

High-Throughput Dynamic Light Scattering (HT-DLS) for Screening Biotherapeutic Formulations

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Introduction

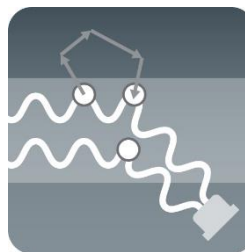
Biotherapeutics account for more than 40% of today's global pharmaceutical research (1). Within the next decade, the output from this investment is expected to lead to an accelerated emergence of novel protein- and antibody-based treatments for a range of diseases. However, the biopharmaceutical development workflow remains complex. Progressing a potential candidate from identification to formulation and through to manufacture is an expensive process. As costs increase exponentially the further a drug moves down the pipeline, failure at an advanced development phase can result in a substantial loss of investment.

Assessing a drug candidate's suitability during pre-formulation is, therefore, essential to minimize the risk of costly downstream failure. Stability is a defining feature of biotherapeutic performance; measuring formulation stability during early-phase screening allows developers to make robust predictions of suitability for further development. Viscosity of a protein is another important predictive parameter as formulations with high viscosity are not suitable for intravenous delivery.

Traditional processes and techniques for the analysis of biotherapeutics often suffer from low throughput, thereby limiting the number of candidates and formulations that can be analyzed. Evaluating many candidates in a multitude of formulations with these current techniques is a costly, labor- and material-intensive process. In many cases, scientists will need to compromise on the size of their "developability" study to meet project timelines. This approach is associated with the risk of not selecting the best candidate and formulation, which could result in issues further downstream.

Recently, automated analytical technology has been developed specifically to address the aforementioned challenges. Automated high-throughput [dynamic light scattering](#) (HT-DLS) utilizing Wyatt Technology's [DynaPro® Plate Reader II](#) helps to streamline the pre-formulation process, and can be used as a standalone instrument or as part of a larger automated formulation workflow. Using very low solution volume, HT-DLS enables rapid, unattended stability assessments of multiple biotherapeutic candidates in a variety of different conditions. Automated analysis frees formulators to focus on more valuable tasks and to rapidly build a comprehensive formulation profile in a fraction of the time that it takes for batch techniques. The level of insight delivered into formulation performance is essential for the implementation of highly efficient development strategies, such as quality by design (QbD).

This article introduces the technology that powers automated HT-DLS and explores its practical applications in enhancing formulation stability investigations. Case studies are presented that illustrate the role of HT-DLS within Freeslate's completely automated prescreening workflow employing the CM3 Biologics Formulation System, and highlight the benefits of employing this approach for rapid formulation screening.



Overcoming the Challenges of Bioformulation with HT-DLS

Success of a commercial biotherapeutic relies on the drug meeting a range of regulatory, performance, and safety targets. For instance, a successful biologic must be stable throughout its lifecycle: from manufacture, through to up to two years' storage, during administration, and during in-vivo drug delivery. Within the bioformulation workflow, dynamic light scattering (DLS) is widely used to provide insight into stability at a very early stage of development.

DLS is a non-invasive, non-perturbative method of measuring protein and particle size distribution in terms of hydrodynamic radius (r_h) across a dynamic size range of 0.2–2500 nm. The fundamental strength of DLS is its ability to determine changes in protein size and conformation across a range of conditions, allowing users to rigorously investigate a candidate's behavioral profile. DLS delivers essential insight into biopharmaceutical formulations, including:

- Gross aggregation of nano to submicron aggregates
- Colloidal and conformational stability studies
- Propensity of aggregation
- Viscosity of high-concentration protein formulations.

Traditional batch DLS is a manual technique suitable for testing a handful of samples, but not hundreds. Automation helps to resolve the challenge of analyzing hundreds of samples, both for DLS and formulation screening as a whole. Automated experiments improve study results by offering higher reproducibility and removing subjectivity from data interpretation. More significantly, automation delivers substantial improvements over manual performance. Automated HT-DLS allows a broad range of conditions to be characterized with minimal manual input and at a fraction of the time of batch techniques. With data generated as rapidly as 10 seconds per well, an experiment covering 385 sample wells consisting of a variety of different candidates, pH values, and ionic strengths can be completed in just 90 minutes.

The DynaPro Plate Reader II employs industry-standard well plates compatible with other plate based pre-formulation techniques. In practice, this means DLS samples are easily transferable to other instruments for multivariate sample analysis. The ability to rapidly scope a drug product's performance under hundreds of different conditions allows scientists to more easily implement design of experiments (DoE) and QbD processes to create better designed biopharmaceuticals with greater commercial potential.

The following examples illustrate the effective application of automated HT-DLS for a number of essential stability investigations.

Measuring gross aggregation

Protein aggregation is a major concern during biopharmaceutical development. Unchecked aggregation reduces the efficacy of a biotherapeutic, affects its manufacturability, and could present a serious immunogenicity risk to a patient.

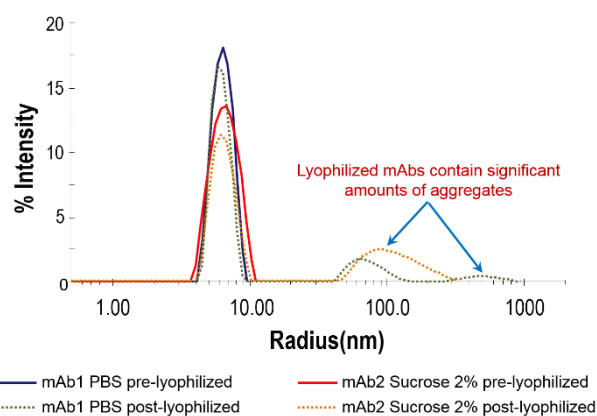


Figure 1: HT-DLS allows users to determine gross aggregation of a protein sample under a range of different conditions. Following lyophilization and reconstitution, a monoclonal antibody (mAb) solution containing PBS is shown to aggregate in the submicron size region.

By monitoring changes in particle size distribution with DLS, formulators are able to assess gross protein aggregation across a dynamic temperature range, pH, or following essential aspects of the formulation process. For example, Figure 1 shows the particle size distribution of two monoclonal antibody (mAb) formulations with and with-

out sucrose, before and after lyophilization, measured using the DynaPro Plate Reader II. Lyophilized samples were reconstituted to the same concentration as pre-lyophilized solutions.

Before lyophilization, the size distributions of both samples were monomodal. The pre-lyophilized solution containing sucrose has a marginally wider distribution peak, which suggests that the small sucrose molecules impart some polydispersity. It is clear that the lyophilized mAbs contain a significant amount of large aggregates, with the PBS sample in particular forming extremely large species post-lyophilization.

Despite the intensity of the post-lyophilized aggregate peaks between 50-200 nm, the number of these aggregates is actually very small. Converting these data to percent mass via [DYNAMICS software](#) reveals that although the lyophilized antibody's primary peak between 4-10 nm has an integrated intensity is only 80%, its total percentage mass was 99.6%. Aggregates in the 50-200 nm range, therefore, account for only 0.04% of the overall protein population. Whether this percentage falls within the limits of suitability depends on the candidate; the role of HT-DLS is to quickly provide the information required for formulators to make this decision.

Measuring conformational changes with thermal stability

DLS also allows formulators to probe other aspects of protein behavior, such as conformational changes across a dynamic temperature range. Figure 2 shows a temperature scan of lysozyme in an acetate buffer of pH 4. Lysozyme proteins fold and unfold readily without aggregation. In this case, the molecular size of the sample increased rapidly around 65°C before reaching a plateau around 90°C. When held at this temperature for multiple readings, the size remained constant, supporting the assumption that the protein is unfolding rather than aggregating.

For an aggregating protein, on the other hand, holding a formulation at a constant temperature and measuring the particle size is an effective way to determine the thermal stability and rate of aggregation. Moreover, DLS systems inherently incorporate coarse static light scattering (SLS) measurements (i.e., the DLS count rate), which allow users to differentiate between folding and aggregation

behavior. SLS is proportional to molar mass, a property that increases during aggregation but remains unchanged during conformational changes. This system provides users invaluable insight into the nature of protein interactions in solution and the transition temperature at which conformational changes give way to aggregation.

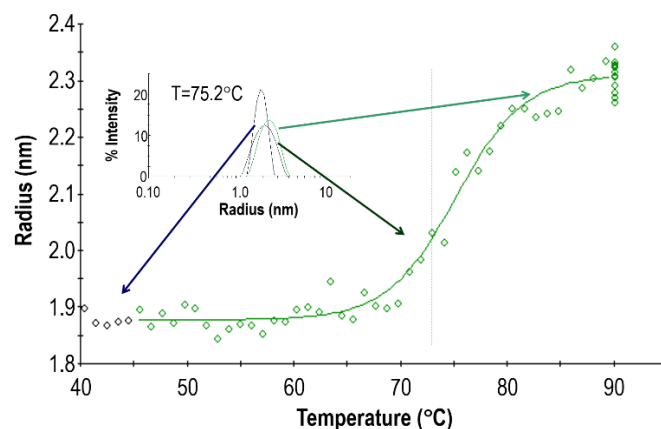


Figure 2: Dynamic light scattering (DLS) is used to measure conformational changes in structure, such as the unfolding of lysozyme across a dynamic temperature range.

Measuring aggregation propensity

HT-DLS also measures aggregation propensity, enabling users to better predict stability at early phases of analysis or to secure more in-depth information of protein behavior around unfolding and aggregation transition points.

Targeting colloidal stability with DLS allows formulators to determine the magnitude of attraction between molecules in solution by measuring the change in a protein's diffusion coefficient as a function of concentration. If the diffusion coefficient decreases with a decrease in concentration, then larger particulates are forming and self-association occurs. If the coefficient increases with concentration, then molecules are likely repelling each other and are stable in solution.

These measurements are described by the diffusion interaction parameter D_1 (also known as k_D). The magnitude of D_1 directly relates to aggregation propensity. When D_1 is greater than 0, repulsive forces dominate and the solution

is more likely to be stable. When D_1 is less than 0, attractive forces dominate and the sample is likely aggregate.

Figure 3 shows one example of how D_1 changes with temperature around the unfolding transition of a mAb sample. At lower temperatures, D_1 and r_h are fairly constant, approximately -10 (mL/g) and 5 nm, respectively. When the temperature exceeds 55°C, however, r_h increases significantly as aggregation begins. Several degrees before the point of aggregation, the value of D_1 decreases sharply. This observation can be rationalized by considering the unfolding behavior of the protein around this point. As proteins unfold, their hydrophobic cores expose and the molecules become self-attractive. D_1 reaches a negative maximum at the onset of aggregation; however, as aggregation progresses, the hydrophobic cores become hidden from the other molecules in solution and D_1 begins to increase again.

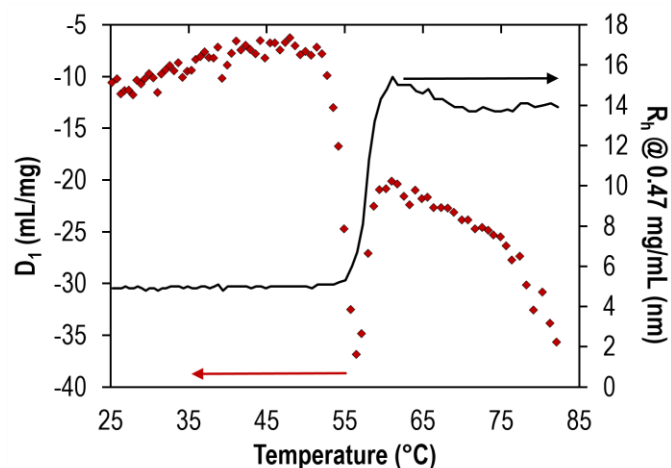


Figure 3: The diffusion interaction parameter, D_1 , decreases prior to the point of aggregation as protein molecules begin to unfold.

Using HT-DLS to determine viscosity

Finally, DLS can be used to assess formulation viscosity to help determine the long-term stability and deliverability of a biotherapeutic. DLS measures viscosity by spiking sample wells with polystyrene beads of a known diameter and radius. The Stokes-Einstein equation is manipulated to determine viscosity. The results from DLS usually correspond well to traditional analytical techniques, such as capillary viscometry. The advantage of employing automated HT-DLS for viscosity measurements is that samples are assessed rapidly at the same time as a variety of other features, adding a further layer of detail to scientists' understanding of their formulations.

High-throughput dynamic light scattering

Dynamic light scattering (DLS) uses a sensitive detector to monitor the intensity fluctuation of scattered light that arises from the Brownian motion of particles in solution. **DYNAMICS** software then analyzes the autocorrelation data to determine the rate of diffusion, which is converted to hydrodynamic radius via the Stokes-Einstein equation.

$$r_h = \frac{k_B T}{6\pi\eta D_t}$$

The Stokes-Einstein equation relates light scattering intensity and Brownian motion to hydrodynamic radius.

The **DynaPro Plate Reader II** carries out high-throughput DLS measurements of the size and interactions of proteins, nanoparticles, and other macromolecules. The laser intensity auto-attenuates by a factor of up to a million in order to accommodate a wide range of particles sizes and concentrations. The device can perform temperature ramps in order to assess thermal behavior and test protein features such as the onset temperature and rate of aggregation. Since the DLS measurements are completed *in situ* in industry-standard microwell plates, liquid handling is minimized. This capability reduces measurement time and eliminates sample carryover, resulting in a typical scan time of less than one hour for a 96 well plate.



Using an automated workflow for formulation preparation and analysis

Standalone automated high-throughput analytical techniques deliver substantial savings in biopharmaceutical characterization and formulation development. However, it is the ability to combine these techniques into a single automated workflow that delivers one of the most powerful innovations in modern formulation testing.

The Freeslate CM3 Biologics Formulation Systems (BFS) shown in **Figure 4** automates all aspects of formulation development including formulation preparation, formulation stressing, analytical sample preparation, and formulation analysis. The standard BFS workflow includes liquid handling, along with proprietary analytics such as viscosity, turbidity, visual particles, and third-party analytics including UV/Vis spectrophotometers and the Wyatt Technology DynaPro Plate Reader II. The entire workflow is managed by LEA (Lab Execution and Analysis) software that allows for fast experimental design and DOE import, automated execution, and streamlined data analysis. The combination of liquid handling capabilities, integrated analytics, and easy import and execution of DOE studies allows the bulk of the formulation screening process to be completely automated, from sample preparation through to complex analysis.



Figure 4. The Freeslate CM3 Biologics Formulation System

The following case study explores how automated workflows, combined with HT-DLS, can expedite biopharmaceutical formulation development while minimizing materials.

Case study: Plate-based high-throughput formulation evaluation

This case study demonstrates the power of automation to quickly evaluate 96 potential formulations. The experiment was designed to have the BFS prepare the formulations, stress the formulations, and collect the data to eliminate non-robust formulations. Robust formulations were then analyzed by size exclusion chromatography-ultra-high performance liquid chromatography (SEC-UHPLC) to quantitate soluble aggregates. All data were collected, stored, and viewed in LEA for rapid evaluation of formulation robustness.

The required stock solutions were loaded onto the BFS deck. The BFS mixed the solutions in the appropriate ratios to evaluate two buffer systems (acetate and histidine) at eight different pH points. These solutions were then diluted to three different concentrations and the API and excipients were added to create 96 formulations in a standard 96-well plate.

Once the formulations were prepared, the BFS transferred the 96 well plate to a UV spectrophotometer for measurement at A350 nm to measure turbidity. The BFS then moved the plate from the spectrophotometer to the HT-DLS DynaPro Plate Reader II. Light scattering data were collected from each well at room temperature before running a temperature ramp up to 68°C and measuring size and polydispersity at each time point. The BFS then removed the 96-well plate from the HT-DLS and transferred it back to the UV spectrophotometer to determine if turbidity had increased as a result of the heat stress.

Figure 5 presents the data generated from this process. After undergoing heat stress in the HT-DLS device, the particle size distribution increased substantially for several formulations, which, combined with turbidity data, indicated that aggregation had occurred. These formulations were identified as unsuitable and removed prior to further analysis by lower-throughput, higher-cost techniques such as SEC-UPLC.

This case study is just one example of an automated workflow for formulation development. The CM3 system can also perform a variety of workflows in both 96-well plates and a variety of standard formulation vials. Automated workflows not only allow users to robustly identify

the most appropriate formulations for further development but, by performing otherwise menial manual tasks automatically, create much more efficient laboratory. This approach enables scientists to rapidly work up a diverse set of formulations in a fraction of the time it would take manually and allows them to focus on formulation design

and characterization. This experiment required only a small amount of API at low volumes and the compatibility of the HT-DLS plate with additional analytical instruments means that the same material is subject to a range of different analytics.

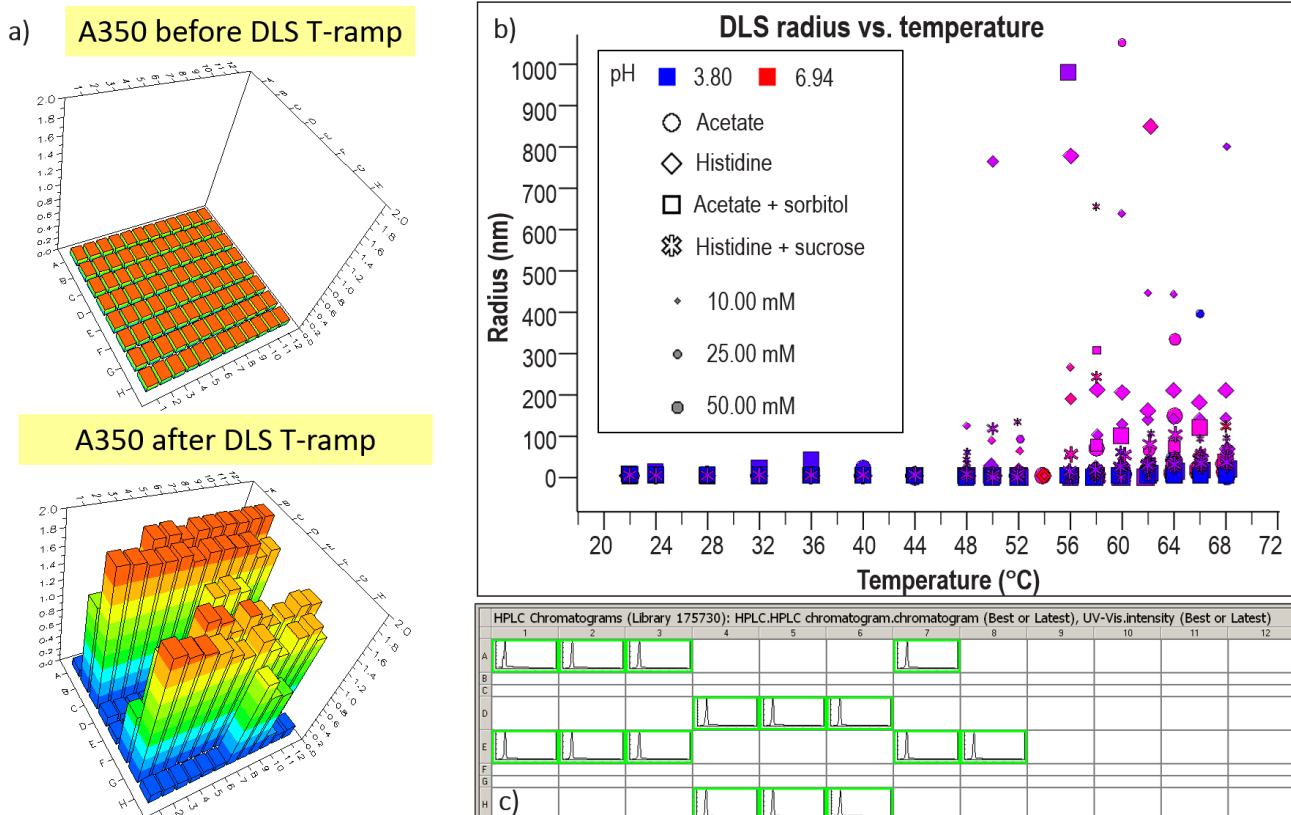


Figure 5. Data generated from the automated workflow. The turbidity data before and after temperature ramp-up is shown in a) The HT-DLS data from the temperature stress tests on a variety of formulations are shown in b) These two data sets allow users to quickly eliminate formulations that are unstable and unsuitable for further development and only analyze robust formulations with lower throughput techniques, such as SEC-UHPLC; c) shows the SEC data as viewed in LEA software, which allows all samples to be viewed in a single window for the quick evaluation of all formulations.

Identifying the best candidates as early as possible

As the pharmaceutical industry targets increasingly complex biologic candidates, automated formulation and analytical workflows play an important role in streamlining the development process. Capable of performing 96 runs in under 45 minutes with completely unattended operation, high performance HT-DLS accelerates analysis time, reduces the need for expert operator supervision, and im-

proves confidence in data quality. Moreover, when combined with the BFS system, it provides the ability fully scope candidate performance and explore a wider design space. Full automation provides developers with the depth of data and resources necessary to implement highly efficient development strategies for better performing, safer biotherapeutics.

Reference

1. R.A. Rader, *BioProcess Int.* 11 (3) 18–27 (2013).



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