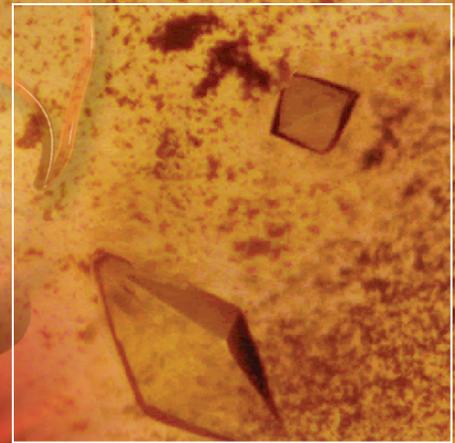


ProSEC 300S

Protein Characterization
columns



Agilent's ProSEC 300S is a silica-based material specifically designed for the analysis of proteins by aqueous size exclusion chromatography. With a proprietary surface modification to remove acidic silanols and reduce interaction effects, the ProSEC packing material shows minimal non-size exclusion effects for a wide range of protein types. The packing material has a nominal 300Å pore size suitable for globular protein analysis and a 5 µm particle size that gives high efficiency for improved resolution, making the new ProSEC 300S an ideal choice for routine protein analyses.

Product features

- **The silica base material** is for use with the aqueous buffers required for protein analysis.
- **Superb reproducibility**, batch to batch, column to column.
- **The high surface stability** ensures that the column is suitable for use within the buffers, pH range 2.0 and 7.5.
- **The modified surface**, which ensures that silanols are removed and protein-column interactions, non-size exclusion effects, are minimized, makes for high sample recovery and reliable protein analysis.
- **A 300Å pore size suited** to the analysis of a wide range of therapeutic and biologically active globular proteins.
- **High mechanical stability** makes for excellent column lifetimes and gives high sample throughput.
- **Two column dimensions** are available to suit multi-detector size exclusion and the analysis of small sample volumes.



Protein Analysis by Size Exclusion Chromatography

The study of proteins is of intense interest due to their role in many biological processes. Isolating, purifying and studying proteins are major goals of many laboratories around the globe.

Proteins are complex molecules that contain ionic as well as hydrophobic and hydrophilic amino acids. Proteins are monodisperse (contain species of a single molecular weight) but are often analyzed as complex mixtures with components that range in size from small to extremely large.

Proteins can be loosely separated into three main groups, globular proteins, fibrous proteins and membrane proteins. Globular proteins or spheroproteins are globe or sphere-like in shape and are generally soluble in water. Nearly all enzymes with major metabolic functions are globular proteins.

Agilent's ProSEC 300S column is designed for the analysis of proteins, particularly globular proteins. The ProSEC 300S column may be used to analyze both natural-state and denatured globular proteins, depending on the conditions of the analysis. Very different elution profiles will be seen before and after denaturing of the protein as the compacted form of the folded tertiary structure is disrupted when the protein unravels. So that its effective size in solution is much larger. It will elute early in a SEC separation.

Calibration with Globular Proteins

For the characterization of proteins of unknown molecular weight the column can be calibrated with proteins.

Flow Rate: 1.0 mL/min
Detection: UV at 280 nm
Injection: 20 μ L @ 2-4 mg/ml.
Sample: Protein samples
Eluent: 50mM KH_2PO_4 - K_2HPO_4 (@ pH 6.8) containing 0.3M NaCl

Materials and Methods

Table 1. Molecular weights of the proteins.

Mw / Daltons	Protein
670,000	Thyroglobulin
155,000	γ -Globulin
66,430	Bovine serum albumin
44,287	Ovalbumin
29,000	Carbonic anhydrase
16,700	Myoglobin
12,384	Cytochrome C
1,423	Bacitracin

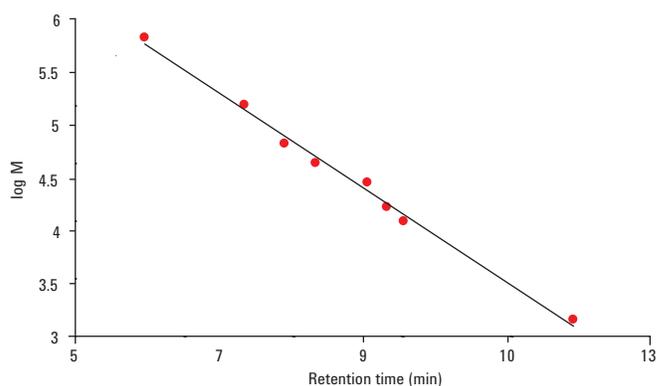


Figure 1. Calibration of the ProSEC 300S column.

Analysis of Globulin Proteins Using ProSEC 300S Columns

The plasma globulins are classified into five fractions by their electrophoretic mobility during serum protein electrophoresis into – α^1 , α^2 , β^1 , β^2 and the γ globulins. Members of the globulin class perform a variety of biological functions, including acting as carrier proteins, performing activities to control inflammation and performing key clotting activities. The γ -globulin protein fraction is composed almost entirely of immunoglobulins.

In this example two proteins, γ -globulin and thyroglobulin, were analyzed by SEC using a ProSEC 300S column. The following conditions were employed for the analysis.

Column: ProSEC 300S (300 x 7.5 mm)
Flow Rate: 1.0 mL/min
Detection: UV at 280 nm
Injection: 20 μ L @ 4 mg/mL conc
Eluent: 0.3M: 50mM KH_2PO_4 - K_2HPO_4 (@ pH 6.8) containing 0.3M NaCl

Figures 2 and 3 show chromatograms of the two proteins.

The γ -globulin material eluted as a multimodal peak in the mid-range of the elution volume of the column, indicating the presence proteins of larger size in the sample, possibly dimers and trimers or aggregates. Thyroglobulin is a large molecule with a high molecular weight that eluted near the exclusion limit of the column with a small subsidiary peak at around 5.5 minutes attributed to the trimer.

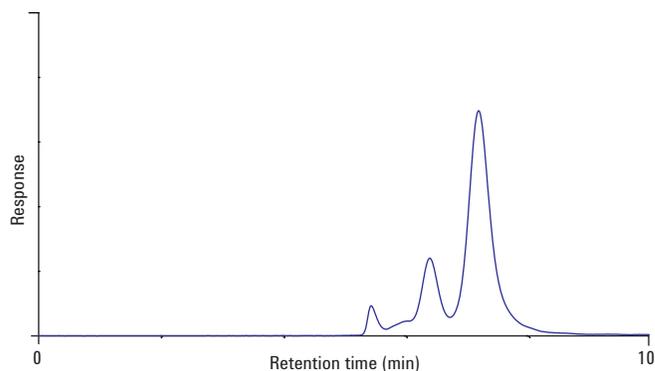


Figure 2. γ -Globulin multimodal peak indicates a complex protein mixture.

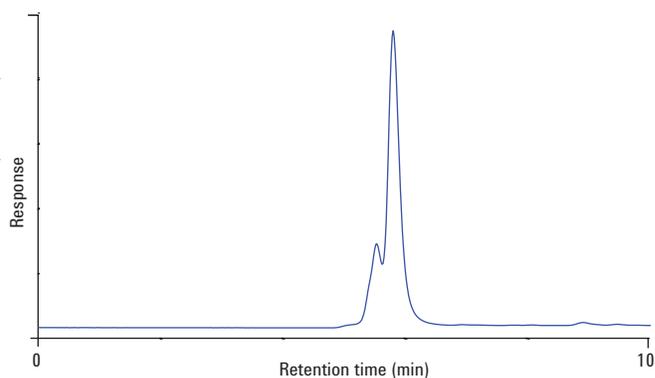


Figure 3. Thyroglobulin of relatively large molecular weight.



Analysis of Albumin Proteins Using ProSEC 300S Columns

Albumins are found in animal tissue, blood plasma, in bacteria and in plants, and include many important proteins with key bio-functions. Two examples of albumins are bovine serum albumin (BSA) and ovalbumin from eggs.

ProSEC 300S columns are designed for protein analysis. Separating molecules on the basis of their size in solution, SEC is an excellent technique for investigating the formation of protein conjugates in solution, such as dimers and trimers. The presence of these aggregates closely affects many of the solution properties of proteins.

In this application, the aggregation of BSA and ovalbumin was analyzed by SEC using a ProSEC 300S column. The following conditions were employed for the analysis.

Column: ProSEC 300S (300 x 7.5 mm)
Flow Rate: 1.0 mL/min
Detection: UV at 280 nm
Injection: 20 μ L @ 4 mg/mL conc.
Eluent: 0.3M: 50mM KH_2PO_4 - K_2HPO_4 (@ pH 6.8) containing 0.3M NaCl

Figures 4 to 5 show chromatograms of the two proteins.

The BSA sample showed the presence of monomer at 7.7 minutes, dimer at 7.0 minutes and a small amount of trimer at 6.6 minutes. With ovalbumin the monomer at 8.2 minutes, dimer at 7.4 minutes and a small amount of trimer at 7.0 minutes were all observed.

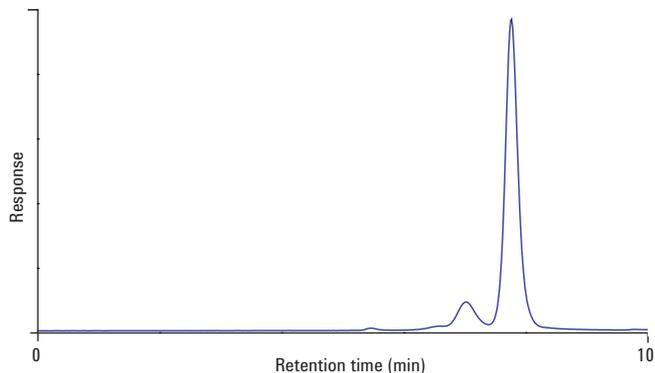


Figure 4. Bovine serum albumin with monomer, dimer, trimer and small amount of aggregation.

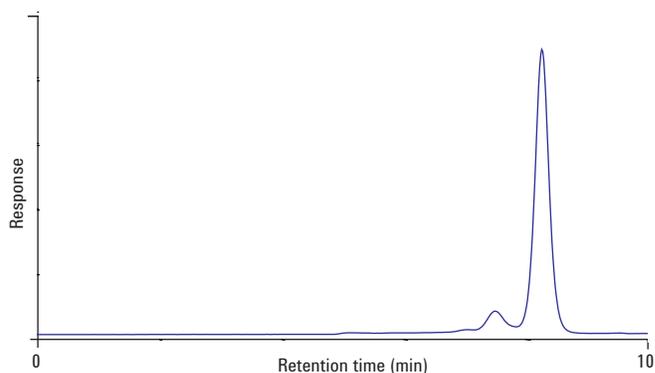


Figure 5. Ovalbumin containing monomer, dimer, trimer and some aggregation.

Analysis of Bovine Serum Albumin by light scattering using ProSEC 300S columns

The ProSEC column can be used in conjunction with a light scattering system to obtain accurate molecular weights for protein molecules separated by size exclusion and to investigate the formation of dimers, trimers and aggregates of protein molecules in solution. The presence of these species can affect the efficacy of proteins in medicinal applications.

To demonstrate the compatibility of the ProSEC 300S column with a light scattering detector, a sample of bovine serum albumin was analyzed.

Column: ProSEC 300S (300 x 7.5 mm)
 Flow Rate: 1.0 mL/min
 Detection: Differential refractive index + 15/90 dual angle light scattering
 Injection: 100 μ L
 Sample: Bovine serum albumin
 Eluent: Water + 120 mM NaCl, 2.7 mM KCl, 10 mM NaH_2PO_4

Figure 6 shows an overlay of the differential refractive index and dual angle light scattering data for the sample, showing monomer, dimer, trimer and aggregation peaks.

Using the light scattering data, it is possible to calculate the molecular weights of each of the species in the sample and to determine the relative proportions of the species.

Monomer: 66,900 Daltons, 88.5%
 Dimer: 34,900 Daltons
 (2.02 x monomer molecular weight), 9.8%
 Trimer: 197,000 Daltons
 (2.94 x monomer molecular weight), 1.2%
 Tetramer: 279,300 Daltons
 (5.17 x monomer molecular weight), 0.5%

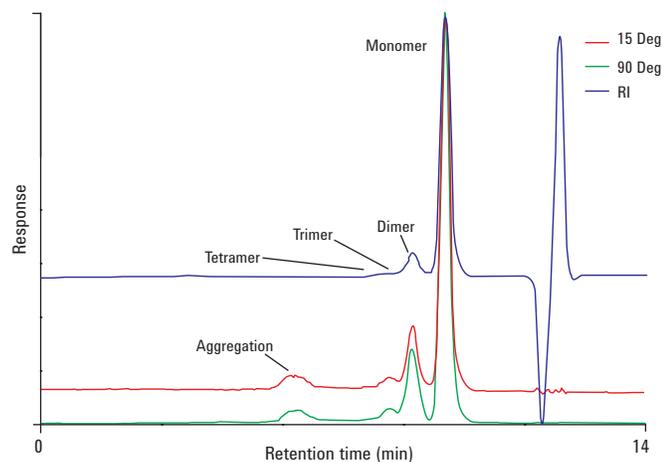


Figure 6. Overlay of differential refractive index and dual angle light scattering sample.

Ordering Information

Parts	Part No.
ProSEC 300S, 300 x 7.5 mm	PL1147-6501
ProSEC 300S, 250 x 4.6 mm	PL1547-5501

Guard Column	Part No.
ProSEC Guard, 50 x 7.5 mm	PL1147-1501
ProSEC Guard, 50 x 4.6 mm	PL1547-1501

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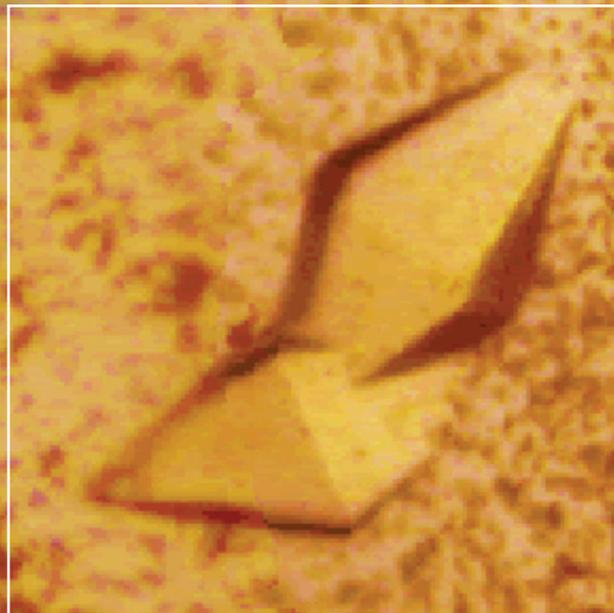
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