Static Light Scattering Analysis of Globular Proteins with Agilent ProSEC 300S Columns

Application Note

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Introduction

In globular proteins the amino acid chain folds to enhance solubility by internalizing hydrophobic regions and placing hydrophilic groups on the surface of the protein. The complex interplay of intramolecular forces between the different groups of atoms determines the overall tertiary structure of the protein molecule. The aqueous solubility of globular proteins allows them to exist in biological fluids as individual molecules or in small clusters, and to accomplish a wide range of critical biological functions. Enzyme catalysis, antibodies for the body's immune system, transport vehicles for other proteins, and DNA repair are just a few examples of globular protein involvement in metabolic processes.

As the name suggests, globular proteins are typically spherical in shape. Common globular proteins include enzymes, bovine serum albumin (BSA), myoglobin, ovalbumin, insulin, α-globulins, β-globulins, γ-globulins, and thyroglobulin. Due to their wide-ranging roles in biological processes, globular proteins have generated great research interest. Structural studies on proteins often involve size exclusion chromatography for purification and to provide information about size, shape and oligomeric state.

Size Exclusion Chromatography (SEC) is an excellent technique for the separation of a protein mixture in solution on the basis of size, allowing analysis of aggregation and oligomerization. Analysis of proteins by SEC is often difficult due to the complex intra- and inter-molecular interactions involved. The ionic groups and propensity of proteins to aggregate further complicate characterization.

Static light scattering detection is an excellent addition to a system for protein analysis as it may be used to generate absolute molecular weight irrespective of any column calibration, and is insensitive to non-size exclusion effects that may occur during analysis. This application note describes the analysis of a series of globular proteins to obtain accurate molecular weights and identify oligomeric and aggregate species using a ProSEC 300S column in conjunction with a Agilent PL-LS 15/90° Light Scattering Detector.

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Methods and Materials

Conditions

Column:	ProSEC 300S, 300 x 7.5 mm (p/n PL1147-6501)
Flow rate:	1.0 mL/min
Temperature:	5 °C
Detection:	UV at 310 nm + PL-LS 15/90°
Injection:	100 mL
Sample:	Proteins
Eluent:	0.1 M KH_2PO_4 containing 0.3 M NaCl, pH 8.0

Results and Discussion

Figures 1-3 illustrate chromatograms for a range of globular proteins. Each of the samples produced multimodal peaks, indicative of polymers and aggregates, demonstrating the complexity of proteins. The results demonstrate the strength of coupling ultra-violet absorption with light scattering.

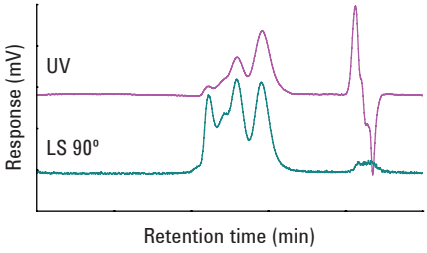

Figure 1. Overlay of UV and light scattering 90° for a sample of γ-globulins, illustrating monomer, dimer, and trimer peaks

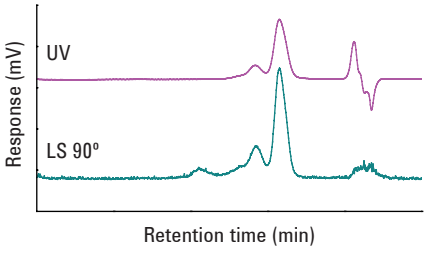

Figure 2. Overlay of UV and light scattering 90° for a sample of BSA, illustrating monomer, dimer, trimer, and aggregate peaks

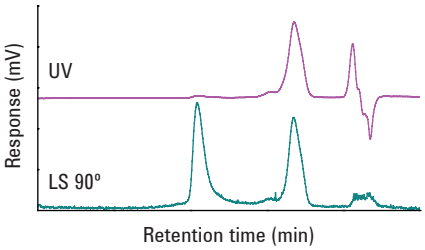

Figure 3. Overlay of UV and light scattering 90° for a sample of ovalbumin, illustrating monomer, dimer, and a small amount of aggregate

Multiple peaks for each of the samples indicate the presence of protein oligomers and/or protein aggregation.

Conclusion

Analysis of globulins using a ProSEC 300S column allowed the separation of proteins on the basis of their size in solution. Measuring the intensity of scattering light in conjugation with ultra-violet absorption identifies low intensity species of high molecular weight such as aggregates that may not be seen in the UV trace. The packing material in the ProSEC columns comprises a specific pore size that allows the analysis of small to larger sized proteins.

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Published in UK, April 8, 2011
5990-7939EN

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