# A Novel Stationary Phase: Capillary-Channeled Polymer (C-CP) Fibers for HPLC Separations of Proteins

**Dwella K. Nelson** and **R. Kenneth Marcus***
Department of Chemistry, Howard L. Hunter Laboratories, Clemson University, Clemson, SC 29635-0973

## Abstract

**A novel
stationary phase is demonstrated for the separation of proteins. Capillary-channeled polymer (C-CP) fibers provide a stationary phase that is characterized by a high surface activity (yielding strong wicking action) and drastically reduced back pressures. Columns prepared by pulling approximately 1200 50-µm diameter polypropylene C-CP fibers through stainless steel tubing with column dimensions of 4.6-mm i.d. and 306-mm length exhibit reversed-phase characteristics in the separation of the proteins. A gradient method [95:5 water–acetonitrile (ACN)/propanol (1:1) to 35:65 water–ACN/propanol] with trifluoroacetic acid added as an ion-pairing agent yields high-quality separations of superoxide dismutase, hemoglobin, hemocyanin, and myoglobin. It is believed that the C-CP fiber stationary phase holds a number of promising traits for applications in both analytical and prep-scale separations of diverse organic species, including a wide range of biomolecules.**

## Introduction

Efficient protein separations are an important element to understanding protein functions as related to cell growth and neoplasia, as well as mechanisms controlling translation of information through cell walls of bacteria and viruses (1). The physiochemical nature of the proteins themselves requires the use of specific separation strategies manipulating the interactions between solutes, solvents, and stationary phases in order to resolve these macromolecules (2). The development of new cost-effective stationary phase materials for "soft" applications that do not destroy the structure of the molecule or perturb biological activity (or both) have been the driving forces for the development of new selective and reliable stationary phases (3).

The three most common approaches for analytical scale separation of proteins are capillary zone electrophoresis (CZE) (4,5), ion-exchange chromatography (IEC) (4,6), and hydrophobic interaction chromatography (HIC) (4,7). Clearly, the strongest case for the use of CZE is found in small-scale, high-resolution analytical separations in which coupling to high sensitivity detection methods can be effectively implemented. Although IEC permits high loading capacities (4), when compared with reversed-phase chromatography (RPC), the resulting resolution is much lower. Sensitivity to the pH of the buffer, the form of the buffer, and the type of stationary phase can have detrimental effects regarding the reproducibility of the separations (8). When hydrophobic stationary phases are selected for IEC, the concentrated salt gradient required in IEC methods causes proteins to bind more strongly to the stationary phase, thus decreasing recovery. This same hydrophobicity is probed in HIC in which salt gradients are used to separate proteins by an adsorption/desorption process (9). The exposed "hydrophobic foot" of the protein adsorbs to the hydrophobic stationary phase at high salt content, desorbing as the salt content decreases during gradient elution (4). Ionic strength, polarity, and solvent strength can affect the degree of denaturization of proteins, which leads to complications in separation capabilities and a loss of biological activity of recovered proteins (4). These difficulties noted, IEC and HIC enjoy wide application. By the same token, methods of greater versatility and lower degrees of complication, among other issues, are continually being developed.

A novel capillary-channeled polymer (C-CP) fiber stationary phase for high-performance liquid chromatography (HPLC) has been described for the separation of diverse organic compounds including polyaromatic hydrocarbons, amino acids, organometallic complexes, and triglycerides employing both isocratic and gradient elution methods (10). Polymeric fibers of different format have been employed as stationary phases for chromatography in the past (11–13). In this application, solid (i.e., not hollow) polymeric fibers of polypropylene having nominal diameters of approximately 50 µm and 8 branched channels running along their periphery are packed into stainless steel tubing (4.6-mm i.d., 305-mm length) for use in separations (10,14). The diameters of the capillary channels range in diameter from approximately 5 to 15 µm on each fiber, but are extremely uniform across the entire fiber population (i.e., on a given spool) (14). The fibers exhibit very strong reversed-phase (RP) (hydrophobic) characteristics, as determined by atomic force microscopy (AFM) with functionalized probe tips (10). Bundles of approximately 1200 fibers are loaded collinearly into the stainless steel tubing, providing flow channels that extend the entire length of the

* Author to whom correspondence should be addressed.

column. As a result, C-CP fiber columns [polypropylene (PP) here] exhibit an approximately 75% decrease in backpressure (650 vs. 2750 psi) in comparison with $C_4$ sphere-packed columns at flow rates of 1.0 mL/min.

We describe a preliminary demonstration of the use of these novel PP C-CP fibers as a stationary phase in the separation of proteins ranging in molecular weight from 12 to 75 kDa. A gradient elution program employing trifluoroacetic acid (TFA) as an ion-pairing agent was readily modified from a published RP-HPLC separation (15,16). The separation of a protein mixture containing superoxide dismutase (SOD), myoglobin, hemoglobin, and hemocyanin occurs with high efficiency and is apparently achieved via a hydrophobic/RP mechanism, as the elution order does not correspond to molecular weight. It is believed that the ability to affect separations under chemically mild conditions with low backpressures on C-CP columns holds a great deal of promise for both analytical and prep-scale protein separations. Other potentially beneficial characteristics include low material costs, ease of fabrication, a wide variety of fiber (polymer) surface characteristics, and the ability to chemically modify those surfaces for improved separations.

## Experimental

### Column preparation

Chromatographic columns were prepared by pulling strands of the polymer fiber through 4.6-mm i.d., 306-mm long stainless steel tubing (Valco Instruments, Houston, TX) (10). This particular format was chosen simply by the ready availability of column hardware of these dimensions. PP fibers (Eastman Chemical, Kingsport, TN) were obtained from a bobbin of fibers measuring more than 1000 m in length. The fibers were manually wound onto a circular wire frame having a diameter of approximately 30 cm to accumulate enough strands to tightly fill the stainless steel tubing. The circularly wound fiber bundle was pulled through the stainless steel tubing by attaching a plastic monofilament (50-lb test fishing line) and passing it through the column. The initial length of the fibers was such that the fiber ends extended past both ends of the stainless steel tube, as shown in Figure 1. The general alignment of the fibers within the column was longitudinally parallel. As a result, broadening by eddy diffusion was expected to be minimal. The fiber lengths were trimmed with a stainless steel razor blade to be flush with the tubing ends; the column ends sealed with 0.75-mm thick, 6.35-mm diameter frits (10-µm pores), and completed with column end fittings (Valco Instruments). Each fiber column consisted of approximately 1200 fibers, having a packing mass of 1.7 grams. Previous column porosity ($\varepsilon_T$) determinations for the polypropylene-fiber columns yielded values of approximately 0.66 (10). The columns were then flushed repeatedly with organic solvent [methanol and acetonitrile (ACN)] and distilled water to remove residual antistatic surfactant coatings applied during the manufacturing process of the fibers.

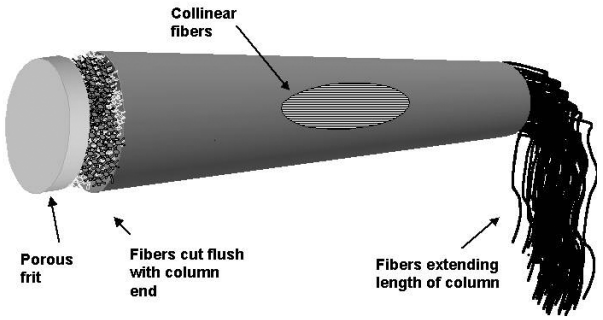


**Figure 1.** Illustration of the C-CP fiber column arrangement (using 4.6-mm i.d. stainless steel tubing and porous frit) during the fabrication process, including cross-sectional exposure of collinear fibers within the column.

### Chromatographic system and operations

The chromatographic system consisted of a Waters (Milford, MA) Model 600S HPLC pump with a 6-port Rheodyne injection valve (Rohnert Park, CA) fitted with a 10-µL injection loop. The C-CP fiber column was mounted in the place of the conventional liquid chromatography column. A Waters 2487 dual wavelength absorbance detector was employed at 216 nm, and the separations were performed at a solvent flow rate of 1.5 mL/min. The chromatograms (absorbance vs. elution time) were generated by the Millennium 32 Chromatography Manager and further processed and managed in the form of Microsoft (Seattle, WA) Excel files.

### Chemicals, reagents, and standards

HPLC-grade water (Fisher Scientific, Pittsburgh, PA) was used for the preparation of all protein solutions. Each protein stock solution was prepared as a 1-ppm (1 µg/mL) solution using 5:95 (ACN–water) containing 0.1% TFA. The four proteins [SOD from bovine erythrocytes (EC No. 232-943-0), myoglobin from horse skeleton muscle (EC No. 309-705-0), hemocyanin from human, and hemoglobin from horseshoe crab] and TFA used in the mobile phase were all purchased from Sigma Aldrich (Milwaukee, WI). The mobile phase for this separation was prepared from HPLC-grade ACN, water, and 2-propanol (Fisher Scientific). The protein test solutions were stored at 6°C.

### Chromatographic separations

The protein test mixture was prepared by mixing 2 mL of each protein stock solution in a 20-mL vial. The column was rinsed with mobile phase 95:5 water and (1:1) propanol–acetonrile for 10 min before each injection. Ultimately, the separation of these four proteins was achieved using a gradient elution of 95:5 to 35:65 water containing 0.1% TFA (v/v)–propanol/ACN (1:1) containing 0.085% TFA) over 70 min at 1.5 mL/min.

## Results and Discussion

### Method development for C-CP separation

Protein retention in RP-HPLC is governed by amino acid constitution, the conformational (i.e., three-dimensional) structure of the protein, and the protein's affinity for the stationary phase being used (4,9,15,17). Unfortunately, proteins frequently undergo denaturation and conformational changes in organic solvents commonly used in RP-HPLC (4). The main objective

here was to successfully separate a protein mixture using the novel C-CP fiber stationary phase with results that would merit further consideration of this stationary phase for RP-HPLC separations of macrobiomolecules. In many protein and peptide chromatographic methods, short alkyl chain stationary phases ($C_2$–$C_6$) are employed to improve adsorption/desorption kinetics and recoveries, along with solvents including water–ACN gradients and ion-pairing agents (3,4,15,16).

Initially, the C-CP fiber separation of proteins was investigated under isocratic conditions employing 100% water, ACN, and propanol, independently, to better understand how the proteins interact with the stationary phase in the presence of different solvents. Use of a 100% water mobile phase resulted in total retention of the proteins. Use of a pure ACN mobile phase produced a single large peak, most likely composed of the total mixture of constituents, with very little retention. Finally, use of propanol solely as the mobile phase yielded one small and slightly retained peak. Additions of various amounts of TFA to the pure solvents induced somewhat greater retention, but did not improve resolution. These responses reflect the general RP characteristics of this nonpolar C-CP fiber stationary phase (PP).

Methods currently used for RP-HPLC of proteins typically employ gradient elutions using ACN and water as mobile phases (15–18). The basic gradient method used for protein separation on this C-CP fiber column was derived from an elution program described by Hearn et al. for protein separations on $C_4$ columns (15,16). The first modification of that method involved incorporation of propanol in the organic phase. Proteins tend to maintain their three-dimensional structure better when propanol is added than with ACN alone (1,18), thus increasing recovery (15). Using a gradient method in which equal amounts of TFA were added to both the aqueous and organic mobile phases, the proteins were fully retained on the column until a 50% ACN content was reached, wherein a single large asymmetrical peak eluted with no separation of the individual components.

Additional modifications to the method of Hearn et al. (16) have included varying the amount of TFA (v/v) added to the mobile phase to compensate for the baseline drift (4) and varying the mobile phase flow rate to enhance the peak shape. Both TFA and heptafluorobutyric acid (HFBA) have been used in many peptide and protein separation studies as hydrophobic ion-pairing and solubilizing agents (3,16,17). The addition of TFA to both aqueous and organic mobile phases is common in protein and peptide separations to enhance hydrophobicity of the stationary phase. TFA also aids in maintaining a more rigid conformation of the protein structure as it elutes from the column (i.e., a lower propensity for denaturation) (4,15). Addition of 0.1% TFA to the aqueous phase alone resulted in the elution of protein mixture as a single band, with no other components observed throughout the remainder of the solvent gradient. Only in the case of adding a small amount of TFA to the organic phase (ACN–propanol) did well resolved peaks evolve (1,4,18). The addition to both of the phases serves to effectively maintain a consistent TFA level throughout the entire gradient.

**Analytical separation of a four-protein mixture**

The eventual optimized gradient elution chromatogram of the four-protein mixture is shown in Figure 2. The solvent gradient ran continuously from a 95:5 water containing 0.1% TFA (v/v)–propanol/ACN (1:1) containing 0.085% TFA (v/v) to a 35:65 aqueous–organic composition over 70 min at 1.5 mL/min. Under these conditions, the inlet pressure of the system was approximately 700 psi. As seen in the Figure, each of the peaks is very well resolved and quite symmetric in profile (Table I). The elution order does not correspond to the analyte molecular weights (31.2, 60, 75, and 17 kDa, respectively). The last eluting component of the mixture (myoglobin) comes off of the C-CP fiber column after approximately 30 min at a 70:30 (aqueous–organic) solvent composition. Based on the previous studies using the PP C-CP columns, the overall character of myoglobin would be expected to be more hydrophobic in nature than the other proteins, under these conditions. Clearly, the degree of hydrophobicity will be affected by the extent of possible protein denaturation under specific solvent conditions. These sorts of determinations are required as the C-CP methodology evolves.

The qualitative aspects of the four-protein mixture are quite encouraging at this point. Table I summarizes the basic characteristics for this particular separation. As is evident in the chromatogram, the peaks are well-separated in time, exhibiting quite good selectivity, in particular. Unfortunately, the high degree of selectivity is not complemented with narrow peak widths, which are on the order of 1 min. It is believed that the breadth of the C-CP peaks is caused by the variation in mobile phase velocities across the different sized capillaries on each fiber, as well as nonidealities in the intrafiber packing. It is possible to use fibers having channels of the same diameter, which will greatly reduce this broadening mechanism. Refinement of the fiber packing process is expected to allow higher densities of fibers to be utilized as well. The consistency of the peak widths over the 30-min separation is encouraging from the point of view of adsorption/desorption kinetics. The combination of high selectivity factors and consistent peak widths yields respectable resolution values, particularly in consideration of the complexities of protein separations and the relatively crude column fabrication methodology used for these investigations. Given the strong tendency for peak tailing for proteins separated by some silica-based RP stationary phases, the relatively high level of peak symmetry observed in each constituent peak of the chromatogram suggests a good deal of promise for future applications. Finally, the peak capacity (PC), which is an approximate measure of the number of peaks that can be resolved under gradient elution conditions, is presented. This value is a function of the peak width and the gradient time ($t_G$). As such, the consistent peak widths yield values that increase as a function of retention time, as would be expected in general. The values themselves, though, point again to a need to reduce the peak widths.

A rather simplistic comparison of the separation of this suite of proteins on a commercial-packed $C_4$ column was performed to get a qualitative comparison of the chemical nature of the surfaces. There was no readily available $C_4$ separation of this precise mixture found in the literature, so the comparison is only relative (in very general terms) and cannot be assumed to be representative of the highest quality of separation that can be achieved when using packed bead columns. Use of the same gradient program on a $C_4$ packed column operating at a flow rate of 1 mL/min caused the termination of the separation because of excess pressure (4000 psi) at 40% organic content point. The protein mixture was

eventually separated on the $C_4$ column, employing the same aqueous mobile phase (water containing 0.1% TFA) and with only an ACN organic mobile phase (containing 0.085% TFA). The gradient method employed was 20–65% organic over 40 min, meaning a start from a more hydrophobic composition that makes direct comparisons difficult. The qualitative figures of merit are reported in Table II, using the same format as in Table I for the C-CP column. The most interesting point of comparison for this set of proteins is the fact that the elution order differs between the two column types. (The actual peak identities were confirmed by single component injections.) In fact, the SOD is the least retained on the C-CP column, yet it shows the highest stationary phase affinity for the commercial column. Although the difference in the organic phase compositions would be expected to cause differences in retention times, it is not likely to be the sole reason for a change in elution order. Here, the differences must be related to the surface interactions. The greatest benefit of the $C_4$ is manifest in the eluting peak widths, which are on the order of one-third of a minute, as opposed to almost a full minute for the C-CP column. These shorter widths, of course, propagate through somewhat higher resolution values and much higher peak capacities. On the other hand, the selectivity values for the C-CP column are quite good by comparison, which bodes well for potential uses in preparative scale work. As would be expected, separations achieved on the short-chain functionalized $C_4$ columns show very good symmetry characteristics that are relatively comparable with those of the C-CP fiber column. Finally, by virtue of the much narrower peak widths, the peak capacity values for the commercial column are much higher than the fiber column. The values here are relatively constant with time, which is a reflection of the generally increasing peak widths across the gradient.

Clearly, rigorous comparisons between column types must be undertaken using well-established (i.e., published) methodologies for the commercial columns. Such comparisons will be made in the future, once the C-CP column packing procedures have been thoroughly evaluated and optimized. Those studies will also look to additional figures of merit, such as column loading and overall analytical throughput.

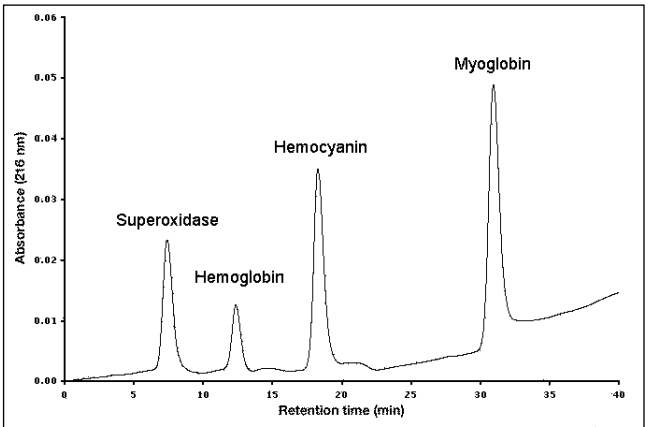


**Figure 2.** Separation of the protein mix containing SOD ($1.8 \times 10^{-4}$M), hemoglobin ($3.4 \times 10^{-4}$M), hemocyanin ($3.0 \times 10^{-4}$M), and myoglobin ($2.8 \times 10^{-4}$M) on a PP C-CP fiber column. UV absorbance detection at 216 nm, flow rate of 1.5 mL/min, with a gradient elution program of 95:5 to 35:65 water containing 0.1% TFA (v/v)–propanol/ACN (1:1) containing 0.085% TFA over 70 min.

**Table I. Figures of Merit for the PP C-CP Fiber Column Separation of Protein Mixture***

| Protein | Retention time ($t_R$) | Retention factor (k′) | Selectivity factor (α) | Peak half-width ($w_{0.5}$) | Resolution (R) | Asymmetry (As) | PC |
|---|---|---|---|---|---|---|---|
| SOD | 7.5 | 2.3 | – | 0.95 | – | 1.4 | 4.6 |
| Hemoglobin | 12.4 | 4.4 | 1.9 | 0.86 | 1.4 | 1.09 | 8.5 |
| Hemocyanin | 18.3 | 6.9 | 1.6 | 0.85 | 1.6 | 1.08 | 13 |
| Myoglobin | 31 | 12.5 | 1.8 | 0.99 | 2.9 | 1.05 | 18 |

* Ten-microliter injection of SOD ($1.8 \times 10^{-4}$M), hemoglobin ($3.4 \times 10^{-4}$M), hemocyanin ($3.0 \times 10^{-4}$M), and myoglobin ($2.8 \times 10^{-4}$M). UV absorbance detection was at 216 nm, flow rate of 1.5 mL/min, with a gradient elution program of 95:5 to 35:65 water containing 0.1% TFA (v/v)–propanol/ACN (1:1) containing 0.085% TFA over 70 min. $t_0$ = 2.3 min. $t_0$ = elution time of unretained solvent, $k' = (t_R - t_0)/t_0$, $\alpha = k'_B/k'_A$ (in elution order), $w_{0.5}$ (in minutes), As = B/A @ 10%, $R = \Delta t_R/w_{avg}$, $PC = t_G/4\sigma_t$, $\sigma_t = w_{0.5}/2.35$.

## Conclusion

The baseline separation of a mixture of four proteins (SOD, myoglobin, hemoglobin, and hemocyanin) was achieved by gradient elution using a novel PP C-CP fiber column. This is an extension of the previous work in the separation of small molecules using inexpensive polypropylene and polyester C-CP fibers (10). Simplistic comparisons with a commercial HPLC column point to some advantageous characteristics, as well as aspects requiring further improvement. Even so, the results from this example suggest a good deal of promise for the separation of other complex biomolecules.

Although it is very early in the development of this methodology, the basic separation characteristics observed to date are promising, though there must be appreciable practical justification for further development. At this point, a few potentially beneficial features have presented themselves for applications in analytical, preparative, and microscale separations: (*a*) surfaces of different hydrophobicity and chemistry are achieved by use of a variety of polymer fibers; (*b*) native fiber surfaces act as the stationary phase, as such the fibers should be more chemically robust than silica beads that are derivatized to achieve hydrophobic character; (*c*) chemical modifications of the surfaces can be affected to achieve different separation characteristics/mechanisms; (*d*) directed flow in the capillary channels yields greatly reduced back pressures in comparison with packed bead columns; (*e*) reduced back pressures make high-speed separations (i.e., flow rates) more practical from the point of pumping hardware; and (*f*) very low cost of the fiber materials (~ $0.15 for the column employed here).

Clearly, much work must be done to demonstrate the efficacy of these potential advantages. Future studies will focus on development of packing methodologies that ensure higher fiber densities with high reproducibility. Potential

**Table II. Figures of Merit for the $C_4$ Column Separation of Protein Mixture***

| Protein | Retention time ($t_R$) | Retention factor (k′) | Selectivity factor (α) | Peak half-width ($w_{0.5}$) | Resolution (R) | Asymmetry (As) | PC |
|---|---|---|---|---|---|---|---|
| Hemocyanin | 12.4 | 5.5 | – | 0.23 | – | 1.05 | 32 |
| Hemoglobin | 16.6 | 7.8 | 1.4 | 0.22 | 3.7 | 1.03 | 44 |
| Myoglobin | 20.7 | 9.9 | 1.3 | 0.7 | 1.9 | 1.08 | 17 |
| SOD | 24.3 | 11.8 | 1.2 | 0.32 | 1.5 | 1.04 | 44 |

* Ten-microliter injection of SOD (1.8 × 10–4M), hemoglobin (3.4 × 10–4M), hemocyanin (3.0 × 10–4M), and myoglobin (2.8 × 10–4M). UV absorbance detection was at 216 nm, flow rate of 1.0 mL/min, with a gradient elution program of 80:20 to 35:65 water containing 0.1% TFA (v/v)–ACN containing 0.085% TFA over 40 min. $t_0$ = 1.9 min.

trade-offs in column geometry (length and inner diameter), including the construction of a microbore/capillary and preparative scale C-CP columns, will be investigated. Only after the assembly methodology is refined can definitive comparisons with commercial columns be performed. Such comparisons may point to deficiencies in one area of separation science while revealing practical advantages in another (e.g., analytical vs. preparative scale). Finally, other C-CP fiber materials, including nylon, polyester, polylactic acid, and ionomeric material, will be evaluated because they offer a very wide range of surface chemistries to affect different modes of separation such as IEC and HIC.

## Acknowledgments

This project was supported in part by the Clemson University, National Science Foundation-sponsored Center for the Advanced Engineering Fibers and Films (CAEFF) under award number EEC-9731680. Helpful discussions with Dr. Shriram Bagrodia of the Tennessee-Eastman Corporation are greatly appreciated.

## References

1. M. Green and K.H. Brackmann. The application of high-performance liquid chromatography for the resolution of proteins encoded by the human adenovirus type 2 cell transformation region. In *HPLC of Proteins and Peptides: International Symposium on HPLC of Proteins and Peptides*. M.T. W. Hearn, F. Regnier, and C.T. Wehr, Eds. Academic Press, New York, NY, 1983, pp. 73–80.
2. J.G. Voet and C. Pratt. *Fundamentals of Biochemistry*. John Wiley & Sons, New York, NY, 2002, pp. 161–91.
3. B.S. Welinder, T. Kornfelt, and H.H. Sorensen. Stationary phases: the achilles heel in HPLC of protein pharmaceuticals. *Anal. Chem.* **67:** 40–43 (1995).
4. R.L. Cunico, K.M. Gooding, and T. Wehr. *Basic HPLC and CE of Biomolecules*. Bay Bioanalytical Laboratory, Hercules, CA, 1998, pp. 67,156, 171–74,192.
5. H.G. Lee, J.L. Tseng, R.R. Becklin, and D.M Desiderio. Preparative and analytical capillary zone electrophoresis analysis of native endorphins and enkephalins extracted from molecular mass of leucine enkephalin. *Anal. Biochem.* **229:** 188–79 (1995).
6. Y. Hue and Peter W. Carr. Synthesis and characterization of new zirconia-based polymeric cation-exchange stationary phases for high performance liquid chromatography of proteins. *Anal. Chem.* **70:** 1734–43 (1998).
7. K.C. O'Connor, S. Ghatak, and B.D. Stollar. Use of hydrophobic interaction chromatography to separate recombinant antibody fragments from associated bacterial chaperone protein Gro EL. *Anal. Biochem.* **278:** 239–41 (2000).
8. L.R. Snyder, J.J. Kirkland, and J.L. Glajch. *Practical HPLC Method Development*. John Wiley and Sons, New York, NY, 1997, pp. 341–46,509–16.
9. S.L. Wu, A. Figueroa, and B. Karger. Protein conformational effects in HIC: retention characterization and the role of mobile phase additives and stationary phase hydrophobicity. *J. Chromatogr. A* **371:** 3–27 (1986).
10. R.K. Marcus, W.C. Davis, B. Knippel, L. LaMotte, T. Hill, D. Perahia, and J.D. Jenkins. Capillary-channeled polymer fibers as stationary phases in liquid chromatography. *J. Chromatogr. A* **986:** 17–31 (2002).
11. M. Czok and G. Guiochon. Aligned fiber columns for size exclusion chromatography. *Anal. Chem.* **53:** 1318 (1981).
12. Y. Yang, Ajoy Velayudhan, C. Ladisch, and M.R. Ladisch. Protein chromatography using a continuous stationary phase. *J. Chromatogr. A* **598:** 169–80 (1992).
13. J. Jinno, J. Wu, H. Sawada, and Y. Kiso. Cellulose acetate fibers as stationary phase in capillary electrochromatography. *J. High Resolut. Chromatogr.* **21:** 617 (1998).
14. W.A. Haile and B.M. Phillips. Deep grooved polyester fiber for wet lay applications. *Tappi J.* **78:** 139–42 (1995).
15. F.E. Regnier. High-performance liquid chromatography of biopolymers. *Science* **222:** 245–52 (1983).
16. M.I. Aguilar and M.T.W. Hearn. High-resolution reversed phase high-performance liquid chromatography of peptides and proteins. *Methods Enzymol.* **270:** 3–26 (1996).
17. J.J. Kirkland, F.A. Truszkowski, and R.D. Ricker. Atypical silica-based packings for high-performance liquid chromatography. *J. Chromatogr. A* **965:** 25–34 (2002).
18. V.E. Turula and J.A. de Haseth. Particle beam LC/FT-IR spectrometry studies of biopolymer conformations in reversed-phase HPLC separations: native globular proteins. *Anal. Chem.* **68:** 629–38 (1996).

Manuscript accepted July 15, 2003.