PSS PROTEEMA — High Resolution Columns for Size Exclusion Chromatography of Proteins

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PSS introduces a new generation of SEC/GPC columns for protein analysis. The PROTEEMA columns fulfill the major separation requirements of proteins with different molar masses. The selective pore size distribution and the narrow and small particle size lead to a high-resolution material for the separation of proteins by size. The PROTEEMA column has remarkable features in terms of resolution of different proteins, as well as aggregates of the same protein. Proteins separate easily for characterization using concentration detectors coupled with on-line light scattering detectors. Molar masses up to 670KD are well below the separation limit of the column.

Introduction

Size exclusion chromatography is a well-established method for the molecular weight analysis of macromolecules. First developed in the early 60s for the characterization of synthetic polymers, the SEC became an overall accepted method for the molecular characterization of polymers.

Proteins of different molar masses may also differ in size or more precisely in their hydrodynamic volume. Therefore, the SEC is able to separate these macromolecules running through adequate SEC columns. Larger molecules elute earlier than smaller molecules because they do not enter all the pores of the stationary phase while smaller molecules do. Natural proteins and peptides are strictly monodisperse and, in general, the molar masses of these samples are well known. In this instance, the SEC is very useful for quality control (associates, impurities etc.) SEC in combination with a light-scattering detector is especially valuable for the molar mass determination of unknown proteins, maybe poly-disperse, that result from biotechnology processes.

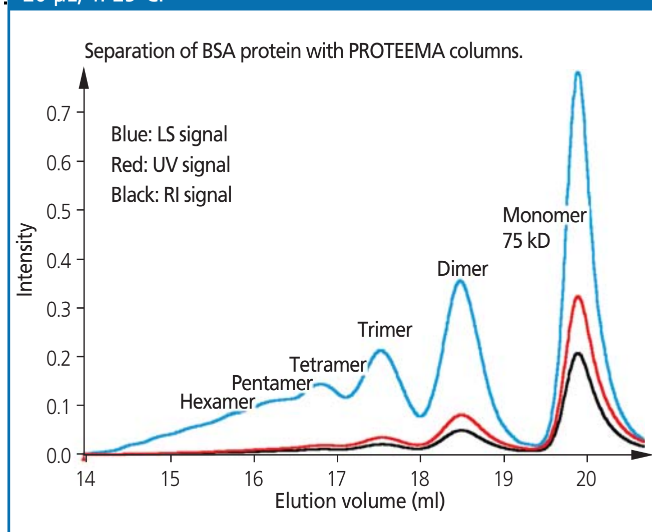
The SEC requires an isocratic mode free of any interactions between sample and stationary phases. This contrasts to the classical HPLC, where the separation requires interaction and mostly happens under a solvent gradient. The separation of high molar mass samples by SEC can be much easier than using HPLC. The SEC requires less complexity in the method development and method robustness is easier to achieve.

The progress in the column development during the last 30 years has expanded the field of application (e.g., feed and food or the pharmaceutical industry), where polydisperse proteins are extensively used. Molar masses range from 100 D up to 5 $\times$ 10^6 D for synthetic polymers and biopolymers like polysaccharides, peptides or proteins.

The quality control of the purity for proteins is a major analytical challenge. One main question is usually, the content of dimer and/or trimer aggregates that occur with the pure monomer of the proteins; another important issue is the determination of protein associates.

This article features performance examples presenting the latest column material developments to fulfill the specific requirements of protein and peptide analysis. The PSS PROTEEMA column is a tailor made (modified silica) material. The selective pore size distribution and the narrow and small particle size leads to a very high resolution for the separation of proteins by size. PROTEEMA is available in 8 $\times$ 300 mm columns filled with 5 micron (μm) particles of a choice of three porosities: 100, 300 and 1000 Angstroms (Å). PROTEEMA columns plate-count are in the range of 80000m^{-1}.

Figure 1: Albumine bovine (BSA) illustrates the separation effectiveness of two connected PROTEEMA columns. Detection with PSS SLD7000 MALLS elucidates aggregates up to the hexamer of the BSA protein. Conditions: Column: PSS PROTEEMA 300Å, 5 μm, 8 $\times$ 300 mm (two columns); Flow-rate: 0.5 mL/min, Concentration: 1 g/L, Injection volume: 20 μL; T: 25°C.



Below, there are two protein separation examples using PSS PROTEEMA columns. They show separations of molecules and their aggregates, as well as separations of proteins of different identity. PROTEEMA columns high resolution will aid your analytical sizing techniques effectively, easily and free from interaction.

Experimental

The measurements were performed using a combination of two PSS PROTEEMA columns. The separated components were detected with the refractive index or a UV detector (concentration detector) of an isocratic Agilent 1100 system, in connection with a PSS SLD7000 multi angle laser light-scattering (MALLS) detector. 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 a sophisticated analysis of protein in aqueous solution.

Experimental conditions

SEC Instrument: Agilent1100 series
On-line light scattering MALLS: SLD7000 (7 angle)
Columns: PSS PROTEEMA, 5 µm, 100 Å, 300 Å, 8 × 300 mm
Solvent: Phosphate buffer pH 6.6 + 0.3 m NaCl
Flow-rate: 1 mL/min, 0.5 mL/min
Injection volume: 20 µL
Concentration: 1 g/L
Temperature: 25 °C

Results

The separation of a BSA protein and its aggregates illustrates the separation effectiveness of a combination of PROTEEMA columns. Figure 1 shows the detection signals of conventional RI and UV concentration detectors together with the signal of the SLD7000 (molar mass sensitive) light-scattering detector. The column combination of two PSS PROTEEMA 300 Å columns enables the baseline separation from the monomer and the dimer and a 2/3 baseline separation of the dimer and the trimer.

The light-scattering detector data better illustrates the separation power of the column combination, which separates the different molar masses up to the hexamer (six times the molar mass of the monomer) without reaching the exclusion limit of the column. Increasing the number of columns or reducing the flow-rate will improve the separation power for the PROTEEMA columns easily.

Figure 2 illustrates the effectiveness of the PROTEEMA column for the separation of different proteins with different molar masses: albumin egg 44 KD; ribonuclease 137 KD; ferritin 450 KD and tyroglobuline 670 KD. It takes about 12 min to get such a protein mixture base line separated. Molar masses from 44 KD up to 670 KD can be detected base line separated without getting into the separation limit of the column.

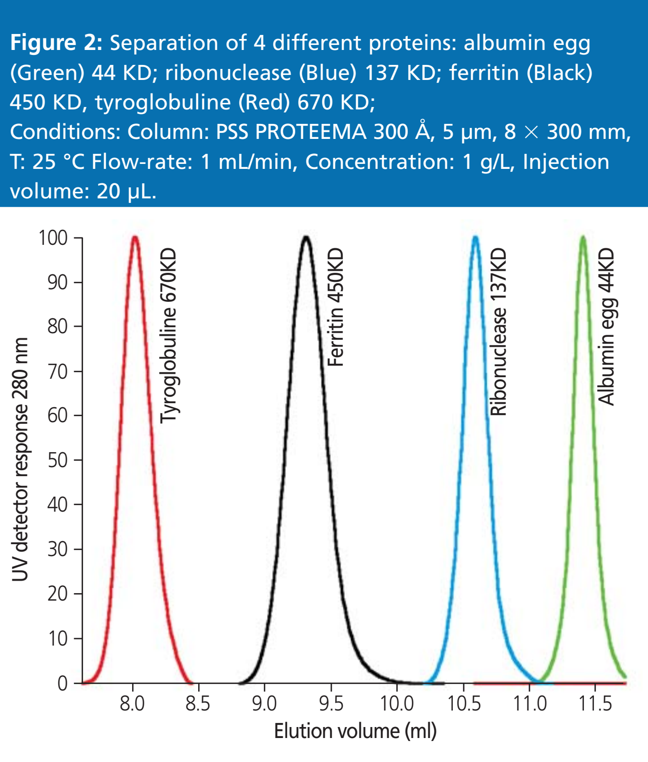


Figure 2: Separation of 4 different proteins: albumin egg (Green) 44 KD; ribonuclease (Blue) 137 KD; ferritin (Black) 450 KD, tyroglobuline (Red) 670 KD;
Conditions: Column: PSS PROTEEMA 300 Å, 5 µm, 8 × 300 mm, T: 25 °C Flow-rate: 1 mL/min, Concentration: 1 g/L, Injection volume: 20 µL.

Conclusion

The PROTEEMA column has remarkable features in terms of resolution of proteins by molecular size regardless of their identity: different protein molecules or aggregates of the same protein.

PROTEEMA columns are optimized for GPC and GPC light-scattering coupling of proteins.

PROTEEMA have a very large molar mass range without any loss of separation power.

PROTEEMA columns are robust, easy to handle and very pressure stable.

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