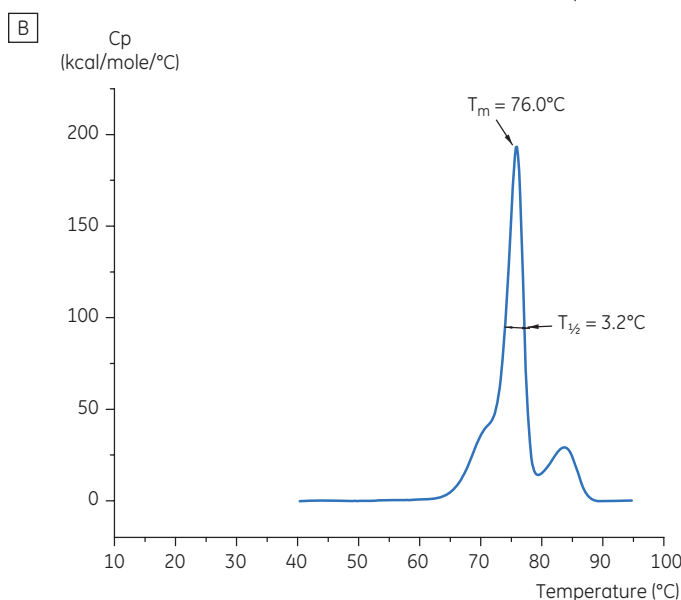
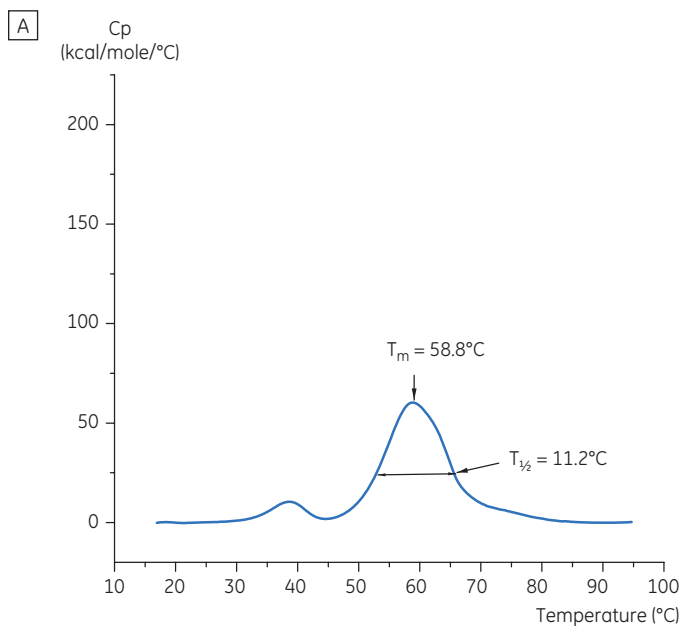


Fig 2. DSC thermograms of antibody X at $t = 0$. (A) dissolved in citrate buffer pH 3.0 (B) dissolved in succinate buffer pH 6.0. T_m and $T_{1/2}$ values are shown.

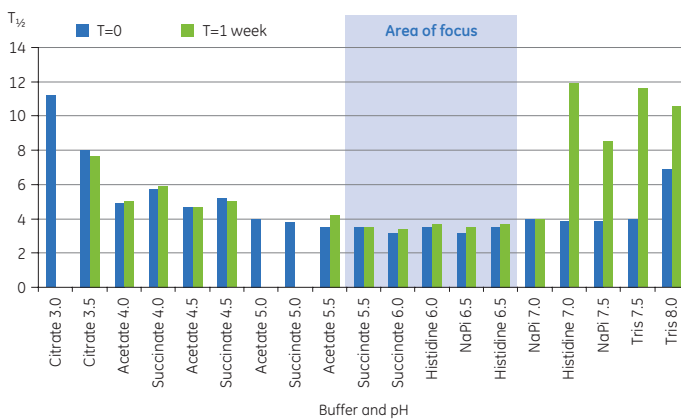


Fig 3. Range of $T_{1/2}$ values for the main T_m transition of antibody X in preformulation buffers at $t = 0$ and $t = 1$ week.

Low pH hold step and neutralization

Citrate buffer at pH 3 is a candidate for use for both the elution of the antibody from the protein A affinity column and for the subsequent low-pH hold step for viral inactivation. Since the majority of proteins tend to become unstable under prolonged exposure to such a low pH, the pH must be raised immediately after the viral inactivation step. The DSC scans of the antibody in buffer at neutral pH and at pH 3 are shown in Figures 4 and 5.

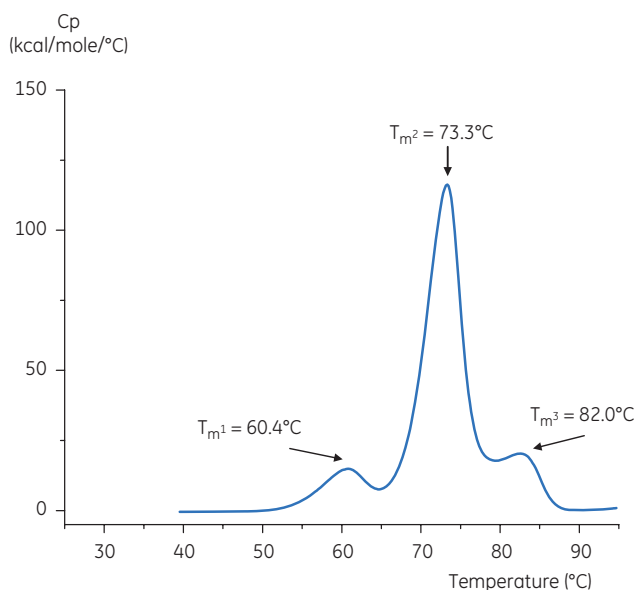


Fig 4. DSC thermogram of antibody in reference buffer (Tris, NaCl, EDTA, pH 7.4). The DSC thermogram shows three thermal transitions. This kind of thermogram pattern is common for antibodies.

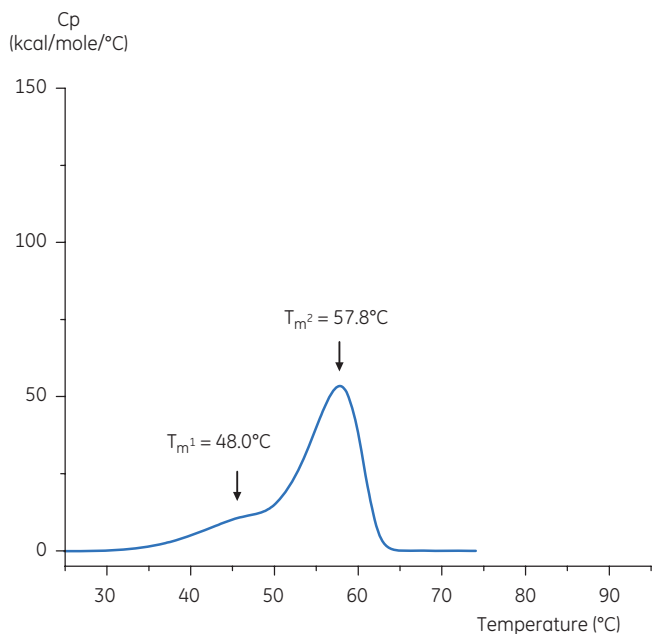


Fig 5. DSC thermogram of antibody in low pH hold buffer (citrate, pH 3.0). The DSC thermogram for antibody in pH 3.0 shows a loss of thermal stability due to decreased T_m values: T_{m1} decreased from 60.4°C to 48.0°C, T_{m2} decreased from 73.3°C to 57.8°C, and T_{m3} was no longer apparent in antibody in the pH 3.0 buffer.

The differences in shape and pattern of the two thermograms demonstrate loss of thermal stability of the antibody in the pH 3.0 buffer. The peak height of the T_{m2} transition is lower, the peak is broader and the definition between the first and second transitions in pH 3.0 is less pronounced as compared to pH 7.4.

To imitate the pH neutralization step, the pH of the antibody solution was adjusted to pH 6.0 with a concentrated Tris solution at pH 9. The thermogram of the resulting antibody solution is shown in Figure 6. Here, an increased thermal stability relative to the antibody in pH 3.0 buffer is seen. The T_{m2} and T_{m3} of the antibody in the pH 6.0 solution are similar to T_{m2} and T_{m3} of the antibody in the reference buffer. The overall shape, peak definition, and peak width for the pH 6 antibodies are also similar to the reference situation at pH 7.4. Neutralization can also be done in the presence of stabilizing excipients, such as histidine. In this case, the thermogram of the antibody neutralized in the presence of histidine was almost identical to Figure 6, indicating that for this particular antibody, histidine did not provide any significant stabilizing effect.

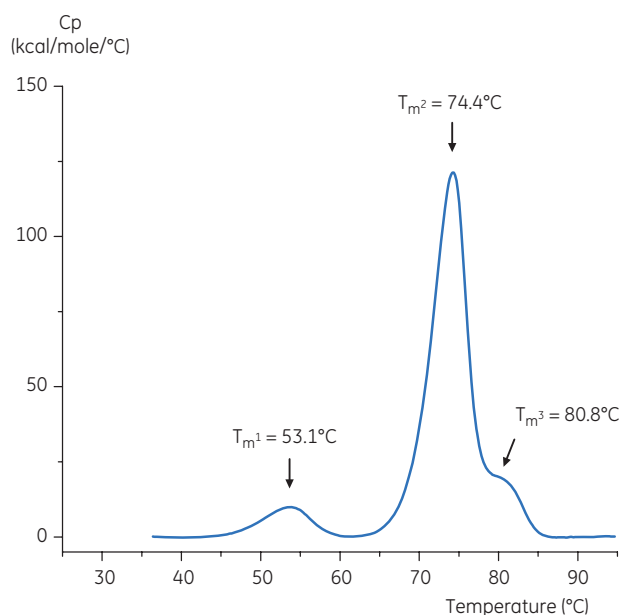


Fig 6. DSC thermogram of antibody after adjustment from pH 3.0 to pH 6.0 with 2 M Tris, pH 9.0.

Conclusion

This study shows that DSC could be used to quickly optimize pH and buffer conditions for preformulation development. These data were used to rank appropriate buffers and pH interval for the subsequent excipient screening, reducing the number of exploratory conditions considerably.

DSC can also be used for checking the stability of an antibody during low pH inactivation hold step and following adjustment of pH from 3.0 to 6.0. This kind of stability information is useful in designing and optimizing processes for biopharmaceutical manufacturing.

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For local office contact information, visit
www.gelifesciences.com/contact

www.gelifesciences.com/microcal

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden



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GE Healthcare UK Limited
Amersham Place
Little Chalfont
Buckinghamshire, HP7 9NA
UK

GE Healthcare Europe, GmbH
Munzinger Strasse 5
D-79111 Freiburg
Germany

GE Healthcare Bio-Sciences Corp.
800 Centennial Avenue, P.O. Box 1327
Piscataway, NJ 08855-1327
USA

GE Healthcare Japan Corporation
Sanken Bldg., 3-25-1, Hyakunincho
Shinjuku-ku, Tokyo 169-0073
Japan