

High Resolution CIEF Protein Separation with New Dynamic Coatings

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INTRODUCTION

Mismatched electroosmotic (EO) flow results in superimposed electroosmotic and Pouseuille flow, which causes loss of resolution during electrophoresis in silica channels. Since capillary isoelectric electrophoresis (CIEF) is an equilibrium technique, both EO flow and force must be minimized to achieve resolution meaningful for proteome level separations, (i.e., comparable to 2-D gels). One of the key challenges of high resolution CIEF separation has been the regulation of EOF, particularly in the basic pH region. It has been often difficult to resolve proteins with high pI values. In this work we use a new acrylamide, N-substituted acrylamide copolymer, EOTrol™, to increase protein resolution in CIEF than that obtained with commercial polyethylene oxide (PEO)-coated or bare silica capillaries. These new dynamic coatings are fairly insensitive to pH changes in the range of pH 3-10, making them ideal for CIEF applications.

MATERIALS AND METHODS

All reagents were purchased from Sigma-Aldrich (St. Louis, MO). Ampholytes (Pharmalyte™) used in this work were made by Fluka (Milwaukee, WI). *E. coli* extract (Proteomic Protein Control--*E. coli*) was obtained from Geno Technology, Inc. (St. Louis, MO). Bare silica capillaries were purchased from Supelco (Bellefonte, PA). DB-WAX capillaries were made by Agilent Technologies, Inc. (Wilmington, DE). All capillaries used were 60.2 cm in overall length with 50 cm effective length (inlet to detector), with an internal diameter of 100 μM. Uncoated silica capillaries were pre-conditioned by rinsing with methanol, 1 M HCl, and 1 M NaOH, with water rinses in between. DB-WAX capillaries were pre-conditioned by treatment with methanol and water only. All rinses were done at 20 psi (138 kPa).

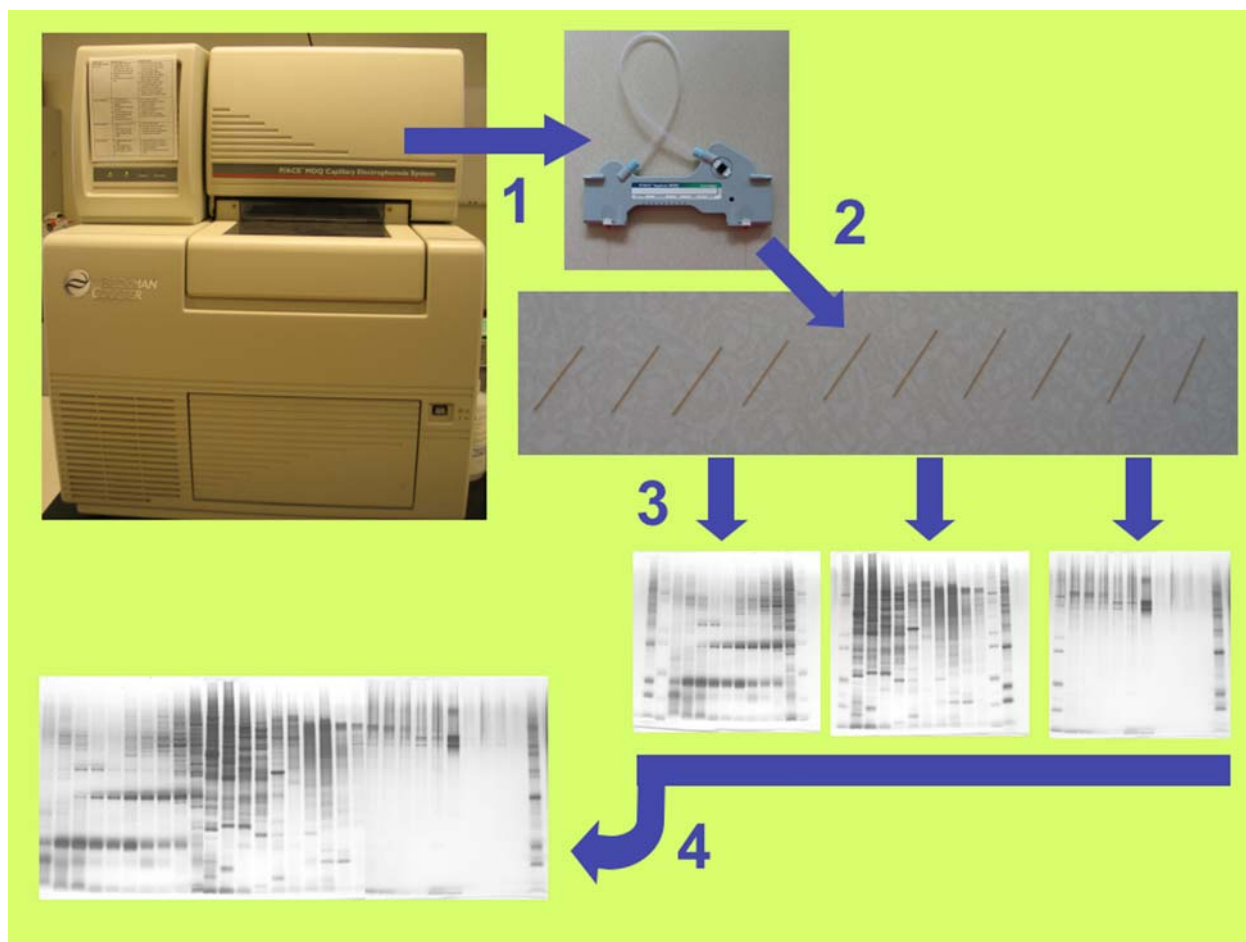
E. coli cellular extract stock solution was prepared by mixing Genotech Protein Control (2 mg) with 0.5 mL 1% SDS, 150 mM DTT. The mixture was vortexed and heated at 95 °C for 5 min. The stock solution was aliquoted and stored frozen (-20 °C) until use. Prior to the CIEF experiments, the stock solution was adjusted to a final total protein concentration of 0.8 mg/mL in 8 M urea, 1% DTT, and 4% CHAPS. Ampholytes 3-10 and marker proteins were also added to this mixture before sample injection.

A Beckman-Coulter P/ACE MDQ Capillary Electrophoresis System (Beckman-Coulter Instruments, Inc., Fullerton, CA) was used in all CIEF experiments. Experiments were carried out with liquid cooling to maintain a run temperature of 25 °C. The capillary was rinsed and filled with the *E. coli* extract by pressure. Separation was carried out by applying a voltage of 25 kV between 10 mM H₃PO₄ and 20 mM NaOH for 130 min.

At the end of the CIEF separation, the capillary was cut up into 2-cm segments. The content of each segment was analyzed using SDS-PAGE (12% Bis-Tris, Invitrogen, Carlsbad,

CA). Protein bands were visualized by silver staining (SilverXpress™ Silver Stain kit, Invitrogen). See illustrations in the panel below.

DISPLAY OF CIEF RESULTS BY SDS-PAGE

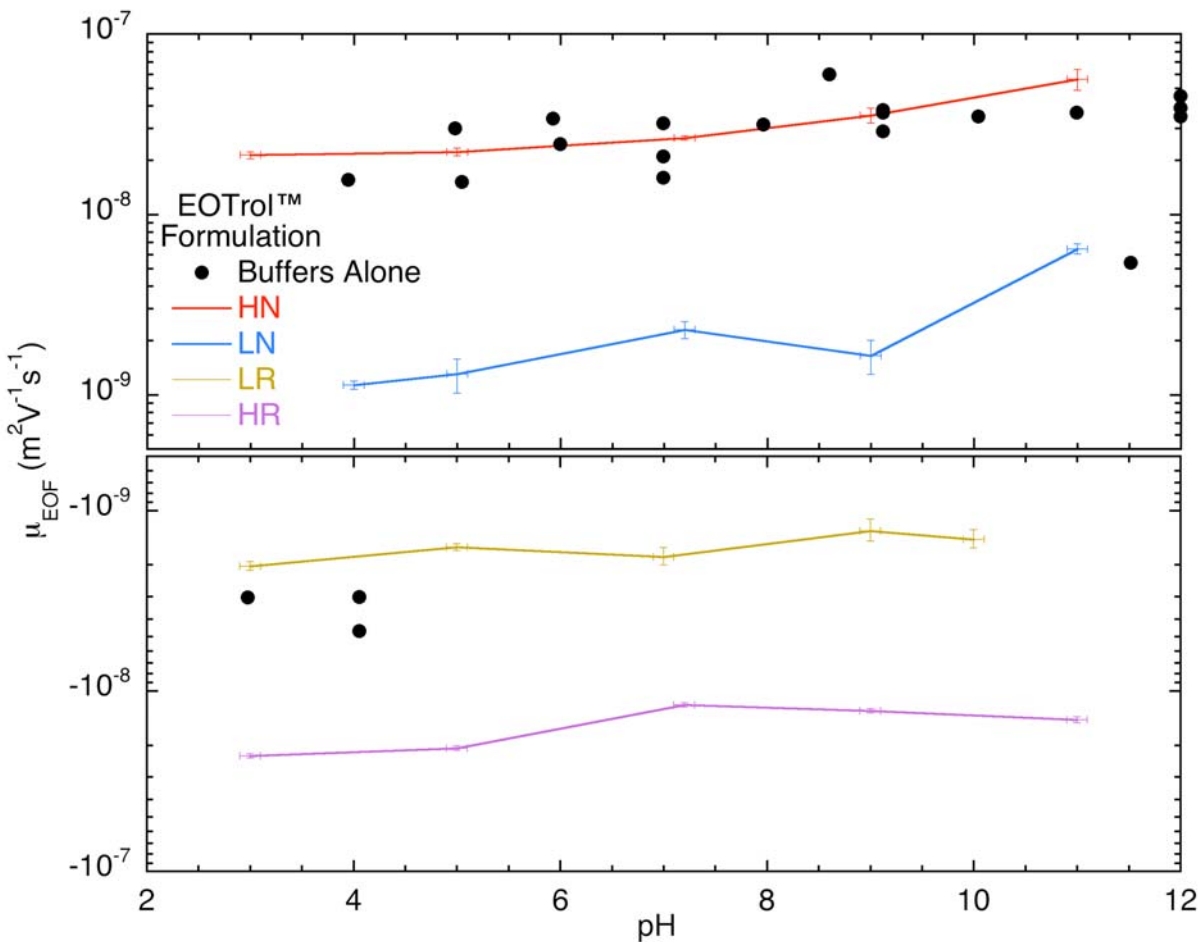


1. Capillary isoelectric focusing (CIEF) is conducted in a Beckman Coulter MDQ™ CE instrument. The capillary cartridge is subsequently removed after focusing.
2. The capillary is then cut into thirty 2-cm pieces (only ten segments shown in the schematic).
3. The contents of capillary segments are separated by size using slab gels.
4. Images from dried silver stained gels are spliced together to provide an overview of the CIEF separation

The EOTrol™ Suite of Dynamic Coatings

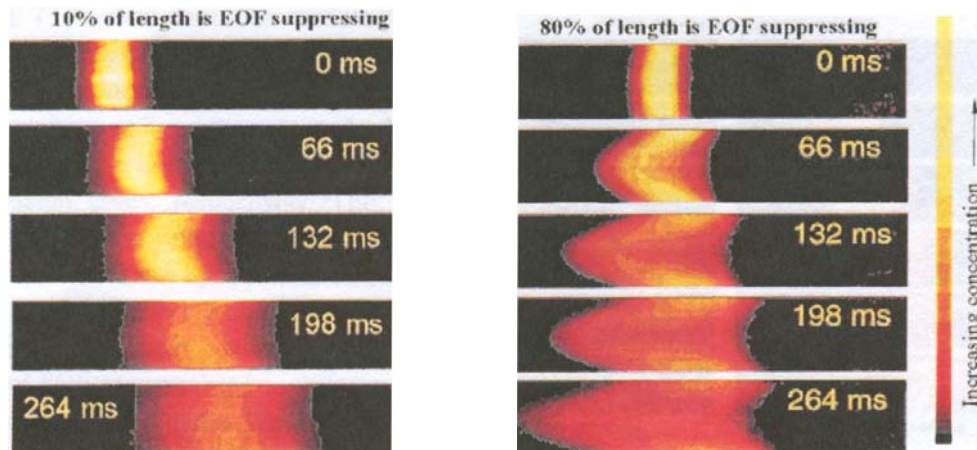
EOTrol™ is a novel suite of acrylamide, N-substituted acrylamide copolymers that serves as dynamic coatings for both CE and microfluidic uses. EOTrol™ provides the following benefits:

- Reduce, eliminate or reverse EOF with uniform coating
- Insensitive to buffer type, pH, chaotropic agents or surfactants
- No absorbance above 250 nm



INCOMPLETE COATING LEADS TO POOR RESOLUTION

It has been reported in the literature¹ that mismatched EOF, resulting from non-uniform coating, leads to more resolution loss than no EOF reduction (see left panels). This points to the need of having a coating that provides uniform, effective EOF suppression, which is particularly crucial for CIEF separation.



¹ Herr *et al.* Anal Chem 2000; 72:1053-1057