

### **53f Multistage Electrophoresis Applications in Protein Analysis**

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In multistage electrophoresis a starting sample is placed in a sample cuvette in a sliding plate. The sample cuvette then becomes the lower half of a shear cell by sliding under an inverted, buffer-filled cavity in an upper plate. A vertical electric field is applied to the shear cell, and separands are collected in the upper half of the shear cell when the plates slide with respect to each other. This process is repeated until all desired separands are collected in up to 20 fractions on the basis of electrophoretic mobility. Rate-based and pseudo-equilibrium based approaches to multistage electrophoresis have been described. Applications of this technology to protein analysis include (1) pH dependent isolation of proteins having specific isoelectric points, (2) concentration of proteins by sequential volume reduction, and (3) debulking of protein solutions to reveal low-abundance constituents. In an example to be presented, a high-mobility, low-abundance protein in human serum was captured in the presence of drastically reduced bulk proteins in the first stage of multistage electrophoresis. The target protein could then be revealed by SDS-PAGE with Ponceau staining without requiring Western-blot analysis and could be gel purified for sequencing. This research was supported by State of Indiana 21st Century Research and Technology Fund contract 092700-0141 and National Aeronautics and Space Administration contract NAS9-98088.