# Synthesis of Hyperbranched Poly(3-methyl-3-hydroxymethyloxetane) and Their Application to Separate Basic Proteins by Adsorption Coated Column

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

[1]*School of Chemistry and Chemical Engineering, University of Ji nan, Jinan 250022, People's Republic of China*
[2]*Research Center of Analysis and Test, Shandong Institute of Light Industry, Jinan 250353, People's Republic of China*

Received 7 August 2008; accepted 13 August 2009
DOI 10.1002/app.31272
Published online 20 January 2010 in Wiley InterScience (www.interscience.wiley.com).

**ABSTRACT:** A series of hyperbranched poly(3-methyl-3-hydroxymethyloxetane)s with different degree of branching were synthesized using $BF_3{\cdot}OEt_2$ as initiator and coated on the inner surface of the fused-silica capillaries. In the pH range of 3–9, the coated capillaries reduced electro-osmotic flow by about four times lower than the bare fused-silica capillary. The coated capillaries also displayed good resistance to adsorption of cationic proteins, providing clean separations of a mixture of Lysozyme, Cytochrome *c*, and Ribonuclease A around pH 3–6 in phosphate buffer. The separation efficiency in terms of peak shape was excellent compared with bare fuse-silica capillary. The separation efficiency of hyperbranched poly(3-methyl-3-hydroxymethyloxetane) with degree of branching of 0.43-coated capillary column for Lysozyme reached $10^6$ plates/m with a resolution of 7.1, and the coated capillary column had good migration time reproducibility. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 116: 2473–2479, 2010

**Key words:** capillary electrophoresis; hyperbranched polyether; protein separation

## INTRODUCTION

Since first developed in the early 1980s, Capillary electrophoresis (CE) has developed into a powerful analytical technique for separations of biomolecules, including proteins, peptides, nucleic acids, and DNA fragments.[1] It has become one of the leading subjects in analytical chemistry field because of the inherent high-efficiency, high-speed performance, and lower consumption of chemicals and samples.[2,3] Unfortunately, proteins often adsorb onto the inner surface of fused-silica capillary because of diverse interactions including hydrophobic interaction, coulombic interaction, and van der Waals interaction.[4] Especially when basic proteins are analyzed, there exists a considerable amount of adsorption because silica is normally negatively charged above pH 3.0.[5] Adsorption leads to tailing of the sample peak, improper migration time, significant zone broadening, poor reproducibility, and makes proteins fail to separate reciprocally in the most severe cases.

Capillary coatings have proved to be good method for controlling electro-osmotic flow (EOF) and avoiding proteins adsorption onto the inner surface of fused-silica capillary. These methods can be categorized into two groups: covalent bonding and physical adsorption methods. The former is most frequently used in the separation of proteins in CE. Hydrophilic polymers such as polyacrylamide,[6] epoxy polymer,[7] and polysaccharides[8] have been coated via bifunctional anchors such as 3-methacryloxypropyltrimethoxysilane,[9,10] polyvinylmethylsiloxanediol,[11] and Grignard reagent.[12] These covalent bonding coatings are generally stable over a wide range of pH offering flexibility in the choice of separation conditions and do not require regeneration between runs. However, the coating process usually consists of multistep reactions, which are quite difficult to control, laborious, and time consuming.

Comparatively, adsorption method is less difficult for preparing coated column because of the simplicity and speed of the coating protocol and used widely in the CE. For adsorption method, the most important thing is the coating materials. At present, a wide variety of traditional linear hydrophilic polymers[13–18] are used as coating materials because these polymers can be strongly adsorbed onto the fused-silica surface by way of coulombic interaction, hydrogen bonding, or van der waals force, Gilges et al. Although many progresses in traditional linear

*Correspondence to:* C. Shou (scq211@163.com).
Contract grant sponsor: Shandong Province Natural Science Foundation; contract grant number: G0645.

coating materials have been made, there are still many problems to resolve.[19,20] For example, the coating can only be used at pH less than 5, and many coating materials are very difficult to coat in the inner of fused-capillary, for their solution concentration viscosity is higher with their solution concentration increase, and the reproducibility of coated columns is poor because the linear polymers have no adequate functional groups to act with silanol groups of fused-silica capillary.

As a new kind of polymer, hyperbranched polymers have received much attention because of their unique chemical and physical properties as well as of their potential applications in additives, coatings, drug and gene delivery, macromolecular building blocks, nanotechnology, and supermolecular science in recent years.[21–23] For their compact structure and multitude of end groups, hyperbranched polymers have several unique properties different from linear polymers. One of their excellent properties is that they exhibit lower viscosity than the corresponding linear counterparts at the same molar mass because of their highly-branched structure preventing the formation of chain entanglements, so it is easy to coat the capillary inner surface. Furthermore, hyperbranched polymers have plenty of groups on their molecule surface that make it easy to bond with silanol groups in the inner surface of the capillaries and form a stable and reproducible coating. Hyperbranched poly(3-methyl-3-hydroxymethyloxetane) (PMHO) is a new kind of hyperbranched polymers, and the relevant articles describe the synthesis and characterization of branched PMHO.[24–27] Because of compact structure and plenty of hydroxyl end-group as shown in Figure 1, it is anticipated that hyperbranched PMHO would be a good kind of coating materials.

According to review of the literature available to us, hyperbranched PMHO have been not coated onto the inner surface of fused-silica capillary in adsorption method to separate basic proteins. In this article, a series of hyperbranched PMHO with different degree of branching (DB) were synthesized and coated on the inner surface of fused-silica capillary in adsorption method to improve proteins adsorption. The results showed that high-column efficiency of $10^6$plates/m was obtained, and good reproducibility of migration time was achieved.

## EXPERIMENTAL

### Materials and equipment

3-Methyl-3-hydroxymethyloxetane was obtained from Jinan Chenghui Shuangda Chemical , (Jinan, Shandong, China). $BF_3{\cdot}O(C_2H_5)_2$ was purchased from Wujiang Zhenrong Chemical, (Wujiang, Jiangsu, China). Dichloromethane ($CH_2Cl_2$) was from Nanjing Chemical Reagent, (Nanjing, Jiangsu, China). Three basic proteins, Lysozyme, Cytochrome *c*, and Ribonuclease A were purchased from Sigma (St. Louis, MO). All other chemicals were of analytical or high-reagent grade. Fused-silica capillary tubing of 75-μm i.D. was obtained from Hebei Yongnian Optical Fiber Factory, China.

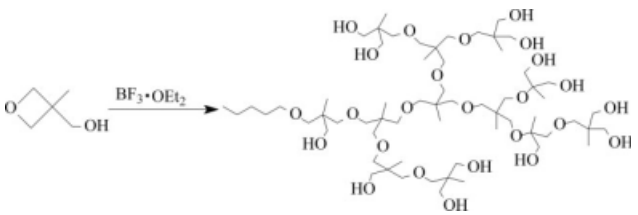


**Figure 1** Synthesis scheme of hyperbranched PMHO.

### Synthesis of hyperbranched PMHO

The polymerization was conducted under a constant stream of nitrogen in the synthesis system. Before performing the reaction, the system was heated to about 100°C and degassed using nitrogen for atleast 20 min. Then, 80 mL of purified $CH_2Cl_2$ and 0.05 mol of $BF_3{\cdot}O(C_2H_5)_2$ (initiator) were added into the flask via syringe, and 0.1 mol of 3-methyl-3-hydroxymethyloxetane(monomer) was introduced through the isobarically funnel within 2 h at the room temperature. After 48 h, the polymerization was quenched with ethanol. The product was then precipitated in distilled water, and the white solid sample was dried at 80°C under high-vacuum, and the sample1 (S1) was prepared. Changing the molar ratio of initiator($I$) and monomer($M$) as $I/M$ 0.05, with the same synthesis method, sample2 (S2) was prepared; Changing the molar ratio of initiator and monomer as $I/M$ 0.01, sample3 (S3) was received. The synthesis scheme of the hyperbranched PMHO was shown in Figure 1.

### Characterization

Fourier Transform Infrared (FTIR) spectra in the range of 400–4000 $cm^{-2}$ were recorded on FTIR TENSOR 27 spectra (Bruker, Germany). KBr pellet was used for sample preparation. $^{13}$C-NMR spectra of hyperbranched PMHO were collected on a Bruker AV400 spectrometer (Bruker AXS, Germany) using $CD_3OD$ as solvent. The DB was calculated from the relative intensity of the $-CH_3$ peaks in proton decoupled-quantitative $^{13}$C-NMR spectra.[28] The molecular weights and their distributions were measured by GPC.

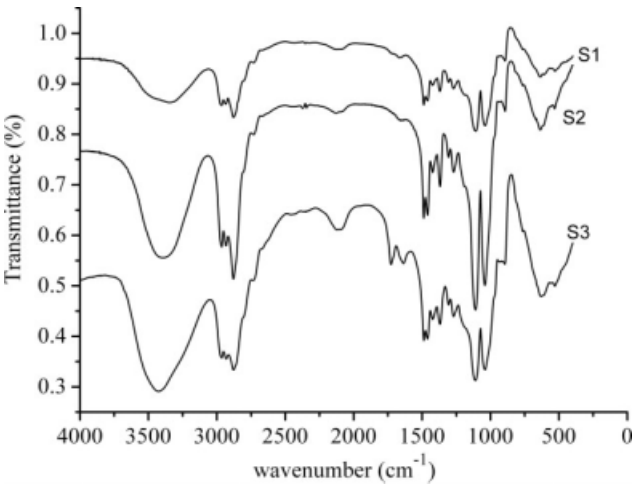


**Figure 2** FTIR spectra for hyperbranched PMHO.

### Procedure of adsorption coated column

First, the fused silica capillary (75 μm i.d., 60 cm total length and 55 cm effective length) was purged with 1*M* sodium hydroxide under 0.2 MPa Nitrogen pressure for 3 h. After rinsing for 10 min with double-deionized water, followed by 10 min with methanol, the capillary was dried with high-purity nitrogen flow. Then, methanol solution at 10% (w/w) hyperbranched PMHO was aspirated into the capillaries for 5 min, and the residual polymers were blowed out of the capillaries under a gentle nitrogen gas flow, followed by 4 h with nitrogen gas flow. With a thin layer of coating deposited dynamically onto the surface of capillaries, the column was heated from 50°C to the final temperature of 150°C at the rate of 5°C min$^{-1}$ and held at the final temperature for 3 h under a gentle nitrogen gas flow in a GC oven.

### Electrophoresis conditions

CE was performed on a Binda1229 CE instrument (Beijing Institute of New Technology Applications, China). The equipment for detection of protein was UV–vis detector, and the proteins were detected at 214 nm. Data collection and processing were accomplished on a N2000 work station (Zhejiang university, China). The 1% aqueous solution of dimethylsulfoxide (DMSO) was used as neuter marker to determine EOF. Protein solutions of 0.2 mg/mL were introduced into the capillary by hydrodynamic injection for a fixed period of time (10–15 s) at constant voltage of 16 kV.

The higher buffer concentrations could lead to large amounts of joule heating that could reduce the separation efficiency. The previous experimental result showed that the joule heating could be neglected while the buffer concentration was below 0.05 mol/L, so phosphate buffer of 0.04 mol/L concentration was used to separate three proteins for getting higher separation efficiency. Each coated column was rinsed with running phosphate buffer for 2 h before using.

## RESULTS AND DISCUSSION

### IR spectroscopy analysis

The FTIR spectra of S1, S2, and S3 were shown in Figure 2. A broad absorption band at 3600–3100 cm$^{-1}$ indicated the presence of plenty of hydroxyl groups. The characteristic peaks at 2960–2850 cm$^{-1}$ were assigned to the methyl and methylene group. Another characteristic peak at 1100 cm$^{-1}$ absorption band was because of C—O—C ether linkage stretching. Other FTIR absorption bands because of —$CH_2$— bending vibration (1470–1430 cm$^{-1}$) and —$CH_3$ bending vibration (1380–1355cm$^{-1}$) could also be observed. The FTIR spectra of S1, S2, and S3 were quite similar because the chemical structure of them resemble each other.

### $^{13}$C-NMR spectroscopy analysis and molecular weights

The $^{13}$C-NMR spectra of S1, S2, and S3 were shown in Figure 3. S1: $^{13}$C-NMR ($CD_3OD$, 400 MHz, δ ppm): 18.36, 17.84, 17.28 m —$CH_3$; 42.70m quaternary carbon atom C; 66.66, 66.77 m —$CH_2OH$; δ = 75.61m —$CH_2OCH_2$—. S2: $^{13}$C-NMR ($CD_3OD$, 400 MHz, δ ppm): 18.43, 17.86, 17.34 m —$CH_3$; 42.72 m quaternary carbon atom C; 66.69, 6679 m —$CH_2OH$; δ = 75.63 m —$CH_2OCH_2$—. S3: $^{13}$C-NMR ($CD_3OD$, 400 MHz, δ ppm): 18.38, 17.81, 17.30 m —$CH_3$; 42.72 m quaternary carbon atom C; 66.59, 66.69 m —$CH_2OH$; δ = 75.56 m —$CH_2OCH_2$—. The $^{13}$C-NMR spectra of S1, S2, and S3 were also quite similar because the chemical structure of them resemble each other.

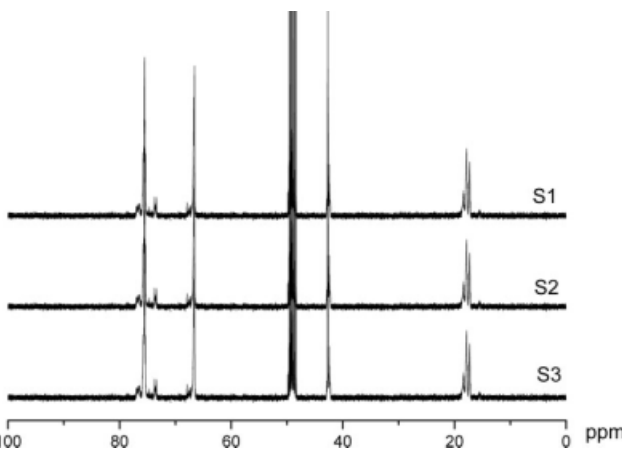


**Figure 3** $^{13}$C-NMR spectra for hyperbranched PMHO.

**TABLE I**
**Polymerization Conditions and Characterization of Hyperbranched PMHO**

| Samples | Molar ratio ($I/M$) | Yield (%) | $M_n \times 10^{-4}$ | Polydispersity | DB |
|---|---|---|---|---|---|
| S1 | 0.5 | 91 | 1.31 | 1.50 | 0.43 |
| S2 | 0.05 | 91 | 1.35 | 1.41 | 0.39 |
| S3 | 0.01 | 93 | 1.43 | 1.35 | 0.21 |

The DB was commonly determined by NMR-spectroscopy on the basis of low molecular weight model compounds, which possessed structures similar to the linear, dendritic, and terminal units in the respective hyperbranched polymers. The DB was obtained by comparison of the intensity of the signals for the respective units. The DB was calculated according to the following equation:

$$\mathrm{DB} = \frac{D + T}{D + L + T}$$

$D$, $T$, and $L$ were the fractions of dendritic, terminal, or linear incorporated monomers in the resulting hyperbranched polymers obtained from integration of the respective signals in NMR-spectra. In this article, the DB was calculated according to the earlier equation. The three peaks near 18.00 ppm are attributed to the dendritic unit ($D$), the linear unit ($L$), and the terminal unit ($T$) in the $^{13}C$ NMR spectra. The DB value could be calculated by integration peak area of the three methyl groups. According to the calculation method,[28] the DB of S1, S2, and S3 were 0.43, 0.39, and 0.21, respectively. The molecular weights of the products were measured by gel permeation chromatography, and the reaction conditions and the characterization results were listed in Table I.

### Electroosmotic flow

One of the goals in developing the coating was to minimize EOF because the negative charges on the capillary surface could cause protein adsorption. But it was necessary to maintain a moderate EOF as it could pump the charged samples across the capillary. In this study, EOF was measured over a range of pH 3.0–9.0 in both coated and uncoated columns, and the results were shown in Figure 4. For an untreated fused-silica capillary, the EOF significantly increased with an increase in pH from 3.0 to 9.0 compared with the gradual increase in the hyperbranched PMHO-coated capillary columns, which showed that the coatings could suppress the EOF effectively. The S1 coated column gave a lower EOF versus the S2 and S3 coated column over the pH range of 3–5, but it was opposite over the pH range of 5–9. The reason was that the low DB in hyperbranched PMHO had enough hydroxyl groups bounding to silanol hydrogen, and resulted in a decrease of the negative charge which was from silicon-hydroxyl (Si-OH) ionized on the inside surface of capillary. A small quantity of unbonded hydroxyl groups of higher DB polymer ionized at higher pH, which made the consistency of electric charge on the inner surface column increase, and zeta potential was reduced.

### Evaluation of coated capillary

The coated columns were evaluated with a mixture of three basic proteins, Cytochrome *c*, Lysozyme, and Ribonuclease A. Figure 5 showed a typical electropherogram of protein mixture in phosphate buffer at pH 5.0 on uncoated and hyperbranched PMHO-coated columns. The protein mixture could not be separated in the uncoated column, for the adsorption result from silanol ionization on the inside surface of capillary, specially. At the same conditions, the protein mixture could be separated successfully in the three hyperbranched PMHO-coated columns. The resolution (Rs) and column efficiency of coated columns were listed in Table II. The results indicated that the DB of 0.43-coated column had good column efficiency and Rs, especially, the column efficiency to lysozyme attained $1.1 \times 10^6$ plates/m.

The coated columns by hyperbranched PMHO showed excellent separation performances, which was strongly related to their molecular structure. Hyperbranched PMHO contained large numbers of

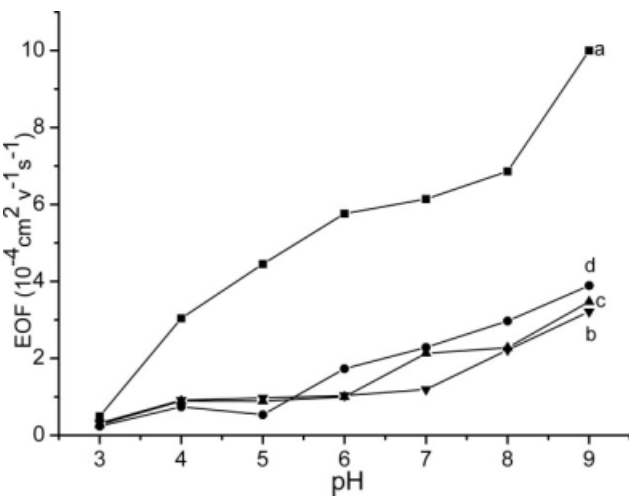


**Figure 4** Effect of the buffer pH on EOF. (a) uncoated fused-silica capillry column; (b) S3-coated column; (c) S2-coated column; (d) S1-coated column. Conditions: capillary 60/55 cm × 75 μm i.D.; buffer, 0.04 mol/L phosphate; operation voltage, 16 kV; hydrodynamic injection 15 s at 10 cm; temperature, 25°C; DMSO as neutral marker.

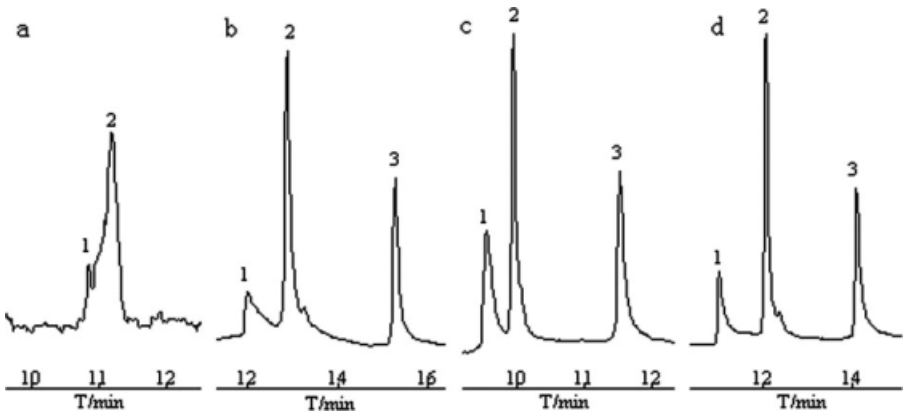


**Figure 5** Seperation of basic proteins by coated capillary columns with hyperbranched PMHO. a, b, c and d same as in Figure 4. Capillary (60/55 cm × 75 μm i.D); 0.04 mol/L phosphate (pH 5.0); operation voltage 16 kV; hydrodynamic injection 15 s at 10cm; temperature, 25°C. Peak identifiction: 1, Cytochrome *c*; 2, Lysozyme; 3, Ribonuclease A.

inner-groups of ether oxygen and end-group of the hydroxyl, which could produce hydrogen bonding effectively with silanol hydrogen. Hence the stable coating was formed. The results in Table II showed that the separation efficiency and Rs were bigger along with the DB of the hyperbranched polyether increased. This was because the average number of molecular periphery hydroxyl group was more, which absorbed to silanol groups existing in the capillary inner wall increased. Thus, the degree of coverage was higher when the polymer was introduced in a capillary.

### Basic proteins separation at different ph

The separation results of basic proteins were shown in Figure 6 and Table III in phosphate buffer at pH range of 3.0 to 7.0. The peak shapes of proteins were good in Figures 6(a–d) around pH 3–6, and the coated column could separate three basic proteins reciprocally. This indicated that the coating was stable and effectively suppressed protein adsorption in the inner fused-silica capillary when the pH of the buffer varifid in the range of 3–6. However, the result was poor at pH 7, and the shape appeared overlaping and broadending of the peaks [Fig. 6(e)]. It was because that only a small part of periphery hydroxyls absorbed to silanol groups in the inner surface of the capillary when the pH was high, which leaded to many adsorption spots to proteins exiting in the inner surface of the capillary. The results in Table III showed higher column efficiency and Rs were obtained on the hyperbranched PMHO-coated column at pH 5.0 in phosphate buffer. Especially, the efficiency for Lysozyme separation reached $10^6$plates/m with a Rs of 7.1 for Lysozyme-Ribonuclease A.

### Reproducibility

The reproducibility of coated capillary columns was evaluated by run-to-run, day-to-day, and column to column. The RSD values for migration time reproducibility of coated columns by hyperbranched PMHO were shown in Table IV. From the Table IV, the migration time reproducibility of coated column by hyperbranched PMHO was <2.8% RSD from run to run, the day-to-day reproducibility was <3.7% RSD,

**TABLE II**
**Column Efficiency and Rs of Basic Proteins in Uncoated and Coated Columns**

| | Column efficiency ($10^5$Plates/m) | | | Rs | |
|---|---|---|---|---|---|
| Columns | Cytochrome *c* | Lysozyme | Ribonuclease A | Rs1 | Rs2 |
| a | – | – | – | – | – |
| b | 0.920 | 3.298 | 3.858 | 1.61 | 2.43 |
| c | 1.826 | 3.746 | 2.614 | 1.75 | 3.37 |
| d | 2.003 | 11.478 | 5.036 | 2.23 | 5.01 |

a, b, c, and d same as in Figure 4; Rs1 and Rs2 were the resolutions between Cytochrome *c* and Lysozyme, Lysozyme and Ribonuclease A, respectively; Same experimental conditions as in Figure 5.

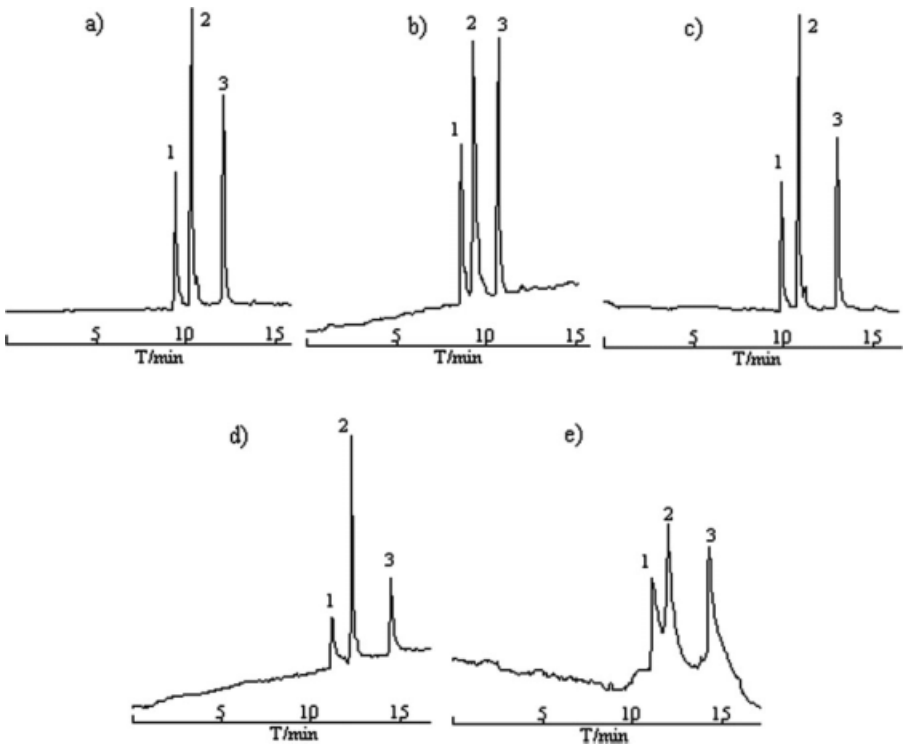


**Figure 6** Seperation of basic proteins by S1 coated column at different pH value. (a) pH 3.0; (b) pH 4.0; (c) pH 5.0; (d) pH 6.0; (e) pH 7.0. Capillary, 53.5/48.5 cm × 75 μm i.D; 0.04 mol/L phosphate; operation voltage 16 kV; hydrodynamic injection 15 s at 10 cm; temperature, 25°C. Peak identifiction: 1, Cytochrome *c*; 2, Lysozyme; 3, Ribonuclease A.

and the column to column reproducibility was <3.4% RSD.

The reproducibility of the coated capillary columns was also evaluated by consecutive protein separation runs and long-time storage. The results showed that the hyperbranched PMHO coating was stable, and its performance was reproducible for more than 400 consecutive runs at pH = 5.0 without obvious loss in separation efficiency. After a month, the separation efficiency of the coated capillary did not decreased obviously. All these indicated that the coated columns by hyperbranched PMHO had good stability.

## CONCLUSION

In this study, hyperbranched PMHO were synthesized and coated the inner wall of fused capillaries. The Preparation procedure of absorption coated column of by hyperbranched PMHO was fast and simple. The coated capillary columns were able to effectively reduce EOF and adsorption of basic proteins, and successfully performed protein separation at the phosphate buffer pH range of 3.0 to 6.0. Efficiencies greater than $1 \times 10^6$ plates were achieved for lysozyme, and $4 \times 10^5$ plates for Cytochrome *c* and Ribonuclease A, and the coated columns by hyperbranched PMHO had good migration time

**TABLE III**
**Column Efficiency and Rs of Basic Proteins in Coated Columns at Different PH Value**

| | Column efficiency ($10^5$Plates/m) | | | Rs | |
|---|---|---|---|---|---|
| pH | Cytochrome *c* | Lysozyme | Ribonuclease A | Rs1 | Rs2 |
| 3.0 | 2.974 | 5.111 | 4.697 | 4.35 | 5.73 |
| 4.0 | 1.943 | 0.906 | 5.205 | 2.15 | 3.29 |
| 5.0 | 4.078 | 11.153 | 4.622 | 3.20 | 7.10 |
| 6.0 | 1.970 | 6.550 | 2.844 | 2.12 | 3.59 |
| 7.0 | 0.329 | 0.226 | 0.182 | 1.31 | 2.76 |

Rs1 and Rs2 same in Table II; Same experimental conditions as in Figure 6.

**TABLE IV**
**Reproducibility of Migration Time for Coated Column (RSD%)**

| | run to run ($n = 6$) | | | day to day ($n = 3$) | | | column to column ($n = 3$) | | |
|---|---|---|---|---|---|---|---|---|---|
| | b | c | d | b | c | d | b | c | d |
| Cytochrome *c* | 2.8 | 2.5 | 2.1 | 3.7 | 3.2 | 2.9 | 3.4 | 3.2 | 2.7 |
| Lysozyme | 2.4 | 2.2 | 1.6 | 3.3 | 2.9 | 2.2 | 2.5 | 1.9 | 1.6 |
| Ribonuclease A | 2.7 | 2.0 | 1.7 | 3.4 | 2.6 | 2.1 | 2.8 | 2.3 | 1.9 |

b, c and d same as in Figure 4; Same experimental conditions as in Figure 5.

reproducibility. The peripheral groups of hyperbranched PMHO with high activity were easy to introduce to other functional groups, which provide a new approach to prepare for various capillary columns with high special selectivity. This study demonstrated the excellent potential of hyperbranched PMHO as coating materials for protein and other biomolecule analysis.

## References


1. Jorgenson, J. W.; Lucas, K. D. Anal Chem 1981, 53, 1298.
2. Liu, Y. J.; Foote, R. S.; Culbertson, C. T.; Jacobson, S. C.; Ramsey, R. S.; Ramsey, J. M. Microcol Sep 2000, 12, 407.
3. Rodriguez, I.; Zhang, Y.; Lee, H. K.; Li, S. F. Y. J Chromatogr A 1997, 781, 287.
4. Dill, K. A. Biochemistry 1990, 29, 7133.
5. Bolt, G. H. Phys Chem 1957, 61, 1166.
6. Hjerten, S.; Kicssling, M. J Chromatogr A 1991, 550, 811.
7. Lin, Y.; Fu, R.; Gu, J. J Chromatogr A 1995, 694, 498.
8. Zhao, Z.; Malik, A.; Lee, M. L. Anal Chem 1993, 65, 2747.
9. Hjerten, S.; Kubo, K. Electrophoresis 1993, 14, 390.
10. McCormick, R. M. Anal Chem 1988, 60, 2322.
11. Schmalzing, D.; Piggee, C. A.; Foret, F.; Carrilho, E.; Karger, B. L. J Chromatogr A 1993, 652, 149.
12. Cobb, K. A.; Dolnik, V.; Novotny, M. Anal Chem 1990, 62, 2478.
13. Wiktorowicz, J. E.; Colbum, J. C. Electrophoresis 1990, 11, 769.
14. Towns, J. K.; Regnier, F. E. J Chromatogr A 1990, 516, 69.
15. Yao, Y. J.; Li, S. F. Y. J Chromatogr A 1994, 663, 97.
16. Gilges, M.; Kleemiss, M. H.; Schomburg, G. Anal Chem 1994, 66, 2038.
17. Busch, M. H. A.; Kraak, J. C.; Poppe, H. J Chromatogr A 1995, 695, 287.
18. Ng, C. L.; Lee, H. K.; Li, S. F. Y. J Chromatogr A 1994, 659, 427.
19. Chiari, M.; Nesi, M.; Sandoval, J. E. J Chromatogr A 1995, 717, 1.
20. Shi, W. F.; Huang, H. Chem J Chin Univ 1997, 8, 1398.
21. Hanselmann, R.; Holter, D.; Frey, H. Macromolecules 1998, 31, 3790.
22. Huang, X.; Wirth, M. J. Macromolecules 1999, 32, 1694.
23. Malmstrom, E.; Hult, A. Macromolecules 1995, 28, 1698.
24. Bednarek, M.; Biedron, T.; Helinski, J.; Kaluzynski, K.; Kubisa, P.; Penczek, S. Macromol Rapid Commun 1999, 20, 369.
25. Hou, J.; Yan, D.; Zhu, X.; Fang, Y. Chem J Chin Univ 1999, 20, 1815.
26. Yan, D.; Hou, J.; Zhu, X.; Kosman, J. J.; Wu, H. S. Macromol Rapid Commun 2000, 21, 557.
27. Mai, Y.; Zhou, Y.; Yan, D.; Lu, H. W. Macromolecules 2003, 36, 9667.
28. Emrich, T.; Chang, H. T.; Frechet, J. M. J. Macromolecules 1999, 32, 6380.