



DETERMINATION OF **PROTEIN PURITY** AND **HETEROGENEITY** BY CAPILLARY GEL ELECTROPHORESIS AND CAPILLARY ISOELECTRIC FOCUSING

INTRODUCTION

Antibodies, protein-based therapeutics, and other recombinant proteins are final products in biotechnological and pharmaceutical industry. Determination of their purity, stability, and heterogeneity is of utmost importance since post-translational modifications as well as degradation processes can change drastically the biological activity of these proteins.

MEASUREMENT METHOD

To determine the purity and heterogeneity of protein samples, two capillary-based methods are proposed – **capillary gel electrophoresis (CGE)** and **capillary isoelectric focusing (cIEF)**. The former is applied when proteins differing in molecular mass must be resolved. The latter is applied when proteins or protein isoforms exhibit almost identical molecular masses, but differ in net charges.

In **CGE**, capillary is filled with a buffered polymer solution. Prior to analysis, protein sample is denatured in the presence of SDS by heating either under reducing (with reducing agent added) or non-reducing conditions. After rapid cooling the sample is injected, high voltage is applied, and proteins start migrating through polymer solution. Similar to SDS-gel electrophoresis in a slab, proteins are separated in a capillary according to their molecular masses due to a sieving effect of the capillary polymer solution. Proteins differing by as little as 4% in molecular mass can be resolved.

In **cIEF**, the entire capillary is filled with a polymer-based ampholyte-sample mixture. At the first stage, named focusing, high voltage is applied, which results in a pH gradient formation and focusing of proteins in narrow zones according to their pI values (where their net charges are equal to zero). At the end of this stage there is almost no any movement in the capillary and current is about zero. During the second stage, named mobilization, the outlet vial is replaced with mobilization solution, high voltage is applied again, and focused zones start moving toward detector point. Depending on selected ampholytes, proteins and protein isoforms differing by as little as 0.04 pI unit can be resolved and quantified.

For both methods a linear regression exists, reflecting the dependence of log Mw of the proteins (for CGE) or their pI values (for cIEF) upon migration time. This is used to determine both parameters of the unknown proteins by adding the corresponding markers to the sample solution.

EQUIPMENT AND REAGENTS

The CAPEL[®]-105M capillary electrophoresis system with a special capillary cassette for the protein analysis is used in all measurements.

System controlling, data acquisition, data processing and output are performed using a personal computer running under "WINDOWS[®] XP" operating system with installed ELFORUN[™] software package.

Appropriate kits of reagents for either CGE or cIEF are available and supplied according to the specified task.

EXAMPLES OF REAL ANALYSES

Method: capillary gel electrophoresis
Sample: recombinant antibody IgG, analysed under reduced (shown in **red**) and non-reduced conditions (shown in **black**)
IS – internal standard,
LC – light chain (25kDa),
HC – heavy chain (55 kDa),
IgG – whole antibody (146 kDa).
Other peaks are antibody degradation products

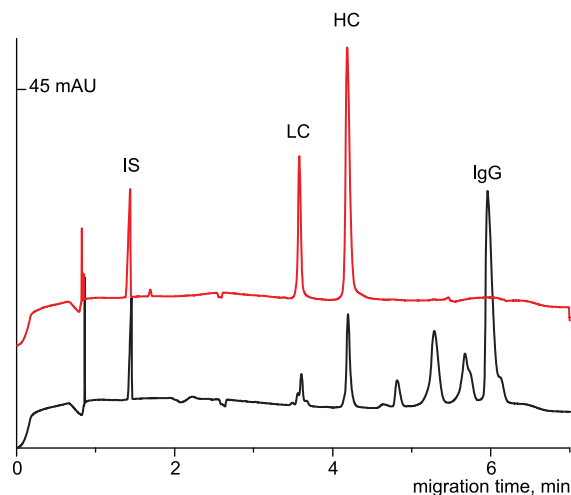
Buffer : special for proteins

Capillary: L_{eff}/L_{tot} 32 / 42 cm;
ID 75 µm

Voltage: - 20 kV

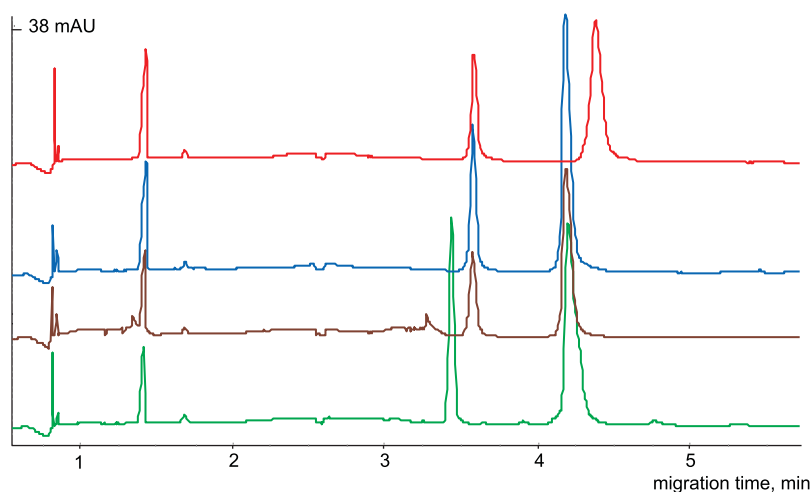
Temperature: 25 °C

Detection: 220 nm

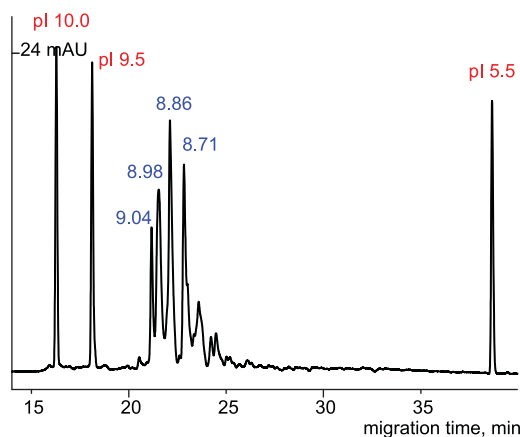




Method: *capillary gel electrophoresis*
Samples: four different IgG preparations, analysed under reduced conditions
Buffer: special for proteins
Capillary: $L_{\text{eff}}/L_{\text{tot}}$ 32 / 42 cm; ID 75 μm
Voltage: - 20 kV
Temperature: 25 °C
Wavelength: 220 nm
Results: peaks stemming from LC and HC are seen. Small differences in Mw can be detected: 2 kDa only in case of LC (**bottom** trace) and 5 kDa in case of HC (**upper** trace)



Method: *capillary isoelectric focusing*
Sample: recombinant IgG preparation
Buffer: special for cIEF
Capillary: coated $L_{\text{eff}}/L_{\text{tot}}$ 32 / 42 cm; ID 50 μm
Voltage: + 25 kV
Detection: 280 nm
Results: four protein isoforms with slightly different pI values were resolved. pI markers, added to the sample (shown in **red**), help to calculate pI values of protein isoforms (shown in **blue**)



Inter-day reproducibility

Method: capillary isoelectric focusing
Sample: protein-based therapeutic
Results: Several isoforms, indicating charge heterogeneity were revealed from the sample, which had earlier appeared to be homogeneous on a slab gel. Sequence of runs was carried out within several days to evaluate reproducibility, which appeared to be on a highest level. Run #5 (shown in **blue**) was performed two days after Run #1 (shown in **red**).

