

# TheReporter

Reprinted from Volume 15, No. 5, 1996

T296025

© 1999 Sigma-Aldrich Co.

For more information, or current prices, contact your nearest Supelco subsidiary listed below. To obtain further contact information, visit our website ([www.sigma-aldrich.com](http://www.sigma-aldrich.com)), see the Supelco catalog, or contact Supelco, Bellefonte, PA 16823-0048 USA.

**ARGENTINA** • Sigma-Aldrich de Argentina, S.A. • Av. Pueyrredon 2446/50 • Piso 5-B • Buenos Aires 1119  
**AUSTRALIA** • Sigma-Aldrich Pty. Ltd. • Unit #2, 14 Anella Avenue • Castle Hill NSW 2154  
**AUSTRIA** • Sigma-Aldrich Handels GmbH • Hebbelplatz 7 • A-1110 Wien  
**BELGIUM** • Sigma-Aldrich N.V./S.A. • K. Cardijnplein 8 • B-2880 Bornem  
**BRAZIL** • Sigma-Aldrich Quimica Brasil Ltda. • Rua Sabara, 566-Conj. 53 • 01239-010 São Paulo, SP  
**CANADA** • Sigma-Aldrich Canada, Ltd. • 2149 Winston Park Dr., Oakville, ON L6H 6J8  
**CZECH REPUBLIC** • Sigma-Aldrich s.r.o. • Pobrezni 46 • 186 21 Praha 8  
**DENMARK** • Sigma-Aldrich Denmark A/S • Vejlegaardsvej 65B • DK-2665 Vallensbaek Strand  
**FINLAND** • Sigma-Aldrich Finland/YA-Kemia Oy • Teerisuonkuja 4 • FIN-00700 Helsinki  
**FRANCE** • Sigma-Aldrich Chimie • Chromatographie Supelco • L'Isle d'Abeau Chesnes - B.P. 701 • 38297 Saint-Quentin Fallavier Cedex  
**GERMANY** • Sigma-Aldrich Chemie GmbH • Geschäftsbereich Supelco • Grünwalder Weg 30 • D-82041 Deisenhofen  
**GREECE** • Sigma-Aldrich (o.m.) Ltd. • 72 Argonafton Str. • 16346 Ilioupoli, Athens  
**HUNGARY** • Sigma-Aldrich Kft. • Nagy Diófa u. 7., IV fl. • H-1067 Budapest  
**INDIA** • Sigma-Aldrich Co. • Survey No. 31/1, Sitharamapalaya • Mahadevapura P.O. • Bangalore 560 048  
**IRELAND** • Sigma-Aldrich Ireland Ltd. • Airton Road • Tallaght • Dublin 24  
**ISRAEL** • Sigma Israel Chemicals Ltd. • Park Rabin • Rohovot 76100  
**ITALY** • Sigma-Aldrich s.r.l. • Via Gallarate, 154 • 20151 Milano  
**JAPAN** • Sigma-Aldrich Japan K.K. • Division Supelco • JL Nihonbashi Building • 1-10-15 Nihonbashi Horidome-cho, Chuo-ku • Tokyo 103  
**KOREA** • Sigma-Aldrich Korea • Samhan Camus Annex, 10<sup>th</sup> Floor • 17-26 Yoido-dong, Yungdeungpo-ku • Seoul  
**MALAYSIA** • Sigma-Aldrich (M) Sdn. Bhd. • 9-2, Jalan 2/128, Taman Gembira • Off Jalan Kuchai Lama • 58200 Kuala Lumpur • Selangor  
**MEXICO** • Sigma-Aldrich Quimica S.A. de C.V. • Calle 6 North No. 107 • Parque Industrial Toluca 2000 • 50200 Toluca  
**NETHERLANDS** • Sigma-Aldrich Chemie BV • Postbus 27 • 3330 AA Zwijndrecht  
**NORWAY** • Sigma-Aldrich Norway • Sandakerveien 102 • N-0483 Oslo  
**POLAND** • Sigma-Aldrich Sp. z o.o. • Szelagowska 30 • 61-626 Poznań  
**PORTUGAL** • Sigma-Aldrich Quimica, S.A. • P.O. Box 131 • Sintra 2710  
**RUSSIA** • Sigma-Aldrich Russia • TOO Techmedbiochem • Makarenko Str. 2/21 • Building 1, Flat 22 • Moscow 103062  
**SINGAPORE** • Sigma-Aldrich Pte. Ltd. • 102E Pasir Panjang Road • #08-01 Citilink Warehouse • Singapore 118529  
**SOUTH AFRICA** • Sigma-Aldrich (pty) Ltd. • CNR Kelly & Ackerman Streets • Southern Life Industrial Park Unit • Unit 16/17 • Jet Park 1459  
**SPAIN** • Sigma-Aldrich Quimica, S.A. • Apt. Correos 161 • 28100 Alcobendas, Madrid  
**SWEDEN** • Sigma-Aldrich Sweden AB • Solkraftsvägen 14C • 135 70 Stockholm  
**SWITZERLAND** • Supelco Switzerland • Industriestrasse 25 • P.O. Box 260 • CH-9471 Buchs  
**UNITED KINGDOM** • Sigma-Aldrich Company Ltd. • Supelco UK • Fancy Road, Poole • Dorset BH12 4QH  
**UNITED STATES** • Supelco • Supelco Park • Bellefonte, PA 16823-0048 • Phone 800-247-6628 or 814-359-3441 • Fax 800-447-3044 or 814-359-3044 • email: [supelco@sial.com](mailto:supelco@sial.com)

H

This article is archived from a past issue of The Supelco Reporter. Information in the article was appropriate at the time of publication, but product specifications, catalog numbers, and availability may have changed over time.

If you have questions about applying methodology described in this article to a current application, please contact our technical service chemists.

 **SUPELCO**

ISO 9001  
REGISTERED

Supelco is a member of the Sigma-Aldrich family. Supelco products are sold through Sigma-Aldrich, Inc. Sigma-Aldrich warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product for a particular use. Additional terms and conditions may apply. Please see the reverse side of the invoice or packing slip.

# Innovative Coating Technology Produces Stable, Hydrophilic CE Columns for Nucleic Acid and Protein Separations

M. Huang, M. Bigelow, M. Byers  
Supelco, Bellefonte, PA USA

*Untreated and unsuitably treated capillary electrophoresis columns can adsorb analytes, preventing reliable and reproducible separations. A self-assembled bilayer coating improves the quality of CE separations. The inner, hydrophobic layer protects Si-O-Si bonds on the column surface from hydrolysis. The outer, hydrophilic layer is inert to analytes, allowing highly efficient and reliable separations of peptides and proteins, DNA fragments, or SDS-protein complexes. The coating is stable at pH 2-10.*

Active groups on the surface of untreated fused silica capillary electrophoresis (CE) columns can adsorb analytes, making separations difficult and inconsistent. Electroosmotic flow (EOF), to which resolution is closely related, can vary because of adsorption at the tubing surface, and this also makes it difficult to obtain reproducible results. In addition, the EOF desired for a separation may not be achievable with the separation medium required with an untreated fused silica column.

An ideal surface coating for CE columns completely deactivates the surface, ensures reproducible separations, is hydrolytically stable over a wide pH range, and allows EOF to be decreased or eliminated, reversed, or stabilized by changing pH. A very promising method for preparing reliable surface coatings consists of treating the capillary surface with a vinyl group containing silane or polysiloxane, then copolymerizing a hydrophilic monomer onto the vinyl/silane layer *in situ*, exposing a hydrophilic polymer coating to the separation buffer and analytes (1-3).

We have improved this two-step method for preparing stable and hydrophilic CE column coatings, based on the formation of a self-assembled bilayer film. The small relative standard deviations in Table 1 indicate the coating is very stable over a wide pH range. The finished CElect™-N column shows virtually no electroosmotic flow from pH 2 to 10.

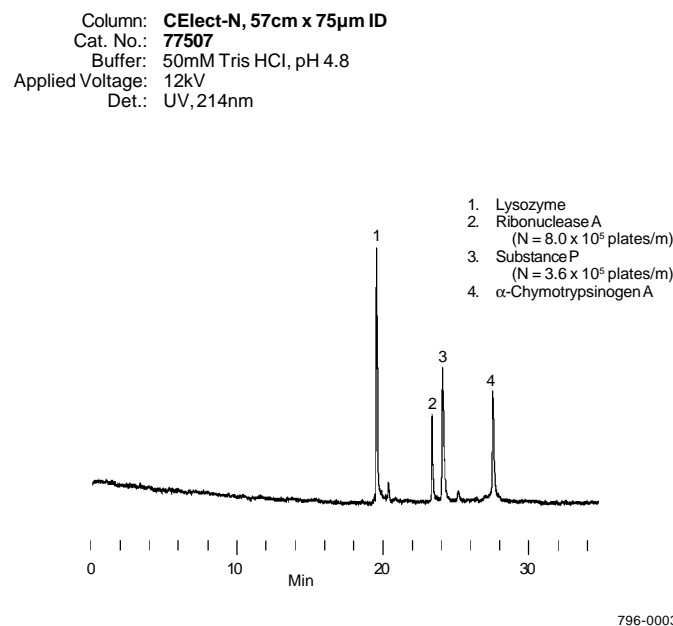
**Table 1. Self-Assembled Bilayer Coating Is Stable Over a Wide pH Range**

	Migration Time (min) <sup>a</sup> , N = 10 runs Lys-Phe (pH 2.0) <sup>b</sup>	Migration Time (min) <sup>a</sup> , N = 10 runs Benzoic Acid (pH 10.0) <sup>c</sup>
	6.77-6.94	13.50-13.88
RSD	0.8%	1.1%

<sup>a</sup>10 min rinse between runs.  
<sup>b</sup>47cm x 75µm column, 50mM phosphate buffer, 15 kV.  
<sup>c</sup>37cm x 75µm column, 25mM borate buffer, -9 kV.

The usefulness of CElect-N columns lies in their stability, hydrophilicity, and absence of EOF. High efficiency peaks for difficult analytes indicate that interaction between the surface and the analytes has been eliminated (Figure A). In contrast, such molecules are readily adsorbed to untreated fused silica (4). Since there is no EOF in CElect-N columns, these columns give much faster and higher efficiency separations of analytes that migrate strongly against EOF, such as ribonucleotides (Figure B). Monophosphate ribonucleotides require more than 2 hours to exit from an uncoated column.

**Figure A. Column with Bilayer Coating Effectively Separates Basic Peptides and Proteins**

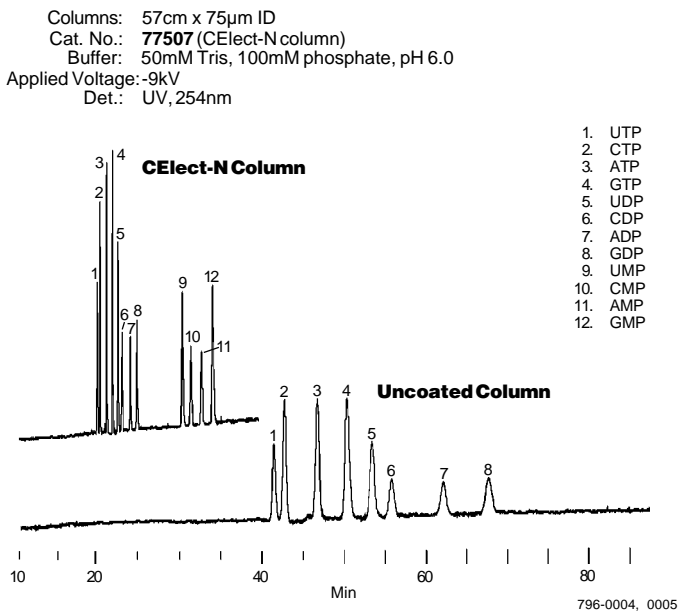


In capillary gel electrophoresis (CGE), an entangled polymer solution produces a molecular sieving effect, so that the separation depends on differences in analyte molecular size and weight. Most polymers used for this purpose are hydrophilic and water soluble, and the hydrophilic polymer coating on a CElect-N column provides a homogenous separation system beneficial to highly efficient separations (Figure C). The efficiency for double-stranded fragment 124 in Figure C, 3.5 x 10<sup>5</sup> plates/m, is very close to the theoretical value. In addition, the absence of EOF eliminates

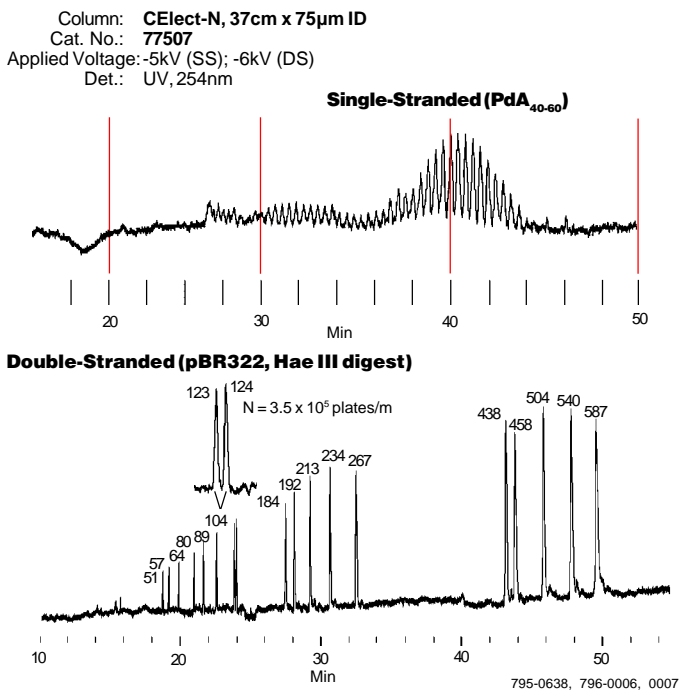
Reprinted from Volume 15, No. 5, 1996

potential disturbance to the separation system. The column-to-column reproducibility of CElect-N columns also is beneficial in DNA separations, particularly in capillary array electrophoresis, where fast, high-throughput DNA sequencing is desired.

**Figure B. Ribonucleotides on CElect-N and Uncoated CE Columns**



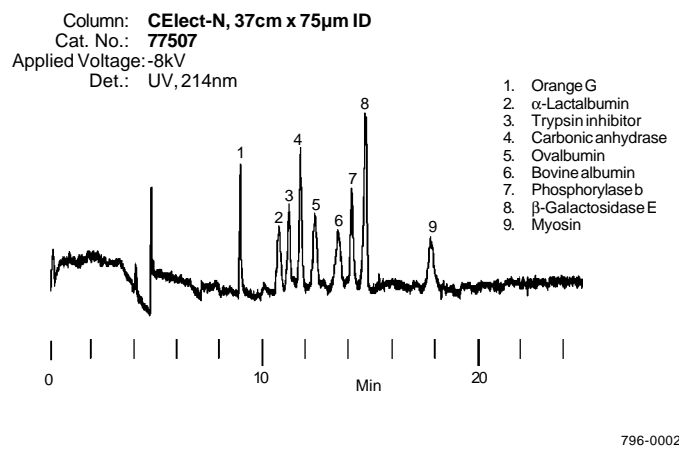
**Figure C. DNA Fragments**



A stable, hydrophilic column with no EOF and an SDS-polymer solution provide a means of conveniently determining protein molecular weight by CE. Figure D shows the high efficiency separation of standard SDS-protein complexes, using a CElect-N column with a UV-transparent SDS-polymer solution. This separation was repeated more than 100 times, with no significant change in any of the migration times (variation ≤ 5%).

Our self-assembly coating method produces stable, hydrophilic columns that are proving very useful for a variety of CE separations, including capillary isoelectric focusing of proteins, CGE of DNA fragments (more than 200 runs with no indication of column failure), and acidic proteins in high pH buffer. Furthermore, the method appears to have great potential for creating a remarkable variety of functionalities in capillary surface coatings. This can help make CE more versatile.

**Figure D. SDS-Protein Complexes**



**Ordering Information:**

Description	Cat. No.
<b>CElect-N Capillary Electrophoresis Columns</b> <sup>■</sup> 1 meter x 363µm OD, pk. of 2.	
50µm ID	<b>75007</b>
75µm ID	<b>77507</b>

For additional information about CElect-N columns, request Bulletin 908. Refer to the current Supelco catalog for other CElect bonded phase columns, CElect column kits, uncoated fused silica tubing, and CE buffers, additives, and accessories.

**References**

1. H. Engelhardt and M.A. Cunat-Walter, *J. Chromatogr.*, **716**: 27 (1995).
  2. M. Huang, *et al.*, *J. Microcol. Sep.*, **6**: 571 (1994).
  3. S. Hjerten, *J. Chromatogr.*, **347**: 191 (1985).
  4. Z. Zhao, A. Malik, and M. L. Lee, *Anal. Chem.*, **65**: 2752 (1993).
- References not available from Supelco.

CElect is a trademark of Sigma-Aldrich Co.

<sup>■</sup>US Pat. No. 5,192,406.

