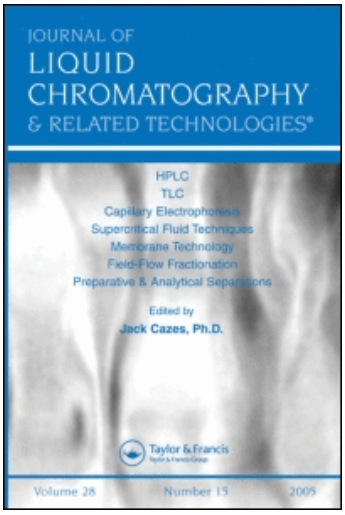

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SEPARATION BEHAVIOR OF AVIDIN AND RELATED SUBSTANCES USING A COMBINATION OF ZWITTERIONIC SURFACTANT AND C_8 PHASE CAPILLARY

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SEPARATION BEHAVIOR OF AVIDIN AND RELATED SUBSTANCES USING A COMBINATION OF ZWITTERIONIC SURFACTANT AND C_8 PHASE CAPILLARY

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ABSTRACT

The separation of mixtures of avidin, streptavidin, and neutravidin were investigated by using combinations of C_8 phase capillary and some surfactants such as anionic, cationic, zwitterionic, nonionic surfactants, polyvinyl alcohol (PVA), and hydroxyl cellulose (HEC) polymers. The combination of zwittergent 3 – 14 and C_8 phase capillary gave the best separation of avidin and its relatives. The satisfactory reproducibility of the peak height and the migration time were achieved by this system. The complete separation of the mixture of avidin, streptavidin, neutravidin, and other three basic proteins was achieved with sharp peaks in pH 5, 7 and 9 of running buffers.

INTRODUCTION

Nowadays, the developments of biotechnology and molecular biology have increased the demand for protein separations. In particular, high resolution, fast analysis, high sensitivity, are required for protein separation. Capillary electrophoresis (CE) has a great potential to meet these requirements.[1]

In CE, a fused silica capillary column is still most commonly used for a separation channel, due to its flexibility, uniform inner diameter, and excellent optical property.[2] The negative charges of silanol groups on the inner surface of fused silica capillary is very important in CE because they produce an electroosmotic flow (EOF), which is one of the motive forces for CE. However, the negative charges often cause the severe adsorption with cationic substances due to the electrostatic interaction. They lead to peak tailing and poor reproducibility.

In the case of protein separations, adsorption by hydrophobic interaction occurred between the inner surface of a capillary and proteins.[3-5] To avoid this adsorption, various approaches have been attempted in the last decade: the use of a low pH,[6-7] a high pH,[8-9] and a high salt concentration;[10-12] permanent coating on the capillary surface;[13-15] and dynamic coating to the capillary surface by additives such as polymer, zwitterionic salt, and surfactant.[16-20] Moreover, as a novel method for protein separations, the use of coated column based on sol-gel chemistry;[23] the combination of hydrophilic coating and polysiloxane-bonded columns;[24] and stable capillary coating with successive multiple ionic polymer layers[25] have been reported.

Various types of the permanent coating capillaries are commercial available now. These capillaries can be classified into a hydrophilic-polar bonded phase column or a hydrophobic type column. The hydrophilic polymer is said to be ideal for protein separations in CE. However, hydrophilic permanent coatings need a careful treatment because the coating is sensitive to the run condition such as pH. Therefore, it is difficult to use hydrophilic type columns in wide range of pH. On the other hand, the hydrophobic coating is relatively stable and can be used in wide range of pH from approximately 3 to 9.

Some attempts with respect to separations of proteins using these coating capillaries[20,24] have been reported. However, most of studies for protein separations were performed on the basis of dynamic coating to an untreated capillary.[22,26-28]

In this paper, we investigated protein separations by using combinations of permanent and dynamic coatings. Samples used in this study were avidin, its relatives, and three other basic proteins. Avidin, which is one of the glycoprotein, is used widely as avidin-biotin technology because of strong binding with biotin.[29] However, avidin and its relatives have strong adsorptivity on the inner surface of the untreated fused silica capillary.

Therefore, it is supposed that the separation of avidin and its relatives by CE using an untreated fused silica capillary is difficult. In this study, the possibility of the combination of permanent capillary and dynamic coating for separation of avidin and its relatives was demonstrated.

EXPERIMENTAL

Apparatus

The commercial coating capillary, CElect™ H150 (C_8 phase, 50 μm i.d.) was purchased from SUPELCO (Bellefonte, USA), and a fused silica capillary (untreated, 50 μm i.d.) was purchased from GL Science (Tokyo, Japan). Spectra phoresis™ 2000 (Thermo separation products, San Jose, USA) was used to apply high voltage, and to detect UV absorbance of samples. Both of C8 phase and untreated fused silica capillary were operated at total length 44 cm (37 cm from inlet to detector). The UV absorbance of the samples was measured at 214 nm. Injection of the sample solutions was performed by hydrodynamic method from positive end for 10 s. A constant voltage +15 kV was applied, but when cationic surfactant was added in the buffer, -10 kV was applied because the direction of migration was reversed.

The preparation of capillary and analyses were performed at 25°C. Benzyl alcohol (0.05 % (v/v)) was used as the EOF marker. The C8 coating capillary was washed with 50 % (v/v) acetonitrile for 2 minutes and methanol for 2 minutes, followed by 2 minutes with a running buffer. An untreated capillary was washed with methanol for 2 minutes, deionized and distilled water for 2 minutes, and 0.1 M sodium hydroxide for 2 minutes, followed by 2 minutes with a running buffer.

Reagents

Avidin from egg white, streptavidin from Streptomyces avidinii, trypsin, lysozyme from egg white, and cytochrome C from horse heart were purchased from Wako Pure Chemical Inc. (Osaka, Japan). Neutravidin™ (biotin-binding protein) was purchased from Pierce (Rockford, Illinois, USA). Sample solutions were prepared by dissolving the above reagents in 0.1 M sodium phosphate (pH 7) at about 1.0×10^{-4} - 5×10^{-5} M. The mixed samples were prepared by mixing equal volumes of each solution.

Buffer Conditions

Sodium dodecylsulfate (SDS, $CH_3(CH_2)_{11}OSO_3Na$) (Sigma, St. Louis, USA), as an anionic surfactant, was prepared to 10 mM with 20 mM sodium phosphate. Polyethylenesorbitan monolaurate (Tween 20) and polyxyethylene 23 lauryl ether (Brij 35), as nonionic surfactants, were also purchased from SIGMA, which were prepared to higher concentration than the critical micelle concentrations (cmc) with 20 mM sodium phosphate, (Tween 20, 0.1 mM and Brij35, 0.5 mM), respectively. Dodecyl trimethyl ammonium bromide (DTAB,

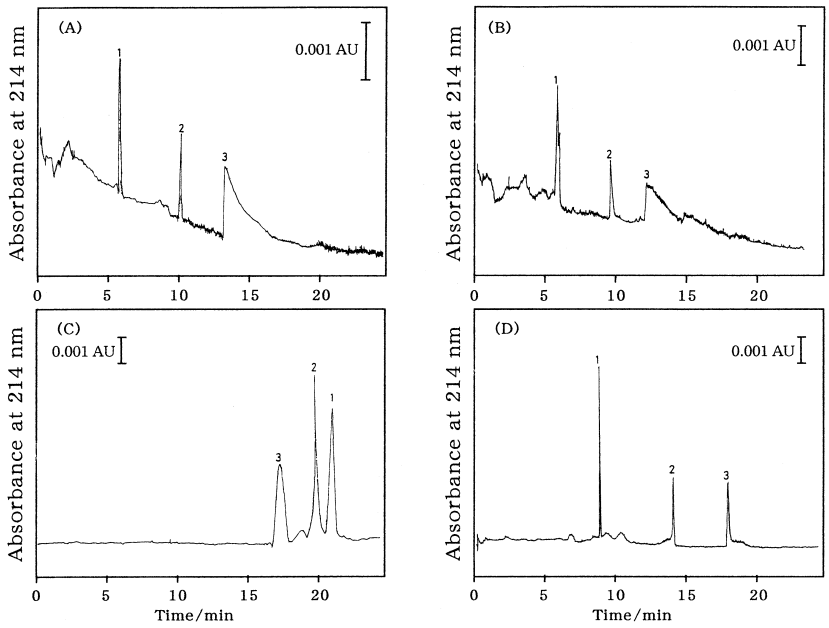

Figure 1. Separation of avidin and its relatives. Sample numbers: (1) avidin; (2) neutravidin; (3) streptavidin. Buffers: (A) 0.05 % PVA and 20 mM phosphate with 0.1 M NaOH; (B) 0.05 % HEC and 20 mM phosphate with NaOH; (C) 6 mM DTAB and 15 mM phosphate; (D) 5 mM zwittergent 3-14 and 15 mM phosphate; Other conditions: same as in Table 1.

$CH_3(CH_2)_{11}N(CH_3)_3Br$) (Sigma) as a cationic surfactant was prepared at 6 mM. Benzyl alcohol (EOF maker), polyvinyl alcohol (PVA) (Hydrozoas grade 99%, average Mw 89,000-90,000) were purchased from Aldrich (Steimheim, Germany).

Hydroxyl cellulose (Cellosize WP 40. Middle viscosity) was from Fluka (Neu-Ulm, Germany). Two hydrophilic polymers were prepared by 0.05 % (v/v) with 20 mM sodium phosphate.

Zwittergent 3-14 ($CH_3(CH_2)_{13}N(CH_3)_2(CH_2)_3SO_3Na$), as a zwitterionic surfactant, was purchased from Calbiochem (La Jolla, USA). All buffers were adjusted to 50mM ionic strength with NaCl.

Table 1

Separation Efficiency and the Reproducibility of Migration Time for Avidin Relatives by CZE

Approaches	% RSD of Proteins	Efficiency Migration Time	(Plates/m)
SDS (n = 3)[a]	Avidin	20.64	4,500
Tween 20 (n = 5)[b]	Avidin	8.35	32,000
	Streptavidin	10.38	17,000
Brij 35 (n = 5)[c]	Avidin	7.68	32,000
PVA (n =5)[d]	Avidin	1.82	280,000
	Streptavidin	4.23	88,000
	Neutravidin	2.89	132,000
HEC (n = 5)[e]	Avidin	1.93	220,000
	Streptavidin	5.23	48,000
	Neutravidin	2.87	112,000
DTAB (n = 3)[f]	Avidin	3.46	120,000
	Streptavidin	6.23	320,00
	Neutravidin	3.34	88,000
Zwittergent 3-14 (n = 5)[g]	Avidin	1.52	330,000
	Streptavidin	2.12	230,000
	Neutravidin	1.97	298,000

a) 10 mM SDS and 20 mM phosphate (pH 6); b) 0.1 mM Tween 20 and 10 mM phosphate with NaCl (pH 5); c) 0.5 mM Brij 35 and 10 mM phosphate with NaCl (pH 5); d) 0.05 % PVA and 20 mM phosphate with 0.1 M NaOH; e) 0.05 % HEC and 20 mM phosphate with NaOH; f) 6 mM DTAB and 10 mM phosphate; g) 5 mM zwittergent 3-14 and 15 mM phosphate; All buffers adjusted to 50 mM ionic strength with NaCl and pH 5 buffer with 0.1M NaOH. Conditions: capillary length, 44 cm (37 cm to detector); applied voltage, 15 kV and that of adding DTAB –10 kV; temperature, 25 °C; sample, 10^{-4} M of each protein

RESULTS AND DISCUSSION

Separation of Avidin, Streptavidin, and Neutravidin by Combinations of C_8 Phase Capillary and Several Surfactants or Hydrophilic Polymers

C_8 phase capillary can suppress adsorption by the decrease of the electrostatic interaction between proteins and capillary wall because the octyl

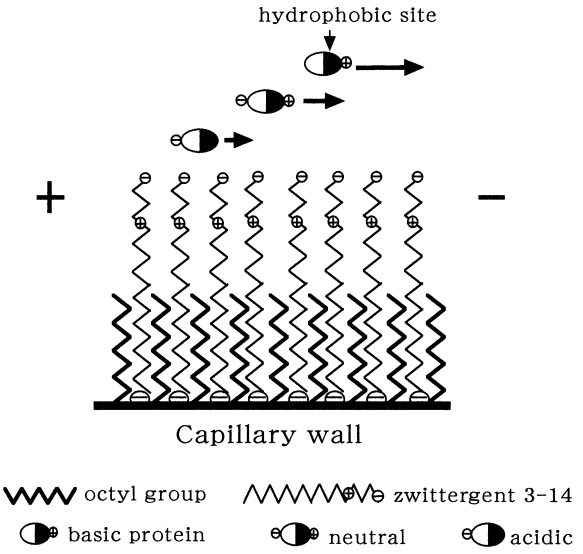

Figure 2. Schematic of C_8 phase capillary and zwittergent 3-14 in the capillary wall.

group of C8 capillary inhibited protein having charges to approach close to the capillary wall. However, it is impossible to suppress adsorption by hydrophobic interaction with protein. Therefore, surfactants or polymers were added to a running buffer in order to have a hydrophilic property on a C_8 modified capillary.

Table 1 shows the separation efficiency and the reproducibility of the migration time of avidin relatives, which were migrated in the combination system of C8 coating capillary and some surfactants (or hydrophilic polymers). The combination of SDS and C8 phase capillary could not separate avidin and neutravidin, because of the strong interaction between negative charges of SDS micelle and positive charges of these proteins. Tween 20 and Brij 35, nonionic surfactant could improve a separation of each protein. However, the RSD of migration time and the separation efficiency were not improved significantly because these surfactants did not completely inhibit absorption of proteins. The addition of PVA, HEC, DTAB, and zwittergent 3-14 gave good results. In particular, zwittergent 3-14 with the C_8 phase capillary gave better results than those by other polymers in both of the RSD of migration time and the separation efficiency.

Figure 1 shows the electropherograms of the mixture of avidin, neutravidin, and streptavidin using four combinations of PVA, HEC, DTAB, and zwittergent with the C_8 phase capillary at pH 5. As using PVA, HEC, and

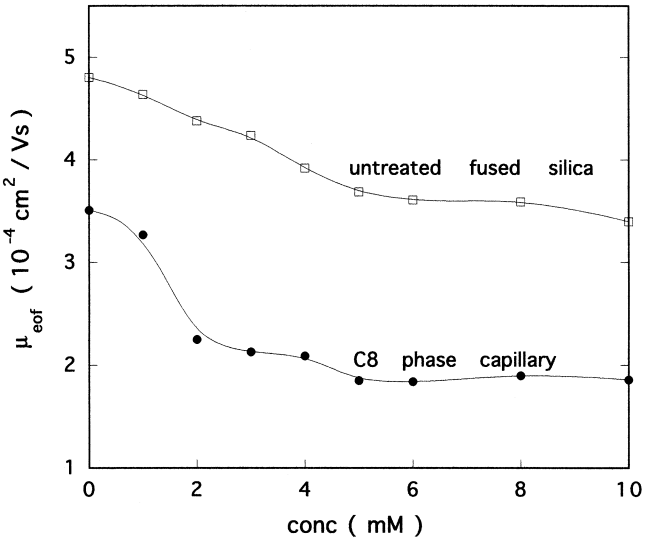

Figure 3. Effect of zwittergent 3-14 concentration on the EOF at pH 7. Conditions: applied voltage, 20 kV; temperature, 25°C; capillary length, 44 cm (37 cm to detector); buffer, 10 mM phosphate buffer adjusted to 50 mM ionic strength with NaCl.

DTAB, the peaks of avidin and neutravidin were relatively sharp. However, the peak of streptavidin was broad, additionally, the RSD value of migration times and the separation efficiency of the peak was worse than that of avidin or neutravidin as shown in Table 1.

In the case of hydrophilic polymers such as PVA or HEC, coatings for the C_8 phase capillary might not be so stable at pH 5. The use of DTAB having positive charges resulted in severe adsorption of streptavidin with negative charges, because of the electrostatic interaction between anionic protein and cationic surfactant coated on the surface in the capillary.

The combination of the C_8 phase capillary and zwittergent 3-14 achieved a complete separation with sharp peaks for three avidin relatives. In this system, alkyl chain of zwittergent 3-14 is thought to be oriented to the octyl groups coated on the surface and a hydrophilic part of the zwitterion on face to the solutions as shown in Figure 2.

Therefore, this zwitterion might suppress both electrostatic and hydrophobic interactions between proteins and a capillary wall. From these advantages using zwittergent 3-14 with C_8 phase, we attempted to characterize the effect of zwitterion on the EOF and protein separations.

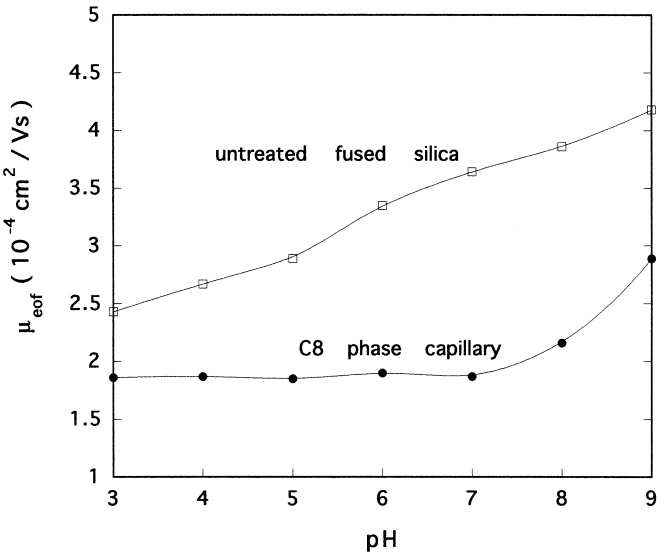

Figure 4. Effect of pH on the EOF. Conditions: as in Figure 3.

Effect of Zwittergent 3-14 Concentration and pH on EOF

Figure 3 shows the effect of the concentration of zwittergent 3-14 on the EOF at pH 7. In the case of an untreated capillary, the mobility of the EOF slightly decreased with the increasing of the concentration of zwittergent 3 - 14 from 0 to 2 mM. A rapid reduction of EOF was obtained at the concentration of zwittergent 3 - 14 from 2 to 4 mM. On the other hand, at the C8 coated capillary, a decrease of the EOF was obtained when the concentration of zwittergent 3 - 14 exceeded 1 mM. The difference in the effect of zwittergent 3 - 14 concentration between the untreated fused silica and C8 phase capillary was predicted due to the effective charges on inner surface of each capillary. That is, the untreated fused silica capillary, has considerable negative charges from dissociation of silanols. This charge would be shielded by the adsorption of zwittergent on the capillary wall. On the other hand, in the case of the C8 coated capillary, since octyl groups are covalent by coated on inner surface of the capillary, the negative charge on the capillary surface is already shielded.

The formation of the second layer with zwittergent 3-14 keeps negative charges at the capillary surface away. Figure 4 shows the effect of pH of a running buffer on the EOF mobility when the concentration of zwittergent 3-14 is 5 mM. In an untreated capillary, an increase of EOF mobility was observed when pH of a running buffer exceeded above 6. On the other hand, EOF mobility of a C_8 coating capillary was about 1.8 (x $10^{-4}$$cm^2$ / Vs) over the range

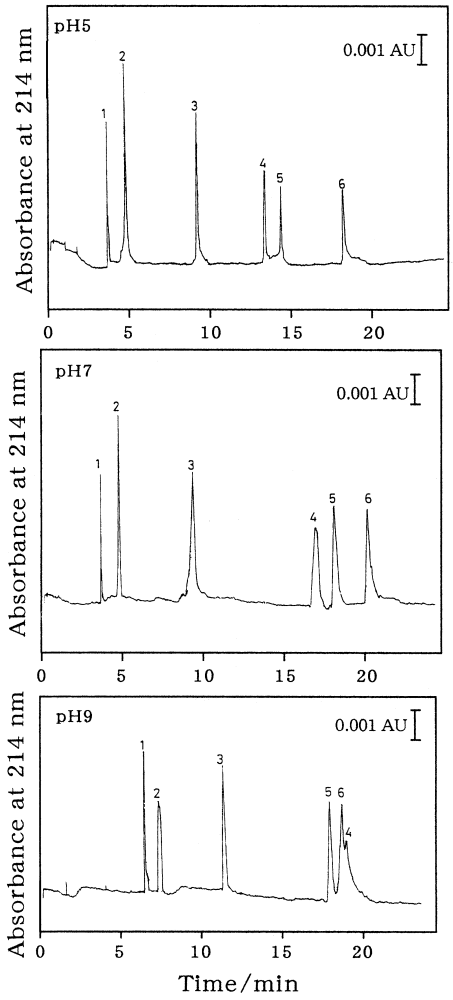

Figure 5. Separation of avidin, its relatives and some basic proteins over a range of pH values. Samples: (1) lysozyme; (2) cytochrome C; (3) avidin; (4) trypsin; (5) neutravidin; (6) streptavidin. Conditions: applied voltage, 15 kV; temperature, 25°C; capillary length, 44 cm (37 cm to detector); buffer, 10 mM phosphate and 5 mM zwittergent 3-14 buffer adjusted to 50 mM ionic strength with NaCl; sample, 5×10^{-5} M of each protein.

of pH 3 to 7. When pH of a running buffer was 8 and 9, the EOF mobility became about 3.0 (x $10^{-4}cm^2$ / Vs) and the RSD of migration time was also stable with < 1%. It was found that this system enabled the use of zwitterionic surfactant at lower concentration and in the wider range of pH than that of an untreated capillary. As shown in figure 3 and 4, the EOF could not be completely suppressed. However, the EOF in zwitterionic surfactant could show a relatively stable value at the wide range of pH. Thus, in this system, it is possible to migrate and detect protein having negative charges if the electrophoretic mobility is smaller than the mobility of EOF.

Separation of Avidin, Its Relatives and Some Basic Protein by Using the Combination of C8 Coated Capillary and Zwittergent 3-14

Figure 5 shows the influence of pH on the electropherograms for a mixture of six proteins, avidin, neutravidin, and streptavidin, cytochrome C, lysozyme and trypsin, by using combination of zwittergent 3–14 with C_8 coating capillary. Migrating times of streptavidin having pI 5-6, and neutravidin having pI 6.3 ± 0.3 decreased rapidly at pH 5 and 7, respectively. Delay of migration time of trypsin was obtained in buffer pH 7. However, all proteins could be detected with relative sharp peaks in the wide range of pH.

The simultaneous separation could be achieved because electrophoretic mobilities of streptavidin and neutravidin having negative charges, were smaller than the mobility of EOF at pH 7 and 9. Thus, the migration system of C8 coating capillary and zwitterion was effective to separate three proteins having different kinds of charges. The averages of the RSD of migration time of six of proteins were 0.5 - 2.0% at pH 5 - 9. Zwittergent 3 - 14 bound with octyl group of C8 coated capillary orientated the hydrophilic part having the both of positive and negative charge on the surface. Therefore, adsorption of proteins with negative and positive charge on the inner surface of a capillary was suppressed by partial electric repulsion under the condition of the alkali, neutral, and acidic pH.

CONCLUSIONS

Depression of adsorption between proteins and inner surface of capillary was performed using combinations of permanent and dynamic coating. The combination of zwitter-ionic surfactant and C_8 coated capillary provided most effective separation for the mixture of avidin, neutravidin, and streptavidin. It was thought that alkyl chain of zwittergent 3–14 was fixed to octyl group and its zwitterion was orientated on the inner surface of C_8 phase capillary. Therefore, it was found that this capillary could be used at lower concentration of zwitterionic surfactant and in a wider range of pH than that of an untreated capillary. Good separations and reproducibility of migration time for a mixture of six proteins were obtained at pH 5, 7 and 9.

REFERENCES

1. P. D. Grossman, J. C. Colburn eds., **Capillary Electrophoresis, Theory and Practice, Part III**, Academic Press, San Diego, 1992.

2. B. L. Karger, F. Foret, in **Capillary Electrophoresis**, N. Uzman, ed., Marcel Dekker, New York, 1993, in press.

3. J. W. Jorgenson, K. D. Lukacs, Science, **222**, 266-272 1983.

4. S. F. Y. Li, **Capillary Electrophoresis: Principles, Practice and Applications**, Elsevier, Amsterdam, 1993, pp. 158-200.

5. K. F. Greve, W. Nashbeh, B. L. Karger, J. Chromatogr. **680**, 15-24 (1994).

6. R. M. McCormick, Anal. Chem., **60**, 2322-2328 (1988).

7. H. Lindner, W. Helliger, A. Dirshlmayer, M. Jaquemar, B. Puschendorf, Biochem. J., **283**, 467-471 (1992).

8. H. H. Lauer, D. McManianigill, Anal. Chem., **58**, 166-170 (1986).

9. Y. Walbroehl, J. W. Jorgenson, J. Microcol. Sep., **1**, 41-45 (1989).

10. J. S. Green, J. W. Jorgenson, J. Chromatogr., **478**, 63-70 (1989).

11. M. M. Bushey, J. W. Jorgenson, J. Chromatogr., **480**, 301-310 (1989).

12. F. A. Chen, L. Kelly, R. Palmieri, R. Biehler, H. Schwartz, J. Liq. Chromatogr., **15**, 1143-1611 (1992).

13. D. Schmalzing, C. A. Piggee, F. Foret, E. Carrilho, B. L. Karger, J. Chromatogr. A, **625**, 149-159 (1993).

14. Y. Liu, R. Fu, J. Gu, J. Chromatogr. A, **723**, 157-167 (1996).

15. K. Srinivansan, C. Pohl, N. Avdalovic, Anal. Chem., **69**, 2798-2805 (1997).

16. S. Hjerten, J. Chromatogr., **347**, 191-198 (1985).

17. M. Gilges, M. H. Kleemiss, G. Schomburg, Anal. Chem., **66**, 2038-2048 (1994).

18. N. J. Clarke, A. J. Tomlinson, Anal. Chem., **69**, 2786-2792 (1997).

19. J. K. Towns, F. E. Rengnier, Anal. Chem., **64**, 2473-2478 (1992).

20. E. Cordova, J. Gao, G. M. Whitesides, Anal. Chem. **69**, 1370-1379 (1997).

21. M. M. Bushey, J. W. Jorgenson, J. Chromatogr., **480**, 301-310 (1989).

22. K. K. C. Yeung, C. A. Lucy, Anal. Chem. **69**, 3453-3441 (1997).

23. J. D. Hayes, A. Malik, J. Chromatogr. B, **695**, 3-13 (1997).

24. M. Huang, D. Mitchell, M. Bigelow, J. Chromatogr. B, **677**, 77-84 (1996).

25. H. Katayama, Y. Ishihama, N. Asakawa, Anal. Chem., **70**, 2254-2260 (1998).

26. M. A. Abubaker, J. R. Petersen, M. G. Bissell, J. Chromatogr., B: Biomed. Appl., **674**, 31-38 (1995).

27. M. A. Strege, A. L. Lagy, Anal. Biochem. **210**, 402-410 (1993).

28. G. Li, D. C. Locke, J. Chromatogr., A, **734**, 375-365 (1996).

29. M. Wilchek, E. Bayer, Anal. Biochem., **171**, 1-32 (1988).

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