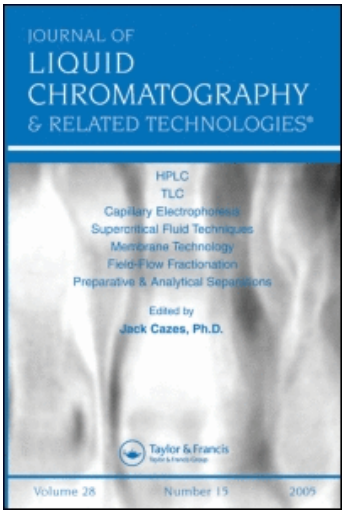

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COMPARISON OF ALKYL-BONDED ALUMINA STATIONARY PHASES FOR PEPTIDE SEPARATIONS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

The separations of synthetic octapeptide mixtures and tryptic digests of larger proteins were compared on columns packed with several alkyl-bonded alumina-based stationary phases of differing chain lengths, particle pore diameters and particle shapes. Employing both standard and optimized mobile phase gradient conditions, it was found that: (1) columns of different lengths (15 cm and 25 cm) packed with the octadecyl-bonded alumina phase gave similar peptide separations; (2) narrow pore (diameter = 11 nm) alumina-based phases gave equal or superior peptide separations compared to those obtained on corresponding wide pore (diameter = 21 nm) phases; (3) octadecyl (C-18) bonded alumina phases gave superior peptide separations compared to those obtained on corresponding octyl (C-8) phases; and (4) alumina-based phases consisting of fused microplatelet particles gave superior separations compared those obtained on corresponding phases consisting of spherical particles. Peptide chromatographic recoveries were also found to be similar on all alkyl bonded alumina-based phases tested. Differences in these effects with those observed for peptide separations on silica-based phases are attributed to the enhanced solute mass transfer properties of the fused microplatelet alumina particles.

INTRODUCTION

The separation of complex mixtures of polypeptides is important in a variety of problems in biochemistry and biotechnology. Reversed-phase high performance liquid chromatography (HPLC) has been shown to be an effective method for such separations, and is now routinely employed in a number of applications, including the isolation and characterization of polypeptides from biological mixtures (1), the preparative purification of synthetically produced peptides (2), and the analysis of peptide mixtures resulting from the enzymatic fragmentation of high molecular weight proteins (3-5). The most widely-used stationary phases for such peptide separations consist of alkyl-bonded porous silica particles. Extensive studies have been performed to determine optimal dimensions and type of surface coverage of the silica particles used in this application (6-10). As a result of these studies, wide-pore (20-40 nm diameter) octyl or octadecyl-bonded silica stationary phases are now generally regarded as being best for use in peptide separations of all types.

Despite the successful use of the silica-based phases for such separations, the inherent hydrolytic instability of alkyl-bonded silica-based phases and the presence of interfering silanol sites on their surfaces have often resulted in short column lifetimes and poor chromatographic reproducibility (6). This has prompted the investigation of non-silica-based phases for peptide separations. Materials investigated for this application have included polystyrene-divinylbenzene copolymers (11), polystyrene (7) and polybutadiene-coated alumina (12). Varied results have been obtained with such phases.

We have recently been investigating peptide separations on an octadecyl-bonded alumina (ODA) stationary phase developed by Wieserman and coworkers (13). This stationary phase is unique not only in that it is based upon alumina rather than silica, but also in that it consists of particles which are not spherical, but are

composed of microplatelets bound together in a symmetrical manner. We have shown that a column packed with narrow pore (11 nm average diameter) ODA particles produces equal or superior separations of octapeptide mixtures and protein tryptic digests, compared to that obtained on a widely-used octadecylsilane (ODS) column (14).

Research performed with silica-based phases have indicated significant effects of alkyl chain length, particle pore size and particle shapes of these phases on the resolution and recovery of peptide mixtures (6-10). In order to investigate analogous effects on alumina-based phases, we fabricated a number of columns packed with alkyl-bonded alumina particles of varying dimensions and types of surface modification, and compared separations of peptide mixtures on these and other more conventional columns. In this paper, we discuss the results of these comparisons.

EXPERIMENTAL

Materials and Apparatus

Lysozyme was obtained from Sigma (St. Louis, MO, U.S.A). The octapeptide standards listed in Table 1 were synthesized in the Protein Chemistry Core Facility of the University of Florida by the solid phase technique, as reported elsewhere (15,16). All solvents used were glass distilled, obtained from E.M. Science (Cherry Hill, NJ, USA). Trifluoroacetic acid (TFA) and N,N-diethylaniline (DEA) were obtained from Aldrich (Milwaukee, WI, USA).

The HPLC system consisted of a Perkin-Elmer Series 410 solvent delivery system, a Rheodyne Model 7125 injector (20 microliter loop) and a Perkin-Elmer Model LC-135 diode array UV-visible detector. Wavelengths monitored were 280 nm for the octapeptide mixtures and 220 nm for the lysozyme tryptic digest. The

TABLE 1

Octapeptide Standards

Octapeptide	Amino Acid Sequence
Lys-Ser-R	Lys-Ser-Ala-Lys-Phe-Nph-Arg-Leu
Lys-Ala-R	Lys-Ala-Ala-Lys-Phe-Nph-Arg-Leu
Arg-Pro-R	Arg-Pro-Ala-Lys-Phe-Nph-Arg-Leu
Ser-Pro-R	Ser-Pro-Ala-Lys-Phe-Nph-Arg-Leu
Lys-Leu-R	Lys-Leu-Ala-Lys-Phe-Nph-Arg-Leu
Leu-Pro-R	Leu-Pro-Ala-Lys-Phe-Nph-Arg-Leu

Nph = p-nitrophenylalanine

mobile-phase flow rate was set at 1 mL/min. Chromatographic data were recorded and processed on a Perkin-Elmer Omega data system.

Columns

Eight chromatographic columns were used in these studies, as described in Tables 2 and 3. The experimental (not available for purchase) columns 1-6 were all prepared at Alcoa Laboratories (Alcoa Center, PA, USA). Columns 1-5 all consist of surface-modified Biotage Unisphere™ alumina particles. The Unisphere particle consists of ~200 nm thick platelets bonded together to form spheroidal particles with open, readily accessible inter-platelet macroporosity and intra-platelet microporosity, as described by Wilhelmy (17,18). Column 6 consists of surface-modified Spherisorb™ A5Y spherical alumina. The Spherisorb alumina for column 6 was purchased from Phase Separations, Inc. (Norwalk, CT, USA). Its

TABLE 2

Stationary Phase Descriptions

Column	Abbreviation	Substrate	Surface Modification	Particle Shape	Column Length (mm)
1	ODA5NP(l)	Al_2O_3	C-18	FMP	250
2	ODA5NP(s)	Al_2O_3	C-18	FMP	150
3	ODA5WP	Al_2O_3	C-18	FMP	250
4	OCA5NP	Al_2O_3	C-8	FMP	150
5	OCA5WP	Al_2O_3	C-8	FMP	150
6	C18A5Y	Al_2O_3	C-18	SPH	150
7	BIORAD	SiO_2	C-18	SPH	250
8	VYDAC	SiO_2	POD	SPH	150

C-18 = monomeric octadecyl; C-8 = monomeric octyl; POD = polymeric octadecyl; FMP = fused microplatelet; SPH = spherical

TABLE 3

Stationary Phase Dimensions

Column	Avg. Particle Diam. (microns)	Avg. Pore Diam. (nm)	Surface Area (m^2/g)	Wt %C
ODA5NP(l)	5.6	11	105	10.1
ODA5NP(s)	5.6	11	105	10.1
ODA5WP	5.6	21	46	4.8
OCA5NP	5.6	11	105	5.2
OCA5WP	5.6	21	46	2.4
C18A5Y	5.0	13	93	n.a.
BIORAD	5.0	9	150	17.5
VYDAC	5.0	30	90	8.5

n.a. = data not available

surface was modified with monomeric octadecyl groups at Alcoa Laboratories by methods described below. Column 7, packed with spherical monomeric octadecylsilane particles was obtained from Biorad Laboratories (Richmond, CA, USA). Column 8, consisting of spherical "Vydac" polymeric octadecylsilane particles, was obtained from The Separations Group (Hesperia, CA, USA).

Monomeric alkyl groups (octyl or octadecyl) were covalently bonded to the Unisphere and Spherisorb alumina particles packed in Columns 1-6 using processes described by Wieserman et al (13) and Haky et al (19).

The housing of all columns consisted of 4.6 mm i.d. stainless steel tubing. All columns were packed by the slurry method described by Snyder and Kirkland (20).

Sample Preparation and Analytical Conditions

The tryptic digest of lysozyme was prepared by the method of Crestfield et al (21) except that mercaptoethanol was replaced by dithiothreitol (8). Solutions of the octapeptide standards were prepared at 1-4 mg/mL in 0.1% trifluoroacetic acid and stored at 0° C.

HPLC analyses of the lysozyme tryptic digest were performed on each column using a linear binary mobile phase gradient consisting of 5-75% solvent B over 45 minutes, where solvent A is 0.1% (v/v) TFA in water and solvent B is 0.1 % (v/v) TFA in acetonitrile.

Gradient ranges for chromatographic analysis of the octapeptide mixture were optimized for each column with Drylab G™ HPLC modelling computer software (LC Resources, Inc., Lafayette, CA U.S.A.), using an arbitrarily-set gradient time of 14.7 minutes. Gradients employed for each column are shown in Table 4. Segmented gradients were occasionally employed to obtain the best overall

TABLE 4

Peptide Peak Capacities and Minimum Resolutions

Column	Gradient For Octapeptides (% B, time)	Average PC For Tryptic Digest	Minimum Resolution For Octapeptides
ODA5NP(l)	21-23%, 3.7 min. 23-28%, 3.3 min. 28-33%, 7.7 min.	87.7	1.20
ODA5NP(s)	17-25%, 14.7 min.	83.8	1.13
ODA5WP	19-27%, 14.7 min.	66.0	1.15
OCA5NP	15-20%, 8.0 min. 20-24%, 6.7 min.	55.5	1.01
OCA5WP	15-17%, 5.0 min. 17-34%, 9.7 min.	43.1	0.82
C18A5Y	17-25%, 14.7 min.	38.8	0.98
BIORAD	27-35%, 14.7 min.	68.9	0.55
VYDAC	19-27% (14.7 min.)	75.5	1.32

octapeptide separations. Details of the optimization process will be reported elsewhere (22). The elution order of the octapeptides was the same for each column, and corresponds to the order in which the peptides are listed in Table 1.

HPLC analyses of 0.1% DEA solutions were performed on the VYDAC, BIORAD, and ODA5NP(l) columns using mobile phases consisting of acetonitrile and water, adjusted to give capacity factors (k' s) of 5-7 for each column. Mobile phase compositions used for each column are shown in Table 5.

TABLE 5

N,N Diethylaniline Peak Asymmetries

Column	Mobile Phase (% Acetonitrile)	Asymmetry
BIORAD	95	7.1
VYDAC	60	5.3
ODA5NP(s)	40	1.2

Calculations

Average peak capacity (PC) values for the lysozyme tryptic digest separations for each column listed in Table 4 were determined by averaging individual peak capacity values calculated for three peaks selected from the beginning, middle and end of each chromatogram, as described by Banes et al (23). The individual PC values were calculated by the equation $PC = (t_l - t_o)/w$, where t_l= elution time in minutes of the last peak in the chromatogram, w = width of the peak of interest at baseline, and t_o = column dead time. The value of t_o was determined by injection of a sample of pure water. Minimum chromatographic resolutions for octapeptide separations shown in Table 4 were calculated by the method described by Snyder et al (24). Chromatographic peak asymmetry factors for the DEA analyses (Table 5) were calculated using the method described by Snyder and Kirkland (20) .

RESULTS AND DISCUSSION

Earlier studies with silica-based phases often compared the separations of complex peptide mixtures on different stationary phases using standard mobile

phase gradients (6-10, 23). On a practical basis, the use of the same mobile phase gradient for each column is an efficient and useful approach, especially for the separation of complex mixtures such as tryptic digests, where optimization of the mobile phase gradient for each column would be inordinately time-consuming. On the other hand, optimizing the separation of a simpler, more defined peptide mixture on each column requires less time, and the resulting optimized separations for each column allows for a valid comparison of the overall selectivities of each of the stationary phases tested. Therefore, in this study, both methods were employed to compare peptide separations on each stationary phase. First a standard mobile gradient was used for the separation of the components of tryptic digest of lysozyme on each column, and average peak capacities were calculated on the basis of the resulting chromatograms. Secondly, optimized mobile phase gradients were developed and used for the separation of a mixture of six octapeptides (Table 1) on each column , and the resolutions between the two closest-eluting peptides (Lys-Ser-R and Lys-Ala-R in all cases) were calculated and compared. These comparisons of average peak capacities and minimum resolutions for each column are shown numerically in Table 4, and in a bar graph format in Figure 1. The use of two sets of test mixtures and experimental conditions allowed for a more complete evaluation of each stationary phase for this application.

Effect of Column Length

Owing to the high molecular weights and resulting low rates of mass transfer of peptides and proteins into the stationary phase, the separations of peptide and protein mixtures on silica-based phases have been found to be less dependent on column length compared to separations of compounds of lower molecular weight (6, 25). This has been found to be especially evident when short gradient times (< 1 hour) are employed (25). These earlier results with silica-based phases appear

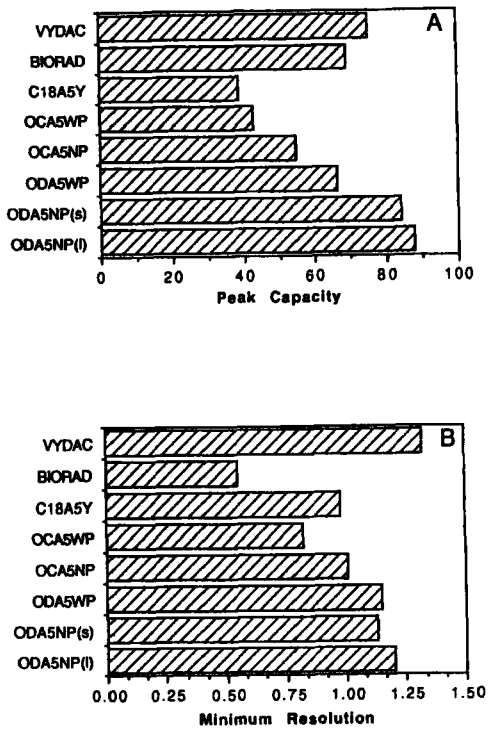

FIGURE 1. Bar graphs showing average peak capacities for lysozyme tryptic digest (A) and minimum resolutions for octapeptide mixture (B) on all columns tested.

to correspond with those found for an alumina-based phase used in the present study.

The data in Table 4 and the graph in Figure 1 show that the average peak capacity for the lysozyme tryptic digest and minimum resolution for the octapeptide mixture are both only slightly higher for the longer ODA5NP(l) column (length: 25 cm) than for the shorter ODA5NP(s) column (length: 15 cm).

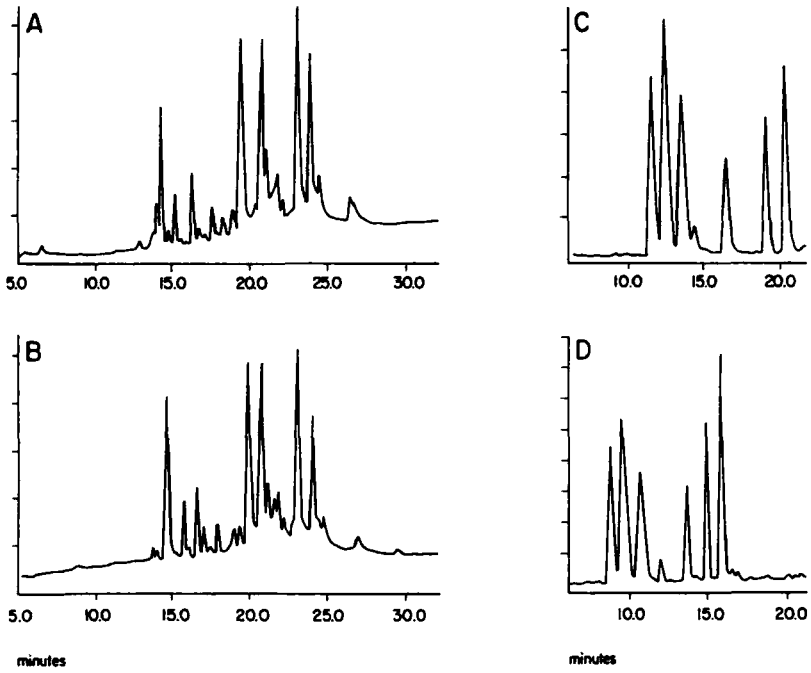

FIGURE 2. Chromatograms comparing peptide separations on long and short columns packed with alumina-based phases. A = lysozyme tryptic digest on ODA5NP(l) column; B= lysozyme tryptic digest on ODA5NP(s) column; C = octapeptide mixture on ODA5NP(l) column; D = octapeptide mixture on ODA5NP(s) column.

Chromatograms of both peptide mixtures on the two columns are indeed quite similar (see Figure 2). These results indicate minimal improvement in chromatographic efficiency and selectivity for peptide separations with increasing column length, consistent with previous studies on silica-based columns.

Effect of Particle Pore Size

Previous studies with spherical silica-based phases have suggested that efficient peptide separations and high chromatographic recoveries require that the pore size

of the phase particles be large enough to allow entry and exit of the solute molecules without encumbrances which can lead to reduced mass transfer rates and peak broadening (6,7,9). Four times the diameter of the analyte has been proposed as being sufficiently large (10). If it is assumed that a peptide exists in solution as a random coil, its diameter in nanometers may be estimated by multiplying the square root of its molecular weight by the factor 0.0816 (26). None of the peptides used in the present study has a molecular weight which exceeds 2500. Therefore, under the criteria described above, the required minimum stationary phase pore size would be $4 \times (2500)^{0.5} \times 0.0816$, or approximately 16 nm. Silica-based phases with average pore diameters nearly twice as large as this are generally used for the separations of peptide mixtures of this type, which ensures that virtually no particles have insuffient pore sizes. However, in the current study, only the alumina-based phases ODA5WP and OCA5WP and the silica-based VYDAC have average pore diameters which exceed 16 nm (see Table 3).

Consistent with earlier studies described above, resolutions, peak capacities and overall chromatographic performance for the separation of both peptide mixtures are indeed superior on the silica-based wide-pore VYDAC column than on the corresponding silica-based narrow pore BIORAD column, as shown by the chromatograms in Figure 3 and the data in Figure 1 and Table 4. Chromatographic peak areas for a representative peptide are also lower for the BIORAD column at all concentrations, indicating reduced recovery (see Figure 4). As discussed earlier, the poorer performance of narrow pore silica-based phases such as BIORAD for peptide separations can at least partially be attributed to its greater resistance to solute transfer in and out of its pores, compared to a wide-pore silica-based phase such as VYDAC. In addition, the greater number of accessible silanol sites on the surface of the higher-surface-area BIORAD particles and the resulting increase in

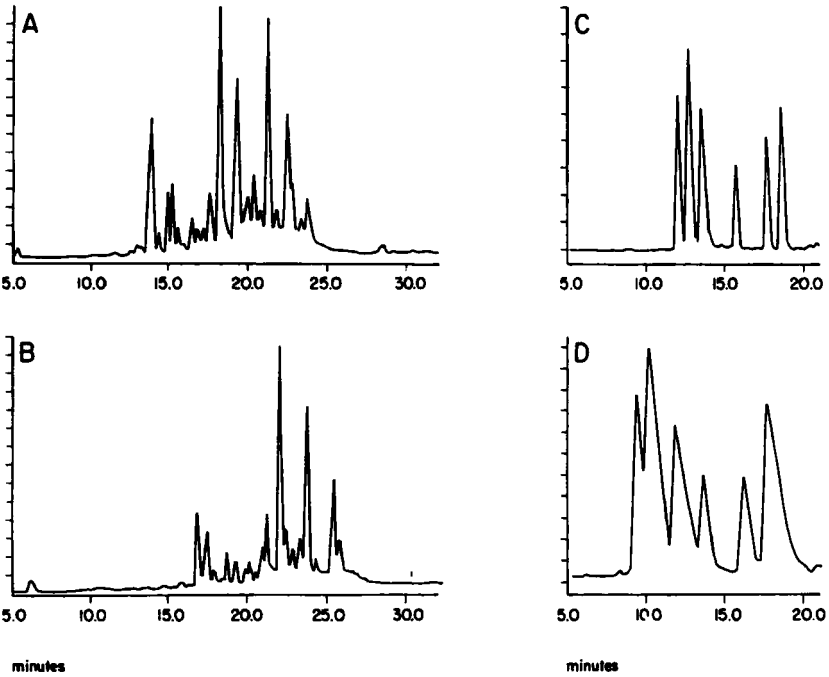

FIGURE 3. Chromatograms comparing peptide separations on wide pore and narrow pore silica-based phases. A = lysozyme tryptic digest on VYDAC column; B= lysozyme tryptic digest on BIORAD column; C = octapeptide mixture on VYDAC column; D = octapeptide mixture on BIORAD column.

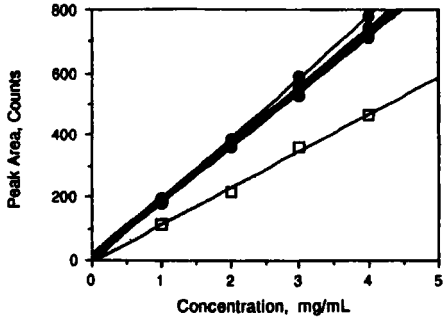

FIGURE 4. Plots of chromatographic peak area vs concentration of octapeptide Leu-Pro-R analyzed. □ BIORAD column; ● all other alumina-based columns.

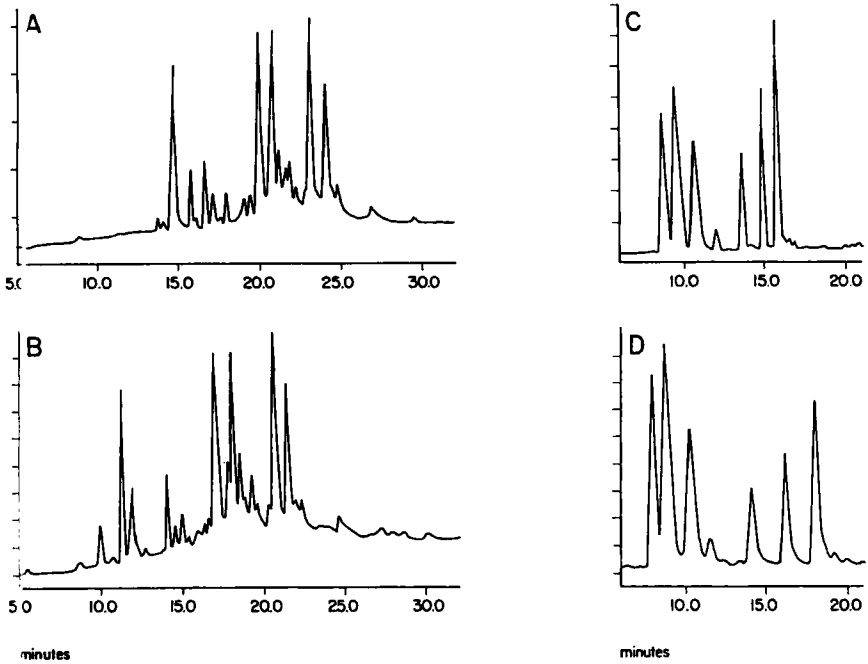

FIGURE 5. Chromatograms comparing peptide separations on wide pore and narrow pore alumina-based phases. A = lysozyme tryptic digest on ODA5WP column; B= lysozyme tryptic digest on ODA5NP(1) column; C = octapeptide mixture on ODA5WP column; D = octapeptide mixture on ODA5NP(1) column.

interfering acid-base interactions may also account for some of the reduced efficiency for peptide separations. The presence of larger numbers of free silanol groups on the BIORAD phase compared to that of the VYDAC phase is indicated by the poorer asymmetry factors obtained on that column for the chromatogram of the basic solute N,N diethylaniline (see Table 5).

In contrast to the results obtained with the silica-based phases, the performances of the narrow-pore ODA5NP and OCA5NP alumina-based columns for the peptide separations are similar or even superior to those of the

corresponding wide pore ODA5WP and OCA5WP columns, as shown for the ODA phases by the chromatograms in Figure 5 and for both OCA and ODA phases by the peak capacity and resolution data in Figure 1 and Table 4. Additionally, chromatographic peak areas for a representative peptide are virtually identical for all of the alumina-based columns at all solute concentrations (see Figure 4), indicating no dependence of chromatographic recovery on pore size.

The excellent chromatographic results for peptide mixtures on the narrow pore alumina columns suggests that solute transfer into and out of the pores of these phases is not a factor which significantly controls chromatographic resolution and peak width for peptide separations on these phases. If this is the case, the high chromatographic selectivity of the narrow pore ODA5NP and OCA5NP phases can be attributed to their high surface areas (Table 3) which would allow for enhanced interaction of solutes with these stationary phases. As discussed later, this effect may also be enhanced by the unique fused-microplatelet particle shape of the alumina-based phases, which can allow greater accessibility of the solutes to the stationary phase.

Unlike silica-based phases, alumina-based materials do not have any acidic silanol groups on their surfaces, and thus interfering acid-base interactions with solutes are completely avoided. This is confirmed by the superior asymmetry factor obtained for DEA on the ODA5NP column compared to that obtained on the silica-based BIORAD and VYDAC columns (Table 5).

Effect of Alkyl Chain Length

Previous studies have indicated that short-chain alkylsilane phases (C2-C8) give enhanced protein and peptide resolutions and recoveries compared to longer-chain C18 materials (6,23,27,28). Recently, this effect has been attributed to the

lower resistance offered by the short-chain alkyl groups to penetration of the pores of the stationary phase particles by the peptide solutes (9). In fact, little differences in peptide selectivities and recoveries between short and long chain akylsilane phases were found when narrow pore particles (< 20nm) were scrupulously excluded from the columns (9). Since pore penetration appears to be less of a controlling factor for peptide resolution and recovery on the fused microplatelet alumina-based phases used in this study, a different type of dependency of alkyl chain length on peptide selectivity might be expected for such phases. This is indeed observed.

The data in Figure 1 and Table 4 and the chromatograms in Figure 6 show that the longer-chain ODA5NP(s) and ODA5WP columns give somewhat better peptide peak widths, peak capacities and overall resolutions compared to those obtained on corresponding shorter-chain OCA5NP and OCA5WP columns. The superior performance of the octadecyl-bonded column is especially evident in the lysozyme tryptic digest separation (see Figures 1A, 6A and 6B). Such results can be attributed to the effects of higher carbon density of the longer-chain stationary phases (see Table 3). As stated earlier, ease of solute pore penetration does not appear to be a controlling factor for peptide separations on the fused-microplatelet alumina-based phases. As a result, the high carbon densities of the octadecyl-derivatized materials and their resulting high lipophilicities have a substantial effect on the separation, allowing for greater interaction of the solutes with the stationary phases compared to that obtained with the lower-density octyl-derivatized phase. Similar alkyl-chain effects have been observed for the separation of low molecular weight compounds on silica-based columns (20,24), when solute pore penetration is also not a controlling factor.

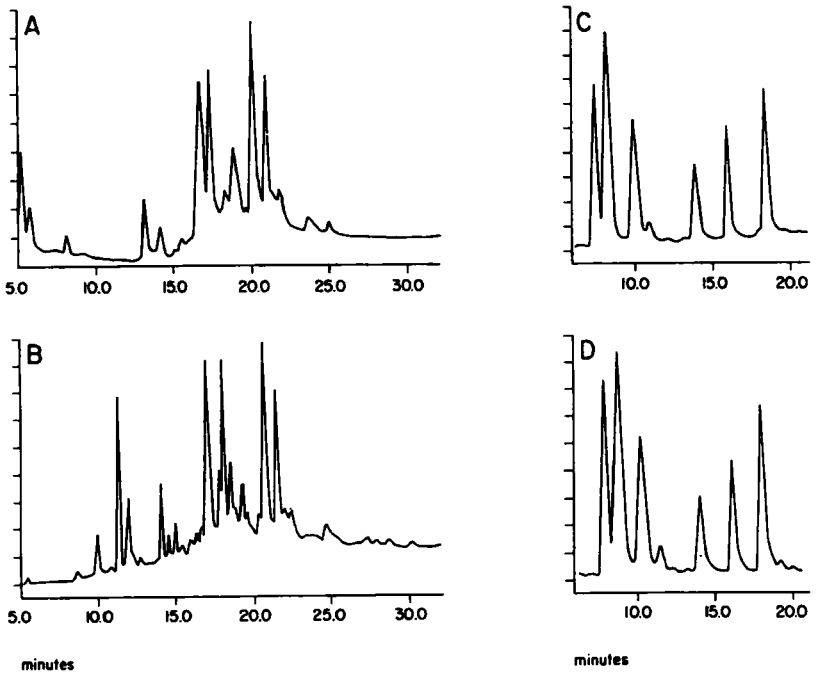

FIGURE 6. Chromatograms comparing peptide separations on octyl and octadecyl bonded alumina-based phases. A = lysozyme tryptic digest on OCA5NP column; B= lysozyme tryptic digest on ODA5NP(s) column; C = octapeptide mixture on OCA5NP column; D = octapeptide mixture on ODA5NP(s) column.

Effect of Particle Shape

It has been suggested that the unique fused microplatelet shape of the alumina stationary phase particles used in this and other studies would allow more efficient solvent flow and solute-stationary phase interactions than that which can be achieved with standard spherical particles, resulting in lower column backpressures, enhanced selectivity and improved chromatographic efficiency (29). Testing the validity of that suggestion requires a direct comparison of the

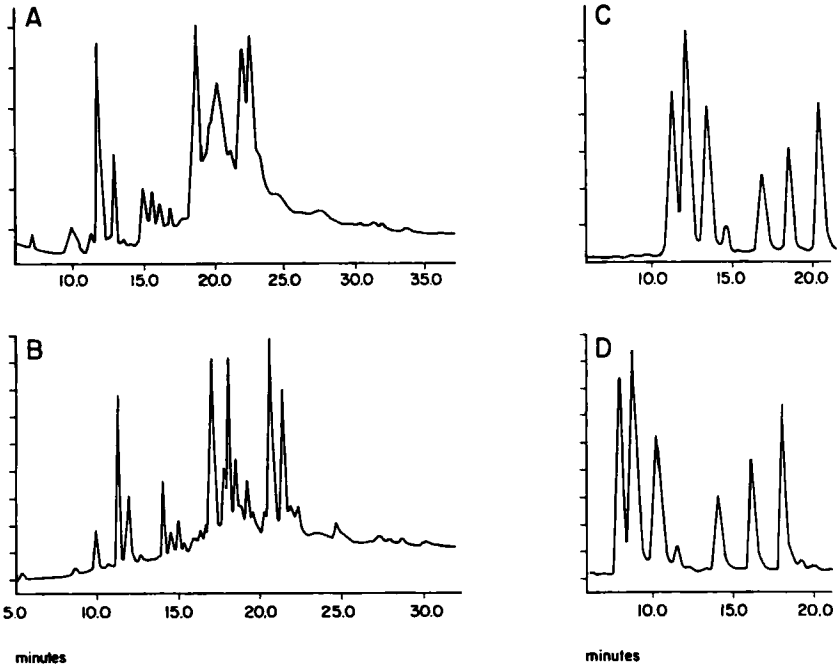

FIGURE 7. Chromatograms comparing peptide separations on spherical and fused-microplatelet alumina-based phases. A = lysozyme tryptic digest on C18A5Y column; B= lysozyme tryptic digest on ODA5NP(s) column; C = octapeptide mixture on C18A5Y column; D = octapeptide mixture on ODA5NP(s) column.

chromatographic properties of spherical and fused-microplatelet alumina (or silica) of similar dimensions and surface modification.

A comparison of the mobile phase flow resistances of columns packed with a spherical alumina-based stationary phase with those packed with similarly-prepared fused microplatelet alumina particles was recently reported (30). That study concluded that solvent flow is indeed more efficient through the fused microplatelet particles, although other factors (e.g., pore size, type of surface modification, etc.) often have greater effects on mobile phase flow resistance than particle shape.

Since the present study utilizes virtually the same set of columns as in the ealier flow resistance investigation, a similar approach can be employed to compare the selectivities of spherical and fused microplatelet particles for peptide separation.

Figure 7 shows chromatograms of the two peptide mixtures on the C18A5Y column, which consists of spherical alumina particles and on the ODA5NP column, which consists of fused microplatelet particles. Both phases had been surface modified by identical chemical processes. These chromatograms and the data in Figure 1 and Table 4 clearly indicate superior peptide peak shapes, resolutions and peak capacities on the fused-microplatelet ODA5NP phase compared to that obtained on the spherical C18A5Y phase. The superior separation of the lysozyme digest on the ODA5NP phase is especially evident, as shown in Figure 7B. This occurs in spite of the somewhat larger particle diameter of the ODA5NP phase than that of C18A5Y (see Table 3), which would normally result in reduced chromatographic selectivity and efficiency (6,7). These and the previously discussed data are consistent with a greater efficiency of solute-stationary phase interactions in fused microplatelet particles compared to spherical particles, resulting in lower mass transfer resistance for peptide separations. When other factors are kept similar, fused-microplatelet particles thus appear to be more selective and efficient for peptide separations than spherical particles, in accord with that which had been previously speculated. However, it should be emphasized that the excellent peptide separations obtained on the spherical-particle VYDAC phase (see Figures 1 and 2 and Table 4) indicates that factors other than particle shape may often have a more important effect on column selectivity than particle shape alone.

CONCLUSIONS

In this study, the usefulness of fused microplatelet alumina-based alkyl-bonded HPLC stationary phases for separations of complex peptide mixtures has again

been demonstrated. Additionally, it has been shown that narrow pore octadecyl-bonded alumina phases generally outperform corresponding alumina-based phases with wider pores or shorter alkyl chain lengths for such separations. These results are in marked contrast to those which have previously been obtained for silica-based phases, where wide-pore stationary phases with short alkyl chain lengths generally have been found to give the best peptide separations.

The superior separations obtained on an alumina-based phase consisting of fused microplatelet particles compared to those obtained on a corresponding phase with spherical particles suggests that the solute mass transfer properties of the fused microplatelet particles are inherently better than those of the spherical particles. These superior mass transfer properties may account for the some of the differences which were observed in the effects of alkyl chain length and particle pore size on separations of peptides on silica and alumina-based stationary phases. Further research is needed to determine whether these enhanced solute mass transfer properties change the effects of other HPLC parameters, such as solvent flow rate and column temperature. Such research is currently underway in our laboratories.

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REFERENCES

1. S.D. Power, M.A. Lochrie, R.O. Poyton, J. Chromatogr., 266: 585 (1983).

2 J. Rivier, R. McClintock, H. Anderson, J. Chromatogr., 288: 303 (1984).

3. K. Kalghatgi, Cs. Horvath, J. Chromatogr., 443: 343 (1988).

4. R.J. Simpson, R.L. Moritz, G.S. Begg, M.R. Rubira, E. Nice, Anal. Biochem., 177: 221 (1989).

5. R.L. Garnick, N.J. Solli, P.A. Papa, Anal. Chem., 60: 2546 (1988).

6. J.D. Pearson, N.T. Lin, F.E. Regnier, Anal. Biochem., 124: 217 (1982).

7. W.G. Burton, K.D. Nugent, T.K. Slattery, B.R. Summers, L.R.Snyder, J. Chromatogr., 443: 363 (1988).

8. M.W. Dong, D. Tran, J. Chromatogr., 499: 125 (1990).

9. N. Tanaka, K. Kimata, Y. Mikawa, K. Hosoyo, T. Akari, Y. Ohtsu, Y. Shiojima, R. Tsuboi, I. Tsuchiya, J. Chromatogr., 535: 13 (1990).

10. L. Colwell and R. Hartwick, J. Liq. Chromatogr., 10: 2721 (1987).

11. D.P. Lee, J. Chromatogr., 443: 143 (1988).

12. J.E. Haky, A. Raghani, B.M. Dunn, J. Chromatogr., 541: 303 (1991).

13. L.F. Wieserman, J.W. Novak, C.M. Conroy, K. Wefers, U.S. Patent 4,786,628 (1988).

14. J.E. Haky, A.R. Raghani, B.M. Dunn, L.F. Wieserman, Chromatographia, 32: 49 (1991).

15. B.M. Dunn, K. Kammerman, K.R. McCurry, Anal. Biochem., 138: 68 (1984).

16. B.M. Dunn, M. Jimenez, B.F. Parten, M.J. Valler, C.E. Rolph, J. Kay, Biochem. J., 237: 899 (1986).

17. R. Wilhelmy, U.S. Patent 4,822,593 (1989).

18. R. Wilhelmy, U.S. Patent 4,900,537 (1990).

19. J.E. Haky, S. Vemulapalli, L.F. Wieserman, J. Chromatogr., 505: 307 (1990).

20. L.R. Snyder and J.J. Kirkland, Introduction to Modern Liquid Chromatography, second edition, Wiley, New York, 1979, Chapters 5 and 7.

21. A.M. Crestfeld, S.Moore, W.H. Stein, J. Biol. Chem., 238: 622 (1963).

22. J.E. Haky, A.R. Raghani, N.D. Ramdial, manuscript in preparation.

23. A.J. Banes, G.W. Link, L.R. Snyder, J. Chromatogr., 326: 419 (1985).

24. L.R. Snyder, J.L. Glajch, J.J. Kirkland, Practical HPLC Method Development, Wiley, New York, 1988, Chapters 2-3.

25. M.A. Stadalius, M.A. Quarry, L.R. Snyder, J. Chromatogr., 327: 93 (1985).

26. G. Guichon, M. Martin, J. Chromatogr., 326: 3 (1985).

27. M.J. O'Hare, M.W. Capp, E.C. Nice, N.H.C. Hooke, B.G.Archer, in High Performance Liquid Chromatography of Proteins and Peptides, M.T.W. Hearn, C.T. Wehr, F.E. Regnier , eds., Academic Press, New York, 1982. pp. 161-172.

28. E.C. Nice, M.W. Capp, N. Cooke, M.J. O'Hare, J. Chromatogr., 218: 569 (1981).

29. R. Stevenson, Am. Biotech. Lab., 8 [2]: 6 (1990).

30. J.E. Haky, N.D. Ramdial, A.R. Raghani, L.F. Wieserman, J. Liquid Chromatogr., 14: 2859 (1991).