

Separation of BSA Protein Aggregates

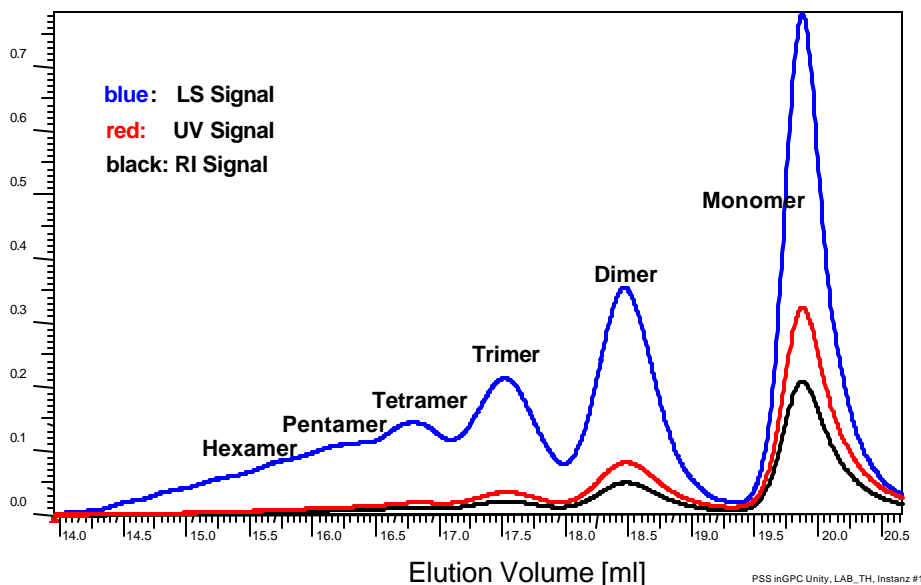


Fig 1. The separation of the BSA protein with two **PROTEEMA** columns leads up to the hexamer using a **SLD 7000 MALLS** Detector; Refractive index and UV detection signals are clearly less sensitive. Increasing the number of columns or reducing the flow rate could improve the separation power of higher aggregates. Data acquisition and processing with **WinGPC Unity** Macromolecular Chromatography Data System

Abstract:

A new generation of SEC/GPC columns is introduced to the field of Protein analysis. The **PROTEEMA** columns are designed to fulfill the major requirements for the separation of Proteins with different molar masses. A selective pore size distribution and a very narrow and small particle size leads to a very high resolution for the separation of proteins by size.

Introduction

The size exclusion chromatography is a well established method for the molecular weight analysis of macromolecules. First developed in the early 60's for the characterization of synthetic polymers, the GPC started a triumphal procession to become an overall accepted method for the molecular characterization of polymers.

The progress in the column development during the last 30 years increased the field of applications constantly. Beginning with the molar mass range from 100D up to 10^6 D for synthetic polymers also biopolymers such like polysaccharides and peptides or proteins. The latest development is a tailor made column material for the specific requirements of protein and peptide analysis. The column based on a modified silica material.

Proteins and peptides are strictly monodisperse and in general the molar mass of these samples are well

known. Proteins are used in many industrial fields e.g. feed & food, or the pharmaceutical industry. The quality control of their purity is one of the major challenges. One of the main questions for the natural or synthetic proteins is the characterization of the pure monomer as well as the aggregate content (dimer, trimer, etc). This characterization can be accomplished by SEC, in combination with a light scattering detector. Biotechnological produced proteins can be polydisperse and the molar mass of the protein and the aggregation products are of interest for the scientists.

The classical HPLC, where the separation of proteins is based on an enthalpic interaction chromatography with a solvent gradient renders the separation of high molar mass proteins very difficult because the enthalpic interaction parameter is not linear for polymers.

The Size Exclusion Chromatography (SEC) separation method is more attractive because it is isocratic, i.e., based on an entropic chromatography free of any interactions between sample and stationary phases. SEC requires less complexity in the method development and easier to achieve method robustness.



Experimental:

The measurements were done on a Agilent 1100 system in combination with a PSS SLD7000, a Multi Angle Laser Light Scattering Instrument (MALLS). The on-line light scattering instrument allows the determination of the absolute molar mass and some structure information about the studied molecules. In combination with the SEC this is a very powerful method for the sophisticated analysis of protein in aqueous solution.

Experimental conditions:

SEC Instrument: Agilent1100 Series;
on-line light scattering: MALLS: SLD7000 (7 angle)
Columns: PSS PROTEEMA, 5µm, 100Å, 300Å, 8x300mm
Solvent: phosphate buffer pH 6,6 +0,3m NaCl
Flow rate: 1ml/min, 0,5ml/min
Injection volume: 20µl
Concentration: 1g/l
Temperature: 25°C

Results

Proteins of different molar mass may also differ in size more precisely in their hydrodynamic volume. If this is so the SEC is able to separate these macromolecules by the run through the SEC column. Larger molecules elute earlier than smaller molecules. Larger molecules don't fit into all the pores of the stationary phase but smaller molecules do.

After the separation the result is detected with a concentration detector (refractive index or a UV

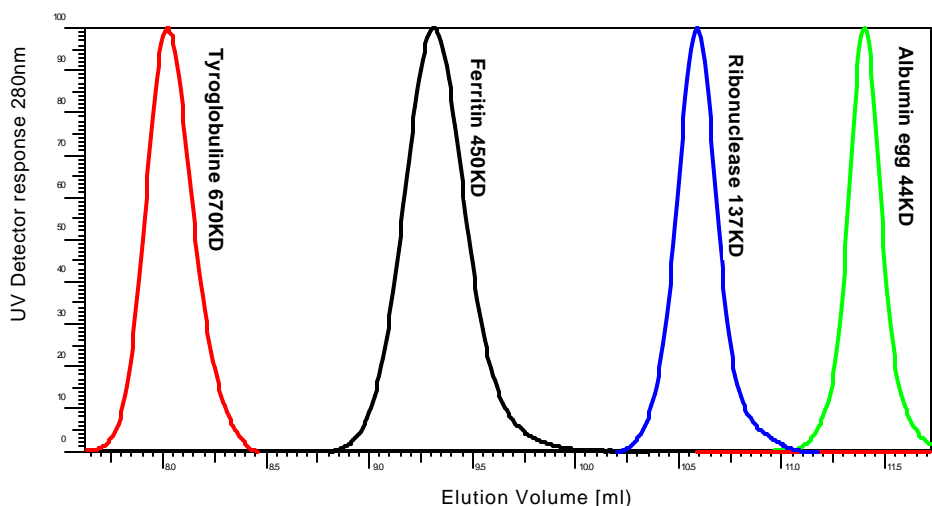
detector). In addition, the separated samples will also be detected by a MALLS instrument:

Figure 1 shows the separation of a BSA protein and the detection difference between the conventional RI and UV concentration detectors compared to a molar mass sensitive light scattering detector. The column combination of two PSS PROTEEMA 300Å columns enables the baseline separation from the monomer and the dimer and a 2/3 baseline separation of the dimer and the trimer. The potential separation power of the column combination is illustrated by the light scattering detector. The column combination separates the different molar masses up to the hexamer (six times the molar mass of the monomer) without getting to the separation limit of the column. Increasing the number of columns or reducing the flow rate could improve the separation power for the PROTEEMA columns easily.

Fig.2 indicates the separation power of the PROTEEMA column for the separation of proteins with different molar masses. Four different Proteins from 44KD up to 670KD can be detected base line separated without getting into the separation limit of the column. So it takes about 12min to get such a protein mixtures base line separated.

Conclusion

The remarkable features of the PROTEEMA column in terms of resolution and pore volume are illustrated in Fig1 and Fig2. The plate counts for these columns are in the range of 80.000m⁻¹. The resolution power is almost unbeatable and together with a light scattering instrument the detection power is excellent.



Separation of four different proteins

Fig 2. Albumin egg green 44KD; Ribonuclease blue 137KD; Ferritin black 450KD, Tyroglobuline red 670KD; PSS PROTEEMA 300Å, 5µm, 8x300mm, T: 25°C



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