Isoelectric Focusing of Standard Proteins, p*I* 4.65–9.6

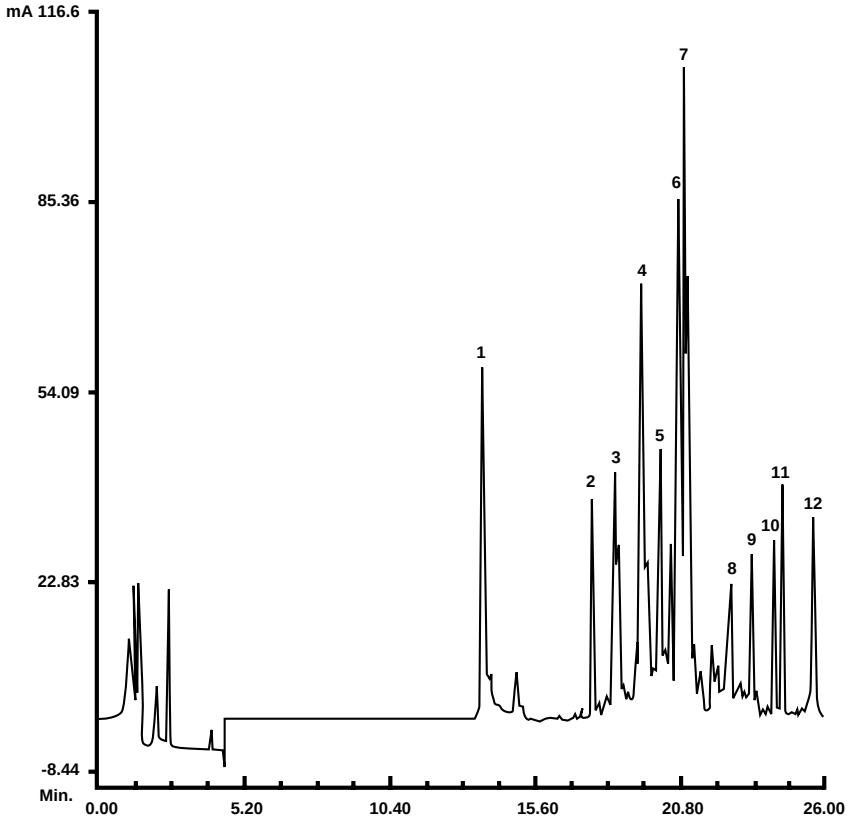

Fig. 1. Separation of standard proteins by capillary isoelectric focusing with pH 3–10 ampholytes using cathodic mobilization. Peak identities: **1,** cytochrome c (p*I* 9.6); **2–4,** lentil lectins (p*I* 8.20, 8.00, 7.80); **5,** human hemoglobin C (p*I* 7.50); **6,** human hemoglobin A (p*I* 7.10); **7,** equine myoglobin (p*I* 7.00); **8,** equine myoglobin minor band; **9,** human carbonic anhydrase (p*I* 6.50); **10,** bovine carbonic anhydrase (p*I* 6.00); **11,** β-lactoglobulin B (p*I* 5.10); 12, phycocyanin (p*I* 4.65).

Analysis Conditions

Sample	Bio-Rad's IEF Standards (catalog 161-0310) diluted 1:50 with Bio-Lyte 3/10 ampholytes (catalog 148-5031)
Instrument	BioFocus® 3000 automated capillary electrophoresis system
Capillary	17 cm x 25 µm, coated
Polarity	positive to negative
Injection time	60 sec
Anolyte	40 mM phosphoric acid (catalog 148-5029)
Catholyte	40 mM NaOH (catalog 148-5028)
Mobilizer	zwitterion solution (catalog 148-5030)
Focusing conditions	240 sec at 10 kV
Mobilization voltage	15 kV
Detection	280 nm
Capillary temperature	20 °C
Autosampler temperature	20 °C

Separation of proteins based on their isoelectric points can be achieved using isoelectric focusing in coated capillaries. By changing the composition of the anolyte or catholyte solutions, the focused proteins can be mobilized past the detector to generate an electropherogram. The resolving power of capillary isoelectric focusing is demonstrated in the separation of Bio-Rad's IEF standards using a wide-range ampholyte mixture (Figure 1); focused zones were mobilized toward the cathode by exchanging the catholyte solution for a zwitterionic mobilization reagent.

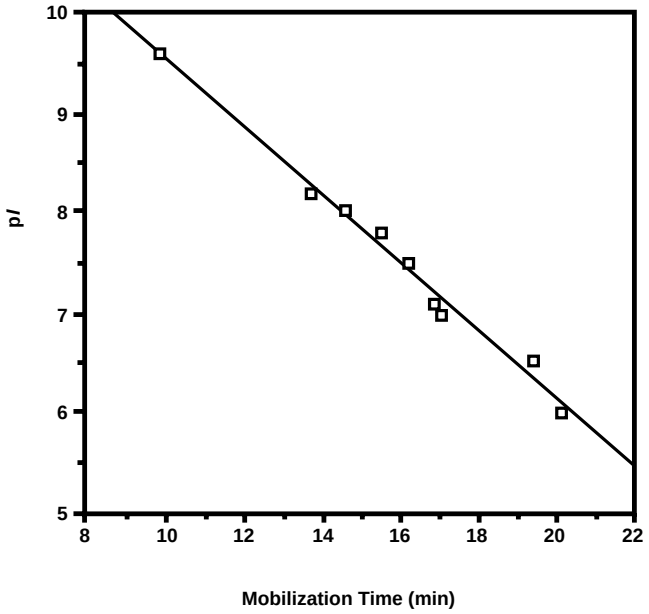

Fig. 2. Isoelectric point vs. mobilization time.

Mobilization times are well-correlated with protein isoelectric points over a pH range of 6–10 (Figure 2), so capillary IEF is a useful method for estimating protein p*I* values. However, addition of internal standard proteins to the sample with p*I* values bracketing that of the analyte is recommended for achieving accurate p*I* estimates.

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