FFE Weber GmbH

ProMetHEUS™ FFE

Native fractionation of human serum by isoelectric focusing free-flow electrophoresis

Introduction

The preparative separation of proteins under native conditions is a tedious problem. Existing methods like the preparative continuous elution PAGE electrophoresis or the preparative PAGE electrophoresis with subsequent band-excision are very time-consuming and labour intensive. Furthermore, reproducibility is low and quantitative recoveries are rare due to the gel-matrix that is used. Free-flow electrophoresis (FFE) is perfectly suited to solve these drawbacks. The isoelectric focusing separation of proteins under native conditions guarantees fast, highly reproducible results in combination with nearly complete sample recoveries. To demonstrate the native separation qualities of FFE, this application note describes the fast separation of human serum proteins by isoelectric focusing under non-denaturing conditions.

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 10°C using the following media:

Anodic stabilization medium (I1) 14.5% (w/w) glycerol; 0.2% (w/w) HPMC; 100 mM H_2SO_4
Separation medium 1 (I2) 14.5% (w/w) glycerol; 0.2% (w/w) HPMC; 17 % (w/w) Prolyte™ 1
Separation medium 2 (I3–5) 14.5% (w/w) glycerol; 0.2% (w/w) HPMC; 17 % (w/w) Prolyte™ 2
Separation medium 3 (I6) 14.5% (w/w) glycerol; 0.2% (w/w) HPMC; 17 % (w/w) Prolyte™ 3
Cathodic stabilization medium (I7) 14.5% (w/w) glycerol; 0.2% (w/w) HPMC; 100 mM NaOH
Counterflow medium 14.5% (w/w) glycerol
Anodic circuit electrolyte 100 mM H_2SO_4
Cathodic circuit electrolyte 100 mM NaOH

The experiments were run in a horizontal separation using a 0.5 mm spacer. A flow rate of ~ 80 g/h (Inlet I1-7) was used in combination with a voltage of 1500 V which resulted in a current of 29 mA. Samples were perfused into the separation chamber using the cathodal inlet at ~ 0.9 g/h. Residence time in the separation chamber was ~ 19 min. Fractions were collected in polypropylene minititer plates, numbered 1 (anode) through 96 (cathode).

Data analysis The pH-values of the individual microtiter plate fractions were measured manually. Horizontal IEF-PAGE analysis of protein fractions was done using a Desaga electrophoresis device HF 210 (Sarstedt) in combination

with Servalyt®-based ultrathin polyacrylamide gels (5% T, 3% C). The gels were cast according to the instructions from Serva. The silver-staining procedure was based on the method of Blum (see references).

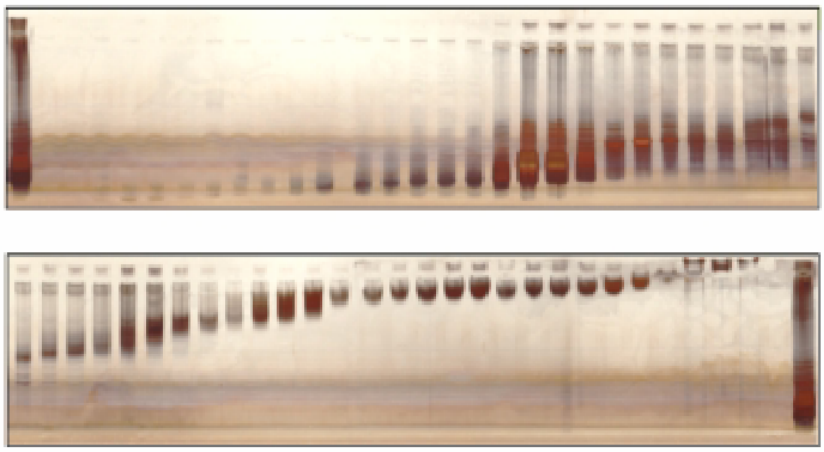

Figure 1: Silver-stained IEF-PAGE analysis of IEF-FFE-fractionated human serum. Top: Lanes from left to right: Crude sample; fraction 17 (pH = 2.88); 18; 19; ...; 42; 43; 44 (pH = 5.93). Bottom: Lanes from left to right: Fraction 45 (pH = 6.03); 46; 47; ...; 65; 66; 67; 69; 71; 73; 75; 77; 79 (pH = 9.96); crude sample.

Results

The silver-stained IEF-gels in Figure 1 exemplify the fast (residence time only 19 min!) fractionation of the human serum proteins by isoelectric focusing FFE under non-denaturing conditions using ProlytesTM-based separation media. The proteins are nicely distributed over the whole pH-range. Even the albumins, which are known to show a broad distribution based on their interactions with all kinds of other proteins are mainly focused to 2 fractions (Figure 2, fraction 34+35).

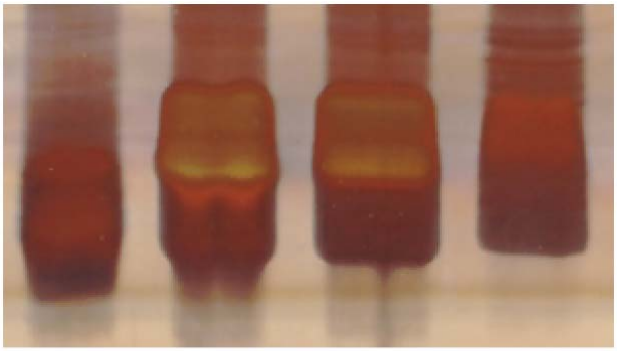

Figure 2: Silver-stained SDS-PAGE analysis of IEF-FFE-fractionated human serum. Enlarged albumin region: fractions 33-36.

Conclusion

FFE in the isoelectric focusing mode is an indispensable tool for the separation of proteins under non-denaturing conditions. The absence of any matrix (like a polyacrylamide-gel) guarantees highly reproducible results combined with quantitative sample recoveries in a fast and straight forward manner.

References

1) Blum, H., Beier, H. and Gross, H. J. (1987) Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels, Electrophoresis 8 93-99.

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