Malvern | Material relationships

ACCELERATING BIOTHERAPEUTIC DEVELOPMENT

STREAMLINING CONCEPT TO MARKET

SOLUTIONS TO SUPPORT DEVELOPMENT AND MANUFACTURE

As market interest and investment in biopharmaceuticals surges and the requirement for novel biological drugs continues to grow, the industry is under increasing pressure to ensure delivery of therapies rapidly yet safely.

Biopharmaceutical development and manufacture, focusing on molecules such as monoclonal antibodies (mAbs), recombinant proteins, vaccines and oligonucleotides, are lengthy and complex processes with very specific analytical challenges. We know that in order to successfully deliver a biotherapeutic to market it is vital to reduce risk as soon as possible, deliver timely and accurate data and increase efficiency by streamlining processes and easing bottlenecks.

Malvern Instruments provides a growing range of solutions focused specifically on your critical path of testing and manufacturing requirements, which take into account the pressures of time and cost along with such factors as sample availability and complexity. These solutions are provided by a suite of instruments which enables access to a wide range of biophysical information on your product, to speed you through characterization studies, formulation development and process development, through to commercial manufacture.

Malvern Bioscience Development Initiative

The past decade has seen biopharmaceuticals become the fastest growing class of therapeutic agent, with increasing numbers of novel molecules entering development every year. Underlying this is a fundamental shift in pharmaceutical industry investment and emphasis. The challenges of developing complex biotherapeutics are considerable, requiring new analytical tools.

Established in 2012, Malvern's Bioscience Development Initiative (BDI) is an independent, agile and entrepreneurial organization, partnering with industry and academia to rapidly identify and assess analytical problems and deliver innovative solutions.

BDI oversees the invention and development of innovative technologies to enable in-depth product knowledge and understanding. This includes the Zetasizer Helix, which combines Malvern's Zetasizer technology with Raman spectroscopy, to analyze higher order structure and aggregated state of therapeutic proteins, allowing correlation of colloidal and structural stability, providing advanced understanding of protein stability.

DEVELOP COST-EFFECTIVE, NOVEL PRODUCTS FASTER

DEVELOPMENT STAGES	KEY STEPS AND REQUIREMENTS	DEVELOPMENT COSTS
CANDIDATE VALIDATION	• Measure of bioefficacy • Indication of developability/ manufacturability • Label-free samples • Easy-to-interpret data • Large sample numbers	$
EARLY FORMULATION DEVELOPMENT	• Stability prediction • Stability indicators • Manufacturability • Optimization of bioactivity • Excipient/formulation screening	$ $
LATE FORMULATION DEVELOPMENT	• Subvisible particles • Aggregation characterization • Absolute viscosity profiling, with shear rate and temperature • Stability indicators • 2°/3° protein structure • Manufacturability • Stability testing • Shelf life stability	$ $ $
PROCESS DEVELOPMENT	• QbD • Biocomparability • Product quality • Container/closure stability • Pilot scale/scale-up • Shelf life stability • Particle characterization	$ $ $ $
MANUFACTURING SUPPORT	• Biocomparability • Particle characterization • Product quality/ consistency • Orthogonal methodology • Process understanding • Root cause analysis • Stability	$ $ $ $ $

PIPELINE SUPPORT

SOLUTIONS TO SUPPORT DEVELOPMENT

CANDIDATE VALIDATION

- Measure of bioefficacy
- Indication of developability/ manufacturability
- Label-free samples
- Easy-to-interpret data
- Large sample numbers

- Advanced binding affinity characterization
- Protein size screening
- Protein viscosity screening
- Automation

EARLY FORMULATION DEVELOPMENT

- Stability prediction
- Stability indicators
- Manufacturability
- Optimization of bioactivity
- Excipient/formulation screening

- Low concentration prediction of stability
- Highly sensitive detection of protein aggregates
- Absolute molecular weight determination of aggregates
- Formulation viscosity screening
- Structural stability screening
- Advanced characterization of protein interaction processes
- Automation

LATE FORMULATION DEVELOPMENT

- Subvisible particles
- Aggregation characterization
- Stability indicators
- 2˚/3˚ protein structure
- Manufacturability
- Stability testing
- Shelf life stability

- Sizing/qualification of subvisible particles
- Highly sensitive detection and characterization of protein aggregates
- Characterization of higher order protein structure
- Absolute viscosity profiling - with shear rate and temperature

PIPELINE SUPPORT

ORTHOGONAL METHODOLOGY | ADVANCED CHARACTERIZATION | ASSAY DEVELOPMENT

- Viscosizer TD
- MicroCal ITC

- Zetasizer Nano
- Viscotek SEC-MALS
- OMNISEC REVEAL
- Viscosizer TD
- MicroCal DSC
- MicroCal PEAQ ITC

- Zetasizer Nano
- NanoSight
- Archimedes
- Zetasizer Helix
- Viscotek SEC-MALS
- Kinexus
- OMNISEC REVEAL

AND MANUFACTURE

PROCESS DEVELOPMENT

- QbD
- Biocomparability
- Product quality
- Container/closure stability
- Pilot scale/scale-up
- Shelf life stability
- Particle characterization

- Sizing/qualification of subvisible particles
- Chemical identification of particles
- Detection and quantification of aggregates over full range
- Advanced characterization of soluble aggregates
- Characterization of higher order protein structure
- Advanced characterization of protein : ligand interaction processes

MANUFACTURING SUPPORT

- Biocomparability
- Particle characterization
- Product quality/ consistency
- Orthogonal methodology
- Process understanding
- Root cause analysis
- Stability

- Advanced particle characterization
- Advanced protein aggregate characterization
- Multiple, complementary technologies to characterize aggregates
- Increased product understanding
- Highly sensitive technologies to characterize product quality attributes

| QbD/DESIGN SPACE | CONTINUOUS IMPROVEMENT

- NanoSight
- Archimedes
- Morphologi G3/G3-ID
- Viscotek SEC-MALS
- OMNISEC REVEAL
- Zetasizer Nano
- Zetasizer Helix
- MicroCal PEAQ ITC
- MicroCal DSC

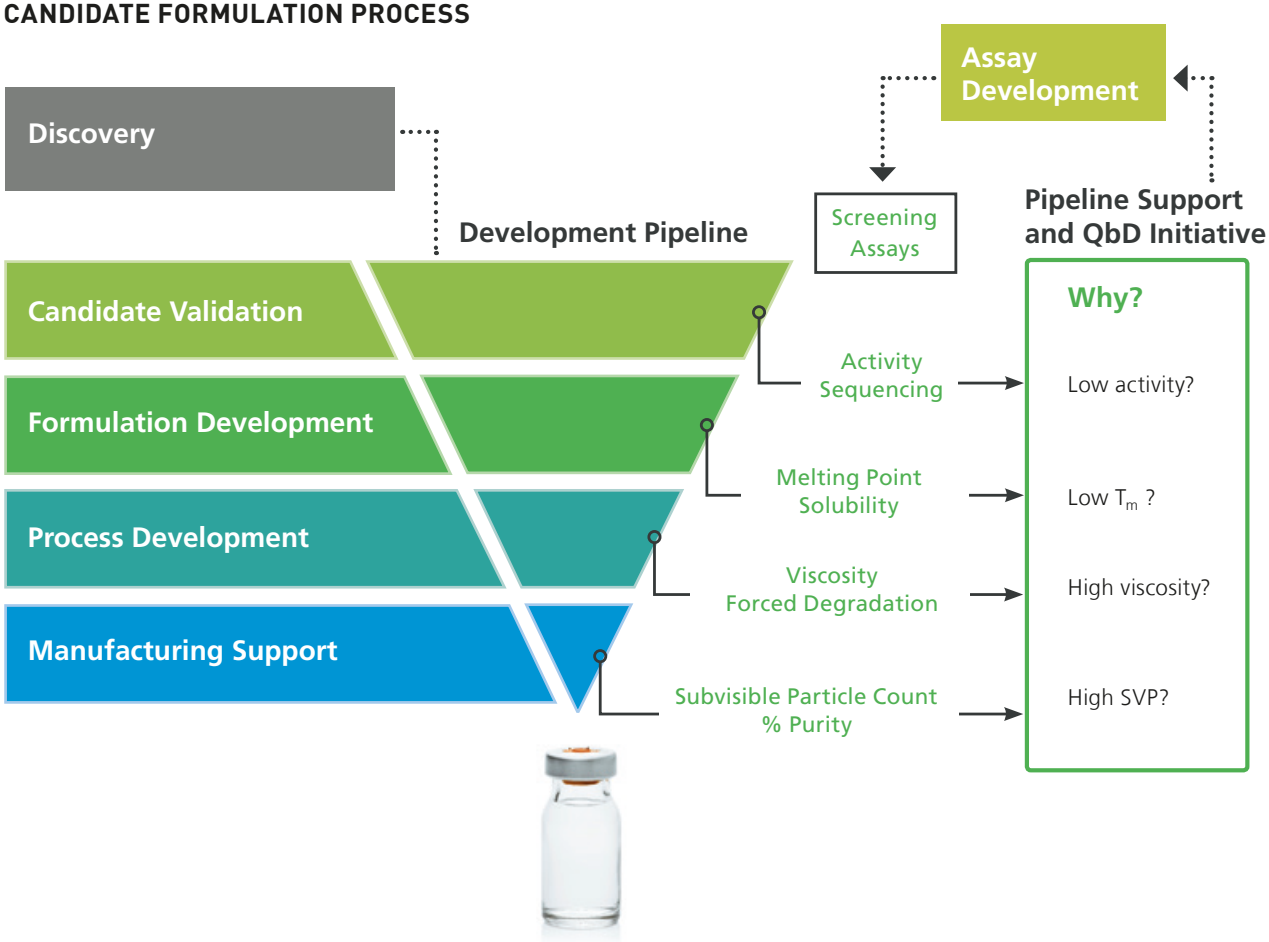
- NanoSight
- Archimedes
- Morphologi G3/G3-ID
- Zetasizer Helix
- Viscotek SEC-MALS
- OMNISEC REVEAL
- Zetasizer Nano
- MicroCal PEAQ ITC
- MicroCal DSC
- Kinexus

PIPELINE SUPPORT

Pipeline support activities run parallel to those of the core development pipeline, providing additional physicochemical analysis and screening. As potential candidates progress, they are subjected to a battery of tests to ensure only the most suitable molecules move on to the next stage. Candidates and formulations that fail to satisfy the predetermined criteria are removed from the main product pipeline and placed in the pipeline support area. More in-depth characterization testing is then performed to understand the reason for such failures. Based upon the data and information obtained from this additional testing, screening assays are further developed to ensure the most relevant and predictive tests are being used in the pipeline and are often implemented to support the development of future products.

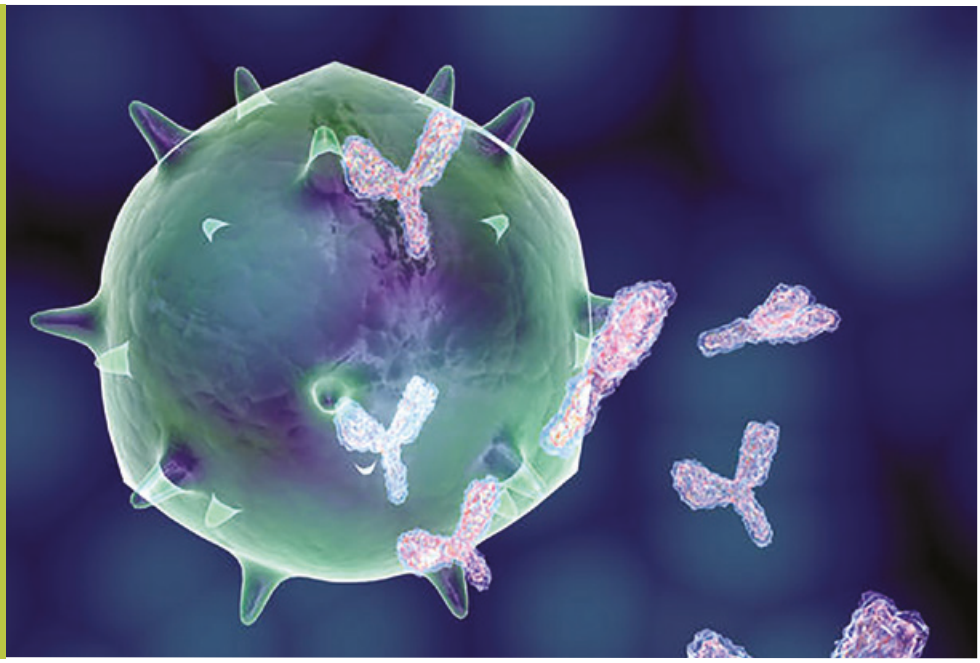
Technologies applied in the pipeline support function are often required to be multifunctional and information-rich, providing detailed product understanding from high quality data. Malvern Instruments offers an array of technologies to provide data-rich analysis of a wide range of protein attributes. These instruments, centered on industry leading technology, driven by Malvern's intuitive software and supported by our global network of technical specialists, provide detailed product understanding to streamline development.

CANDIDATE FORMULATION PROCESS



CANDIDATE VALIDATION

- Bioactivity
- Hydrodynamic size (D_H)
- Viscosity (η)



Candidate validation studies aim to quickly identify and validate the most suitable candidates, screening for attributes such as biological activity and developability. Early removal of potentially problematic molecules reduces time and costs.

MicroCal Isothermal Titration Calorimeters (ITC) allow direct, label-free measurement of binding affinity and thermodynamics in a single experiment, enabling the accurate determination of binding constants (K_D), reaction stoichiometry (n), enthalpy (ΔH) and entropy (ΔS). These parameters provide a complete thermodynamic profile of the molecular interaction. This supports rational design and optimization of candidates to ensure a highly efficacious product.

The Viscosizer TD provides automated screening of candidates for both hydrodynamic size and relative viscosity. Utilizing Taylor Dispersion Analysis with UV-detection, the Viscosizer TD provides size data for the target molecule unaffected by other components. In addition, relative viscosity screening identifies candidates exhibiting abnormally large increases in viscosity as a function of concentration, even at low concentrations. Consequently, potentially problematic candidates displaying a propensity to self-associate can be removed early in the development pipeline.

MicroCal PEAQ ITC Automated:

- All binding parameters (affinity, stoichiometry, enthalpy and entropy) in a single experiment
- High signal-to-noise increases confidence in data quality and relevance of generated affinity and thermodynamic parameters
- Identification of stabilizing excipients
- Automated washing (with detergent) of the sample cell and titration syringe assists in producing high quality reproducible data
- Fully automated with capacity to run 4× 96-well plates unattended

Viscosizer TD:

- Screens attributes including molecular size, conformational stability and self-association, solubility and relative viscosity
- Low concentration (µg quantities) and ultra-low volume requirements (sizing 40 nL, viscosity 6 µL)
- Automated, multi-sample analysis with precise environmental control for sample storage and measurement
- Label-free characterization of target molecules in highly complex solutions, from small molecules to peptides and proteins and also samples containing mixtures of these species

EARLY STAGE FORMULATION DEVELOPMENT – I

DEVELOPING STABLE FORMULATIONS

- Zeta potential (ZP)
- Second virial coefficient (B_{22})
- DLS interaction parameter (K_D)

The ability to predict product stability early in the development pipeline, with the use of low concentration formulations, can streamline early formulation development.

> ICH stability testing guidelines (Q5C) are written with the understanding that the only way to measure the stability of biopharmaceuticals is to perform long-term stability studies under true-to-life storage conditions.

Long-term stability studies cannot be performed for every potential formulation at an early stage. The stability prediction parameters (B_{22}, K_D, ZP) delivered by the Zetasizer Nano are invaluable for screening purposes, ensuring only the most suitable formulations are taken forward, reducing development time and costs.

Zeta potential is a measure of intermolecular electrostatic interactions. Higher zeta potential increases repulsion amongst molecules, thereby minimizing the formation of native aggregates. While native aggregates are often reversible, their presence is a significant risk factor for the formation of denatured aggregates which are generally non-reversible.

The second virial coefficient (B_{22}) is determined by intermolecular interactions such as electrostatic and hydrophobic bonding, while the DLS interaction parameter, (K_D) is affected by both thermodynamic and hydrodynamic interactions. More positive B_{22} and K_D values are indicative of more stable formulations, so both of these parameters are effective predictors of stability. B_{22}, K_D and ZP can all be obtained using a Zetasizer Nano, with its best-in-class concentration range and broadest buffer composition capability.

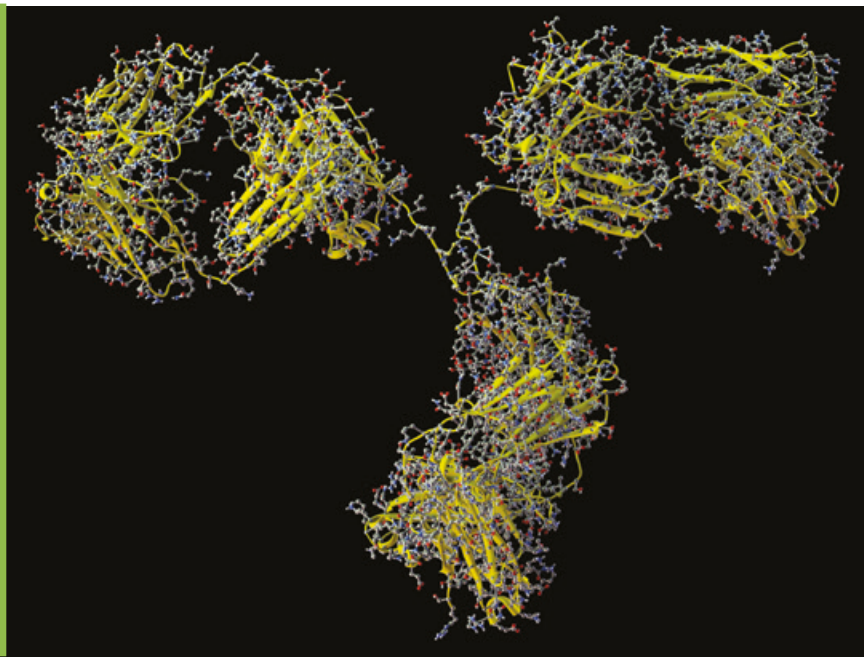
Zetasizer Nano range:

- Rapid measurements, providing a multitude of analytical data
- Industry-leading technology, with exceptional sensitivity and compatibility with broad range of sample attributes and protein concentrations
- Fully automated with capacity to run 4× 96-well plates unattended (Zetasizer APS)
- Intuitive software, simplified data analysis, built-in procedures and calculators specifically for proteins
- 21CFR compliant capability
- Applicable throughout development pipeline and beyond

EARLY STAGE FORMULATION DEVELOPMENT – II

DETECTION AND CHARACTERIZATION OF PROTEIN AGGREGATES

- Hydrodynamic size (D_H)
- Molecular weight (MW)
- Intrinsic viscosity (IV)



Protein aggregation in biopharmaceuticals is of particular concern for the industry, reducing product efficacy and stability and increasing immunogenic risk. Malvern's highly sensitive protein aggregation characterization tools allow complete evaluation of therapeutic proteins and early identification of potential stability concerns.

The Zetasizer determines a protein's hydrodynamic size as well as detecting the presence of larger aggregated species. This solution is ideal for early formulation stability screening activities.

The addition of multiple detector systems to Size Exclusion Chromatography (SEC) provides more characterization of protein aggregates, improving product understanding and supporting biocomparability studies. Multiple angle light scattering (MALS) and OMNISEC REVEAL measure absolute molecular weight (MW) of each aggregated species, allowing them to be characterized by the number of monomer units they contain.

OMNISEC REVEAL also contains an intrinsic viscometer, providing protein structural parameters, and RI/UV detectors, providing concentration information, thus enabling measurement of polydispersity and determination of hydrodynamic size. These data are obtained from a detector suite that can be connected to an existing HPLC-SEC system, without compromising existing SEC use or functionality.

The Viscosizer TD provides an unmatched molecular size range, enabling sizing of small peptides and large proteins, or even mixtures of the two. The use of specific UV wavelength detection and matched sample buffer allows selective measurement of target molecules, without interference from buffer components. Consequently, samples can be analyzed without dilution or filtration. Taylor Dispersion Analysis (TDA) provides data that is both complementary and orthogonal to many other sizing technologies, giving confidence and robustness to formulation characterization activities.

Zetasizer Nano range:

- Industry-leading technology, with exceptional sensitivity and compatibility with broad range of sample attributes and protein concentrations
- Fully automated with capacity to run 4× 96-well plates unattended (Zetasizer APS)
- Intuitive software to streamline workflows with simplified data analysis and built-in expert advice for confident decision-making
- 21CFR compliant capability
- Applicable throughout development pipeline and beyond

OMNISEC REVEAL / Viscotek SEC-MALS range:

- Highly sensitive light scattering detector to identify low levels of aggregates with low sample requirements (100 ng)
- Quick and seamless integration with existing SEC systems
- Compatible with vials or 96/384-well plates, with optional temperature controlled autosampler

Viscosizer TD:

- Screens attributes such as molecular size, conformational stability and self-association
- Low concentration measurements (µg quantities) and with ultra-low sample volumes of 40 nL
- Automated, multi-sample analysis with precise environmental control for sample storage and measurement
- Sample analysis without dilution or filtration, unaffected by the presence of a small amount of aggregates, excipients or surfactants

EARLY STAGE FORMULATION DEVELOPMENT – III

UNDERSTANDING AND CONTROLLING FORMULATION VISCOSITY

- Viscosity (η)

The production of high concentration yet low viscosity biotherapeutic formulations is one of the key challenges faced by the biopharmaceutical industry. Viscosity issues often accompany the desired high concentration/low frequency doses of parenteral administration. High viscosities can be unsuitable for injection and cause challenges during bioprocessing. Discovering too late that a particular formulation has a high viscosity can have significant cost implications.

The Viscosizer TD can accurately and reproducibly discern differences between the relative viscosities of different protein formulations at low concentrations and therefore identify abnormal viscosity-concentration profiles early. Automated relative viscosity screening of early stage formulation candidates with Viscosizer TD can be performed using incredibly low sample volumes.

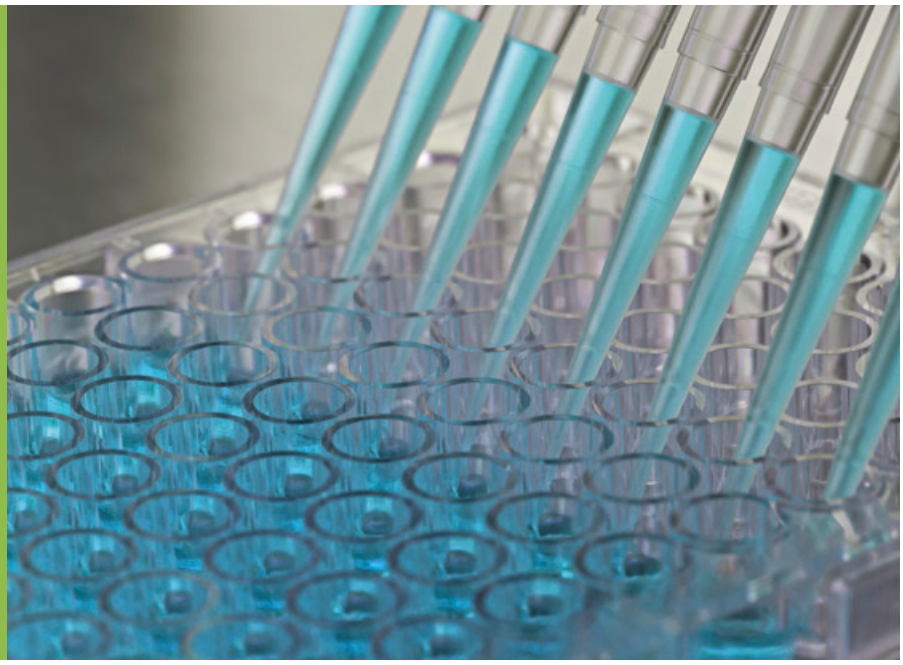
Viscosizer TD:

- Screens attributes including molecular size, conformational stability and self-association, solubility and relative viscosity
- Low concentration (µg quantities) and ultra-low volume requirements (viscosity 6 µL)
- Automated, multi-sample analysis with precise environmental control for sample storage and measurement
- Label-free characterization of target molecules in highly complex solutions, from small molecules to peptides and proteins, and also samples containing mixtures of these species

EARLY STAGE FORMULATION DEVELOPMENT – IV

CHARACTERIZING STRUCTURAL STABILITY TO AID PREFORMULATION DEVELOPMENT

- Melting temperature (T_M)
- Aggregation temperature (T_{agg})



Thermal stability is a widely used parameter for measuring protein stability, enabling screening of different formulations and comparisons of different candidates. Malvern Instruments provides two technologies which offer complementary data to evaluate the thermal stability of selected candidates and formulations.

MicroCal differential scanning microcalorimeters (DSC) provide fast and accurate determination of melting transition midpoint (T_M) and changes in enthalpy (ΔH), as indicators of thermal stability. These changes occur as the protein unfolds, allowing DSC to detect denaturing events. Any increase in T_M seen when comparing native and modified forms during formulation screening, can be associated with an increase in stability.

The aggregation temperature (T_{agg}), can be measured using the Zetasizer Nano by performing thermal ramps up to 90°C and collecting data at predetermined temperatures.

Low volume cuvettes minimize sample volume requirements, while automated screening can be performed in 96-well and 384-well plates. The inherent sensitivity of dynamic light scattering (DLS) to the presence of aggregates allows very small changes to be detected and subtle differences between formulations and candidates to be reported. These differences may arise from the formation of aggregates in response to thermal stress and are a direct indication of protein stability.

DSC and DLS are complementary technologies which combine perfectly to evaluate the stability of biotherapeutics. While DSC detects unfolding of the protein structure and the resultant change in heat capacity, DLS detects changes in protein size as a result of aggregate formation. The combination of these two techniques provides insights into changes induced by protein instability resulting from thermal stress. Together, DLS and DSC can provide a highly robust determination of formulation and candidate stability, critically supporting the development process.

Zetasizer Nano range:

- Industry-leading technology, with exceptional sensitivity and compatibility with broad range of sample attributes and protein concentrations
- Fully automated with capacity to run 4× 96-well plates unattended (Zetasizer APS)
- Intuitive software to streamline workflows with simplified data analysis and built-in expert advice for confident decision-making
- 21CFR compliant capability
- Applicable throughout development pipeline and beyond

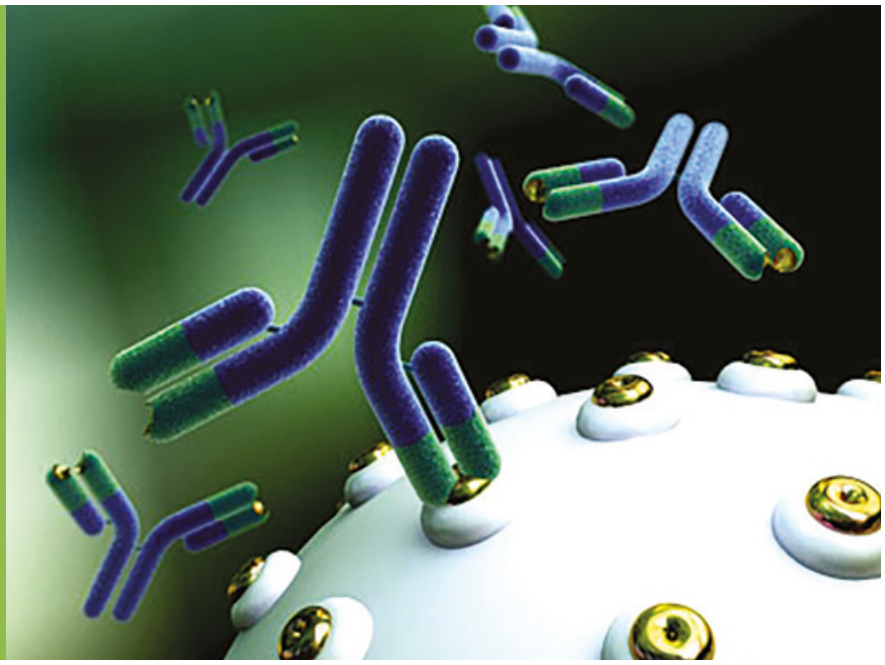
MicroCal DSC range:

- Compatible with a variety of sample types, including high concentration, colored and turbid, as well as a broad range of solvents and buffers
- Rapid identification of formulation conditions, utilizing label-free, universal stability assay
- Simple assay development
- Full automation using standard 96-well plate format ensures high capacity and easy loading, with thermostatically-controlled storage of up to 6 plates

EARLY STAGE FORMULATION DEVELOPMENT – V

ASSESSMENT AND OPTIMIZATION OF BIOACTIVITY

- Binding affinity (K_D)
- Enthalpy (ΔH)
- Entropy (ΔS)



Candidate optimization is often driven by studying the affinity of interactions between candidate and target molecules. However, thermodynamic variables underlying these interactions, such as ΔH and ΔS, are also fundamental to this process and provide deeper insights into the drivers for such interactions. MicroCal PEAQ ITC calorimeters have the sensitivity and throughput for efficient determination of all the binding parameters that may guide candidate optimization and formulation development activities.

MicroCal isothermal titration calorimeters (ITC) all allow direct, label-free, in-solution measurement of binding affinity and thermodynamics in a single experiment, enabling the accurate determination of binding constants (K_D), reaction stoichiometry (n), enthalpy (ΔH) and entropy (ΔS). These provide a complete thermodynamic profile of the molecular interaction, enabling the user to go beyond binding affinities and elucidating the mechanisms which underlie molecular interactions. Hydrogen bonding, measured by ΔH, is often a more effective predictor of efficacy than hydrophobic interactions, measured by ΔS, and so more effective therapeutics usually focus on optimizing ΔH.

ITC permits a multi-dimensional approach, where the contribution of enthalpy and entropy to affinity is used to screen for the most effective biological candidates, support engineering to design better biotherapeutics or ensure biological activity is maintained during formulation screening.

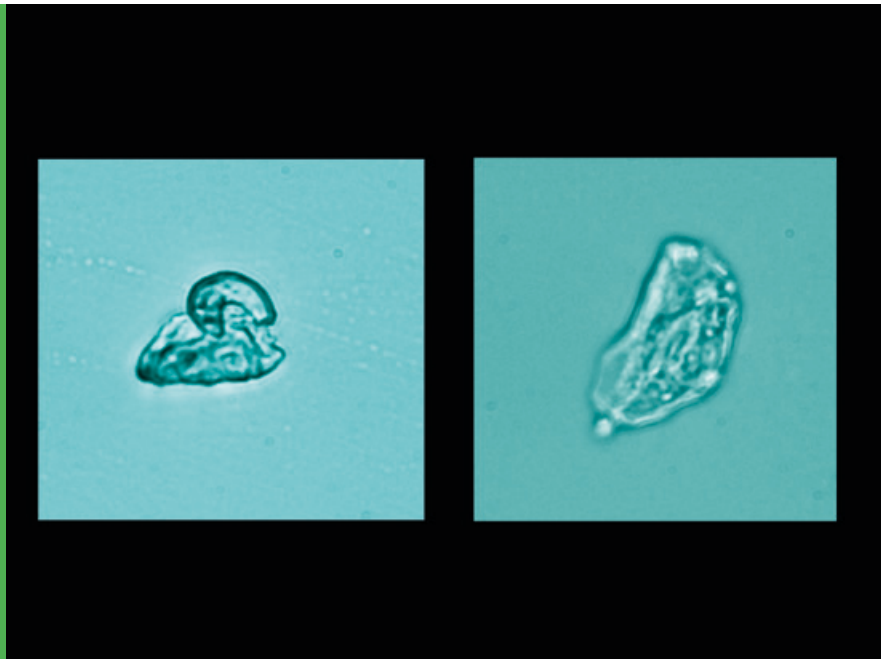
MicroCal PEAQ ITC:

- All binding parameters (affinity, stoichiometry, enthalpy and entropy) in a single experiment
- High signal-to-noise increases confidence in data quality and relevance of generated affinity and thermodynamic parameters
- Identification of stabilizing excipients
- Automated washing (with detergent) of the sample cell and titration syringe assists in producing high quality reproducible data
- Fully automated with capacity to run 4× 96-well plates unattended

LATE STAGE FORMULATION DEVELOPMENT – I

DETECTION AND CHARACTERIZATION OF SUBVISIBLE PARTICLES

- Particle size (200 nm – 100 µm)
- Particle concentration



Subvisible particles are of major concern to biopharmaceutical manufacturers since they can impact the efficacy and immunogenicity of the active molecule.

Guidance from the FDA *(Guidance for Industry: Immunogenicity Assessment for Therapeutic Protein Products - 2014)*, concerning the assessment of immunogenic potential states:

> *"…the use of any single method for assessment of aggregates is not sufficient to provide a robust measure of protein aggregation"*

and

> *"Excipients should be evaluated for their potential to prevent denaturation and degradation of therapeutic protein products during storage"*

Quantification of subvisible particles is required for injectables (USP <787> and USP <788>). Importantly, guidance from the FDA states:

> *"…it has been recognized that subvisible particulates in the size range of 0.1-10 microns have a strong potential to be immunogenic, but are not precisely monitored by currently employed technologies"*

NanoSight products utilize nanoparticle tracking analysis (NTA) to characterize subvisible particles from 10 nm - 1000 nm in solution.

Based around a high-resolution camera and specially designed software, NTA measures Brownian motion of each individual particle to determine hydrodynamic size.

The result is high-resolution particle size distributions, within a known sample volume, allowing the concentration of particles to be determined. The particle-by-particle approach is particularly appropriate for polydisperse samples. The size range and particle information provided by NTA offers data complementary to other sizing tools such as the Zetasizer and provides a bridge to the particle sizing capabilities of the Archimedes.

Archimedes uses the technique of resonant mass measurement (RMM) to detect, weigh, count and size particles in the size range 50 nm to 5 µm. This size range is of great importance within the biopharmaceutical industry due to the immunogenic risk of such particles.

The fundamental particle property measured by the Archimedes is buoyant mass from which particle size can be calculated. This makes RMM a method which is truly orthogonal to all other particle sizing tools currently available. In addition, RMM can distinguish between particles that are positively buoyant and those that are negatively buoyant. This is hugely important in the arena of pre-filled syringes where Archimedes can discriminate between proteinaceous material and contaminants such as silicone oil.

NanoSight range:

- Number-weighted concentration and high resolution size distributions in the size range 10 nm – 2000 nm
- Visual validation of results gives extra confidence
- Minimal sample preparation
- Fluorescence capability, allowing differentiation of sub-populations

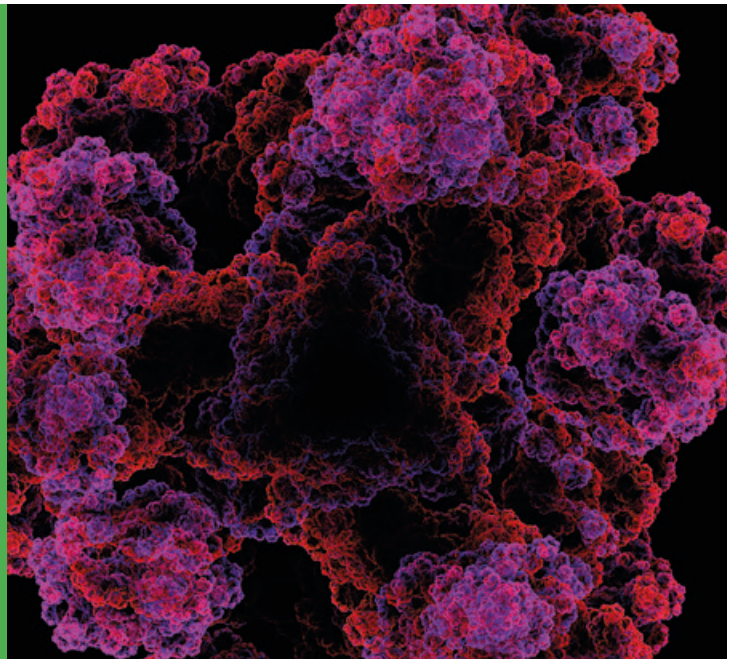
Archimedes:

- Unique and accurate particle counting capability in the size range 50 nm – 5 µm
- Low sample consumption: only 100 µL
- Differentiation between negatively buoyant and positively buoyant particles
- Capable of measuring high concentration samples (to 10^9 particles/mL) and high viscosity samples (to 100 cP)

LATE STAGE FORMULATION DEVELOPMENT – II

ADVANCED CHARACTERIZATION OF STRUCTURAL AND COLLOIDAL STABILITY

- Hydrodynamic size (D_H)
- Higher order protein structure (2° & 3° structure)
- T_M, T_{agg}
- van't Hoff enthalpy



Elucidation of the aggregation process is key to product understanding and aids evaluation of process impact, thereby enabling more effective implementation of Quality by Design (QbD). The Zetasizer Helix combines the DLS and zeta potential capabilities of the Zetasizer with Raman Spectroscopy to provide advanced in-depth knowledge of protein stability. Whilst DLS enables highly sensitive monitoring of protein size and aggregated state, Raman spectroscopy enables sensitive monitoring of both secondary and tertiary structure markers, thereby providing highly detailed analysis of higher order structure. This ability to correlate both colloidal and structural stability enables the Zetasizer Helix to provide unique insights into protein folding, unfolding, aggregation, agglomeration and oligomerization.

In addition, Zetasizer Helix can derive T_M and van't Hoff enthalpies, complementing DSC and providing detailed insights into the protein structural motifs contributing to observed thermodynamic behavior.

Three different experimental modes can be implemented: Sample Series for comparing and evaluating biosimilarity, Thermal Ramps to evaluate thermal stability and Thermodynamic Behavior and Isothermal Incubations to analyze and measure the kinetics of structural and size changes. Consequently, the Zetasizer Helix is a highly versatile technology, providing valuable product knowledge and understanding at all stages of a product life cycle, from development through to commercial manufacture.

Zetasizer Helix:

- Study formulations without (or with minimal) dilution
- Understand mechanisms of aggregation and oligomerization
- Identify Critical to Quality product attributes
- "Expertise Optional" data analysis software provides trend results without making expert-level decisions
- Advanced data analysis tools include a full suite of spectroscopic analysis tools from pre-treatment to multivariate analysis
- In-depth analysis of secondary and tertiary protein structure

LATE STAGE FORMULATION DEVELOPMENT – III

ADVANCED CHARACTERIZATION OF AGGREGATES

- Hydrodynamic size (D_H)
- Intrinsic viscosity (IV)
- Molecular weight (MW)

The formation of protein aggregates can be detected by a wide range of analytical technologies. However, to perform more detailed analysis of the aggregate species, a combination of regular SEC-HPLC with advanced detection systems, such as Viscotek SEC-MALS or OMNISEC REVEAL, is often applied. These detectors, which can be placed in-line with existing SEC instruments, provide absolute MW, hydrodynamic size, polydispersity and percentage composition of each individual species within a sample. Such information-rich data is beneficial in the understanding of protein aggregates, allowing full characterization of each aggregate species.

Absolute MW enables each species to be classified by the number of monomer units present. This MW is absolute and does not contain the inherent error present when using conventional calibration for MW determination. The measurement of intrinsic viscosity (IV) gives valuable information regarding the structure of each species within a sample. Taken together, the product information and understanding obtained from the use of Viscotek SEC-MALS or OMNISEC REVEAL detector systems supports the development of stable biotherapeutics and can be used to demonstrate biocomparability during the development of biosimilars.

OMNISEC REVEAL / VISCOTEK SEC-MALS range:

- Low sample requirements (100 ng)
- Highly sensitive light scattering detector to detect low levels of aggregates
- Quick and seamless integration with existing SEC systems
- Workflow-based software eases handling of large amounts of data
- Optional temperature controlled autosampler
- Compatible with vials or 96/384-well plates

LATE STAGE FORMULATION DEVELOPMENT – IV

ADVANCED VISCOSITY PROFILING

- Viscosity (η) - Shear rate (γ) relationship
- Viscosity (η) – Temperature (T) relationship

During late stage development, candidate biotherapeutics are often formulated at their target high concentration to meet the required dose requirements. Within the design space there will be specified viscosity limits for the drug product to reflect manufacturability, administration and storage. It is important to understand that as protein concentration increases the inherent viscosity properties of the solution become complex in nature, as evidenced by a viscosity-concentration plot showing a typical exponential increase at high concentrations.

In the low concentration regime, a single viscosity measurement is representative of the solution characteristics, i.e. typical Newtonian behavior. However, in the high concentration regime where protein molecules interact with each other in solution, the measured viscosity will depend upon the measurement conditions, such as the applied force or shear rate, i.e. non-Newtonian behavior.

For accurate and robust absolute viscosity characterization of high concentration protein solutions, a rheometer is required.

Advanced viscosity profiling enables formulators to produce viscosity versus shear rate and/or temperature curves that provide a rheological fingerprint of the formulation, allowing specifications to be set from manufacturing to storage to delivery.

In manufacturing, various shear rates and temperature variations will exist in the process, arising from changes in tubing dimensions, pumping mechanisms and filtration steps. Inappropriate viscosity profiles can result in manufacturing issues and/or problems during administration of injectables. For successful manufacture and administration of high concentration parenteral drugs, it is important that formulators assess the viscosity profiles of late stage formulations as a function of shear rate and temperature. This knowledge is vital to support final formulation studies and the progression to initial process development activities.

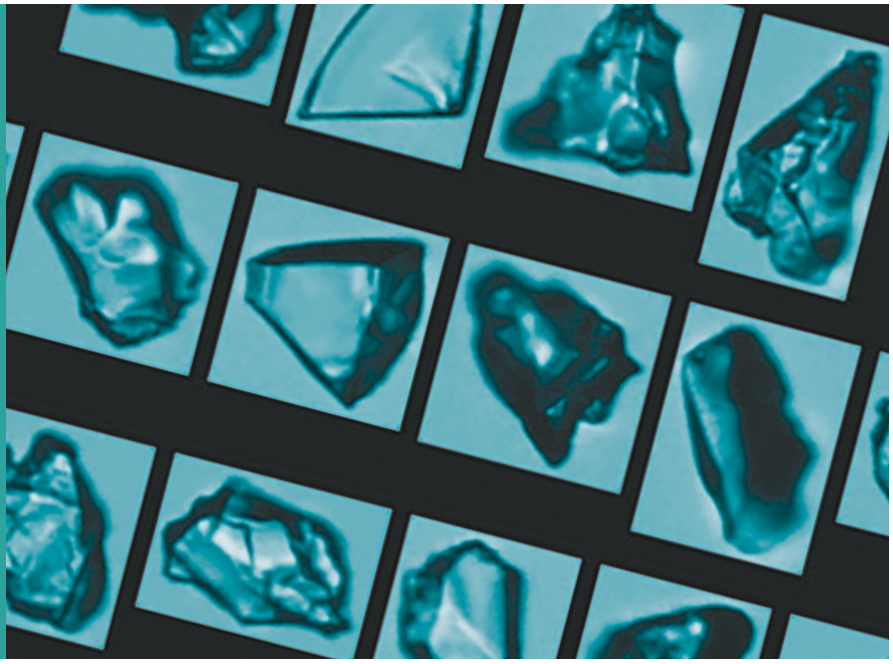
Kinexus rheometer range provides:

- Both strain rate and stress-controlled operation to access measurement regimes that are representative of real conditions over the product lifetime
- Unique rheometer software interface based on sequence-driven Standard Operating Procedures (SOPs) and full user guidance to enable ease of use and consistent rheological testing
- Fast, accurate and high resolution temperature control for thermal profiling and thermal cycling measurements
- Range of measurement geometries specifically designed for testing complex fluids over a wide shear rate range
- Kinexus Ultra+ minimizes sample use wherever possible for limited volume and critical samples

PROCESS DEVELOPMENT – I

SUBVISIBLE PARTICLE DETECTION AND QUANTIFICATION

- Subvisible particle size
- Subvisible particle shape



Regulatory agencies view immunogenicity as a major risk when it comes to the safety and efficacy of biotherapeutics. Subvisible particles are widely considered to be a significant cause of immunogenic reaction to parenteral drugs, so their detection, enumeration and characterization is of great importance, as recently highlighted in USP <787>. By utilizing RMM, Archimedes can detect, weigh, count and size particles in the size range 50 nm to 5 µm, filling a gap in current particle sizing capabilities, improving subvisible particle characterization and supporting immunogenic risk assessment for biotherapeutics.

USP <788> specifically requires parenterally-administered therapeutics to be assessed for the number of particles they contain which are ≥ 10 µM & ≥ 25 µM.

The use of manual light microscopy to count contaminant particles is often a means to comply with USP <788>. However, limitations such as human error and time pressures make this method less than ideal.

Malvern's Morphologi G3 provides fast, accurate and highly repeatable measurement of subvisible particles in the absence of operator subjectivity enabling the rapid classification of particles into user-specified size and shape brackets.

Morphologi G3:

- Particle attributes characterized: size, shape, transparency and number/ concentration
- Sizing from 2 µm – 1000 µm
- Automated SOP control for unattended operation and reproducible results
- Advanced manual microscope control mode, providing increased value as a shared resource
- Rapid automatic particle counting

Archimedes:

- Unique and accurate particle counting capability in the size range 50 nm – 5 µm
- Low sample consumption: only 100 µL
- Differentiation between negatively buoyant and positively buoyant particles
- Capable of measuring high concentration samples (to 10^9 particles/mL) and high viscosity samples (to 100 cP)

PROCESS DEVELOPMENT – II

FOREIGN PARTICLE / CONTAMINANT IDENTIFICATION

- Subvisible particles (SvP)
- Chemical identification

A significant increase in the number of subvisible particles present in a formulation requires investigation of their root cause and source. The first step in any such investigation is the identification of the contaminants. Morphologi G3-ID provides solutions that not only count and size particles, but also chemically identify them thereby greatly facilitating deviation resolution activities.

Archimedes uses RMM to measure the buoyant mass of particles and therefore calculate their size within the range 50 nm to 5 µm. The ability to measure buoyant mass provides Archimedes with the means to distinguish particles based on their positive or negative buoyancy within a particular medium. In biotherapeutic formulations, Archimedes can distinguish between negatively-buoyant protein aggregates and positively-buoyant silicone oil, a significant contaminant arising from the use of prefilled syringes. Silicone oil leaching from container closure systems can be quickly identified as a potential cause for failure to meet quality specifications thus reducing investigation time.

For a more comprehensive assessment of contaminants, the Morphologi G3-ID combines automated static microscopy with Raman spectroscopy to chemically identify individual particles of interest.

By automatically comparing the recorded spectra against a library of known compounds, it is possible to identify particles from many different sources. This combination of imaging and Raman spectroscopy provides identification of many commonly-used bioprocessing materials. Identification of the contaminant source greatly facilitates deviation resolution and root cause analysis, ultimately reducing the time and cost of such investigations.

Archimedes:

- Unique and accurate particle counting capability in the size range 50 nm – 5 µm
- Low sample consumption: only 100 µL
- Differentiation between negatively buoyant and positively buoyant particles
- Capable of measuring high concentration samples (to 10^9 particles/mL) and high viscosity samples (to 100 cP)

Morphologi G3/G3-ID:

- Measures particle size, shape and chemical identity in one platform
- Positively identifies the chemical composition of materials commonly used within bioprocessing
- Automatic selection, targeting and chemical classification of 1,000s of individual particles
- Export function for third party forensic library investigations

PROCESS DEVELOPMENT – III

BIOCOMPARABILITY

The ability to demonstrate batch-to-batch consistency (biocomparability) is a crucial aspect of many stages in the lifecycle of a biotherapeutic. During late stage process development and the clinical phase, biocomparability is evaluated to demonstrate the consistency of manufactured batches and to provide confidence in the control of bioprocessing parameters. A broad range of physicochemical and biochemical properties are used to demonstrate biocomparability, including protein size, polydispersity, aggregation state, aggregation species, secondary/tertiary protein structure, T_{agg}, T_M, binding affinities and particular matter content. Malvern Instruments' product portfolio can analyze and evaluate a wide range of product attributes to underpin biocomparability activities.

The International Conference for Harmonisation (ICH) states:

> *"An inherent degree of structural heterogeneity occurs in proteins….. the manufacturer should define the pattern of heterogeneity of the desired product and demonstrate consistency with that of the lots used in preclinical and clinical studies"* (International Conference on Harmonisation - Q6)

and

> *"When validating processes, or implementing manufacturing changes, batch variability needs to be assessed"* (International Conference on Harmonisation - Q5)

More specific ICH guidance requests the assessment of higher order protein structure:

> *"…sponsors should consider all relevant characteristics of the proposed product (e.g. …secondary, tertiary, and quaternary structure…) to demonstrate that the proposed product is highly similar to the reference product…"* (Guidance for Industry: Scientific Considerations in Demonstrating Biosimilarity to a Reference Product (Feb 2012)).

and

> *".. following a manufacturing process change, manufacturers should attempt to determine that higher order structure (secondary, tertiary, and quaternary structure) is maintained in the product …"* (*International Conference on Harmonisation - Q5E*).

Through the implementation of Raman spectroscopy, the Zetasizer Helix provides highly detailed information about the secondary and tertiary structure of protein drugs. Such information includes fractional secondary structure determination, e.g. α-helix, β-sheet and random coil, and specific markers for the aromatic amino acids tyrosine and tryptophan, providing information on tertiary structural changes. When combined with the sizing and polydispersity data obtained from DLS, this allows a specific profile for a particular protein therapeutic to be developed and used as a highly sensitive measure of comparability. It is therefore a valuable tool during continuous improvement activities or the development of biosimilars.

Zetasizer Helix:

- Study formulations without (or with minimal) dilution
- Understand mechanisms of aggregation and oligomerization
- Identify Critical to Quality product attributes
- "Expertise Optional" data analysis software provides trend results without making expert-level decisions
- Advanced data analysis tools include a full suite of spectroscopic analysis tools, from pre-treatment to multivariate analysis
- In-depth analysis of secondary and tertiary protein structure

MicroCal DSC:

- High quality, reproducible output data that is ideal for monitoring batch to batch variability
- Compare T_M of each structural domain to help identify where in the biotherapeutic any change may have occurred
- Compatible with a wide variety of biotherapeutics and buffer conditions
- Full automation using standard 96-well plate format ensures high capacity and easy loading, with thermostatically controlled storage of up to 6 plates
- Easy to use data analysis software

PROCESS DEVELOPMENT – IV

UNDERSTANDING AND EVALUATING PROCESS IMPACT

Quality by Design (QbD) is a concept which is key within the biopharmaceutical industry. It is defined by the ICH as:

> *"A systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management"*
> *(ICH, Q8 (R2)).*

QbD aims to ensure that bioprocessing parameters are developed using a rational approach, focusing on process impact and product quality. Underpinning QbD principles is product understanding. This requires the detection and evaluation of process impact and therefore the identification of potential impact on key product quality attributes and stability requirements. In support of QbD initiatives, Malvern Instruments provides advanced characterization tools to enable sensitive and highly detailed understanding of product attributes and thus the impact of bioprocessing parameters and variables.

In manufacturing, product is exposed to shear rates and temperature variations as the fluid path utilizes different tubing dimensions, different pumping mechanisms and filtration steps. In order to understand the impact of these processing steps, it is important to understand the product viscosity profile, as viscosities will be dependent on the shear stress and rate.

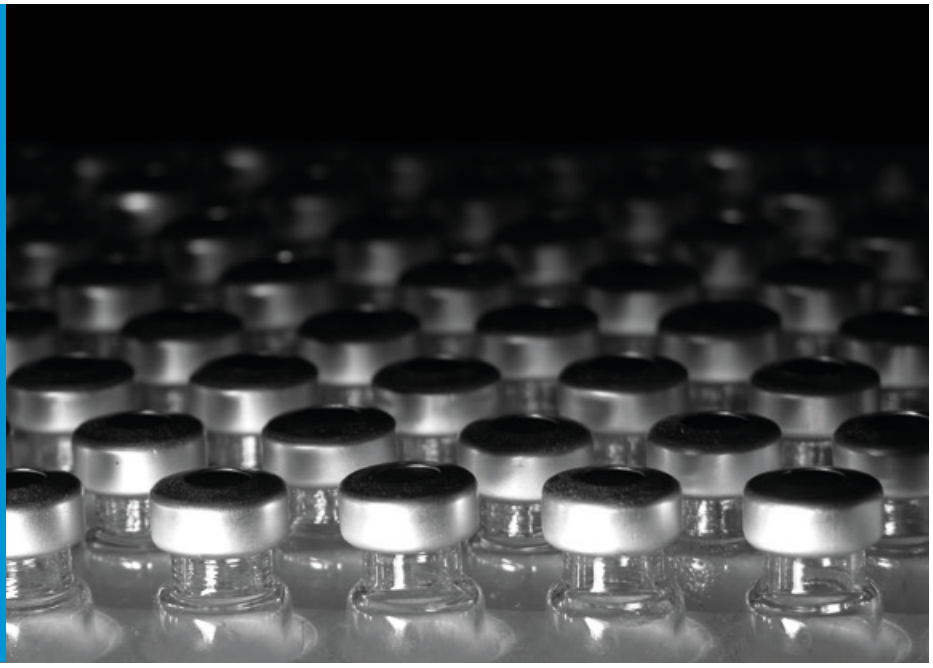
The ability to characterize the rheological properties of a biotherapeutic in response to relevant bioprocessing conditions, using a Kinexus rheometer, provides a greater understanding of process impact. In addition, shear stresses can be reproduced in laboratory environments to study degradation products and pathways in response to manufacturing.

The Zetasizer range enables highly sensitive DLS analysis for the identification of protein aggregates, detecting small aggregates long before other technologies are able to, and providing confidence that any small change in the protein size distribution will be exposed. Consequently, this technique is a very useful tool for the early detection of aggregation onset as the result of bioprocessing stresses. By combining the technical capabilities of the Zetasizer with Raman spectroscopy, Zetasizer Helix provides both protein size information and high order protein structural information, allowing detailed evaluation of bioprocessing impact on key attributes for protein stability. Furthermore, by assessing both protein structure and aggregation state, degradation pathways and the aggregation mechanism can be studied. This is a key aspect of QbD, supporting the development of processing steps and parameters that ensure the biotherapeutic satisfies quality and safety attributes.

At the other end of the aggregate size scale are subvisible particles. Protein aggregates larger than 100 nm will likely be excluded from SEC-HPLC columns, while those below 2 µm are often missed from microscopic flow imaging analysis. Therefore, to provide the full range of protein aggregate detection and quantification, NanoSight and Archimedes together can detect, measure and count protein aggregates between 30 nm and 5 µm. Accounting for these often-overlooked particles allows a full assessment of a protein's aggregated state and therefore a much more complete evaluation of process impact.

Conventional SEC-HPLC is a recognized benchmark for the measurement of soluble aggregates, although calibration against molecular weight standards results in an inherent error in molecular weight measurements. By connecting Malvern Instruments' advanced detectors such as the Viscotek SEC-MALS or multi-detector OMNISEC REVEAL in-line, additional product characteristics can be extracted from the separated protein species, including absolute molecular weight determination. Such information is vital for improving product knowledge and understanding, therefore evaluating process impact.

MANUFACTURING SUPPORT



Following commercial release, biopharmaceutical products often require characterization methods to support manufacturing operations. These methods are used in addition to the validated QC analytical technologies used to release product to market. Activities that usually require additional characterization methods include technology transfer, continuous improvement projects, deviation and root cause analysis and improved product and process understanding for legacy products.

Malvern's product portfolio can be relied upon in manufacturing support activities to provide understanding and evaluation of a broad range of product attributes and characteristics. Such knowledge is used to demonstrate batch-to-batch consistency (biocomparability) of products from different manufacturing facilities or with different bioprocess parameters and provides product understanding to support activities such as deviation impact assessments.

Product attributes that may be assessed include:

- Hydrodynamic size
- Higher order protein structure
- Subvisible particle quantification up to 5 µm
- Particle/contaminant chemical identification
- % content of soluble aggregates
- Molecular weight of separated species
- Viscosity
- Protein : ligand binding affinity
- Thermal stability

Each of these attributes is directly related to product quality and safety. All are important components of any manufacturing support activity

BIOSIMILARS

Biosimilar molecules are engineered to be as similar as possible to an innovator product. They require detailed characterization by a broad range of analytical techniques to demonstrate this similarity. Regulatory guidelines also require that they undergo side-by-side comparison with the originator product.

Higher order structure, thermal stability, in-solution homogeneity, protein size distribution, aggregation state and target binding characteristics are among the quality attributes that must be accurately measured to demonstrate the similarity of the biosimilar and innovator products. Malvern Instruments' portfolio offers a range of solutions to address these challenges.

GLOBAL SUPPORT

We understand that when you purchase a Malvern product, it is only the first stage of a collaborative relationship that lasts for the lifetime of the instrument. We want you to achieve the maximum return from your investment. At Malvern, we are committed to providing the highest quality support, from simply answering occasional questions and supplying software updates through to delivering a comprehensive support package with priority call-out and fixed costs of ownership.

Within our organization we not only have scientists who are leaders in their chosen fields, but also the accumulated knowledge and experience of more than 45 years in materials characterization across a very broad range of industries. Our customers can access this global technical and applications expertise through a variety of convenient channels and in formats structured to ensure the widest possible availability anywhere in the world. Dedicated Helpdesk teams provide telephone and email support ensuring you get a complete answer to any question you may have.

Our specialists deliver training via convenient online e-learning, classroom participation at a Malvern facility or on-site in your workplace. We help with validation documentation, provide expert applications support and offer a range of service contracts appropriate to individual needs.

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