Predicting monoclonal antibody stability in different formulations using the UNit

Introduction
Over the last few decades there has been a paradigm shift in the world of therapeutic medicines. Small molecules, such as aspirin and penicillin, no longer dominate the market place and block buster biologics such as Humira (Abbott), Remicade (Centocore) and Herceptin (Genentech) are now leading the way.

A biologic therapeutic is typically a protein, the building blocks and machinery of life. Proteins are fundamentally more complicated than small molecules as they are (relatively) large macromolecules with a high degree of complexity of structure and function. Typically administered in solution, the molecules are held together by a combination of short range interactions within the molecule and with the solvent, and longer range forces such as electrostatics.

Proteins, particularly antibodies, have highly complex native conformations, each having unique chemical and physical properties. In order to develop therapeutic proteins as safe and economically feasible medicines they are required to have adequate robustness to endure the wide range of thermal, mechanical and chemical stresses to which they are exposed during manufacture, storage, transport and administration.

This is usually achieved through either engineering the protein candidate to generate a compound which has the required intrinsic stability, or by altering the matrix in which the protein exists by changing its composition.

The process of optimizing the solvent conditions (formulation) of protein drug candidates can be an extremely difficult and time consuming practice. The optimal formulation will provide a protective environment which will maintain the molecular conformation and minimize any degradation products resulting from exposure to conditions that arise during the various stages of the manufacturing process. These conditions include variations in temperature, different solution pHs, freeze–thawing, freeze–drying, spray–drying, reconstitution and agitation [1].

The stresses encountered can cause proteins to degrade via both physical and chemical pathways. Examples of chemical degradation include hydrolysis, deamidation and oxidation, which are usually managed through the use of low temperatures, protection from light or addition of antioxidants that reduce their rates of occurrence. By far the most problematic are the physical degradation pathways of which aggregation and unfolding are the two most prevalent and disadvantageous.

After identifying the degradation pathways, possibly during the preformulation stage, it is necessary to find suitable mechanisms to circumvent their occurrence. For drugs which are to be administered parenterally, changes in the composition of the formulation (other than altering the solution pH or protein concentration) most commonly include the addition of a wide variety of excipients. With such a wide variety of excipients available to protect against the numerous causes of protein degradation, making the choice of which ones to use for further development can be extremely challenging. Analytical methods traditionally used to screen colloidal and conformational stability of the selected drug candidate in these formulations are typically slow and require large volumes, which limits the number of formulations which can be characterized in early development.
Long-term stability studies of the formulated drug substance under different stresses are required to test the shelf life of a drug substance, which are time consuming and require large amounts of protein. As such, it is not feasible to carry them out for every different formulation, especially as there may be no appropriate formulation at the end of the test period. Therefore, it is of specific interest to find a method of analyzing proteins rapidly that can generate a predictive measure of the stability of the protein after an extended period.

Since long term stability studies typically take eighteen months to three years, a long time in the drug development pipeline, scientists have sought alternative routes to allow them to screen more formulations more quickly. This allows them to more easily narrow the number of final formulations selected for long term stability studies. The most common route for this is known as forced degradation, in which proteins are subjected to extreme handling processes, such as extremes in temperature, agitation, light exposure, oxidation, multiple freeze-thaw cycles. These processes are all known to cause degradation in protein biopharmaceuticals which often result from practical applications during the drug substance lifetime, such as shipping, storage, accidental freezing or losses in excipient stability or purity. These extreme handling processes are thought to degrade proteins far more rapidly than in long term storage and the resultant degradation routes can be used to predict which formulations will yield the optimal stability of the biologic.

Typically techniques such as SDS-PAGE (Sodium dodecyl sulphate polyacrylamide gel electrophoresis), SEC (size exclusion chromatography) and DLS (dynamic light scattering) are used to characterize the conformation and aggregation state of the protein during these studies. Models relating to the unfolding and aggregation of proteins can allow a degree of extrapolation to occur such that a researcher is able to predict the formulations which degrade most quickly. The degradation rates obtained for formulations at elevated temperature will allow prediction of degradation (albeit at a slower rate), when stored long term at reduced temperatures. Supplementary to this are other predictive measures where parameters indicative of the thermal stability of the protein can be shown to correlate with both accelerated stability measures and the reduction in the monomer fraction or activity following long term storage [2].

Here, we measure the melting temperature ($T_m$) and aggregation onset temperature ($T_{agg}$) of a protein in various formulations. We propose that where these values are higher for one formulation than another there will be less statistical probability during storage for the protein to occupy an unfolded state, reducing its potential to aggregate.

Typically techniques such as DSC (differential scanning calorimetry), intrinsic protein fluorescence, DSF (differential scanning fluorimetry), LS (light scattering – both static (SLS) and dynamic (DLS)) and self-interaction chromatography (SIC) are used to characterize conformational and colloidal thermal stability, but often these are low throughput, or require large volumes and multiple cleaning steps. DSC is one of the most commonly used techniques to compare thermal stability of proteins, however it takes a significant amount of time and material, limiting its throughput capability. Further, usable concentrations are typically low (<5 mg/mL) as the severe aggregation observed during the thermal treatment of high concentration proteins often clogs the instrument and makes its use difficult and impractical. It is often important to test proteins in formulation solutions at concentrations used for delivery which are often high when subcutaneous injection is preferred.
Alternative high throughput technologies such as DSF can be used to characterize conformational stability of proteins [2], but this has drawbacks as it uses an extrinsic dye which can interact with and affect the protein behavior and aggregation characteristics.

New technology developments have endeavored to navigate these limitations by reducing sample volume requirements, increasing throughput and automation and combining the simultaneous acquisition of information regarding sample conformational and colloidal stability. The UNit instrumentation combines intrinsic protein fluorescence, DSF and static light scattering and offers a complete protein stability platform to characterize both conformational and colloidal stability.

Without prior knowledge of the degradation pathway of a protein, simultaneously measuring both conformational and colloidal stability of the same sample provides a broad range of information, which is crucial when choosing the correct formulation for a product. While in some cases, the thermal conformational stability has been shown to correlate with the monomer loss of a protein during storage, in other cases it has not and instead the aggregation rate at 70 °C has [2]. As such, a complete picture is required to reliably predict the most stable formulation for the protein of study.

**Practical considerations – formulation around degradation**

Proteins are not renowned for their stability in solution. They are well known to be prone to aggregation, even when stored at 4 °C. There are several common routes by which this degradation might occur, including aggregation, fragmentation, and chemical modification. Physical degradation paths include denaturation, adsorption to surfaces, aggregation and precipitation. The risk of following these pathways can be mitigated by careful and considered choice of the pH, ionic strength, buffer and excipients for a drug substance or drug product formulation.

There are many excipients approved by the regulators that are commonly used to formulate protein drugs [3]. These excipients can be categorized into the following: buffering agents, amino acids, osmolytes, sugars and carbohydrates, proteins and polymers, salts, surfactants, chelators and anti-oxidants, preservatives and specific ligands [4] [5]. The choice of excipients to be used is generally based on prior knowledge of the degradation pathways of the protein of interest.

In this study we aim to demonstrate how the measured thermal stability can predict the long term aggregation characteristics of a monoclonal antibody. In this work the protein used, MAb1, was obtained through the Bioprocessing Research Industry Club (BRIC) consortium of the Biotechnology and Biological Sciences Research Council (BBSRC) of the United Kingdom of Great Britain and Northern Island (UK).

**The UNit as a high throughput protein stability screening platform with different formulations**

The UNit instrument is designed for high throughput, low sample volume investigations into protein stability. The instrument uses a proprietary multi well sample plates called the UNi with each well requiring only 9 µL of sample and capacity for 48 samples to be investigated in a single experimental run. The instrument uses thermo-electric heating and cooling for precise control of sample temperature allowing thermal ramps or isothermal type experiments to be performed.

The sample is simultaneously illuminated with multiple wavelengths of laser light allowing simultaneous acquisition of both fluorescence and static light scattering data which is analyzed in the bespoke UNit Analysis software to gain detailed information on both the conformational and colloidal stabilities.
**Typical UNit data for different formulations**

Typical data from the UNit thermal ramp experiment, with two different formulations of the same protein are shown in Figure 1. The different features are highlighted and include information regarding: the initial conformational and colloidal state of the protein around room temperature; aggregation onset temperature ($T_{agg}$) and any further aggregation events; single or multiple melting temperatures ($T_m$); and any differences in solubility of aggregates (precipitation).

144 formulations were screened in one day with the 2 instrument and the resultant information was analyzed in a variety of different ways to understand the different stabilities of each of the individual formulations.

After careful digestion of the wealth of information provided, a final choice of twelve different formulations was selected to be taken forward for further study. These twelve formulations were selected to have a range of different stabilities, both conformationally and colloidal. The final 12 selected were taken on to be studied under a variety of different storage conditions, typical of a normal short and long term stability study and in accordance with ICH guidelines. Comparative information was gathered using size exclusion chromatography (SEC), dynamic light scattering (DLS) and UV concentration measurements to characterize the aggregation and fragmentation throughout the study.

A selection of time points were chosen at which stage each of the samples were measured on all of the techniques. Initially, samples were measured at weeks 0, 1, 2, 4 and at regular intervals after. Storage conditions of -80 °C, -20 °C, 4 °C and 40 °C were used in the study.

**Materials**

A monoclonal antibody, MAb1 was formulated at a concentration of 1 mg/mL in all experiments.

Solvent conditions were varied and the prepared buffers included glycine, Na acetate, Na citrate and Tris at a range of pHs from 2.2 to 10.6. Excipients included NaCl, trehalose, arginine/glutamate (Arg/Glu), polysorbate 80 (Tween 80) and sorbitol. All chemicals and reagents were sourced from SigmaAldrich, UK. Stocks of buffers were prepared at 100 mM.

**Methods**

Following the predictive phase, twelve formulations were chosen for long term stability testing. At each time point in the test, one aliquot of sample was used from each of the different storage conditions for all measurements on all instruments.
The UNiT:
The 144 samples that were included in the predictive screen were measured on the UNiT in three experimental runs. In each run, 9 µL of each sample was loaded into a UNiT and placed in the UNiT instrument. A stepped thermal ramp was carried out between 15 and 95 °C with the protein intrinsic fluorescence and static light scattering signals being recorded every 1 °C, with a hold time of 60 s at each temperature to allow equilibration of the samples before measurement started. An exposure time of 1000 ms was used and the two lasers in the system were attenuated to their maximum value (266 nm laser – filter 4; 473 nm laser – filter 3).

During the stability testing phase the 12 chosen formulations from the four different storage conditions were measured in triplicate using the same experimental settings as during the predictive screening. Each of the formulation buffers was also measured in singlicate to record any degradation of the buffers over time, at each of the storage conditions at each time point. All data was analyzed initially using the UNiT Analysis software version 2.0.4 and melting temperatures, aggregation onset temperatures, the initial light scattering signal and the initial fluorescence ratio (350:330) was extracted from the data where appropriate.

Dynamic Light Scattering:
40 µL of each sample at each time point was measured in singlicate using the Malvern Zetasizer Nano S. Data was analyzed in the provided software to characterize the degree of aggregation or fragmentation in solution.

Size Exclusion Chromatography
Size exclusion chromatography was carried out using an Agilent 1100 Series system that was fitted with an autosampler, a column heater and a variable wavelength detector (flow cell path length = 10mm). The column used with this system was a TOSOH G3000 SWXL (with a guard column), which had a 7.8 mm ID and a length of 30 cm. The mobile phase consisted of phosphate buffered saline at pH 7.4. A flow rate of 0.8 mL/min was used, with an injection volume of 20 µL, a temperature of 25 °C. The detection wavelength was set to 280 nm, and the samples were at a concentration of 1 mg/mL. Agilent total recovery HPLC glass vials were used and the system had a run time of 50 minutes.

Prior to loading onto the column 70 µl of the samples were centrifuged at 21,100 x g for 20 min to remove larger aggregates and 30 mL of the supernatant was placed in the sample vials.

Results and Discussion
Predictive Stage Results
In the predictive screen 144 different formulations were measured on the UNiT as described earlier, and analyzed using the UNiT analysis software. The 266 nm SLS data was used to represent the aggregation stabilities rather than 473 nm due to its increased sensitivity at these concentrations. The raw spectra data were processed to obtain a value of the ratio between the fluorescence intensity at 350 nm and 330 nm which gives an indication of the degree of solvent exposure of the tryptophan residues in the protein and correlates with the unfolding. The data can be processed in a range of different ways and the ratio was chosen as it appeared to give the clearest representation of the conformational data in this case.

The data from all 3 runs was collated and is presented in Figure 2, where the integrated area of the 266 nm scattered light versus temperature can be seen in (a) and fluorescence intensity ratio (350/330 nm) in (b). The data presented in Figure 2(a) suggests that all formulations begin in a monomeric state as there is no significant difference between the initial SLS values for any of the formulations (integrated SLS intensity 15–20 °C). There is a large variation in the derived aggregation onset temperatures (T$_{agg}$) and differences can also be seen in the degree of aggregation (how much the curve increases). Figure 2(b) shows that there are a range of different initial values in the fluorescence ratio data (averaged between 15 – 20 °C) suggesting that the proteins are initially present in a range of conformational states and also a variety in the T$_{m}$ values with some samples showing multiple T$_{m}$’s.
After initially running this large, yet rapid screen each formulation was analyzed to select the final 12 formulations to take on for further study during the long term storage. At this stage, although detailed analysis was not required, the differences in colloidal and conformational stabilities can be seen visually from the curves presented in Figure 2 according to the characteristics identified in Figure 1.

Following analysis of the initial 144 formulations, the features highlighted in Figure 1 were compared and used to select 12 further formulations from the 144. These 12 were selected to cover a range of different stabilities, including demonstrating the effect of particular excipients. Initial tests were also run using orthogonal techniques highlighting that a formulation containing 1 M sorbitol, whilst compatible with the UNit and showing significant stabilizing effects in the conformational data, was incompatible with other techniques due to limitations with the sample viscosity. As such this excipient was omitted from the final screen and replaced with formulations with no excipient (other than NaCl).

The 12 formulations were also chosen to allow comparisons to be made between differences in pH, ionic strength, buffering agent and additive with different storage conditions and forced degradation methods. These are listed and color coded according to their comparative differences along with their ‘formulation number’ in Table 1.

**Initial, time 0 measurements**
Following the final selection of 12 formulations, samples were prepared in bulk and aliquots made for each of the time points, under each of the storage conditions required. These were then grouped (set of formulations 1 – 12 plus the 12 buffers) and transferred to their respective storage temperatures. One set was retained and measured on the UNit and SEC and these were used as the week 0 time point measurements.

**Table 1: 12 final formulations selected for further study**

<table>
<thead>
<tr>
<th>#</th>
<th>Conc. &amp; Buffer</th>
<th>pH</th>
<th>[NaCl]</th>
<th>Additive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10 mM glycine,</td>
<td>ph 2.2</td>
<td>0 mM NaCl</td>
<td>no additive</td>
</tr>
<tr>
<td>2</td>
<td>50 mM glycine,</td>
<td>ph 2.2</td>
<td>50 mM NaCl</td>
<td>no additive</td>
</tr>
<tr>
<td>3</td>
<td>50 mM Na citrate,</td>
<td>ph 3.6</td>
<td>50 mM NaCl, 100 mM trehalose</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>50 mM Na acetate,</td>
<td>ph 3.6</td>
<td>50 mM NaCl, 100 mM trehalose</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>50 mM Na acetate,</td>
<td>ph 4.6</td>
<td>50 mM NaCl, 0.005 mM Tween 80</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>50 mM Na citrate,</td>
<td>ph 6.2</td>
<td>50 mM NaCl, 100 mM Arg/Glu</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>50 mM Na citrate,</td>
<td>ph 6.2</td>
<td>50 mM NaCl, 0.005 mM Tween 80</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>50 mM Na citrate,</td>
<td>ph 6.2</td>
<td>50 mM NaCl, no additive</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>50 mM Na acetate,</td>
<td>ph 6.2</td>
<td>50 mM NaCl, 100 mM trehalose</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>50 mM Tris,</td>
<td>ph 7.5</td>
<td>50 mM NaCl, no additive</td>
<td></td>
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<tr>
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<td>50 mM Tris,</td>
<td>ph 8.2</td>
<td>50 mM NaCl, 100 mM Tween 80</td>
<td></td>
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<tr>
<td>12</td>
<td>50 mM glycine,</td>
<td>ph 7.5</td>
<td>50 mM NaCl, 100 mM Arg/Glu</td>
<td></td>
</tr>
</tbody>
</table>

Each of these 12 samples was measured in triplicate on the UNit with the 12 buffers measured in singlicate making up one run containing a total of 48 samples. Time constraints of the technique allowed the samples to be measured only in singlicate on the SEC.

An example of the triplicate measurements of each of the 12 formulations measured on the UNit can be seen in Figure 3 with the SLS and fluorescence ratio data overlaid to show any cooperative behavior or otherwise between the aggregation and conformational changes. The slight difference in one of the triplicate measurements for formulation 11 may be attributed to the pH being very close to the pl of MAb1.
Parameters which describe the differences between the formulations, were extracted from the curves in Figure 3 and plotted in bar chart form. Figure 4 shows the variation in the initial 266 nm SLS and initial fluorescence ratio values, averaged between 15 and 20 °C. Figure 5 shows the corresponding $T_{agg}$ and $T_m$ values. All values presented in these figures are averages of the triplicate measurements and the error bars are the standard deviations.

There is no significant variation observed between the initial values of the static light scattering across the 12 measured formulations. The SLS reflects the average molecular weight of the components of the sample and therefore the signal is proportional to the state of aggregation. Due to the insignificant variation in the data it is highly probable that the samples were monomeric at room temperature at week 0.

Similarly, in the initial fluorescence ratio data, which reflects differences in the tertiary structure, very little difference can be seen between the samples. This is with the exception of formulations 1 and 2, which reflects samples that are at low pH and appear to have a degree of acid induced unfolding. The difference in the ionic strength between the two samples most probably accounts for this apparent difference in tertiary structure.

Whilst the differences seen here are relatively small, it is expected that larger differences will be seen between the 12 different formulations as the samples degrade in quality and aggregate over the course of time under the different storage conditions.
The data presented in Figure 5 demonstrates that both $T_{agg}$ (top) and $T_m$ (bottom) are formulation dependent. This is likely to be due to a combination of the sample pH affecting the structure of the protein, and in some cases the excipients and buffer conditions providing some protection against thermally induced aggregation. It is also apparent in the unfolding data (fluorescence, bottom) that lower pH separates domain unfolding [6].

On the UNit single measurements were made of the buffers and triplicate measurements of the different formulations containing MAb1 with 2 separate runs for 4 and 40 °C on the first day and 2 separate runs with -20 and -80 °C on the second day. One difference in the two measurements was that with the SEC, whilst automated in its measurements samples had to be removed from their storage temperatures (two temperatures per experiment) and allowed to sit at room temperature for the duration of the experiment, whereas with the UNit, samples were removed from their storage temperature one condition at a time, were loaded into the UNi and the experiment began within 5 – 10 minutes of removal.

As in Figure 4 and Figure 5, the UNit data was parameterized such that the initial integrated SLS intensity, the initial integrated fluorescence intensity ratio, $T_{agg}$ and $T_m$ values were extracted for each time point and each storage temperature. These numbers were then plotted versus time point and the 12 formulations were compared. After only four weeks the most pronounced trends could be seen between formulations 1 and 2, where the discrimination in the formulations was ionic strength; 3 and 4, where buffer species varied; and 6, 7, 8 and 9, which were identical apart from addition of four different excipients. At this stage particular interest is focused on the differentiation of stability with samples that are formulated in different buffer species.

**The effect of buffer species**
Formulations 3 and 4 are almost identical, in that each are at pH 3.6, contain 50 mM NaCl and 100 mM trehalose, with the only difference being the buffering species (both buffers at 50 mM). In formulation 3 a citrate buffer is used and in formulation 4 an acetate buffer is used. There is evidence presented in the literature that suggests that citrate, a multivalent carboxylate buffer, causes antibody self-association and can trigger filament formation in antibodies [6] [7].

Measurement after storage
After the initial measurement on week 0 the aliquots were transferred to their relevant storage temperatures (-80, -20, 4 and 40 °C). Time constraints imposed by the throughput of the HPLC meant that for the samples characterized by SEC it was necessary that they were run in one long experiment. This was carried out over a period of two days, with the first day consisting of the samples stored at 4 and 40 °C and the samples stored at -80 and -20 °C on the second day.
Acetate however, is a monocarboxylate and as such does not trigger association or does not do so to the same extent [7]. Whilst this may cause an effect when the formulations are in solution at 4 and 40 °C, the effects may be different when stored at -20 and -80 °C as the principle cause of degradation when frozen is likely to be the mobility of the protein through the partially frozen lattice and local concentration gradients of both protein and excipients.

**Figure 6** shows the calculated initial SLS intensity, the initial fluorescence and the aggregation onset temperatures for formulation 3, with the citrate buffer. There is a pronounced change in the protein that has been stored at 40 °C during the four weeks of storage. Storage at this temperature can be treated as an accelerated degradation condition. The changes include an overall increasing trend in the initial SLS intensity indicating that the proportion of aggregated protein in solution is increasing over time (the drop at week 2 may be attributed to a non-uniform dispersion of aggregates in the solution).

A similar trend can be observed in the initial fluorescence intensity ratio which indicates increased occupancy of the denatured state. A drop in $T_{agg}$, indicating a reduction in the thermal aggregation stability over time is also observed. Other storage temperatures show slight changes (highlighted in blue) which are less significant after four weeks and the 4 °C samples are expected to follow a similar trend as the elevated temperature data but at a slower rate.

**Figure 7** shows comparative data to **Figure 6**, but for formulation 4, which is the acetate buffer. In this case the area highlighted in red in formulation 3, where there was significant evidence of degradation, is left in place for this formulation (4, acetate). However the data for formulation 4 (acetate) does not show the same degradation as with formulation 3 (citrate). Here all four storage temperatures are highlighted in blue, which show overall little change in the initial SLS intensity, slight increases in initial fluorescence (the most pronounced for 40 °C albeit small compared to formulation 3) and no dramatic decrease in $T_{agg}$ for any of the four storage conditions.

What can be said is that there is a notable increase in the uncertainty on the determination of the aggregation onset temperature for all of the measured samples, which can be attributable to either a smaller magnitude aggregation event (see **Figure 3**, formulation 4 for example), or a less uniform distribution in colloidal stability of the samples.
Figure 7: Initial SLS (a), initial fluorescence (b) and $T_{agg}$ (c) for formulation 4. 40 °C Here no data points are seen in the red highlighted area as were seen for formulation 3.

**Comparative SEC data**

Comparative data from the SEC is presented in Figure 8 for all 12 formulations at week 4. The figure shows the percentage remaining of the main peak indicating the fraction of remaining monomer after incubation under a range of conditions. The most significant degradation from the initial monomer peak at week 0 is for formulations 1, 2, 3 and 4 stored at 40 °C.

Figure 8: Comparative data from SEC analysis for all 12 formulations, here the most significant degradation in formulations 3 and 4 is highlighted in red for samples which are stored at 40 °C. Other storage temperatures highlighted in blue.

Comparison of formulations 3 and 4 (citrate and acetate, respectively) again indicates that there is a far greater reduction in amount of monomer for formulation 3 than 4 at 40 °C, although only a small difference in sample stability is observed at 4 °C. This is in agreement with the UNit data presented in Figure 6 and Figure 7. The changes measured for these formulations at -80 and -20 °C show the opposite trend to that seen for those stored in solution, with formulation 4 degrading more than formulation 3. This may be due to the effects of being in the solid state as opposed to being in solution and potentially highlights separate and independent degradation routes which will be explored in more detail elsewhere.

**The utility of high-throughput screening to predict degradation in storage**

The SEC data provides information on the amount of monomer remaining after storage of MAb1 under various conditions. After 4 weeks of being stored at 4 °C the remaining monomer fraction of MAb1 in the 12 formulations has been ranked where the formulation with the highest residual monomer content is ranked 1.

High-throughput screening on the UNit at the outset of the storage allowed determination of melting temperatures, $T_m$, for different domains of MAb1 in each of the formulations. These values were similarly ranked such that the most stable (highest $T_m$) was ranked as 1, and the least stable as 12.
When the SEC and the UNit measurements are plotted against each other, as in Figure 9, a correlation can be observed such that the sample with the highest melting temperature at the start of the process has the highest remaining percentage of monomer after four weeks of storage in solution.

Analysis of this data suggests that the dominant pathway for degradation of the protein under these conditions is caused by unfolding and aggregation of the fAb region of the antibody.

The UNit has been demonstrated here to be a useful tool in formulation screening. With its excellent correlation between the predictive Tm and resultant data from SEC, an industry standard, after 4 weeks storage at 4 °C shown in Figure 9. It was high-throughput allowing up to 144 samples to be screened in one day on a single instrument. It required very small volumes – only 9 µL per well. There were no microfluidics or auto sampler involved resulting in no cleaning steps and no clogging. It was simple to set up, requiring far less man power. Finally UNit is incredibly data rich, giving many metrics and a more complete picture of stability (Tm, Tagg, Initial SLS, Initial conformation used here are only a few) making it an ideal tool for carrying out high-throughput screening and measurement of proteins in a wide range of different and diverse formulations.

**Summary and Conclusions**

Prediction of a therapeutic protein’s propensity to aggregate as early as possible in formulation is advantageous and optical probes of conformation and aggregation are particularly well suited to this application. They are rapid, use little material and can simultaneously measure multiple stability indicating parameters. By measuring more stability indicating parameters it has been shown in the literature that the predictive power can be increased as different degradation routes correlate with different predictive parameters [2]. Without a priori knowledge of the degradation route of a protein the more complete the characterization the lower the risk of choosing the wrong formulation.

**References**


