An improved strategy for the preparation of octadecylated silica monolith capillary column with high homogeneity was proposed. Column performance was evaluated by nanoscale HPLC. The design for constructing an integrated nanoelectrospray emitter on the octadecylated silica monolith capillary column was first introduced. In comparison with the separated configuration where the emitter is connected to monolithic capillary column by the aid of a zero dead volume union, the integrated capillary column has the inherent advantage of the minimized extracolumn volume thus providing improved separation quality. The performance of the integrated monolithic capillary column was evaluated by separation of BSA tryptic digest, and peak capacity of 313 with a 50-cm column was obtained. The high separation performance allowed highly confident identification of 662 distinct proteins by analysis of tryptic digest of 0.5 μg of Saccharomyces cerevisiae proteins. The higher separation efficiency by a 60-cm monolithic capillary column increased the proteome coverage with identification of 1323 proteins through assignment of 5501 unique peptides over 400-min gradient elution. Molecular & Cellular Proteomics 5:454–461, 2006.

The ultimate goal of proteomics is to study biological processes comprehensively by the systematic analysis of the proteins expressed in living systems (1). The basis for proteome analysis requires resolution of proteins followed by identification of the resolved proteins. Separation by two-dimensional PAGE is the most widely used methodology in proteomic research (2, 3) as it combines two orthogonal separations to obtain efficient resolution of complex protein mixtures. However, two-dimensional PAGE has limitations such as difficulty to be automated and incompatibility with proteins of extreme pI values and molecular weight, low abundance proteins, and membrane-associated or -bound proteins (4–6). Recently shotgun methodology based on nanoscale HPLC (nano-HPLC) has emerged as an attractive alternative for proteome analysis because of its speed, ease of automation, and compatibility with mass spectrometry (7, 8).

Separation columns for nano-HPLC are usually fabricated by packing particulate beads with a controlled range of diameters and pore size. Particles of smaller diameters are preferably used to achieve a better efficiency, although they are hindered by the increase of backpressure. Recently the concept of monolithic column has been well established in separation technology (9–13). The monolithic structure eliminates the interstitial voids in particulate columns, thus leading to fast mass transfer kinetics during separation. Moreover the enhanced permeability of monolithic rods results in a much lower backpressure, enabling the choice of longer columns to achieve increased separation performance (14, 15). The pores present in the monolithic columns are key parameters affecting separation and permeability. As recommended by IUPAC, pores less than 2 nm in diameter are termed “micropores,” those with diameters between 2 and 50 nm are termed “mesopores,” and those greater than 50 nm in diameter are termed “macropores.” Silica monoliths and synthetic polymeric monoliths represent two major families. Although polymer-based monoliths have such advantages as good biocompatibility and wide application range of pH values, they also undergo shrinking or swelling in organic solvents and may contain domains of micropores negatively affecting the efficiency and peak symmetry. Silica monoliths are porous rods consisting of a silica skeleton with interconnecting macropores. Inside the skeleton a large number of mesopores is present. The macropores can provide fast flow, whereas the mesopores determine the surface area of the monolithic rod, which is necessary for a high maximum loadability of the column.

By connecting with a prepared, replaceable emitter, the monolithic capillary column can be readily interfaced with ESI-MS (16–19). Although the separated emitter design (separate format) is convenient and flexible, the connection of the tapered ESI emitter via a union will increase extracolumn volume and decrease separation efficiency. To circumvent this problem, a dead volume-free configuration was developed by Leinweber et al. (20) where the plain cut end of the monolithic capillary was directly used as the spray emitter. However, this design is not well applicable to nano-ESI because of its inferior sensitivity.

Constructing an effective nano-ESI emitter directly on the capillary outlet (integrated capillary) is preferred because of its...
Integrated Monolithic Column for Proteome Analysis

inherent advantages (21). In this study, a novel strategy was proposed concerning optimization of tailoring mesopores of silica monolith inside a capillary. The performance of the octadecylated silica (ODS) monolithic stationary phase was evaluated by nano-HPLC. Thereafter a facile design for fabricating an integrated ESI emitter on monolithic silica capillary column was proposed. In comparison with the separated emitter design for monolithic columns, the integrated format showed superiority in the nano-HPLC/nano-ESI-MS/MS analysis of protein digest. In addition, the integrated monolithic capillary column was also applied to the analysis of the Saccharomyces cerevisiae proteome, and the potential of utilizing longer monolithic capillary column for highly efficient proteomic analysis was further demonstrated.

EXPERIMENTAL PROCEDURES

Sample Preparation—Tryptic digests of S. cerevisiae proteins and BSA (Sigma) were used to evaluate the performance of nano-HPLC/nano-ESI-MS/MS, and the tryptic samples were prepared according to protocols published elsewhere (7, 22, 23).

Preparation of Silica Monolithic Capillary Column with Octadecylated Stationary Phase—The fused silica capillary for nano-HPLC was obtained from Polymicro Technologies (Phoenix, AZ), and the preparation of native silica monolith inside a capillary may refer to procedures reported elsewhere with modifications (24, 25). In detail, the polymerization mixture containing 1.06 g of poly(ethylene glycol) (molecular weight = 10,000; Aldrich), 4.5 ml of tetramethylothsilicate, and 10 ml of 0.01 M acetic acid was violently agitated to promote hydrolytic reaction in an ice bath for 45 min. The resultant transparent sol was then charged into the pretreated capillary followed by immersion of the capillary into a thermostated bath at 40 °C for 24 h, thus leading to the formation of the macropores of the monolith. Here the composition and acid concentration of the starting solution and the reaction temperature are the key parameters that determine the macropore morphology. The mesopore formation requires further tailoring of the internal pore structure of the monolith in the wet state that can be completed by exchanging the fluid phase with basic solution. In our experiment, the basic environment was produced by thermal decomposition of aqueous urea solution at elevated temperatures; thus the microporous gel skeletons become reorganized into skeletons with mesopores. As a consequence of the independent control of macropore and mesopore sizes, a silica gel monolith with sharply distributed continuous pores in discrete size ranges can be fabricated. After calcination at 330 °C for 25 h to remove the organic moieties, the double pore silica monolith was prepared and ready for subsequent modifications. Surface octadecylation of the monolithic silica capillary was done by continuously delivering silanization solution through the column for about 6 h at 80 °C. The silanization solution contained 10% (w/v) octadecyl(dimethylchlorosilane (ABCR, Karlsruhe, Germany) in anhydrous N,N-dimethylformamide with a small amount of 2,6-lutidine as catalyst. The end capping procedure to minimize residual silanols was carried out by using trimethylchlorosilane (ABCR) as silanization reagent for another 6 h. The thus prepared monolithic ODS capillary column was subsequently flushed with N,N-dimethylformamide and methanol prior to use.

Fabrication of Integrated Nanospray Emitters for Monolithic ODS Capillary Columns—The integrated emitter was prepared by directly tapering the tip from the outlet of the monolithic ODS capillary column. A monolithic capillary column of the appropriate length was chosen and mounted on a Waters 510 HPLC pump. The outlet of the monolithic capillary was heated to adhere to a bare capillary by using a butane torch, and the tapered tip from the monolithic capillary was prepared by drawing the bare capillary. Note that the monolithic capillary was always flushed with water to ensure that the emitter remained clog-free during the process. Thereafter an orifice diameter of 3–5 μm was cut, and the monolithic ODS capillary column integrated with an ESI emitter was then ready for use.

Nano-HPLC and Nano-HPLC/Nano-ESI-MS/MS—Nano-HPLC experiments with UV detection were carried out on a split injection HPLC system consisting of two Shimadzu LC-10ATvp pumps (Kyoto, Japan), a diode array detector for on-column detection (Beckman), and an inject valve (model 7125, Rheodyne) fitted with a T-union, which serves as a splitter, with one end connected to the monolithic capillary column and the other end connected to a flow restrictor that is a 50-μm bare capillary. The nano-HPLC/nano-ESI-MS/MS experiments were performed by interfacing a surveyor MS pump to a Finnigan LTQ ion trap mass spectrometer (Finnigan MAT, ThermoFinnigan, San Jose, CA), Purified water with the addition of 0.1% formic acid was used as mobile phase A, and 0.1% formic acid in acetonitrile was used as mobile phase B. Here a polyethyleneetherketone microcross was used, the fourth point of which was connected with a platinum wire and used to supply a spray voltage of 1.8 kV as described elsewhere (23). The effective flow rate through the monolithic capillary column was 100–300 nL/min, and the heated desolvation of capillary was set at 200 °C. For the analysis of BSA digest, the mass spectrometer was set so that one full MS scan was followed by four MS/MS scans on the four most intense ions from the MS spectrum, whereas for S. cerevisiae, one full MS scan was followed by six MS/MS scans.

Data Analysis—All MS/MS spectra were searched against a protein database using the Sequest algorithm (26). The database for BSA was created in house, and the yeast database was downloaded from a website (ftp://genome-ftp.stanford.edu/yeast/data_download/sequence/genomic_sequence/orf_protein/orf_trans.tasta.gz). Trypsin was set as the enzyme for database search. All output results were combined together using the BuildingSummary software to delete keratins and the redundant data (27). For the analysis of BSA, the Sequest results were only filtered by the cross-correlation score (Xcorr). The peptides were considered positive identifications if their Xcorr was above 1.9 for singly charged peptides, 2.2 for doubly charged peptides, and 3.75 for triply charged peptides. For the analysis of yeast sample, one more criterion was used to filter the search results: ΔCn cutoff values were ≥0.1.

RESULTS

Homogeneity of the Monolithic Structure—Our strategy for optimization of mesopore tailoring can produce monolithic silica structure of high homogeneity. By flushing the wet gel with aqueous urea solution, the solvent exchange step was completed by thermal decomposition of urea solution at elevated temperatures to produce an alkaline pH environment homogeneously around the whole monolithic structure. Also the pH increased as time evolved, strengthening the monolithic structure and tailoring the mesopores. Consequently a uniform monolithic silica structure inside a capillary was achieved, and its homogeneity was characterized by scanning electron microscopy as illustrated in Fig. 1.

Separation of benzene homologues in nano-HPLC was used to characterize the performance of the prepared monolithic ODS capillary column. The solutes were eluted in the order of thiourea < benzene < toluene < ethylbenzene < propylbenzene < butylbenzene according to their increased

Molecular & Cellular Proteomics 5.3 455
hydrophobicities. A linear relationship between the logarithm of the capacity factor (log $k'$) and the carbon number of the solutes was also observed. Column efficiencies of about 90,000 plates/m for these solutes were obtained. The Van Deemter plot obtained by treating the experimental data showed that plate height at higher flow rates increases very slightly, indicating the existence of a unique through-pore channel in the monolithic silica capillary columns.

The Integrated Monolithic Silica ODS Capillary Column for Highly Efficient Proteome Analysis—The photographs of the integrated monolithic capillary column prepared in this study are shown in Fig. 2. In this design, fabrication of an integrated ESI emitter requires only a small length of separation column; thus the performance of the separation column can be easily maintained by fabricating a new emitter if the prepared emitter is broken. Such a feature is important for the integrated monolithic column to be widely accepted in proteomic analysis. For integrated packed capillaries, however, the emitter failure also destroys the separation column, presenting an obstacle for its routine application.

The integrated monolithic ODS capillary column is a valuable tool for highly efficient proteome analysis, and a theoretical measure of its liquid chromatographic separation capability is peak capacity. Peak capacity is defined as the number of peaks that can be separated with a resolution of unity in a given time interval, and it is determined by Equation 1 (28),

$$n_c = \frac{L}{4\sigma}$$  
(Eq. 1)

where $L$ is the total time over which the peptides elute and $\sigma$ is the average standard deviation of the peaks. In the nano-HPLC/ nano-ESI-MS/MS analysis of BSA tryptic digest, improved separation efficiency of the integrated monolithic column was obtained. By examining the elution profile of the moderate intensity peptide of KLVNELTEFAKTCVADSHAGCEK.S extracted from the separation chromatogram, the peak capacity of 313 can be theoretically measured for a 30-cm monolithic capillary column.
(see Fig. 3). However, peak capacity was only 250 when the same monolithic column was interfaced for analysis to a tapered ESI emitter from a 5-cm capillary of 25-μm inner diameter (see Fig. 4), i.e. 20% of the separation efficiency has been sacrificed by this undesirable peak broadening effect. The performance of a 12-cm integrated capillary column packed with 5-μm ODS-AQ was also evaluated with BSA digest as the model sample under gradient elution within 58 min. The peak of the same peptide had 2σ of 0.14 min, and a peak capacity of 214 was obtained. Thus the separation performance of a 30-cm monolithic capillary column is higher than the 12-cm ODS-AQ stationary phase.

S. cerevisiae has been the subject of a wide variety of proteomic analyses. Fig. 5 is the base peak chromatogram for separation of the tryptic digest of 0.5 μg of yeast proteins with a 30-cm integrated monolithic capillary column. The mobile phase flow is ~160 nl/min, and gradient ramp time from 10–35% B is 98 min. Symmetric peaks were observed through the entire separation. The MS/MS data were searched against the S. cerevisiae proteome database using Sequest software, and a total of 662 distinct proteins were identified through assignment of the 1933 unique peptides. However, the 12-cm ODS-AQ packed column enabled only 564 different S. cerevisiae proteins to be identified through
assignment of 1485 peptides under identical experimental conditions. Thus, a 60-cm integrated monolithic capillary column was prepared, and its peak capacity was estimated by loading 0.2 pmol of BSA digest for nano-HPLC/nano-ESI-MS/MS analysis. The eluted peaks had an average $2\sigma$ of 0.30 min over the 120-min gradient elution, thus yielding a peak capacity of 400.

In total, 112 unique peptides in the digested product were detected and matched the 94.23% of total sequence of BSA.

Fig. 6 shows the base peak separation of tryptic digest of 0.5 $\mu$g of S. cerevisiae proteins with a 60-cm integrated monolithic capillary column. By filtering the MS/MS data with the above conservative criteria, a total of 5501 unique peptides were assigned for identification of 1323 proteins from the S. cerevisiae proteome by effective gradient elution in 400 min.

**DISCUSSION**

In contrast to polymer-based monoliths, the mesopore structure determining the surface area of silica monolith is independently tailored. Solvent exchange with aqueous ammonium solution is an efficient way for converting micropores to mesopores (12, 13); however, monolithic silica structure
may be disrupted if it is flushed directly with basic solution. The incorporation of urea in the polymerization solution to generate ammonia (29) is an attractive alternative, although the silica-sol-gel polymerization is influenced to some extent. Our improved strategy is superior to the above two protocols because ammonium hydroxide is substituted with aqueous urea solution for tailoring mesopores of the monolithic structure. The urea solution is of neutral pH and friendly to the monolithic structure, while the increased pH that occurs at elevated temperatures is necessary for mesopore formation. Therefore, the mechanical strength of the monolithic silica structure as well as the through-pore channel allowing mobile phase fast flow-through is well maintained in this process.

Reversed-phase LC-mass spectrometry using an ESI interface has become a routine tool for proteomic analysis. The nano-ESI emitter uses the pulled capillary with a very small spraying orifice and a very low flow rate, which in turn leads to very small droplet sizes and provides higher MS detection sensitivity. The most used interface requires the aid of a zero dead volume union; although easy and flexible to be implemented, it introduces extracolumn void volume, which will in turn degrade the separation quality. In an effort to minimize the undesirable peak broadening, the integration of the ESI emitter directly on the separation column seems to be an attractive alternative as has been used for particulate-packed capillary columns by packing stationary phases into a bare capillary with a prepulled emitter. However, fabrication of on-column integrated ESI emitters for monolithic columns has
Integrated Monolithic Column for Proteome Analysis

to be completed only in the final step. The high torch temperature for drawing the tips will disintegrate part of the monolithic bed (~1.5 cm), therefore water pumping is introduced to flush the silica debris off and ensure that emitters remain clog-free. In addition, ESI tips having very thin walls will hence be produced, possibly providing higher detection sensitivity than the equivalent diameter orifices with thick walls.

The integrated monolithic ODS capillary column showed improved separation efficiency in nano-HPLC/nano-ESI-MS/MS analysis of BSA tryptic digest by excluding the undesirable peak broadening effect caused by the separate emitter capillary. In addition to a reduction in extracolumn volume, the integrated design is also free of extracolumn pressure as is often the case for a separate format in which ESI emitters of thin diameter are coupled. The separation performance of a 30-cm monolithic capillary column was higher than that of the 12-cm ODS-AQ, yet half of the column pressure needed for the packed column was needed to maintain similar flow rates. Although peak capacity of unit separation length is somewhat lower than that for 5-μm ODS-AQ, monolithic columns are still attractive because they can be readily prepared either at any length to yield much higher peak capacity than a particulate column or of thinner capillaries to enhance detection sensitivity.

Monolithic ODS capillary columns were demonstrated to be a powerful tool for highly efficient proteomic analysis as they are capable of offering the unique combination of high hydraulic bed permeability, high separation efficiency, and large surface area, a combination of which cannot be realized with any type of particulate stationary phase. The 30-cm monolithic ODS capillary column allowed 662 S. cerevisiae proteins to be identified through assignment of the 1933 unique peptides. The monolithic column is superior to a particulate column because the higher separation efficiency of the former decreases the dynamic range limitations of a single mass spectrum and the possibility of minor species coeluting with major peptide components, which in turn enhances the effectiveness of peptide detection and identification and increases the S. cerevisiae proteome coverage.

According to the relationship recently described by Shen et al. (30), the longer the separation length, the higher the peak capacity. The highly efficient separation of BSA digested products by the 60-cm monolithic capillary column further increases the number of unique peptides identified, leading to increased coverage for matching the protein sequence. In contrast to the relatively less complex BSA digests, the high efficiency of a long column is more desirable for resolving extremely complex mixtures such as a globular enzymatic digest. Twice as many proteins and unique peptides were identified when the trypsin-digested sample of S. cerevisiae was analyzed, and the S. cerevisiae proteome coverage was raised to an extent that is comparable to two-dimensional separation (7). In conclusion, the longer integrated monolithic capillary column with surpassed peak capacity can be a very powerful tool for highly efficient proteomic analysis.

* This work was supported by National Natural Sciences Foundation of China Grant 20327002, China State Key Basic Research Program Grants 001CB510202 and 2004CB520804, and the Knowledge Innovation Program of the Dalian Institute of Chemical Physics (to H. Z.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
† To whom correspondence should be addressed. Tel.: 86-411-84379610; Fax: 86-411-84379620; E-mail: hanfazou@dicp.ac.cn or zouhfa@mail.dlptt.in.cn.

REFERENCES

1. Aebersold, R., and Goodlett, D. R. (2001) Mass spectrometry in proteomics. Chem. Rev. 101, 269–295

2. Shevchenko, A., Jensen, O. N., Pontierjevikov, A. V., Sagliocco, F., Wilm, M., Vorn, O., Mortensen, P., Shevchenko, A., Boucherie, H., and Mann, M. (1996) Linking genome and proteome by mass spectrometry: large-scale identification of yeast proteins from two dimensional gels. Proc. Natl. Acad. Sci. U. S. A. 93, 14440–14445

3. Perrot, M., Sagliocco, F., Mini, T., Monrobiot, C., Schneider, U., Shevchenko, A., Mann, M., Jeno, P., and Boucherie, H. (1999) Two-dimensional gel protein database of Saccharomyces cerevisiae (update 1999). Electrophoresis 20, 2280–2298

4. Gygi, S. P., Corthals, G. L., Zhang, Y., Rochon, Y., and Aebersold, R. (2000) Evaluation of two-dimensional gel electrophoresis-based proteome analysis technology. Proc. Natl. Acad. Sci. U. S. A. 97, 9390–9395

5. Corthals, G. L., Wasinger, V. C., Hochstrasser, D. F., and Sanchez, J. C. (2000) The dynamic range of protein expression: a challenge for proteomic research. Electrophoresis 21, 1104–1115

6. Molloy, M. P. (2000) Two-dimensional electrophoresis of membrane proteins using immobilized pH gradients. Anal. Biochem. 280, 1–10

7. Washburn, M. P., Wolters, D., and Yates, J. R. (2001) Large-scale analysis of the yeast proteome by multidimensional protein identification technology. Nat. Biotechnol. 19, 242–247

8. Shen, Y. F., Zhao, R., Berger, S. J., Anderson, G. A., Rodriguez, N., and Smith, R. D. (2002) High-efficiency nanoscale liquid chromatography coupled on-line with mass spectrometry using nanoelectrospray ionization for proteomics. Anal. Chem. 74, 4235–4249

9. Zou, H. F., Huang, X. D., Ye, M. L., and Luo, Q. Z. (2002) Monolithic stationary phases for liquid chromatography and capillary electrochromatography. J. Chromatogr. A 954, 5–32

10. Gusev, I., Huang, X., and Horvath, C. (1999) Capillary columns with in situ formed porous monolithic packing for micro high-performance liquid chromatography and capillary electrophoresis. J. Chromatogr. A 855, 273–290

11. Peters, E. C., Petro, M., Svec, F., and Frechet, J. M. J. (1997) Molded rigid polymer monoliths as separation media for capillary electrochromatography. Anal. Chem. 69, 3646–3649

12. Minakuchi, H., Nakaniishi, K., Soga, N., Ishizuka, N., and Tanaka, N. (1996) Octadeclsilanlated porous silica rods as separation media for reversed-phase liquid chromatography. Anal. Chem. 68, 3498–3501

13. Ishizuka, N., Minakuchi, H., Nakaniishi, K., Soga, N., Nagayama, H., Hosoya, K., and Tanaka, N. (2000) Performance of a monolithic silica column in a capillary under pressure-driven and electrodrevined conditions. Anal. Chem. 72, 1275–1280

14. Cabrera, K. (2004) Applications of silica-based monolithic HPLC columns. J. Sep. Sci. 27, 843–852

15. Ikeyama, T., Dicks, E., Kobayashi, H., Morikasa, H., Tokuda, D., Cabrera, K., Hosoya, K., and Tanaka, N. (2004) How to utilize the true performance of monolithic silica columns. J. Sep. Sci. 27, 1292–1302

16. Premstaller, A., Oberacher, H., Walcher, W., Timperio, A. M., Zolla, L., Chervet, J. P., Causoogiu, N., van Dorselaer, A., and Huber, C. G. (2001) High-performance liquid chromatography-electrospray ionization mass spectrometry using monolithic capillary columns for proteomic studies. Anal. Chem. 73, 2390–2396

17. Huang, X. A., Zhang, S., Schultz, G. A., and Henion, J. (2002) Surface-alkylated polystyrene monolithic columns for peptide analysis in capillary liquid chromatography-electrospray ionization mass spectrometry. Anal. Chem. 74, 2336–2344

460 Molecular & Cellular Proteomics 5.3
18. Barroso, B., Lubda, D., and Bischoff, R. (2003) Applications of monolithic silica capillary columns in proteomics. *J. Proteome Res.* 2, 633–642
19. Luo, Q. Z., Shen, Y. F., Hixson, K. K., Zhao, R., Yang, F., Moore, R. J., Mottaz, H. M., and Smith, R. D. (2005) Preparation of 20-μm-i.d. silica-based monolithic columns and their performance for proteomics analyses. *Anal. Chem.* 77, 5028–5035
20. Leinweber, F. C., Schmid, D. G., Lubda, D., Wiesmuller, K. H., Jung, G., and Tallarek, U. (2003) Silica-based monoliths for rapid peptide screening by capillary liquid chromatography hyphenated with electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. *Rapid Commun. Mass Spectrom.* 17, 1180–1188
21. Martin, S. E., Shabanowitz, J., Hunt, D. F., and Marto, J. A. (2000) Sub-femtomole MS and MS/MS peptide sequence analysis using nano-HPLC micro-ESI Fourier transform ion cyclotron resonance mass spectrometry. *Anal. Chem.* 72, 4266–4274
22. Wolters, D. A., Washburn, M. P., and Yates, J. R. (2001) An automated multidimensional protein identification technology for shotgun proteomics. *Anal. Chem.* 73, 5883–5890
23. Delahunty, C., and Yates, J. R. (2005) Protein identification using 2D-LC-MS/MS. *Methods* 35, 248–255
24. Xie, C. H., Hu, J. W., Xiao, H., Su, X. Y., Dong, J., Tian, R. J., He, Z. K., and Zou, H. F. (2005) Electrochromatographic evaluation of a silica monolith capillary column for separation of basic pharmaceuticals. *Electrophoresis* 26, 790–797
25. Xie, C. H., Hu, J. W., Xiao, H., Su, X. Y., Dong, J., Tian, R. J., He, Z. K., and Zou, H. F. (2005) Preparation of monolithic silica column with strong cation-exchange stationary phase for capillary electrochromatography. *J. Sep. Sci.* 28, 751–756
26. Eng, J. K., McCormack, A. L., and Yates, J. R. (1994) An approach to correlate tandem mass-spectral data of peptides with amino-acid-sequences in a protein database. *J. Am. Soc. Mass Spectrom.* 5, 976–989
27. Jin, W. H., Dai, J., Li, S. J., Xia, Q. C., Zou, H. F., and Zeng, R. (2005) Human plasma proteome analysis by multidimensional chromatography prefractonation and linear ion trap mass spectrometry identification. *J. Proteome Res.* 4, 613–619
28. Giddings, J. C. (1991) *Unified Separation Science*, p. 105, John Wiley & Sons, New York
29. Tanaka, N., Kobayashi, H., Ishizuka, N., Minakuchi, H., Nakanishi, K., Hosoya, K., and Ikegami, T. (2002) Monolithic silica columns for high-efficiency chromatographic separations. *J. Chromatogr. A* 965, 35–49
30. Shen, Y. F., Zhang, R., Moore, R. J., Kim, J., Metz, T. O., Hixson, K. K., Zhao, R., Livesay, E. A., Udseth, H. R., and Smith, R. D. (2005) Automated 20 kpsi RPLC-MS and MS/MS with chromatographic peak capacities of 1000–1500 and capabilities in proteomics and metabolomics. *Anal. Chem.* 77, 3090–3100