



# Analysis of Albumin Proteins using Agilent ProSEC 300S Columns

## Application Note

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### Introduction

There are many types of globular protein loosely defined by their physical characteristics such as solubility. Albumins are proteins that are easily soluble in water and in 50% saturated ammonium sulphate solutions. Albumins are found in animal tissue, blood plasma, in bacteria and in plants, and include many important proteins with key functions in the body. Three examples of albumins are bovine serum albumin (BSA) obtained from bovine plasma, lactalbumin from milk and whey and ovalbumin obtained from eggs.

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). 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 and is of interest in biomedical applications.

In this note, the aggregation of BSA, lactalbumin and ovalbumin in buffer solution was analyzed by SEC using a ProSEC 300S column.

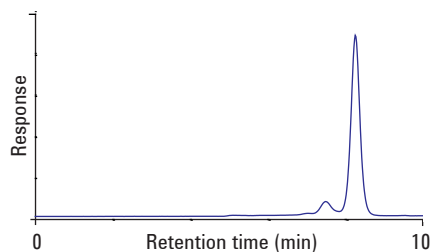


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## Methods and Materials

### Conditions

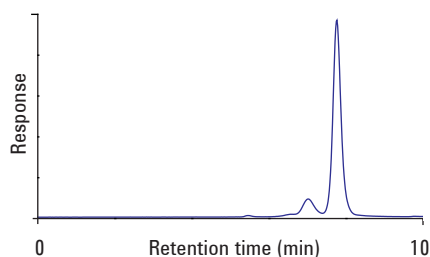
Sample: Proteins  
Column: ProSEC 300S,  
300 x 7.5 mm  
(p/n PL1147-6501)  
Eluent: 0.3M: 50mM  $\text{KH}_2\text{PO}_4$ -  
 $\text{K}_2\text{HPO}_4$ , pH 6.8,  
containing 0.3M NaCl  
Flow Rate: 1.0 mL/min  
Inj Vol: 20  $\mu\text{L}$   
Sample conc: 4 mg/mL  
Temp: 25  $^{\circ}\text{C}$   
Detection: UV at 280 nm



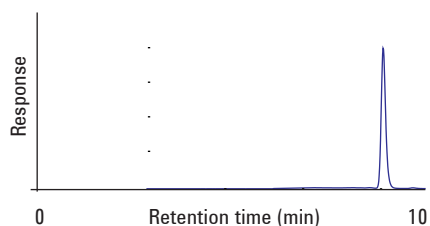
**Figure 3. Ovalbumin containing monomer, dimer and trimer**

## Results and Discussion

Figures 1 to 3 show chromatograms of the three proteins. The BSA sample showed the presence of monomer at 7.7 minutes, dimer at 7 minutes and a small amount of trimer at 6.6 minutes. For lactalbumin, only the monomer was observed eluting at around 9 minutes. With ovalbumin, the presence of monomer at 8.2 minutes, dimer at 7.4 minutes and a small amount of trimer at 7 minutes, was revealed.



**Figure 1. Bovine serum albumin with monomer, dimer and small amount of trimer**



**Figure 2. Lactalbumin with monomer only**

## Conclusion

A single ProSEC 300S column is able to resolve monomer, dimer and trimer peaks for a series of globular albumin proteins and gives an indication of the aggregation of these species, an important solution property that affects the biomedical application of proteins. 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 proteins.

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