Analysis of Globulins using Agilent ProSEC 300S Columns

Application Note

Introduction

Globulin is a general term used to describe proteins that are insoluble in water or highly concentrated salt solution but may be dissolved in lower concentrations of salt solution. This is a wide-ranging class of proteins, for example all of the proteins found in human plasma are globulins except for the albumin proteins. Globulins are normally classed by their electrophoretic mobility during serum protein electrophoresis into five fractions – α1, α2, β1, β2 and the γ globulins. Members of the globulin class perform a variety of biological functions, including membrane proteins that act as carriers, proteins involved in the control of inflammatory processes and proteins that perform clotting functions.

The γ-globulin protein fraction is composed almost entirely of immunoglobulins. Thyroglobulin is a protein found in the thyroid gland and in the blood, and β-lactoglobulin is present in milk and bovine whey protein. These three materials have very different molecular sizes and weights and may be separated into fractions by size exclusion chromatography.

ProSEC 300S columns are designed for protein analysis and may be used to investigate a wide range of globular proteins including the albumins by size exclusion chromatography (SEC). By separating molecules on the basis of their size in solution, SEC is an excellent technique for separating proteins.

In this note γ-globulin, thyroglobulin and β-lactoglobulin were analyzed in buffer solution by SEC using a ProSEC 300S column.
Methods and Materials

Conditions

Column: ProSEC 300S, 300 x 7.5 mm (p/n PL1147-6501)
Eluent: 0.3M: 50mM KH2PO4 - K2HPO4 (@ pH 6.8) containing 0.3M NaCl
Flow Rate: 1.0 mL/min
Inj Vol: 20 µL
Sample Conc: 4 mg/mL
Temperature: 25 °C
Detection: UV at 280 nm

Results and Discussion

Figures 1 to 3 show chromatograms of the three proteins.

The γ-globulin material eluted as a multimodal peak in the mid-range of the elution volume of the column, indicating the presence of a complex mixture of proteins. The β-lactoglobulin protein is small in size and eluted as a single peak at around 5.5 minutes. Thyroglobulin is a large molecule with a high molecular weight that eluted near the exclusion limit of the column; the small peak at around 5.5 minutes was a partial exclusion peak.

Conclusion

A single ProSEC 300S column analyzed a selection of globulin proteins on the basis of their size in solution and molecular weight, showing differences between the nature of the materials. The ProSEC 300S column contains a packing with a surface modified for compatibility with proteins, ensuring that true size exclusion is obtained with minimal unwanted interaction affects. The pore size of the packing has been specifically selected to allow the analysis of a wide range of small to medium-sized protein.