Fig. 1. Malvern Zetasizer systems.

Light scattering techniques support formulation development

Researchers develop methods to test protein stability under a variety of conditions.

Les chercheurs de développer des méthodes pour la stabilité des protéines d'essai sous une variété de conditions.

Forscher Testproteins Stabilität unter einer Vielzahl von Bedingungen.

Researchers from Novozymes Biopharma and Malvern Instruments have been collaborating using light scattering techniques to demonstrate the short- and long-term stability of the novel recombinant human albumins Albucult and Recombumin.

These rAlbumins have been developed and optimised to deliver a safe, stable and regulatory-compliant product for therapeutic formulations.

The teams applied the resolving and detection capabilities of size exclusion chromatography light scattering (SEC-LS) and dynamic light scattering (DLS) offered by Malvern systems to monitor the stability of these Novozymes products.

The work is described in an article 'Tools for evaluating the stability of human recombinant albumins used in human therapeutics'. *See: www.malvern.com/formulation-development*

Novozymes Biopharma develops and manufactures high quality, animal-free and regulatory-compliant recombinant ingredients and technologies to support the development of innovative pharmaceutical products.

Understanding and manipulating protein behaviour is a central element of the associated research and a core company expertise. The work described here focused on the use of light scattering techniques to test protein stability under a variety of conditions.

Malvern's Zetasizer APS, Zetasizer Nano ZS and Viscotek TDAmax SEC-LS (Fig. 1) were used in studies that examined the effects of pH, temperature and concentration on oligomerisation and aggregation behaviours. The results can be used to demonstrate short-term stability and predict shelf life.

Both SEC-LS and DLS were found to be useful for the rigorous testing required to understand and confirm protein stability under various conditions. SEC-LS allows detailed characterisation of the type of oligomers or aggregates present in samples of therapeutic proteins. DLS provides a rapid measurement that allows rapid comparison of a number of sample conditions and to indicate the presence of large aggregates and small oligomers or aggregates.

Malvern offers a growing range of systems for protein characterisation. The Zetasizer Nano is one of the most widely applied systems for measuring particle size and molecular size using dynamic light scattering, and protein mobility (zeta potential) by electrophoretic light scattering, while the Zetasizer APS offers robust, simple to operate, dynamic and static light scattering and automates the measurement of samples in industry standard 96- or 384-well plates.

The Viscotek TDAmax is a complete, research grade, temperature controlled, multi-detector SEC system suitable for the molecular weight and molecular size determination of proteins and other macromolecules.

Novozymes' animal-free, recombinant albumin (rAlbumin) range helps pharmaceutical drug and medical device manufacturers deliver safe and affordable product improvements in a variety of applications.

DLS Microrheology

With the recent launch of the new top of the range Zetasizer Nano ZSP system (Fig. 2), Malvern Instruments has added dynamic light scattering (DLS) Microrheology to its portfolio of materials characterisation techniques.

To introduce this powerful technique, the company has published an in-depth paper that explains the background to microrheology, and how it can be used to investigate the rheological properties of even the most weakly structured fluids, using sample volumes on the microliter scale. An 'Introduction to DLS Microrheology' includes data for protein (bovine serum albumin (BSA)) and polyethylene oxide (PEO) solutions that illustrate the significance and usefulness of the method. *See: www.malvern.com/DLS-Microrheology-paper*.

Microrheology is a relatively new analytical methodology that has been the subject of increasing academic study over the past 15 years, and is of growing interest to those researchers working at the forefront of rheological characterisation. It involves tracking the motion of colloidal tracer particles dispersed in a complex fluid sample, in order to extract the viscoelastic properties of the system.

DLS Microrheology has attributes that are particularly advantageous for characterising low viscosity samples, such as polymer or protein solutions, and extends the measurement range and application of rheology well beyond the regimes accessible with even the most sophisticated mechanical rheometers.

A central advantage is that it can access the very high frequencies, or short timescale measurements, that are needed to characterise these weakly structured fluids.

Furthermore, data can be obtained with very small sample volumes so the technique is well-suited to the analysis of high value protein solutions, for example.

'An Introduction to DLS Microrheology' provides a general overview of microrheology techniques, before going on to focus on DLS Microrheology and the underlying theory. Practical aspects of microrheology are discussed in some detail, with guidance on method development and sample preparation, both of which are particularly important factors for robust measurements. The paper concludes with some experimental data that demonstrate applicability.

Fig. 2. Malvern's new Zetasizer Nano ZSP makes the technique of DLS Microrheology accessible to a more laboratories.

These data show how DLS Microrheology, combined with conventional rotational rheology, can significantly extend the measured viscoelastic spectrum for polymer solutions for characterisation of short timescale dynamics, and demonstrate its value for protein solution characterisation.

The results indicate that the development of solution viscoelasticity can be used to investigate the onset of protein aggregation in denaturing BSA solutions, as well as assessing solution viscosity as a function of concentration to determine onset of non-Newtonian flow properties.

Meanwhile, following the completion of an early access programme, Malvern Instruments has launched the Morphologi G3-ID particle characterisation system to the global market.

The system combines automated particle imaging with the chemical identification of individual particles using Raman spectroscopy. This fully automated system measures particle size, shape and chemical identity in a single platform.

The Morphologi G3-ID (Fig. 3) is suited to solving complex particle characterisation problems where particle size and shape do not provide sufficient information.

The recent early access programme was designed to enable users to explore specific applications and to allow full optimisation of the product features.

Key applications for which it is proving especially valuable are in determining component-specific particle properties of mixtures and blends, such as the particle size of drug ingredients in nasal sprays and asthma inhalers, and in the identification of unknown or suspicious particles in injectable or parenteral products, for example.

The Morphologi G3-ID is designed to meet a wide variety of needs and to enable all users, from particle characterisation scientists with limited spectroscopy experience right through to experienced spectroscopists, to gain an in-depth understanding of particulate samples. Simple SOP operation takes the user from integrated sample dispersion for dry powders through to size, shape and chemical analysis, with automatic selection, targeting and chemical classification of thousands of individual particles. Measurements can be made on dry powders, suspensions and membrane filters.

For more information, visit
www.biopharma.novozymes.com
or www.malvern.com

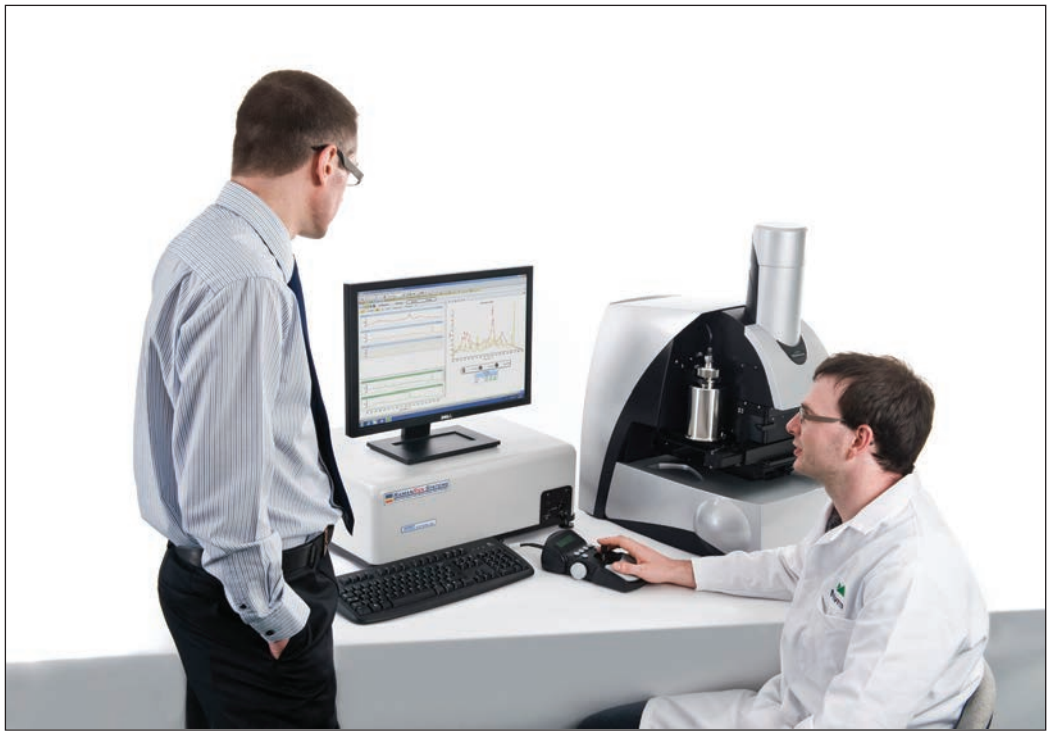

Fig. 3. The Morphologi G3-ID particle characterisation system from Malvern Instruments.

This article first appeared in *EUROLAB, December 2012*. www.scientistlive.com