FFE Weber GmbH

ProMetHEUS™ FFE

Fractionation of human serum proteins by free-flow electrophoresis: Workday-stability of the fractionation pattern

Introduction

Free-flow electrophoresis (FFE) is a versatile fractionation technology that offers nearly unrestricted compatibility to post-fractionation work-up by 2D-electrophoresis,MS ,L C-MS,fr action-concentration, etc . Its main application field is the fractionation of complex protein samples in the isoelectric focusing (IEF) mode. Typically,a high resolution in combination with a great reproducibility can be reached. To demonstrate the latter quality, particularly the reproducibility of the results in the course of a workday,this application note describes the stable and nearly identical outcome of an experiment,tha t was run for several hours: the fast IEF-fractionation of human serum proteins.

Methods

Sample preparation

Human serum from clotted male whole blood (Sigma) was diluted 1:30 with separation media (see below). Traces of the red,acidic dye 2-(4- Sulfophenylazo)-1,8-dihydroxy-3,6-naphthalenedisulfonic acid (SPADNS,Aldrich) were added to ease the optical control of the migration of the sample within the separation chamber. Final protein concentration was approximately 2.5 mg/ml.

Free-flow electrophoresis

FFE separations were conducted at 15˚C using the following media:

Anodic stabilization medium (I1+2) 14.5% (w/w) glycerol; 42% (w/w) 8M urea; 0.03% (w/w) HPMC; 150 mM H_2SO_4
Separation medium (I3+4) 14.5% (w/w) glycerol; 42% (w/w) 8M urea; 0.03% (w/w) HPMC; 19% (w/w) ProlyteTM 2, 10 mM DTT, 1% (w/w) CHAPS
Cathodic stabilization medium (I5-7) 14.5% (w/w) glycerol; 42% (w/w) 8M urea; 0.03% (w/w) HPMC; 100 mM NaOH
Counterflow medium 14.5% (w/w) glycerol; 42% (w/w) 8M urea
Anodic circuit electrolyte 100 mM H_2SO_4
Cathodic circuit electrolyte 100 mM NaOH

The experiments were run in a horizontal separation chamber using a 0.4 mm spacer. The separation chamber was artificially narrowed from 10 cm to approximately 3 cm by the application of the stabilization media via inlets I1,I2,I5 ,I6,and I7 instead of

merely inlets I1 and I7. A flow rate of ~ 80 g/h (Inlet I1-7) was used in combination with a voltage of 500 V which resulted in a current of 22 mA. Samples were perfused into the separation chamber using the middle inlet at ~ 1 g/h. Residence time in the separation chamber was ~ 15 min.
Fractions were collected in several polypropylene microtiter as well as minititer plates, numbered 1 (anode) through 96 (cathode) during more than 5 hours.

Data analysis

The protein fractions were analysed by SDS-PAGE using an XCell SureLock‘ Mini-Cell (Novex) in combination with precast NuPAGE“ Novex 4-12% Bis-Tris gels. Silver-staining of the proteins was carried out using the SilverQuest‘ kit (Novex).

Results

Typically,the fractionation of protein samples using the 2 outer inlets for stabilization media leads to a "net width” of the ProTeamTM FFE separation chamber of approximately 7 cm corresponding to 70 fractions.We decided to reduce this width to approximately 3 cm corresponding to 30 fractions by using 5 out of 7 inlets for stabilization media. This was done for the following purposes: FFE being understood as a pre-fractionation technology prior to 2D electrophoresis or LC-MS should provide a reduced number of fractions. In addition,the residence time of the sample should be decreased,in other words the sample throughput should be increased.

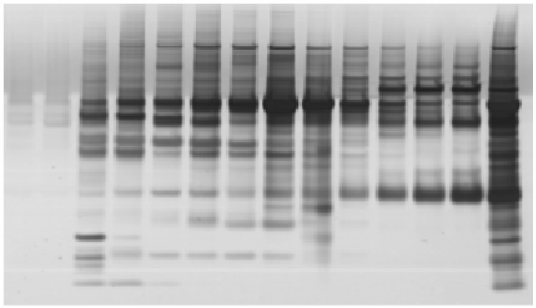

Figure 1: Silver-stained SDS-PAGE analysis of the acidic region of IEF-FFE-fractionated human serum. Lanes from left to right: Fraction 32 (pH = 4.6); 33; ...; 43; 44 (pH = 7.1); unprocessed serum.

The silver-stained SDS-gel in Figure 1 exemplifies the fractionation of the human serum proteins by isoelectric focusing FFE using ProlytesTMbased separation media. The fact that a lot of protein bands just occur in one or two lanes indicates the high resolution of the FFE fractionation.

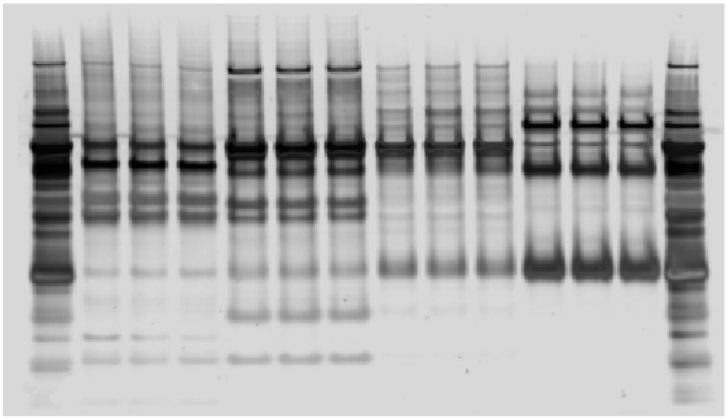

Figure 2: Excellent workday-stability of the FFE-fractionation-pattern: Silver-stained SDS-PAGE analysis of selected fractions of IEF-FFE-fractionated human serum. Lanes from left to right: Unprocessed serum; 350 (@ fraction 35 collected at t = 0); 35200; 35300; 380; 38200; 38300; 410; 41200; 41300; 440; 44200; 44300; unprocessed serum.

In addition,this implies that most of the protein bands that seem to be present in more than 2 consecutive lanes are either different isoforms having different pI-values or are different proteins with similar size,which are undistinguishable due to the limited resolving power of the small SDS-gel. The relatively broad distribution of the albumin band can be explained by several isoforms,too.

In addition, albumine is well known to interact with several other proteins which also could lead to suboptimal resolution. To show the perfect workday-stability of the results the fractionation of human serum was carried out for more than 5 hours,which should represent the typical daily run time of a ProMetHEUS™ FFE instrument.

Fractions were collected at distinct time points and analysed by SDS-PAGE (Figure 2). The comparison of the individual band patterns impressively shows the quasi-identical results. A slight shift of the migration paths of a few millimetres inevitably would have led to massive differences in the "fingerprints" of the bands!

Conclusion

The fractionation of human serum by free-flow electrophoresis in the isoelectric focusing mode using ProlytesTM-based separation media is a descriptive example of the excellent stability of the fractionation pattern in the course of a laboratory workday. Furthermore it shows the excellent resolution of the instrument even in the "narrow separation chamber" mode using limited amounts of HPMC.

References

1) Weber,G. and Bo_ek,P. (1998) Recent developments in preparative free flow isoelectric focusing, Electrophoresis 19(10) 1649-1653.