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Application Note SI-02008

Light Scattering Analysis of BSA with ProSEC 300S Columns

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Introduction

Albumins are globular proteins easily soluble in water and 50% saturated ammonium sulphate solutions. They are found in animal tissue, blood plasma, bacteria and plants, and include many important proteins with key functions in the body.

ProSEC 300S columns are designed for protein analysis and are used to investigate a wide range of globular proteins, such as bovine serum albumin, by size exclusion chromatography (SEC). Separating molecules on the basis of their size in solution, SEC is an excellent technique for investigating the formation of protein conjugates in solution. The presence of conjugates can change the efficacy of proteins in clinical research applications and influence the likelihood of proteins forming single crystals suitable for x-ray crystallographic studies, an area of key interest. The ProSEC 300S can be used with a light scattering system to obtain accurate molecular weights for protein molecules.

To demonstrate the compatibility of the ProSEC 300S column with a light scattering detector, a sample of bovine serum albumin was investigated using a chromatography system coupled to a Varian detector module.

Sample: Bovine serum albumin
Column: ProSEC 300S (300 x 7.5 mm)
Eluent: Water + 120 mM NaCl, 2.7 mM KCl, 10 mM NaH₂PO₄
Flow Rate: 1.0 mL/min
Inj Vol: 100 µL
Temp: 25 °C
Detector: Varian differential refractive index + 15/90 dual angle light scattering

Results and Discussion

Figure 1 shows an overlay of the differential refractive index and dual angle light scattering data for the sample, showing monomer, dimer, trimer and aggregation peaks. The results illustrate the power of light scattering coupled to SEC in protein characterization.

*These data represent typical results.
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Using the light scattering data, it is possible to calculate the molecular weights of each of the oligomers in the sample and to determine the relative proportions of the species, as follows.

Monomer: 66900 Daltons, 88.5%
Dimer: 134900 Daltons (2.02 x monomer MW), 9.8%
Trimer: 197000 Daltons (2.94 x monomer MW), 1.2%
Tetramer: 279300 Daltons (5.17 x monomer MW), 0.5%

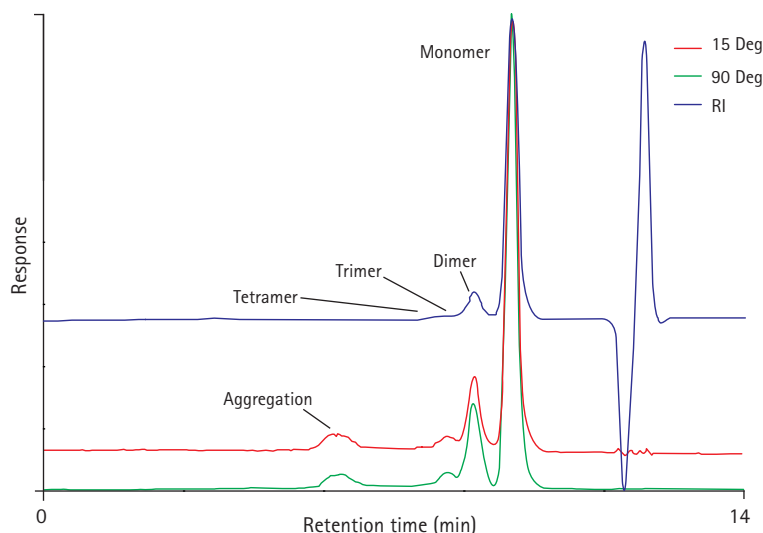


Figure 1. Overlay of differential refractive index and dual angle light scattering sample.

Conclusion

An SEC system combining ProSEC 300S columns with the Varian light scattering detector provides valuable information on the composition of proteins.

ProSEC 300S is a silica-based packing with a surface modified for compatibility with proteins, ensuring that true size exclusion is obtained with minimal unwanted interaction affects. The nominally 300Å pore size allows the analysis of a wide range of small to medium-sized proteins.

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