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Conditioned Nonpolar Capillary Electrophoresis Columns Reduce Equilibration Time in Protein Separations

M. Huang

Conditioned CElect columns have much shorter equilibration times than conventional polyalkylsiloxane-bonded columns. They are ready to use, relieving the analyst from the time-consuming conditioning step and providing consistent results from the onset. Analysts can achieve very efficient protein separations from a CElect H-type column, either by incorporating a surfactant in the buffer solution or by rinsing the column with Supelcoat PS2 surfactant prior to sample injection, and using a Tris-HCl/phosphate buffer at pH 6.

It is generally acknowledged that bonded capillary electrophoresis (CE) columns minimize interactions of proteins with surface silanol groups. Supelco uses a bonding procedure in manufacturing hydrophobic (H-type) columns that results in chemically stable hydrophobic bonded phases that are inert to most solutes (1). In addition, the electroosmotic flow (EOF) is reduced and is less dependent on buffer pH (2). The EOF is based on the formation of an electrical double layer on the capillary surface, resulting in the transport of the buffer solution toward the negative electrode.

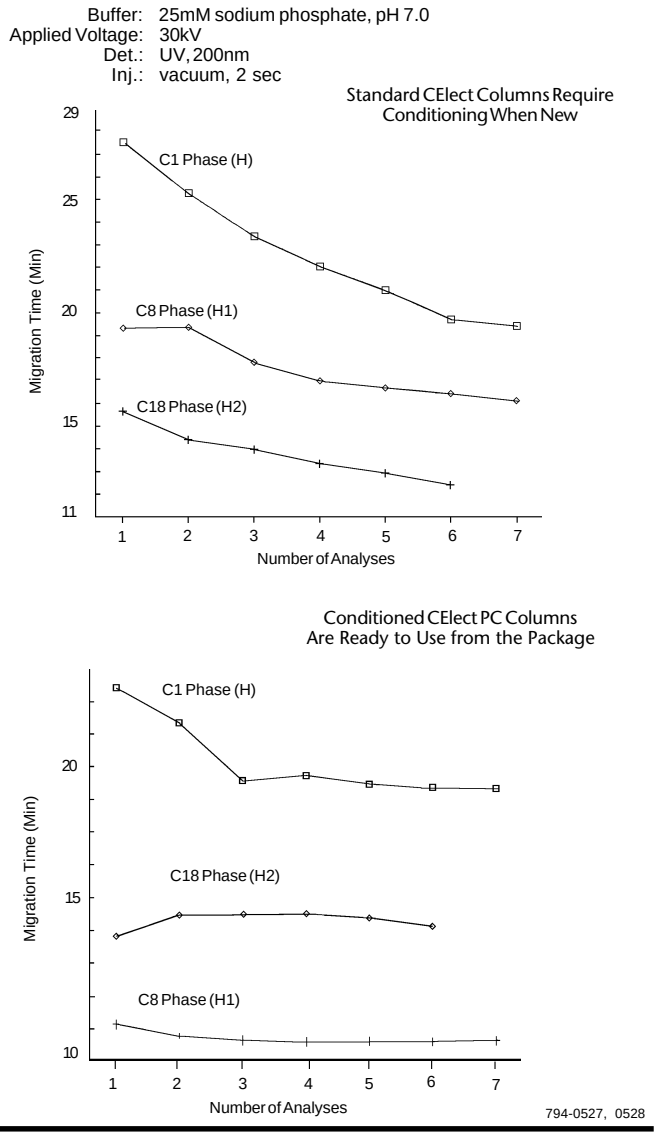
When hydrophobic phases are used in CE separations, the EOF typically changes drastically in the first few analyses. Studies have shown that the migration time of a neutral marker used to measure EOF decreases during the first 5-10 runs before stabilizing. Figure A1 shows the migration times for the neutral marker benzyl alcohol vs. the number of injections on unconditioned CElect-H, H1, and H2 columns. The migration times steadily decreased to reach stable values after about 10 injections.

Two factors slow the equilibration of unconditioned columns. First, since the hydrophobic stationary phase is poorly wetted by the aqueous buffer, it takes time for buffer ions to penetrate the capillary inner surface. Thus, the EOF increases slightly with the continuing formation of the double layer. Additionally, residual siloxane groups (Si-O-Si) may be hydrolyzed upon contact with the buffer solution to form Si-OH groups, further increasing the EOF.

Supelco introduces a new line of conditioned CE columns that eliminate the analyst's need for column conditioning. Conditioned CElect™ H-type capillary electrophoresis columns are ready to use, providing consistent results from the onset. Our proprietary conditioning of these polyalkylsiloxane-bonded nonpolar columns reduces equilibration time to a maximum of three injections (Figure A2).

We analyzed a basic protein test mix using two conditioned hydrophobic columns — CElect-H275 (C18 polymer) and CElect-H50 (C1 polymer). First we added Brij®-35 surfactant to the buffer

Figure A. New CElect Columns Eliminate the Need for Conditioning



at a concentration well below the critical micelle concentration. The hydrophobic portion of the surfactant molecule adsorbed strongly to the nonpolar bonded phase. Adding the surfactant to a buffer with a pH of 6.0 allows high efficiency and good peak shape on a conditioned CElect-H275 column (Figure B). We

analyzed the same mix on a conditioned CElect-H50 column (Figure C) after rinsing the column with a solution containing Supelcoat™ PS2 polymer surfactant, a new comb-like material (3). Again, the separation was favorable.

Unlike other surfactants (e.g., SDS, Brij-35, and Tween® 20), it is not necessary to add Supelcoat PS2 polymer surfactant to the buffer solution. This, together with the use of a near-neutral pH, reduces the chance of protein denaturation. We recommend that the column be rinsed with Supelcoat PS2 polymer surfactant before each injection to ensure full coverage of the surfactant on the bonded phase surface. In addition, rinsing the column before each injection creates a fresh surface and enhances the reproducibility of protein migration times.

While hydrophobic columns are ideal for protein and peptide separations when using select buffer solutions (including those at neutral pH), our hydrophilic bonded phase CElect-P1 columns are useful for analyses using simple buffers at low pH levels (4). We continue to offer unconditioned columns for applications in which they are useful. Contact our Technical Service Department.

Figure B. Proteins (Buffer Includes Brij-35 Surfactant)

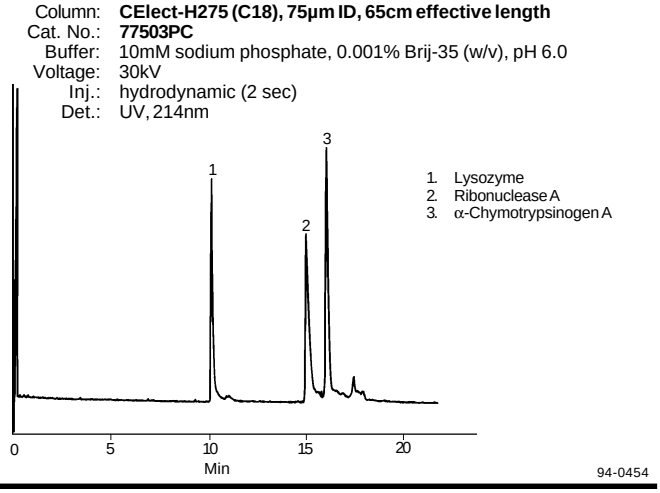
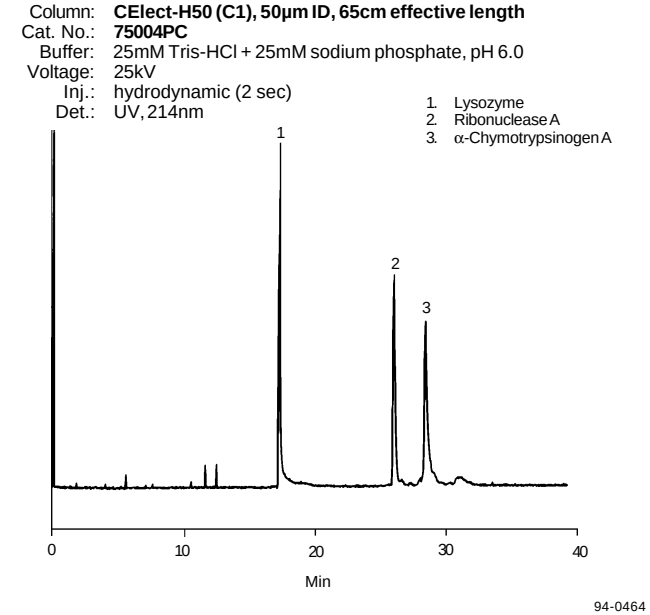


Figure C. Proteins (Column Rinsed with Supelcoat PS2 Polymer)



Ordering Information:

CElect Capillary Electrophoresis Columns■
363µm OD x 1 meter, pk. of 2

Description	ID (µm)	Cat. No. Conditioned	Cat. No. Unconditioned
Hydrophobic (C1)			
H50	50	**75004PC**	**75004**
H75	75	**77504PC**	**77504**
Hydrophobic (C8)			
H150	50	**75002PC**	**75002-U**
H175	75	**77502PC**	**77502**
Hydrophobic (C18)			
H250	50	**75003PC**	**75003**
H275	75	**77503PC**	**77503**
Hydrophilic			
P150	50	_	**75001**
P175	75	_	**77501**

Ordering Information:

Hydroxypropylmethyl Cellulose
25g **H4649-25G**

Methyl Cellulose
100g **M0262-100G**

Supelcoat PS2 Polymer Surfactant
1% volume/volume in water: isopropanol (95:5)
25mL **47449**

Brij-35 Surfactant 500g **P6052-500G**

Neutral Marker
Benzyl alcohol, 0.01% weight/weight in water.
1mL **47450**

For more information, request Bulletin 886, *Strategies for Reliable Peptide Separations on Stable Coated CE Columns* (ChromFax No. 194886).

References

1. Dougherty, A.M., et al, J. Liq. Chromatogr., 14 (1991), 907-921.
2. Dougherty, A.M. and M.R. Schure, *Capillary Electrophoresis Technology*, Chapter 12, N.A. Guzman (ed), Marcel Dekker, NY, 1993.
3. Huang, M., D. Mitchell, and M. Bigelow, paper submitted to Journal of Chromatography for publication of the proceedings of the 5th Annual Frederick Conference on Capillary Electrophoresis (1994).
4. Supelco Bulletin 886, *Strategies for Reliable Peptide Separations on Stable Coated CE Columns.*

■US Pat. No. 5,192,406.

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