



Applications of Differential Scanning Calorimetry (DSC) in the Development of Liquid Formulations for Protein Biopharmaceuticals

Application Note

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Ultrasensitive Calorimetry for the Life Sciences™



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There is a strong demand on the market for protein biopharmaceuticals that are formulated as liquids. They are easier for the physicians to handle and cheaper for the biopharmaceutical industry to produce compared to lyophilisates, as no lyophilization step is needed. Optimally the biopharmaceutical drug product (DP) should have a shelf life of at least 2 years, however, recombinant proteins have limited chemical or physical stability in liquid formulations. Having a multitude of biotechnology products currently in development, it is necessary to use technologies like differential scanning calorimetry to efficiently deliver optimized liquid formulations. This application note presents the function that DSC has in the process of liquid formulation development.

1. Introduction

In recent years, the biopharmaceutical industry has started a magnitude of monoclonal antibody development projects for different clinical indications^{1,2}. Recombinant proteins of commercial interest, including monoclonal antibodies, need additional properties beyond their biological activity to be able to be developed into successful biopharmaceuticals. Particularly, they need to be resistant against chemical degradation and they need to be physically stable in a given environment, without any tendency to aggregate^{3,4}. Furthermore, they need a favorable serum half-life and should present no or very little immunogenic properties⁵.

Apart from being more expensive to produce, the lyophilized drug product (DP) also needs to be reconstituted by the physician, which may even take 10-20 minutes before it can be parenterally administered to the patient. Simplified administration is the main driving force pushing the biopharmaceutical industry to focus their resources on developing liquid formulations instead of conventional lyophilized DPs. However, there are some technical issues that need to be overcome with regard to liquid formulations. The main challenge is to keep the protein biopharmaceutical stable in a liquid formulation by maximizing the physical stability and minimizing chemical degradation. It is especially challenging to develop biopharmaceuticals for subcutaneous (s.c.) applications because in this case the liquid formulations should have as high protein concentrations as possible due to the limitation of injection volume (1-1.5 ml)⁶.

This induces huge pressures on the formulation development teams to deliver fine tuned liquid formulations that optimally suit each biopharmaceutical. Structure and stability analysis of recombinant proteins, in particular monoclonal antibodies, has gained considerable importance during recent years and is an indispensable prerequisite for the development of formulations for biopharmaceuticals. A variety of techniques have evolved which can be used to gain structural information and information on protein stability. Differential Scanning Calorimetry (DSC) has become one of the key physicochemical methods to study the stability of protein biopharmaceuticals⁷⁻¹¹.

Special liquid formulation development programs have been designed to efficiently deliver optimized liquid formulations for biopharmaceuticals. A general scheme of a typical liquid formulation development process is shown in Figure 1.

Biopharmaceutical Liquid Formulation Development Approach

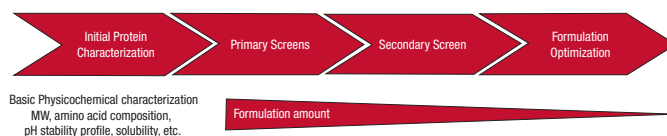


Figure 1. The liquid formulation development process.

Differential scanning calorimetry (DSC) has proved to be especially valuable in the early phases of liquid formulation development where it is preferable to rapidly reduce the number of formulations attempted to save drug substance (DS) and time spent on complex analytics.

2. Experimental Conditions

The DSC experiments were performed using a VP-DSC microcalorimeter (MicroCal) with a scan rate of 1.5 deg/min. Samples were degassed for 5 minutes before analysis. The reference cell of the calorimeter was filled in all experiments with a buffer corresponding to the sample buffer. Buffer baselines were subtracted from the protein scans and the molar heat capacity was used in the data analysis. The temperature induced unfolding of all proteins were checked for reversibility by comparing the heating and reheating DSC scans (data not shown). In all cases, no reversibility was found. The unfolding temperatures were acquired by analyzing the calorimetric profiles according to a two-state transition model. The optimal signal to noise ratio was found when using an antibody concentration of 100 µg/ml (Figure 2). Although higher concentrations did not impact the unfolding temperature it induced strong exothermic heat due to the aggregation of the protein after unfolding.

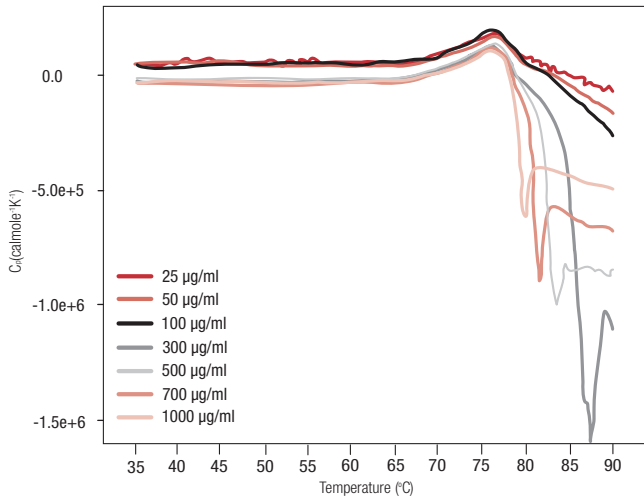


Figure 2. Unfolding transitions of Antibody 1 (IgG1) at different concentrations.

3. DSC in the Initial Protein Characterization Phase

In the Initial Protein Characterization phase of a specific antibody liquid formulation development program (Figure 3), DSC is used for comparing the overall stability of the antibody to other antibodies currently in development. As can be seen in Figure 3, large differences can be found in unfolding temperatures between different antibodies.

Biopharmaceuticals are, like most proteins, very sensitive to

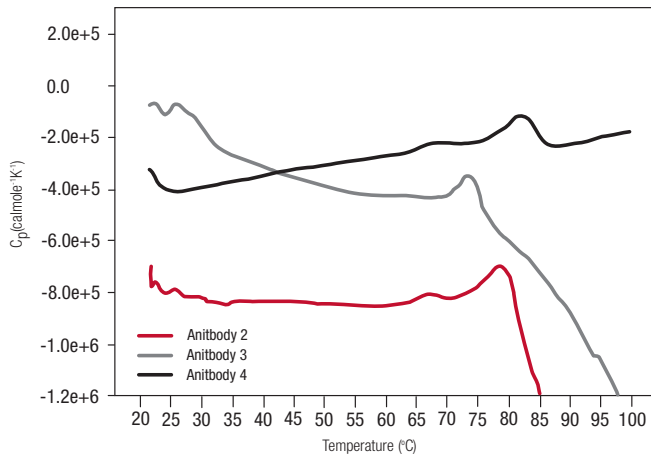


Figure 3. Unfolding transitions of Antibody 2 (IgG4; 200 µg/ml), Antibody 3 (IgG1; 100 µg/ml), Antibody 4 (IgG1; 100 µg/ml).

pH and it is essential to find the optimal pH to maximize stability. DSC has been found to be an efficient tool to optimize the pH since information can be gained without performing 2 and 4 week accelerated stability studies. Although most antibodies typically show the highest stability between pH 5.5-6 it is still important to characterize the optimal pH because deviations have been found. Figure 4 & 5 presents the pH profile of a fusion protein and Antibody 2. As can be seen, the optimal pH for the fusion protein (Figure 4) was pH 5 giving a T_m value of 92.8 °C. The fusion protein was sequentially destabilized at pH values above pH 5 but was especially sensitive to low pH values. At pH 4 no unfolding could be observed (data not shown). Figure 5 shows a typical pH profile of an antibody. The highest stability was acquired at pH 5.5 and 6 giving a T_m value of 78.5 °C.

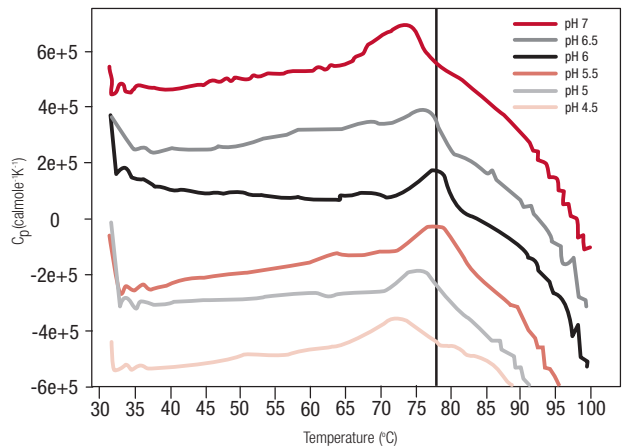
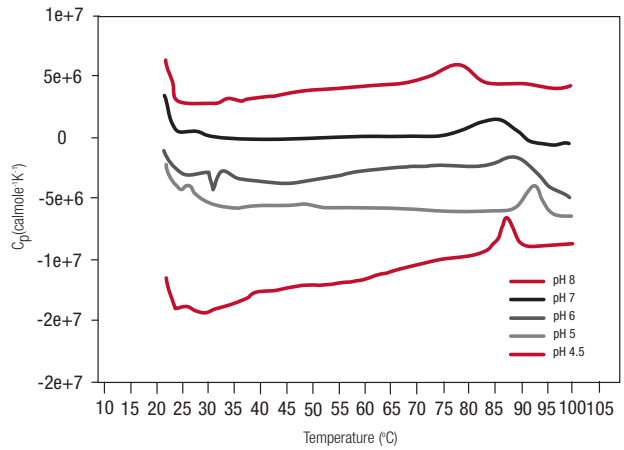


Figure 4 & 5. The pH- profile of a fusion protein (100 µg/ml; Figure 4) and Antibody 2 (100 µg/ml; Figure 5).

Figure 6 presents data on the chemical and physical stability of Antibody 2 as a function of time and pH (Initial Protein Characterization Phase). The pH stability profile study shows that after incubating Antibody 2 for two and four weeks at \leq pH 5 and at 40°C marked changes in the stability occur. DSC could distinguish the optimal pH condition for this monoclonal antibody by only using the T=0 samples (Figure 5), giving similar results as the other methods. Furthermore, the DSC identified the \geq pH 6.5 values as destabilizing, something that was not obvious in the 2 and 4 week timeframe but would rather have needed a 8 to 12 week stability study to be elucidated. In other words the DSC results correspond very nicely with the other methods that were used in this study to optimize the pH of the formulation buffer.

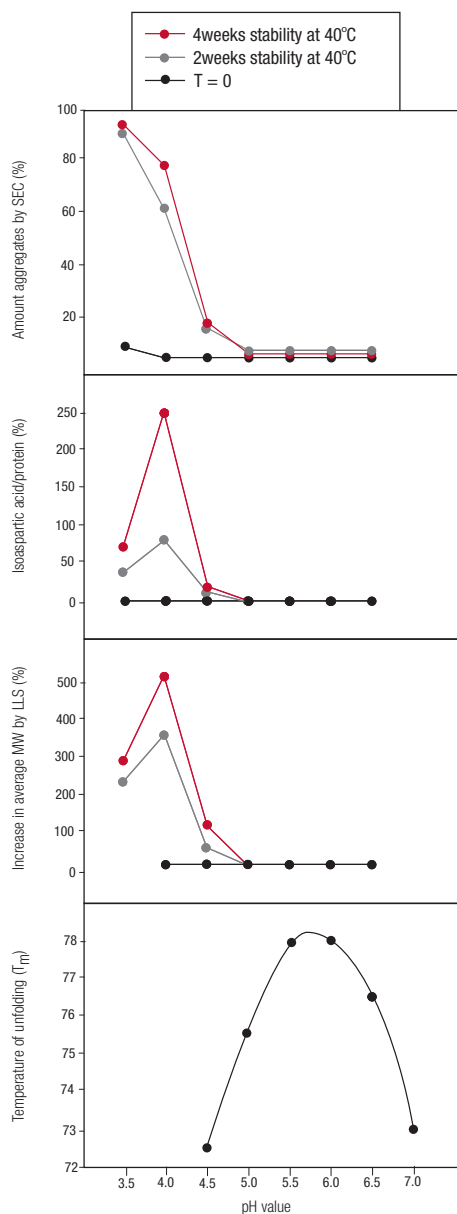
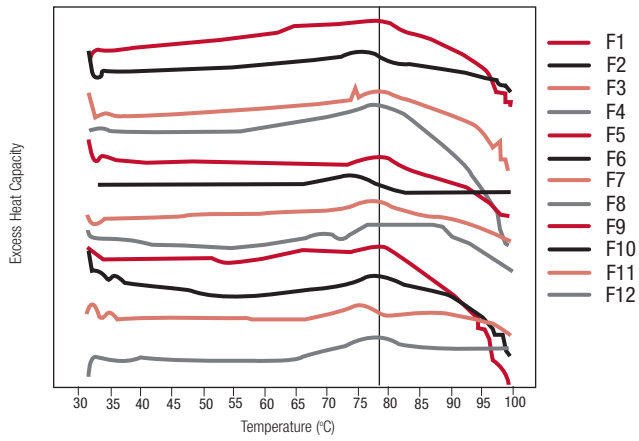


Figure 6. The impact of pH on the stability of antibody 2 as studied by size exclusion chromatography (aggregation), formation of isoaspartate, laser light scattering (increase in average molecular size) and DSC (T_m). The isoaspartate value is calculated according to the increase in the amount of isoaspartate/ protein (mol isoaspartate / mol protein x 100).

The benefit of DSC is that only the T = 0 samples need to be analyzed to indicate stability because the heating of the protein is in itself stress inducing. Typically, if the native conformation of a protein is stabilized it can resist thermal stress to a higher degree (unfolds at a higher temperature) than a protein that is destabilized. Hence, with DSC, it was not necessary to put samples on long term stability at various temperatures to acquire information on the impact of a specific liquid formulation on protein stability.

4. DSC in Liquid Formulation Development (Primary Screen)

DSC was used in the primary screen of Antibody 2 together with other biophysical methods including, size exclusion chromatography (SEC), laser light scattering (LLS), capillary electrophoresis (CE) and SDS-PAGE to select the most promising liquid formulations. The samples were put on an accelerated stability program and analyzed after 4 weeks. DSC was found to be especially successful at identifying formulations that had a negative impact on Antibody 2 but could also identify good liquid formulations. By analyzing the T = 0 samples DSC identified all four liquid formulations (formulation 2, 6, 8 and 11) in the primary screen that were regarded as dissatisfactory according to the other analytical techniques. Figure 7 presents thermograms and Figure 8 presents the unfolding temperatures (T_m) of Antibody 2 in liquid formulation 1-12 from in the primary screen. The unfolding of Antibody 2 in formulation 9 gave a markedly deviating unfolding pattern and was due to this reason regarded as a critical formulation.



5. Conclusions

DSC is a technology that is especially valuable in the early phases of protein characterization and formulation development. Critical data about protein stability can be rapidly acquired and used as a guide to support and speed up liquid formulation development projects.

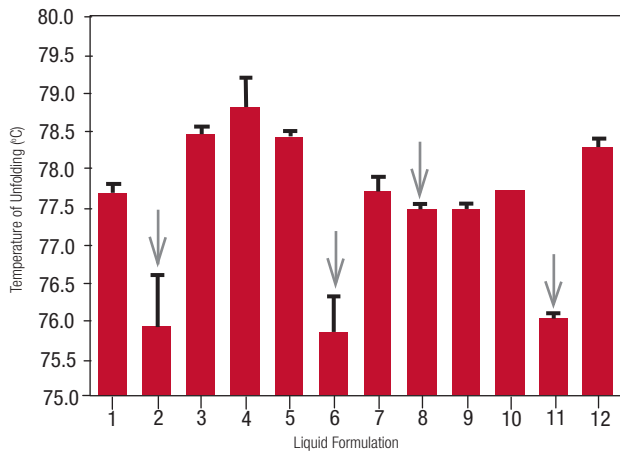
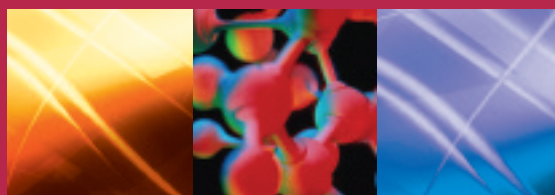


Figure 7 & 8. Thermograms and T_m values of Antibody 2 (100 µg/ml) from the primary screen. The grey arrows indicate which formulations were regarded as dissatisfactory according to the other analytical techniques that were used in this study.

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