Application Note 005 e

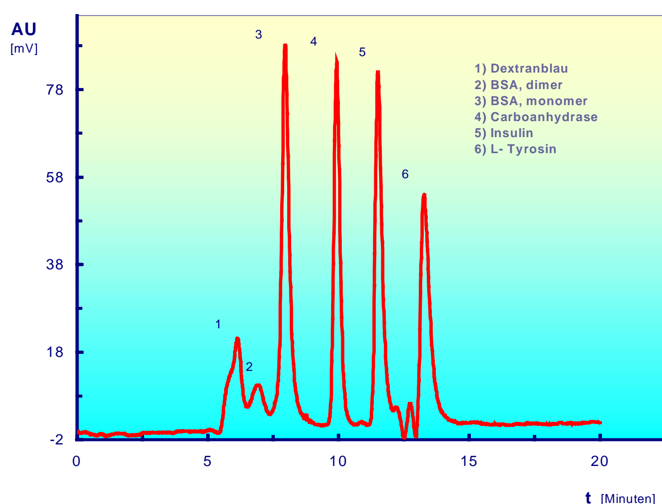
Analytik + HPLC GmbH

Determination of the Molecular Weight of Proteins via Capillary-HPLC

- Steric Exclusion Chromatography (SEC) -

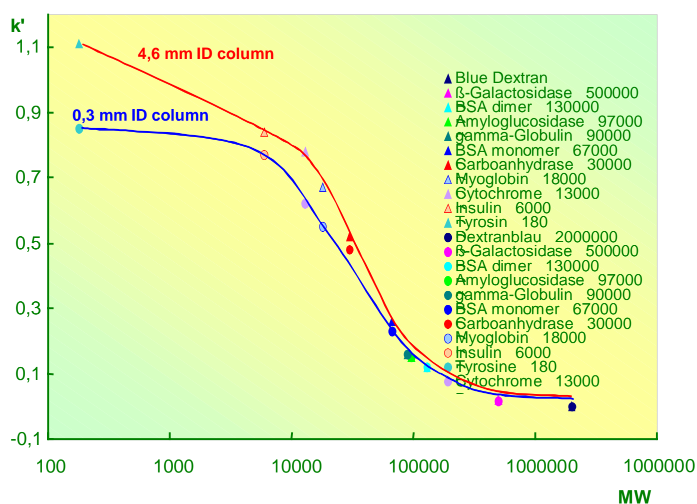
Electrophoresis in polyacrylamide gels (PAGE) and liquid chromatography are two complementary methods for protein separation and characterization in biochemical research in general and specifically in analysis of the proteome of various organisms.
Disadvantages of electrophoretic techniques include labor intensive setup, long run times, failure to detect peptides and small proteins (≤ 10 000 D), variable reproducibility and often the loss of enzyme activity due to the use of denaturing reagents. All of these disadvantages are readily compensated for by the advantages of HPLC: simple setup, rapid separations, quantitative and reproducible analysis of peptides and proteins at much higher sensitivities without denaturation.

Fig. 1 ***SEC of a protein mixture***



Stationary Phase: TOSOH Super SW 2000; **Column Dimensions**: 300 mm x 0.5 mm; **Eluent**: 0.1 M Na-Phosphat, pH 6.8, 0.1 M NaCl; **Flow Rate**: 0.35 mm/s; **Temperature**: RT; **Detection**: 206 nm (UV); **Flow Cell**: 4 nL / 0.2 mm; **Injection**: 300 nl of a protein mixture (0.1 – 0.4 mg/mL)

Fig. 2 ***MW-determination of proteins via SEC***



Stationary Phase: TOSOH Super SW 2000, 4 µm **Column dimensions:** 300 mm x 4,6 mm or 0.3 mm; **Eluent:** 0.1 M Na-Phosphat, pH 6.8, 0.1M NaCl; **Flow rate:** 0.35 mm/s; **Temperature:** RT; **Detector:** 206 nm (UV); **Flow cell:** 15 µl / 10 mm or 4 nl / 0.2 mm; **Injection:** 10 µl or 300 nl protein solution (~ 0.1-0.5 mg/mL)

Roughly 2 ng protein per spot must be applied for the detection of proteins via silver staining in a 2-dimensional gel. In contrast, only about 1.5 ng total protein (in a volume of 100-300 nl) are sufficient for the detection of protein peaks on a 300 x 0.3 mm microbore column. Such columns have a lower detection limit of less than 0.02 ng protein per peak (at a signal to noise ratio of ≥ 3).

A capillary SEC column can be readily and rapidly calibrated via the injection of one or more mixtures of different protein standards (Fig. 1, 2). Thereafter, it is a simple matter to inject the “Proteins of Interest“ and to determine their relative molecular weights by comparison to the standards. (ref.: C. Klein, P. Földi, LaborPraxis, 02, 34-38 (2002).

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