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Separate Proteins, Chiral Compounds, and Anions Using a Stable, Bonded, Positively Charged Capillary Electrophoresis Column

M. Huang

Positively charged coatings can be used to mask the silanol groups on the capillary tubing surface, reverse electroosmotic flow, and stabilize the flow with buffer pH changes. Typical applications of these coatings in capillary electrophoresis (CE) include protein separation, ion analysis, and CE/MS of peptides and proteins. Reported methods to prepare these types of columns, however, cannot produce columns which are hydro-lytically stable over a wide pH range, or reproducible between columns or runs. Using a unique synthesis method, Supelco has developed a bonded, positively charged CE column — CElect-Amine — which is stable over a wide range of buffer pH.

In capillary electrophoresis (CE), electroosmotic flow (EOF) is closely related to solute migration time and analyte resolution. The rate and direction of EOF can be controlled by varying the density and the type of charge on the capillary surface. One way to optimize CE separations is to control EOF through charged surface coatings. Preparation of positively charged column surfaces, which cause a reversal in EOF, is discussed in several studies. Applications include the use of an aminopropyltrimethoxysilane (APS) bonded phase for CE/mass spectrometry (1), a polyethyleneimine coated column for protein separations (2), and dynamically coated, positively charged surfaces for separating anions, synthetic compounds, and biopolymers.

The APS bonded phase is unstable during long-term use. The dynamic coating method for a positively-charged phase is not suitable for some applications, such as CE/MS, fraction collection, and analyses involving larger amounts of organic solvents. The stable CElect™-Amine capillary electrophoresis column overcomes these obstacles. It features high crosslinking and bonding to the fused silica surface, and is stable over a wide range of buffer pH (2.5 to 8.8).

Figure A shows changes in migration time from one analysis to the next on an APS bonded phase column. These changes occurred because the bonding is unstable under typical CE running conditions. However, a stable CElect-Amine column exhibited highly reproducible migration times.

We separated three basic proteins using a CElect-Amine column (Figure B). At low buffer pH, a repulsive Coulombic force exists between the positively charged amine coating and basic proteins with a net positive charge. Therefore, the interaction of these proteins with the capillary surface is eliminated, and high efficiency separation can be achieved. Because EOF is reversed, the elution order is from low pI protein to high pI protein.

Figure A. Migration Time vs. Number of Analyses: CElect-Amine Column and APS Column

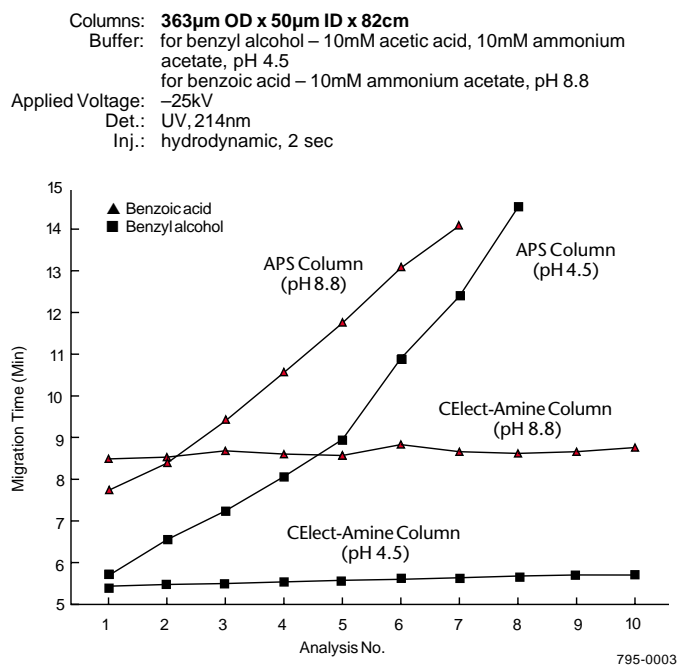
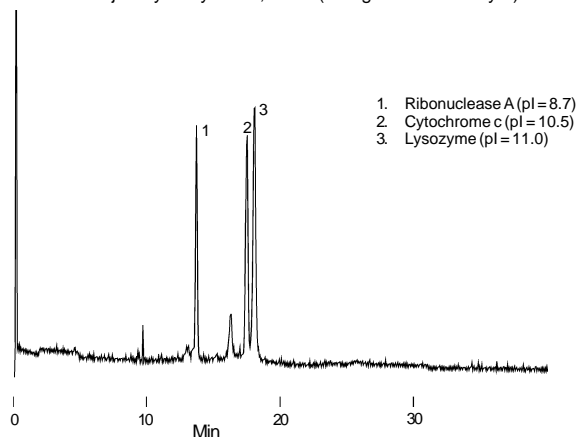


Figure B. Basic Proteins on a CElect-Amine Column

Column: 363 μ m OD x 50 μ m ID x 82cm
 Cat. No.: 75006
 Buffer: 50mM Tris-HCl, pH 4.7
 Applied Voltage: –20kV
 Det.: UV, 215nm
 Inj.: hydrodynamic, 1 sec (~2mg/mL each analyte)



CE shows great potential to resolve enantiomers. Chiral selectants (e.g., cyclodextrins) can be simply added to the running buffer to achieve separation. We used a CElect-Amine column and 2,6-dimethyl- β -cyclodextrin to separate chiral drugs. With a CElect-Amine column, the elution order of the enantiomers can be reversed, compared to a bare fused silica column. If the minor enantiomer elutes before the major enantiomer, potential interference of the tail of the larger peak with the smaller peak can be avoided (Figures C and D), giving a more accurate measurement of optical purity.

CE also provides simple, fast, high efficiency analyses of inorganic ions. An often-used CE method for anion separation involves the use of chromate, pyromellitate, or phthalate as electrolytes for indirect UV detection and the use of an EOF modifier to direct EOF from cathode to anode. A positively charged, amine-coated

column can replace the EOF modifier in anion analysis, giving the user more freedom in method development (Figure E).

CElect-Amine columns exhibit superior stability compared with commonly-used APS bonded phase columns, providing advantages for separating proteins, enantiomers, and inorganic ions.

Ordering Information:

Description	Cat. No.
CElect-Amine Capillary Electrophoresis Columns*	
363 μ m OD x 1 meter, pk. of 2	
50 μ m ID	75006
75 μ m ID	77506

Figure C. Epinephrine Enantiomers

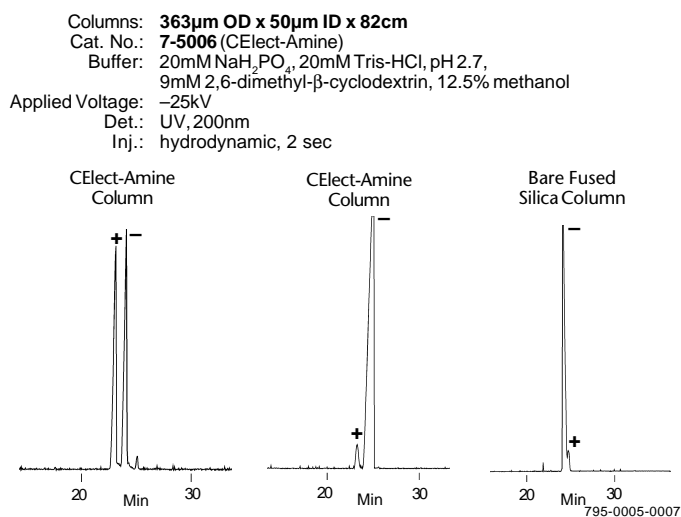


Figure D. Ephedrine Enantiomers

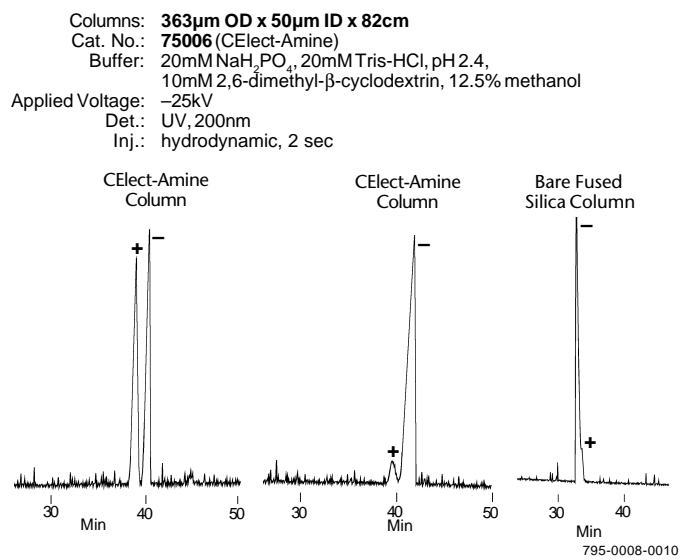
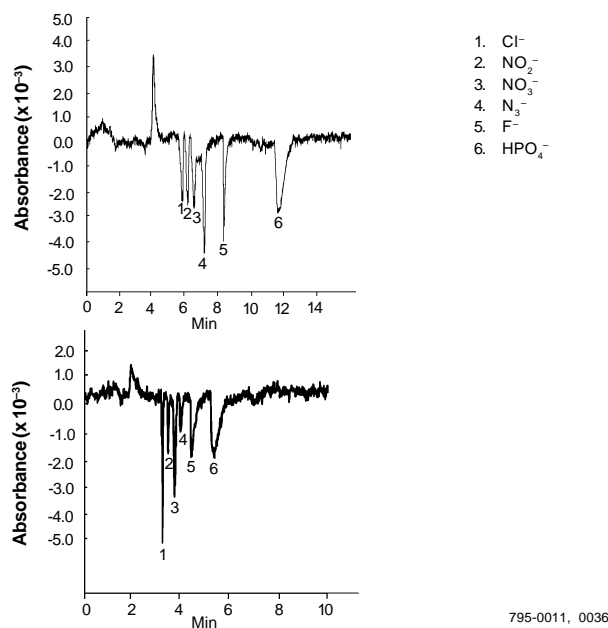


Figure E. Anions on a CElect-Amine Column

Column: **363 μ m OD x 50 μ m ID x 82cm**
 Cat. No.: **75006**
 Buffer: top - 3.5mM 1,2,4-benzenetricarboxylic acid, 0.5% triethanolamine, 10% methanol (pH to 7.5 w/ 1.0M NaOH)
 bottom - 5mM sodium chromate, pH 7.5
 Applied Voltage: -17.5kV (top) or -25kV (bottom)
 Det.: UV, 254nm
 Inj.: hydrodynamic, 3 sec (0.1mM each analyte)



References

- Moseley, M.A., L.J. Deterding, K.B. Tomer, and J.W. Jorgenson, *Anal. Chem.* **63**: 109 (1991).
- Towns, J. K. and F. E. Regnier, *J. Chromatogr.* **516**: 69 (1990).

References not available from Supelco

*US Pat. No. 5,192,406.

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