



DETERMINATION OF PROTEINS CONCENTRATIONS AND THEIR MOLECULAR WEIGHTS BY CAPILLARY GEL ELECTROPHORESIS

INTRODUCTION

The method enables fast separation of proteins according to their molecular weights (Mw) with subsequent protein quantification in protein-containing samples.

MEASUREMENT METHOD

Capillary electrophoresis for the separation and determination of proteins is based on the differential migration of SDS-protein complexes in a narrow fused-silica capillary filled with a low viscous gel, under the influence of the applied electric field. Detection of proteins is performed based on their own absorbance at 220 nm. Due to the presence of a low viscous gel SDS-protein complexes are separated only according to their Mw and thus it enables also to determine Mw of an unknown protein(s).

ADVANTAGES OF THE CAPILLARY ELECTROPHORESIS METHOD

Compared with protein separation with SDS-PAGE, capillary electrophoresis has several important advantages:

- Full automatization
- Direct protein quantification
- Absence of coloring
- Low analysis cost
- Short analysis time

EQUIPMENT AND REAGENTS

The "CAPEL[®]-105/105M" capillary electrophoresis system is used in measurements.

Data acquisition, collection, processing and output are performed using a personal computer running under "WINDOWS[®] 2000/XP" operating system with installed dedicated software package for acquisition and processing of chromatography data.

All reagents must be of analytical grade or higher.

Sample preparation, capillary conditioning and analysis are done according to the detailed protocol, included in the kit.

EXAMPLE OF A REAL ANALYSIS

Buffer: for SDS-protein complex analysis

Capillary: $L_{\text{eff}}/L_{\text{tot}}$ 31/40 cm; ID 75 μm

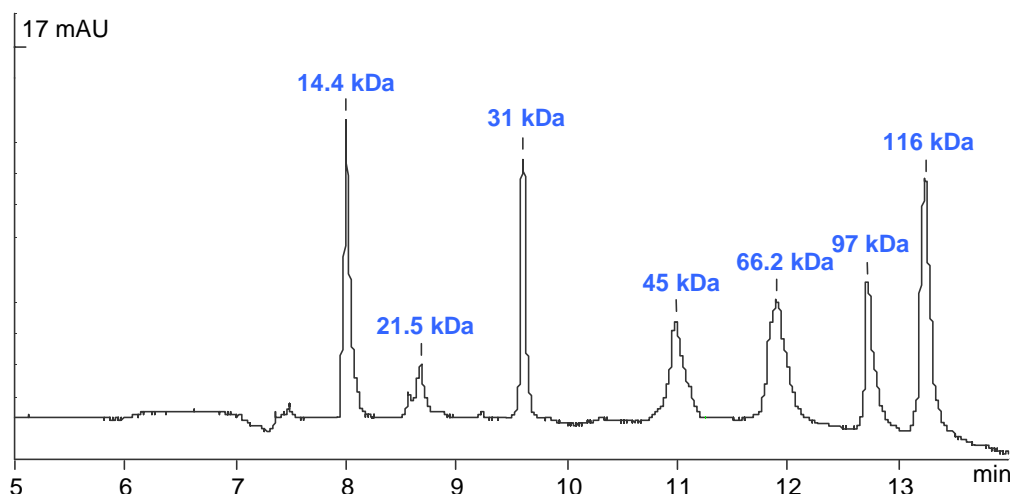
Injection: 15 kV for 15 sec

Voltage: - 25 kV

Temperature: +25 °C

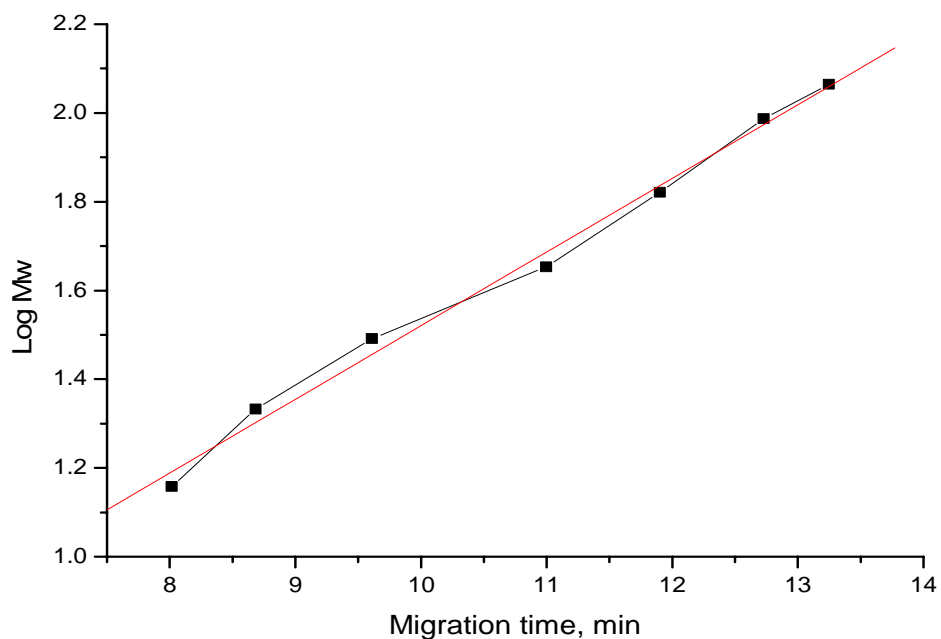
Detection: 220 nm

Sample: standard proteins with different Mw (from 14.4 kDa to 116 kDa)





DEPENDENCE OF $\text{LOG } M_w$ OF PROTEINS FROM THEIR *MIGRATION TIME*



This dependence enables direct determination of molecular weight of the unknown protein(s) based on its migration time.

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