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# Protein Separations Using Capillary Electrophoresis with Rationally Designed Modifications on the Capillary Surface

M. Huang

*Analysts using fused silica capillary electrophoresis columns to study proteins are finding that modifying the column surface reduces sample adsorption and improves separation. The modification of columns used in three CE modes is described in this article.*

Capillary electrophoresis (CE) is a promising technique for protein separation. Typical applications include unknown sample analysis, process analysis, purity assays, binding studies, and pI and molecular weight determinations. CE offers high efficiency, minimal sample consumption, a choice of operation modes, and simple method development.

However, the active surface of a fused silica column can cause adsorption of protein molecules, leading to tailing peaks, poor reproducibility, and reduced protein recovery. To diminish adsorption and control electroosmotic flow (EOF), which results from the surface charge, modification of the column surface becomes necessary (1). We used specially modified capillary columns for protein separations in three CE modes: capillary zone electrophoresis (CZE), capillary isoelectric focusing (CIEF), and capillary gel electrophoresis (CGE).

## CZE

Polysiloxane typically has been used in CE to deactivate the fused silica column surface (2). Hydrophobic interactions between this nonpolar phase and protein molecules, however, discourage the use of this type of column. One solution is to include a nonionic surfactant — such as Supelcoat™ PS1 — in the running buffer to cover the polysiloxane-bonded layer and create an additional hydrophilic top layer. We coated a CElect™ H150 polysiloxane-bonded column with Supelcoat PS1 surfactant by rinsing the column with a dilute aqueous solution. Both basic and acidic proteins were separated in one run (Figure A), eluting with symmetric peak shape. EOF was reduced, compared to bare fused silica columns, but was still strong enough to elute negatively charged proteins which migrated against the EOF.

## CIEF

In CIEF, we used the same strategy and column as in CZE, except that we added 0.1% Supelcoat PS1 surfactant to the ampholyte solution. The separation procedure was:

1. Rinse the column with 5% ampholyte solution (Sigma® Ampholine, pH 3.5 - 9.5) under high pressure for 3 min.
2. Inject the protein (0.1 mg/mL) and ampholyte (5%) mixture under high pressure for 6 sec.
3. Inject 5% ampholyte solution under low pressure for 10 sec, then apply voltage to start the focusing and elution.

**Figure A. Separation of a Protein Mixture by CZE**

Column: CElect-H150, 67cm x 50µm ID  
 Buffer: 25mM Tris-HCl + 25mM sodium phosphate buffer, pH = 6.0  
 (0.001% Supelcoat PS1)  
 Voltage: 20kV  
 Det.: UV, 214nm

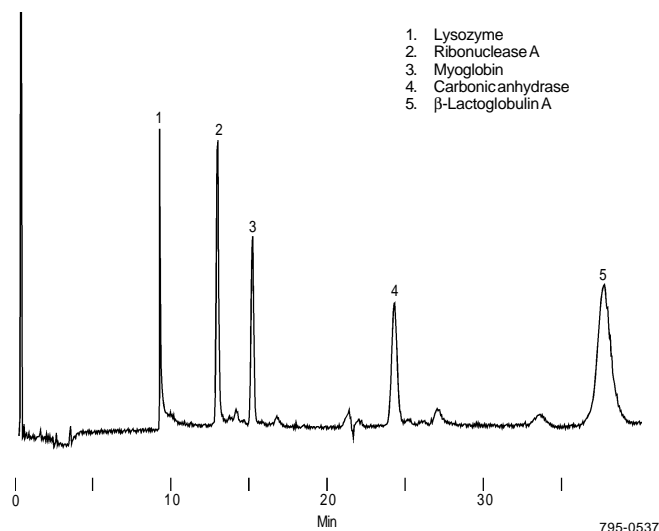
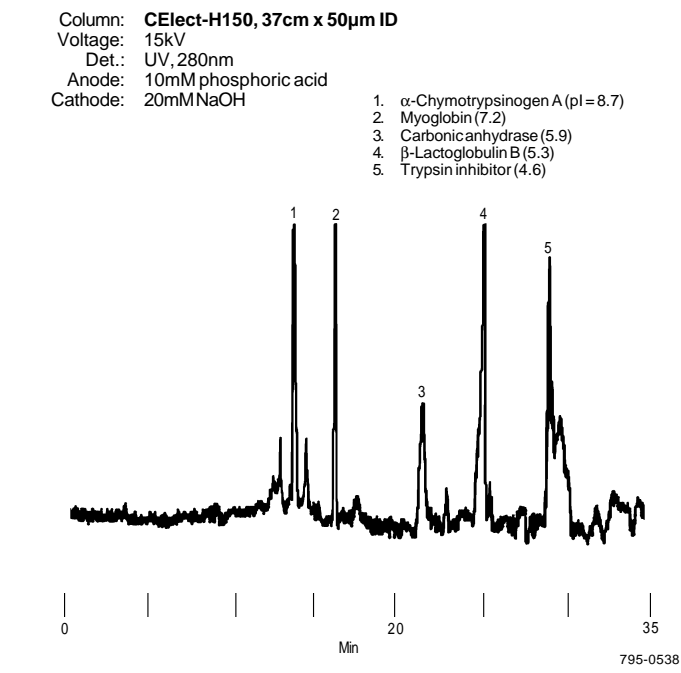
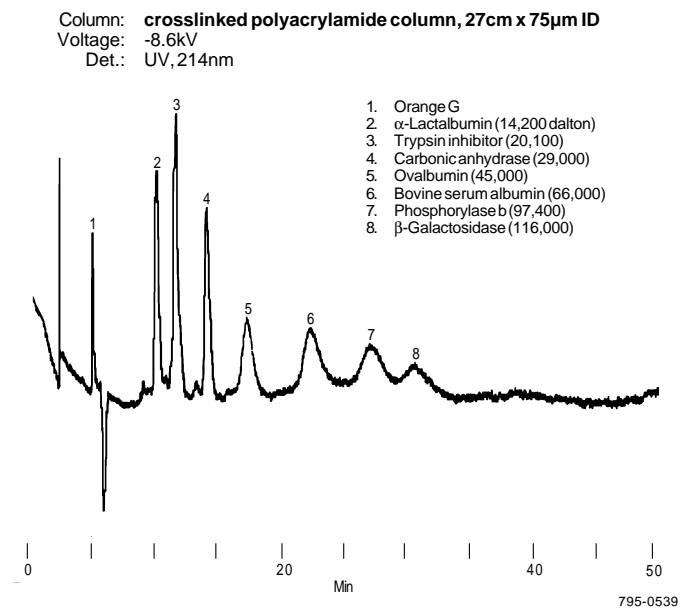


Figure B shows the results. The plot of protein pI vs. migration time was approximately linear, with regression ( $r^2$ ) = 0.963. A significant advantage to using this procedure was that it was completed in one step (i.e., isoelectric focusing and the elution of sample band to detector were performed simultaneously). Another advantage was that the addition of the nonionic Supelcoat PS1 surfactant prevented the focused protein from precipitating.

## CGE

We permanently bonded hydrophilic polymer layer to the capillary surface through an *in situ* polymerization method. The surface was first treated with a vinyl group containing trimethoxysilane, by self-assembly (3). Then a mixture of acrylamide, bisacrylamide, and free radical initiator in methylene chloride was coated onto the surface. The coated column was heated to 120°C for 2 hours to form a copolymer bonded to the capillary surface (4). The resulting column was hydrophilic, with almost no EOF.

In CGE, the separation depends on the sizes of the solutes when they migrate through a gel matrix, providing a sieving effect. Figure C is an electropherogram of a mixture of SDS-protein complexes from a crosslinked polyacrylamide-coated capillary column filled with an entangled polymer solution. Since the size of an SDS-protein complex depends on the molecular weight of the protein,

**Figure B. CIEF of a Protein Mixture****Figure C. CGE of SDS-Protein Complexes****References**

1. Weinberger, R., *Practical Capillary Electrophoresis*, Academic Press, 1993.
2. Dougherty, A.M., C.L. Woolley, D.L. Williams, D.F. Swaile, R.O. Cole, and M.J. Sepaniak, *J. Liq. Chromatogr.* 14:907-921 (1991).
3. Huang, M., E. Dubrovackova-Schneiderman, M.V. Novotny, H.O. Fatunmbi, and M.J. Wirth, *J. Microcol Sep* 6:571-576 (1994).
4. Huang, M., W.P. Vorkink, and M.L. Lee, *J. Microcol Sep* 4:233-238 (1992).

References not available from Supelco.

\*US Patent No. 5,192,406.

the separation of the SDS-protein complexes in CGE gives information about protein molecular weight.

These diverse results show that CElect H-type columns provide excellent results for protein analyses by CZE and CIEF. We now offer conditioned (PC) CElect columns that give reproducible separations from the onset (Reporter, Vol. 14, No. 1). We continue to offer unconditioned columns for our customers using methods developed around these columns, and for those performing applications for which unconditioned columns are required.

**Ordering Information:**CElect Columns\*, 363 $\mu$ m ID x 1 meter, pk. of 2

CElect Column	ID ( $\mu$ m)	Cat. No. Unconditioned	Cat. No. Conditioned
<b>Hydrophobic (C1)</b>			
H50	50	75004	75004PC
H75	75	77504	77504PC
<b>Hydrophobic (C8)</b>			
H150	50	75002-U	75002PC
H175	75	77502	77502PC
<b>Hydrophobic (C18)</b>			
H250	50	75003	75003PC
H275	75	77503	77503PC

**Supelcoat PS1 Polymer Surfactant**

1% solution in water:isopropanol (95:5).

Description	Cat. No.
25mL	custom

**Brij® 35 Surfactant**White, waxy powder. MW: 1197.57;  $A_{280}$  (5% solution): <0.1 AUFS; pH (10% solution): approx. 3.0

Description	Cat. No.
500g	P6052-500G

**SDS Protein Standards**

Vial contains:  $\alpha$ -lactalbumin (MW 14,200), trypsin inhibitor (MW 20,100), carbonic anhydrase (MW 29,000), ovalbumin (MW 45,000), albumin (MW 66,000), phosphorylase b (MW 97,400),  $\beta$ -galactosidase (MW 116,000), and myosin (MW 205,000).

Description	Cat. No.
Vial	M2789-1VL

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