# Preparation and Characterization of Hyperbranched Polyester Capillary Columns Used for the Separation of Basic Proteins

**Chongqi Shou,[1] Zhiliang Zhang[2]**

[1]*College of Chemistry and Chemical Engineering, University of Jinan, Ji'nan 250022, China*
[2]*Research Center of Analysis and Testing, Shandong Institute of Light Industry, Ji'nan 250353, China*

Received 2 April 2008; accepted 24 August 2008
DOI 10.1002/app.29224
Published online 10 November 2008 in Wiley InterScience (www.interscience.wiley.com).

**ABSTRACT:** A series of hyperbranched polyesters with pentaerythritol as the core were synthesized and coated on the inner surface of fused-silica capillaries by chemical bonding. Three kinds of basic proteins were selected for studying the behavior of the adsorption to fused-silica capillaries. Comparative studies of the coating materials were conducted, and the experimental results showed that the coated columns with hyperbranched polyesters could suppress the electroosmotic flow greatly and effectively prevent adsorption in the pH range of 4–6; they were superior to capillaries coated with traditional hydroxypropyl cellulose. Furthermore, research was conducted to study the effect of hyperbranched polyester generation on the column efficiency. The results showed that higher column efficiency was obtained on a capillary column coated with the sixth generation of the hyperbranched polyester at pH 5.0. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 111: 2141–2147, 2009

**Key words:** hyperbranched; proteins; separation techniques

## INTRODUCTION

As a separation technique, much progress has been made in the theory, instrumentation, and application of capillary electrophoresis (CE) in the past 20 years.[1–3] It is thought that CE could be applied successfully to the separation of basic proteins and could be superior to other separation techniques such as liquid chromatography. However, the application of the technique to basic proteins is complicated by the adsorption of proteins onto the capillary wall, which results in sample peak tailing and broadening with reduced separation efficiencies and resolution. Moreover, the adsorption changes the $\zeta$ potential and produces improper migration times and poor peak reproducibility.[4–6]

To make CE a more practical technique suitable for routine analysis, particularly in the field of basic protein separation, various attempts have been made to eliminate this adsorption and optimize the separation of basic solutes. The reported approaches include the use of extreme pH buffers, high ionic strength buffers, zwitterionic buffers, additives in buffers, and coated columns. Among these approaches, coating the capillary surface with a suitable polymer seems to have various advantages, such as stability and reproducibility.[7] Capillaries have been coated with various methods in previous studies.[8–23] These methods can be categorized into two groups: covalent bonding and adsorption. So far, the former has been the most frequently used in the separation of proteins by CE. At present, the coating materials are mostly traditional linear polymers,[8–17] such as polyacrylamide, poly(ethylene propylene glycol), and poly(vinylpyrrolidinone) epoxy polymer. Although much progress in traditional linear coating materials has been made, there are many problems without resolution,[24,25] such as coating difficulties due to rapidly increasing viscosity corresponding to a higher solution concentration[26,27] and poor reproducibility due to inadequate functional groups to bond with silanol groups on the inner surface of the capillaries.

Hyperbranched polymers are a new kind of functional polymer. One of their most applicable properties is their lower melt viscosity due to their spherical shape in comparison with corresponding linear counterparts of the same molar mass; therefore, it is easy to coat the capillary inner surface. Furthermore, hyperbranched polymers have plenty of groups on their molecular surface, which make bonding with silanol groups in the inner surface of the capillaries easy, and they form a stable, reproducible coating. According to a review of the literature available to us, hyperbranched polyesters with

*Correspondence to:* Z. Zhang (zhangzhiliang0531@163.com).

Contract grant sponsor: Shandong Province Natural Science Foundation; contract grant number: G0645.


© 2008 Wiley Periodicals, Inc.

pentaerythritol as the core have not been coated on the inner surfaces of fused-silica capillaries by chemical bonding to separate basic proteins. In this study, a series of hyperbranched polyesters with pentaerythritol as the core were synthesized, and they were first coated on the inner surfaces of fused-silica capillaries by chemical bonding and applied to improve protein adsorption. The results showed that a high column efficiency of $10^6$ plates/m was obtained, and good reproducibility of the migration time was achieved.

## EXPERIMENTAL

### Reagents and apparatus

#### Apparatus

Fourier transform infrared spectra in the range of 400–4000 $cm^{-2}$ were recorded with a Tensor 27 (Bruker Optik GmbH, Ettlingen, Germany). KBr pellets were used for sample preparation. $^1$H-NMR spectra of hyperbranched polyesters were collected on a Bruker AV400 spectrometer with hexadeuterated dimethyl sulfoxide as the solvent. CE was performed on a Binda 1229 CE instrument (Beijing Institute of New Technology Applications, Beijing, China). Data collection and processing were accomplished on an N2000 work station (Zhejiang University, Zhejiang, China). The electroosmotic flow (EOF) and proteins were detected by column ultraviolet absorbance at 214 nm.

#### Materials

Pentaerythritol acid pentaerythritol and *p*-toluene sulfonic acid were purchased from the Chinese Medical Group (Shanghai Medicament Co., Ltd., Shanghai, China). 2,2-Bis(hydroxymethyl)propic and hydroxypropyl cellulose were obtained from Dongying Saimeike Co., Ltd. (Dongying, China), and Jinan Linuo Co., Ltd. (Jinan, China), respectively. Dimethyl sulfoxide (Tianjin Dacheng Chemical Co., Ltd.) was a chromatogram-grade reagent and was used to detect the EOF of the capillaries. Cytochrome C, lysozyme, and ribonuclease A were all obtained from American Sigma Co., Ltd. The other chemicals were analytical-grade or high-reagent-grade.

Fused-silica capillary tubing (Hebei Yongnian Optical Fiber Factory, Hebei, China) with a 75-μm i.d. was used for the preparation of the capillary column. The optical window was made by the removal of a small section of the polyimide coating from the fused-silica capillary. The total length of the coated capillary was about 55 cm with an effective separation length (distance from the injection position to the detection position) of 35.5 cm. Double-deionized water was used in the preparation of the coated capillary and buffer. The samples were prepared by the dissolution of the appropriate proteins in the running buffer solution with a concentration of 0.5 mg/mL. In this work, the phosphate buffers were prepared by the dissolution of a weighed amount of sodium dihydrogenphosphate in water and the addition of sodium hydroxide to adjust pH values.

### Preparation of the hyperbranched polyesters with pentaerythritol as the core

2,2-Bis(hydroxymethyl)propic (90.0 g), 0.46 g of *p*-toluene sulfonic acid, and 2.0 g of pentaerythritol were added to a four-necked flask. The mixture was heated to 140°C and kept for 2 h at that temperature. After the impurities were removed, the final product was the fourth generation of the hyperbranched polyester (G4). With the same synthesis method, the fifth generation of the hyperbranched polyester (G5) and the sixth generation of the hyperbranched polyester (G6) were obtained by changes in the ratio of 2,2-bis(hydroxymethyl)propic to pentaerythritol. The IR and NMR spectra of G4, G5, and G6 were quite similar because their chemical structures were similar.

IR (KBr pellet, ν, $cm^{-1}$): 3250–3500 (—OH), 1735 (C=O), 1302, 1035 (C—O), 874 (—$OCH_2$), 2980, 2880, 1380 (—$CH_3$), 2935, 1440 (—$CH_2$—). $^1$H-NMR (hexadeuterated dimethyl sulfoxide, 400 MHz, δ, ppm): 1.12 (—$CH_3$), 1.39 (—$CH_2$—), 3.42 (—$CH_2$—O*H*), 4.11 (—COO—C$H_2$—), 4.61, 4.92 (—OH).

The structure of the hyperbranched polyesters is shown in Figure 1.

### Preparation of the coated capillary columns

#### Preparation of the hydroxypropyl cellulose bonded coated columns

The capillary was treated with γ-glycidoxypropyltrimethoxysilane (γ-GPS) for 30 min after the pretreatment,[28] and the remaining γ-GPS was then removed by purging with nitrogen with heating at 90°C for 60 min. After this, 5 mL of hydroxypropyl cellulose (0.4%) and boron trifluoride etherate were passed by the capillary at room temperature for 30 min before the solvent was removed with a vacuum. The reaction was carried out at 90°C under nitrogen pressure.

#### Preparation of the hyperbranched polyester bonded coated columns

The typical coating process was as follows. First, the inner capillary was rinsed with hydrofluoric acid for 5 min at room temperature and flushed with double-deionized water and methanol for 15 min. The capillary was dried with a nitrogen purge with heating at 105°C for 60 min in a gas chromatography oven. Second, the capillary was treated with γ-GPS for 30 min, and the remaining γ-GPS was then

Figure 1 Structure of the hyperbranched polyester with pentaerythritol as the core.

removed by purging with nitrogen with heating at 90°C for 60 min. Finally, a 5-mL hyperbranched polyester solution (10%) was passed by the capillary for 30 min, and the remaining hyperbranched polyester solution was removed by purging with nitrogen. Then, the capillary was heated from 50 to 150°C at the rate of 5°C/min and held at 150°C for 3 h with a nitrogen purge through the column in a gas chromatography oven. The reaction scheme is shown in Figure 2.

Figure 2 Scheme of the hyperbranched polyester bonded to the fused-silica capillary.

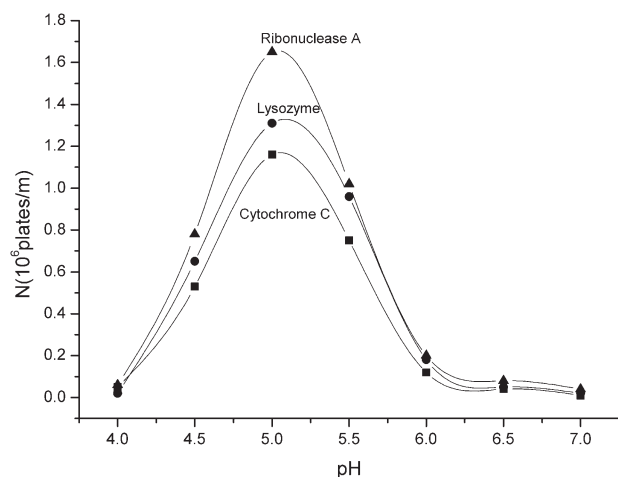


**Figure 3** Effect of the buffer pH on the column efficiency ($N$; conditions: capillary, 51 cm × 75 μm i.d. G5 hyperbranched polyester coated fused-silica capillary column; buffer, 0.04 mol/L phosphate; operation voltage, 18 kV; hydrodynamic injection, 15 s at 10 cm; temperature, 25°C).

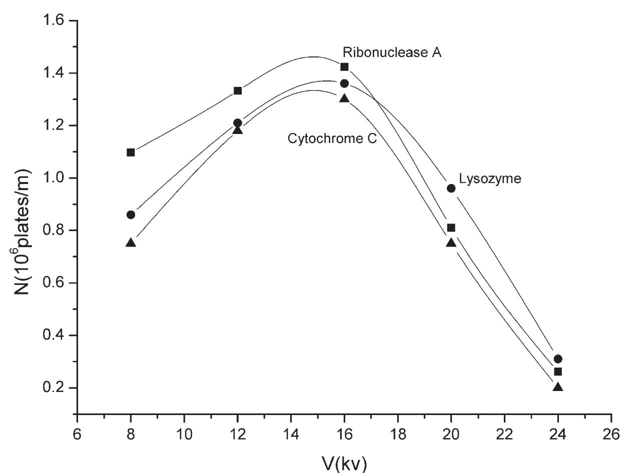


**Figure 4** Effect of the buffer operation voltage ($V$) on the column efficiency [$N$; conditions: capillary, 51 cm × 75 μm i.d. G5 hyperbranched polyester coated fused-silica capillary column; buffer, 0.04 mol/L phosphate (pH 5.0); hydrodynamic injection, 15 s at 10 cm; temperature, 25°C].

### Electrophoresis conditions

Protein solutions of 0.5 mg/mL were introduced into the capillary by hydrodynamic injection at a constant voltage (10–20 kV) for a fixed period of time (10–15 s). A 1% aqueous solution of dimethyl sulfoxide was used to detect the EOF. Each coated column was sequentially rinsed with 1 mL of methylene chloride, methanol, and double-deionized water for 30 min each and with a running phosphate buffer for 24 h before use.

## RESULTS AND DISCUSSION

### Effect of the running buffer pH

The pH of the running buffer has a great influence on the column efficiency. Therefore, it is necessary to study this factor deeply. The running buffer pH effect on the efficiency of a G5-coated column is shown in Figure 3. As the pH increased, the column efficiency increased rapidly. At pH 5.0, the column efficiency reached the maximum between $1.1 \times 10^6$ and $1.7 \times 10^6$ plates and decreased quickly with an increase in the pH of the running buffer. For this reason, it would be better to choose pH 5.0 for maximal column efficiency.

### Effect of the operation voltage

The ideal separation in CE is usually obtained with a voltage as high as possible to obtain the best separation in the shortest time. High voltages lead to difficult heat dissipation during the electrophoretic separation because of the Joule effect. Joule heating is known to affect the EOF and the efficiency of the separation. For this reason, it is important to choose the most appropriate voltage in the electrophoretic separation. A study was made with different voltages varying from 8 to 24 kV in the experiment. In Figure 4, we can observe that the column efficiency of the G5-coated column increased with an increase in the operation voltage; the column efficiency reached its maximum with the operation voltage up to 15.8 kV and then decreased rapidly with higher voltages. Therefore, 15.8 kV was selected as the operation voltage in the separation experiment.

### EOF

The EOF is dependent on the magnitude of the ζ potential across the double-layer surface, which is

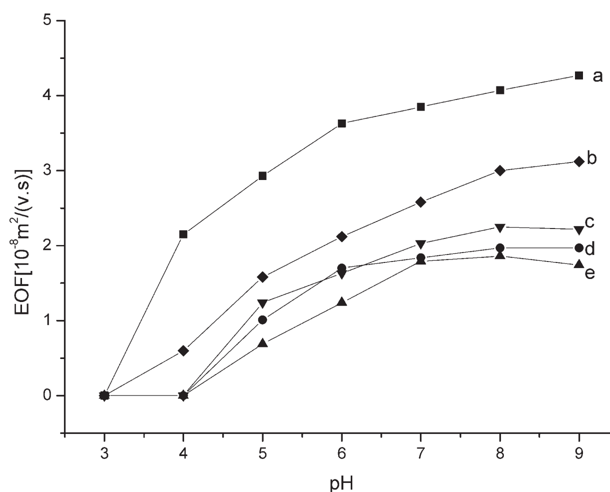


**Figure 5** Effect of the buffer pH on EOF. (a) uncoated column, (b) hydroxypropyl cellulose coated column, (c) G4-coated column, (d) G5-coated column, and (e) G6-coated column (conditions: capillary, 51 cm × 75 μm i.d.; buffer, 0.04 mol/L phosphate; operation voltage, 18 kV; hydrodynamic injection, 15 s at 10 cm; temperature, 25°C; neutral marker, dimethyl sulfoxide).

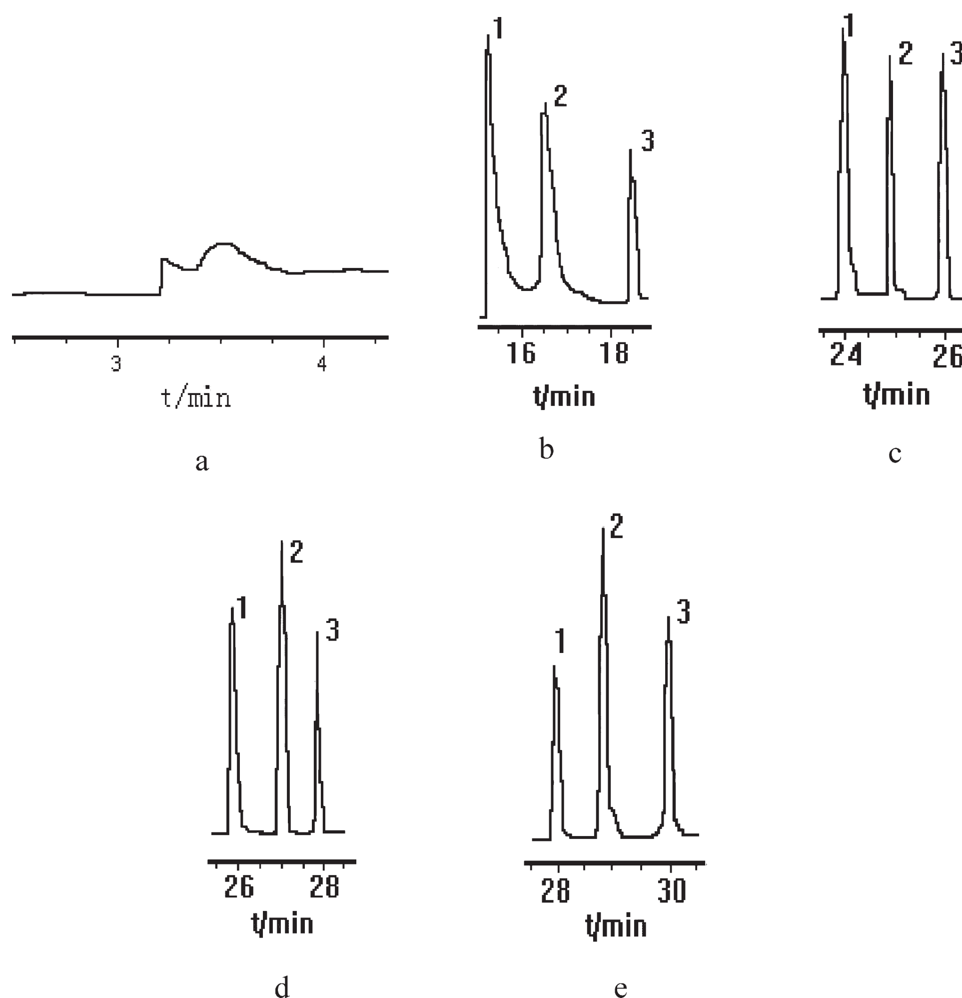


**Figure 6** Separation of basic proteins on coated capillaries: (a) uncoated column, (b) hydroxypropyl cellulose coated column, (c) G4-coated column, (d) G5-coated column, and (e) G6-coated column [conditions: capillary, 51 cm × 75 μm i.d.; buffer, 0.04 mol/L phosphate (pH 5.0); operation voltage, 18 kV; hydrodynamic injection, 15 s at 10 cm; temperature, 25°C]. The peaks are assigned to (1) cytochrome C, (2) lysozyme, and (3) ribonuclease A.

dependent on the charge density at the capillary surface. An increase in silanol ionization at a high pH results in an increase in the EOF. Thus, the magnitude of the EOF reflects the properties of the column inner surface and the effect of the surface treatment. The EOF in the coated capillary was measured as a function of the pH and compared to results obtained with an uncoated capillary (Fig. 5). For an untreated fused-silica capillary, the EOF significantly increased with an increase in the pH from 3.0 to 9.0, in contrast to the gradual increase in the hyperbranched polyester coated and hydroxypropyl cellulose coated capillaries. Moreover, the total EOF decreased more significantly in the hyperbranched polyester coated capillary versus the hydroxypropyl cellulose coated capillary, and this indicated that

**TABLE I**
**Column Efficiency of the Coated Capillaries**

| Coated column | Efficiency ($10^6$ plates/m) | | |
|---|---|---|---|
| | Cytochrome C | Lysozyme | Ribonuclease A |
| Hydroxypropyl cellulose coated column | 0.04 | 0.06 | 0.07 |
| G4-coated column | 0.65 | 1.02 | 1.13 |
| G5-coated column | 0.82 | 1.61 | 2.26 |
| G6-coated column | 1.22 | 2.36 | 3.18 |

The experimental conditions were the same as those in Figure 6.

**TABLE II**
**Reproducibility of the Migration Time for the Coated Columns (RSD %)**

| | Run to run (n = 6) | | Day to day (n = 3) | | Column to column (n = 3) | |
|---|---|---|---|---|---|---|
| | Hydroxypropyl cellulose coated column | G5-coated column | Hydroxypropyl cellulose coated column | G5-coated column | Hydroxypropyl cellulose coated column | G5-coated column |
| Cytochrome C | 1.5 | 0.4 | 2.1 | 1.5 | 1.8 | 0.8 |
| Lysozyme | 0.8 | 0.3 | 1.7 | 0.6 | 1.3 | 0.5 |
| Ribonuclease A | 2.1 | 0.9 | 3.2 | 0.9 | 1.5 | 0.6 |

The experimental conditions were the same as those in Figure 6.

most of the silanol groups were covered. At the same time, the coated column suppressed the EOF more effectively with the generation of hyperbranched polyesters increasing.

### Separation of proteins

The coated capillary was evaluated by the separation of three basic proteins: cytochrome C, lysozyme, and ribonuclease A. Figure 6 and Table I show the efficient CE separation of these three basic proteins. Figure 6 shows that the hyperbranched polyester coated and hydroxypropyl cellulose coated capillaries could separate the three basic proteins reciprocally in comparison with the uncoated capillary. The column efficiency of the hyperbranched polyester bonded coated capillary was better than that of the hydroxypropyl cellulose bonded coated capillary. The reason was that the hyperbranched polyesters had more functional groups than hydroxypropyl cellulose on the molecular surface. As hyperbranched polyesters were coated to the inner surface of the fused-silica capillary, the formed coating was more integrated and stable and could effectively prevent absorption between the inner surface of the fused-silica capillary and the basic proteins. As a result, the column efficiency was higher.

Moreover, the column efficiency of the coated column became better with the generation increasing. The reason is possibly that the hydroxyl amount of the hyperbranched polyesters on the surface was increased quickly with the generation increasing, and the higher generation hyperbranched polyesters had more chances to interact with the anionic silanols in the inner surface of the fused-silica capillary; this made it easier for them to form stable coatings.

### Reproducibility

The coated capillary column was evaluated for run-to-run, day-to-day, and column-to-column reproducibility. The relative standard deviations (RSDs) for the migration time reproducibility of the columns coated by the G5 hyperbranched polyester and hydroxypropyl cellulose are shown in Table II. The migration time reproducibility of the G5-coated column was $<0.9\%$ RSD from run to run, the day-to-day reproducibility was $<1.5\%$ RSD, and the column-to-column reproducibility was $<0.8\%$ RSD. The RSD values for the migration time reproducibility of the G5-coated column were lower than those of the hydroxypropyl cellulose coated column. These low values suggest that the coating method was very reproducible.

## CONCLUSIONS

A new type of coating for CE was developed with hyperbranched polyesters, and this coating could effectively depress the EOF and prevent basic protein absorption to the fused-silica capillary surface in comparison with the traditional polymer coating. Efficiencies greater than $1 \times 10^6$ plates were achieved for three basic proteins, and the column coated by the hyperbranched polyester had good migration time reproducibility. This coating method successfully solves basic protein absorption to the inner surface of a fused-silica capillary and could be a powerful tool for separating biological molecules in the field of biochemistry in the future.

### References


1. Zhao, Z.; Malik, A.; Lee, M. L. Anal Chem 1993, 65, 2747.
2. Kuhr, W. G.; Monnig, C. A. Anal Chem 1992, 64, 389.
3. Novotny, M. V.; Cobb, K. A.; Liu, J. Electrophoresis 1990, 11, 735.
4. Jorgenson, J. W.; Lukacs, K. D. Anal Chem 1981, 53, 1298.
5. Towns, J. K.; Regnier, F. E. Anal Chem 1992, 64, 2473.
6. Hjerten, S.; Kubo, K. Electrophoresis 1993, 14, 390.
7. Run, J. X.; Claire, V. M. J Chromatogr A 1996, 730, 289.
8. Hjerttn, S.; Johansson, M. J Chromatogr 1991, 550, 811.
9. Cobb, K. A.; Dolnik, V.; Novotny, M. Anal Chem 1990, 62, 2478.
10. Schmalzing, D.; Piggee, C. A. F.; Foret, E. J Chromatogr A 1993, 652, 149.

11. Herren, B. J.; Alestine, S. G.; Harris, J. M. J Colloid Interface Sci 1987, 115, 46.
12. Bruin, G. J. M.; Chang, J. P.; Kuhlman, R. H.; Zegets, K. J Chromatogr 1989, 471, 429.
13. Nashabeh, W.; El Rassi, Z. J Chromatogr 1991, 559, 367.
14. Lee, M. L.; Zhao, Z.; Malik, A. Anal Chem 1993, 65, 2741.
15. Mcormick, R. M. Anal Chem 1988, 60, 2322.
16. Towns, J. K.; Bao, J.; Regnier, F. E. J Chromatogr 1992, 599, 227.
17. Liu, Y.; Fu, R. N.; Gu, J. J Chromatogr A 1995, 694, 498.
18. Gilges, M.; Kleemiss, M. H.; Schomburg, G. Anal Chem 1994, 66, 2038.
19. Busch, M. H. A.; Kraak, J. C.; Poppe, H. J Chromatogr A 1995, 695, 287.
20. Ng, C. L.; Lee, H. K.; Li, S. F. J Chromatogr A 1994, 659, 427.
21. Emmer, A.; Jansson, M.; Roeraade, J. J Chromatogr 1991, 547, 544.
22. Muijselaar, W. G. H.; Bruijin, C. H. M. M.; Everaerts, F. M. J Chromatogr 1992, 605, 115.
23. Towns, K.; Regnier, F. E. Anal Chem 1991, 63, 1126.
24. Chiari, M.; Nesi, M.; Sandoval, J. E. J Chromatogr A 1995, 717, 1.
25. Shi, W. F.; Huang, H. Chem J Chin Univ 1997, 8, 1398.
26. Sun, P.; Landman, A. J Chromatogr 1994, 685, 303.
27. Lu, Y.; Lin, D.; Wei, H. Y. Acta Polym Sinica 2000, 4, 411.
28. Malik, A.; Zhao, Z.; Lee, M. L. Microcol Sep 1993, 5, 119.