ProteomeLab™ XL-A/XL-I Protein Characterization System

Native Condition Protein Characterization

A growing demand for protein, antibody, and small molecule therapeutics is challenging the bio-pharmaceutical industry to improve drug discovery processes and accelerate time to market, reduce drug development costs, and ensure optimum dose delivery. To satisfy these demands, high-throughput screening assays are including steps to validate "hits" and reduce the number of "false positives" in lead series. More robust methods for protein characterization are being employed to avoid the expense of adjusting a drug formulation during pre-clinical / clinical trials and minimize the risk of side effects by quantifying lot-to-lot variations in product comparability testing.

Beckman Coulter's **ProteomeLab™ XL-A/XL-I Protein Characterization System** with native condition capability provides the ideal solution to satisfying the demands of the drug discovery process by characterizing a protein's behavior in free solution under physiological concentrations, temperatures and buffer conditions. The XL-A with absorbance optics provides the selectivity and sensitivity to measure protein behavior between 190 – 800 nm. The XL-I adds an additional optical system based on Rayleigh Interference Optics for use in UV absorbing buffers that provides higher concentration capability and greater precision.

An operating system that does not require calibration or use of standards enables measurements based on a redistribution of a sample's molecular mass. Simple yes/no answers provide the flexibility to get quick, top level answers to questions regarding:

- *Heterogeneity / Aggregation*
- *Interacting / Self-Associating systems*
- *Stoichiometry*
- *Molecular Conformation*

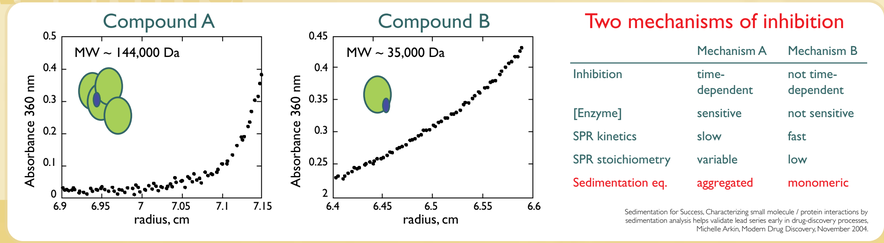
The ProteomeLab XL-A or XL-I provides the ideal solution to questions of protein characterization by uniquely measuring therapeutic targets as interacting elements instead of in isolation or bound to a substrate. In addition, it provides a complementary approach to traditional instrument methods and technologies like size exclusion chromatography, surface plasmon resonance, and mass spectrometry.

SCREENING

Early Lead Validation

Interacting Systems

Understanding the binding of small molecule antagonists helps validate lead series early in the drug-discovery process. In high-throughput drug discovery projects, initial "hits" are often identified by the inhibition of protein function. "False positives" arising from functional screening can include compounds that act through high stoichiometry binding or protein aggregation. By contrast, compounds that have well-defined stoichiometry and binding site are more likely to generate successful drug leads. Sedimentation analysis offers a straightforward approach for characterizing binding stochiometry and affinity early in the drug discovery process and at the same time identifies binding artifacts or anomalies.



Two compound series shown to result in a relative change in the sedimentation profile of the molecular weight complex. The aggregate species is clearly shown to produce an exponential-like profile.

Two mechanisms of inhibition

	Mechanism A	Mechanism B
Inhibition	time-dependent	not time-dependent
[Enzyme]	sensitive	not sensitive
SPR kinetics	slow	fast
SPR stoichiometry	variable	low
Sedimentation eq.	aggregated	monomeric

Sedimentation for Success, Characterizing small molecule / protein interactions by sedimentation analysis helps validate lead series early in drug-discovery processes, Michelle Arkin, Modern Drug Discovery, November 2004.

Sedimentation analysis provides a complementary approach to analyze and confirm stoichiometry and mechanism of assembly.

PRE-CLINICAL/CLINICAL

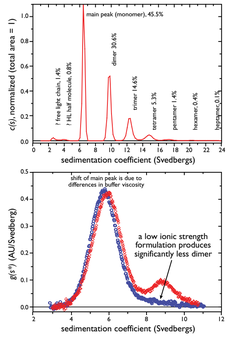
Better Lead Optimization

Aggregation

Characterizing biopharmaceuticals prior to pre-clinical and clinical trials can save millions of dollars in development costs by eliminating expensive reformulations. Aggregation, which affects bioactivity, pharmacokinetics, and immunogenicity is of particular interest in the area of protein therapeutics. High dose protein drugs, such as monoclonal antibodies administered subcutaneously, require formulations at high concentration (>50 mg/mL) which can exacerbate the potential for aggregation. In addition to stability issues, concerns from small amounts of aggregates generating an immune response is causing a growing regulatory interest for including complementary methods of analysis beyond the industry standard of size exclusion chromatography.

Sedimentation analysis is ideally suited for quantifying protein aggregation with measurements made in free solution under physiological conditions and temperatures, typically in the same formulation buffer and concentrations as the stored product.

Bioactivity

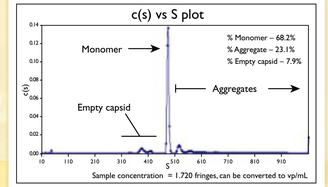


Characterizing the Aggregation and Conformation of Protein Therapeutics. John S. Philo, American Biotechnology Laboratory, October 2003.

Sedimentation coefficient distribution for a highly stressed monoclonal antibody sample showing aggregates at levels as low as 0.1%, by weight.

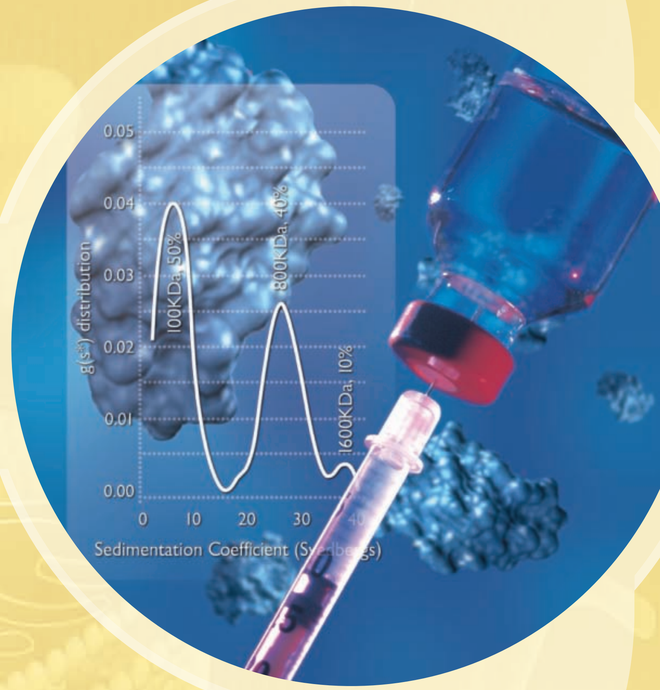
Analysis of two different formulations of an antibody reveals differences in aggregation that could not be seen by size exclusion chromatography.

Immunogenicity



Viral Vectors & Vaccines Conference, Nov. 2004, Steven Berkovitz, Ph.D., Biogen Idec.

Assessing adenovirus homogeneity employed in a gene therapy delivery system allows for determination of the relative concentrations of both monomer and aggregate.

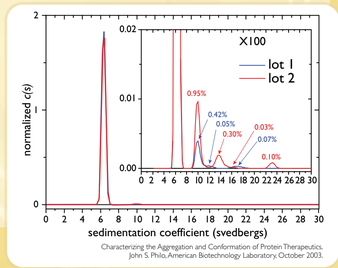


QUALITY CONTROL

Demonstrated Comparability Testing

Conformation

Characterizing the homogeneity of biopharmaceuticals provides an efficient means of demonstrating that samples from different manufacturing lots, or material from different purification processes, all contain the same molecular conformation. Sedimentation analysis uses a unique approach for demonstrating comparability by precisely measuring sedimentation coefficients to reveal detailed information on molecular conformation and aggregate level and types of protein therapeutics. Indeed, one of the benefits of this approach is that sedimentation coefficients are absolutely calibrated (not relying on molecular standards) and can be measured to a precision of +/- 0.2% or better for comparisons within the same run and +/- 0.5% run-to-run.



Characterizing the Aggregation and Conformation of Protein Therapeutics. John S. Philo, American Biotechnology Laboratory, October 2003.

Comparability of conformation and aggregation for two different manufacturing lots of a monoclonal antibody with results from both lots showing good homogeneity (98.6% main peak or better) and the same molecular conformation based on reproducible sedimentation coefficients (6.339 S for lot 1 and 6.335 S for lot 2).

www.beckmancoulter.com/xla

P-10119B B2006-7120-MG-3