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Comparison of polybutadiene-coated alumina and octadecyl-bonded silica for separations of proteins and peptides by reversed-phase high-performance liquid chromatography

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ABSTRACT

The chromatographic retention and separations of proteins and peptides on a novel polybutadiene-coated alumina (PBDA) high-performance liquid chromatographic stationary phase are compared to those obtained on a widely-used polymeric octadecylsilane (ODS) phase. Using acetonitrile–water mobile phase gradients containing 0.1% trifluoroacetic acid, the average peak capacities (which are inversely proportional to average peak widths) and peak resolutions obtained for chromatograms of mixtures of ribonuclease A, cytochrome *c*, lysozyme and carbonic anhydrase are five times lower on a column packed with PBDA than on one packed with ODS. Irreversible adsorption causes increases in column back-pressure during successive analyses of protein solutions on PBDA phases and 50% reductions in protein peak areas on the PBDA phase compared to ODS. In contrast to those results, peak capacities, resolutions and peak areas for synthetic octapeptides on the PBDA and ODS phases are more comparable to each other. Chromatographic capacity factors of 31 low-molecular-weight organic compounds on PBDA and ODS columns are shown to correlate well. The critical concentrations of organic modifier required to elute proteins and octapeptides from PBDA columns are lower than that required for ODS, but still correlate linearly with corresponding values from ODS columns. It is concluded from these results that the retentions of peptides, proteins and smaller molecules on both the PBDA and ODS phases are governed by similar hydrophobic interaction mechanisms, while peak broadening due to mass transfer resistance increases more rapidly with solute size on the PBDA stationary phase than it does on ODS. The increase in solute mass transfer resistance with solute size on the PBDA column is attributed to solute interactions with the uniquely-shaped PBDA particles.

INTRODUCTION

There has recently been much interest in the development of new stationary phases for high-performance liquid chromatography (HPLC) which have greater hydrolytic stability and fewer interfering surface acidic sites (*e.g.*, silanols) than for the commonly used alkyl-bonded silica phases. Much of this research has involved polymeric materials, or polymer-coated silica and alumina. Examples of such stationary phases include polystyrene–divinylbenzene (PRP) [1], polystyrene-coated silica [2,3], and polybutadiene-coated alumina [4].

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Although various applications of polymeric and polymer-coated stationary phases for the separations of low-molecular-weight compounds (*i.e.*, mol.wt. <300) have been reported [2,4,5], chromatographic peak symmetries and efficiencies for such separations are often lower than those obtained on alkyl-bonded silica phases. This has been attributed to the lower solute–solvent mass-transfer rates associated with polymeric phases. In spite of these limitations, many of these phases have been found to be useful in separating higher molecular weight compounds, such as proteins and peptides. Excellent separations of such compounds on wide-pore polymeric and polymer-coated phases, such as polystyrene–divinylbenzene [6], polystyrene [7], polystyrene-coated silica [3] and polymeric octadecylsilane [8] have been reported. Peak shapes and chromatographic efficiencies on these phases are often superior to those which can be obtained on monomeric alkyl-bonded silica.

A number of researchers have investigated the properties of polybutadiene-coated alumina as a stationary phase for reversed-phase HPLC [4,9–12]. Wieserman *et al.* [12] and Wilhelmy [13] developed such a phase (PBDA) which is unique in that it consists of polybutadiene-coated porous alumina particles which are not perfectly spherical, but rather are composed of microplatelets bound together in a highly symmetrical, spheroidal manner. Although this stationary phase has recently been employed for the estimation of octanol–water partition coefficients by reversed-phase HPLC [11], the potential of PBDA for separating high-molecular-weight compounds such as proteins and peptides has not been fully investigated. In this paper, we report on the application of this unique PBDA phase for the reversed-phase separation of proteins and peptides, and compare separations obtained on the PBDA phase with those obtained on a more commonly-used octadecylsilica (ODS) material.

EXPERIMENTAL

Materials

The protein standards ribonuclease A, lysozyme, carbonic anhydrase and cytochrome *c* were obtained from Sigma (St. Louis, MO, U.S.A.). The octapeptide standards listed in Table I were synthesized in the Protein Chemistry Core Facility of the University of Florida by the solid-phase technique, as reported elsewhere [14,15].

TABLE I

OCTAPEPTIDE STANDARDS

Nph = *p*-Nitrophenylalanine.

Octapeptide	Amino acid sequence
Ala-Pro-R	Ala-Pro-Ala-Lys-Phe-Nph-Arg-Leu
Leu-Pro-R	Leu-Pro-Ala-Lys-Phe-Nph-Arg-Leu
Ser-Pro-R	Ser-Pro-Ala-Lys-Phe-Nph-Arg-Leu
Lys-Ala-R	Lys-Ala-Ala-Lys-Phe-Nph-Arg-Leu
Lys-Arg-R	Lys-Arg-Ala-Lys-Phe-Nph-Arg-Leu
Lys-Asp-R	Lys-Asp-Ala-Lys-Phe-Nph-Arg-Leu
Lys-Leu-R	Lys-Leu-Ala-Lys-Phe-Nph-Arg-Leu
Lys-Ser-R	Lys-Ser-Ala-Lys-Phe-Nph-Arg-Leu

All solvents used were glass distilled, obtained from E. M. Science (Cherry Hill, NJ, U.S.A.). Trifluoroacetic acid (TFA) was obtained from Aldrich (Milwaukee, WI, U.S.A.).

Apparatus

The HPLC system consisted of a Perkin-Elmer Series 410 solvent-delivery system, a Rheodyne Model 7125 injector (20-μl loop) and a Perkin-Elmer Model LC-135 diode array UV–visible detector. Unless otherwise specified, the wavelength monitored was 280 nm, and the mobile phase flow-rate was set at 2 ml/min. Chromatographic data were recorded and processed on a Perkin-Elmer Omega data system.

The PBDA column used in this study was obtained from Biotage (Charlottesville, VA, U.S.A.). It was packed with an Alcoa Unisphere polybutadiene-coated alumina stationary phase. The Unisphere alumina particle consists of *ca.* 200 nm thick platelets bonded together to form spheroidal particles with open, readily accessible inner-platelet macroporosity and inter-platelet microporosity. These particles had a mean diameter of 8 μm and a medium pore size of 24 nm. The polybutadiene was coated and immobilized on the alumina surface using processes similar to those described by Bien-Vogelsang *et al.* [4]. The dimensions of the PBDA column were 250 mm × 4.6 mm I.D.

The Vydac ODS column packed with polymeric C_{18} bonded silica was obtained from the Separations Group (Hesperia, CA, U.S.A.). The packing had nominal particle diameter and pore size of 5 μm and 30 nm, respectively. The dimensions of the ODS column were 150 mm × 4.6 mm I.D.

Analytical conditions

Solutions of the protein and octapeptide standards were prepared at 1–4 mg/ml in 0.1% trifluoroacetic acid and stored at 0°C. HPLC analyses of the protein standards were performed on each column using a linear mobile phase gradient from 10 to 70% B over 20 min, where solvent A is 0.1% (v/v) TFA in water (pH 2.0) and B is 0.1% (v/v) TFA in acetonitrile. HPLC analyses of the octapeptide standards were performed on each column using the same solvents A and B and a linear gradient from 10–46% B over 24 min.

Six successive analyses of a solution of ribonuclease A (2 mg/ml in 0.1% TFA) on the PBDA column caused initial backpressures to rise *ca.* 80 p.s.i. (see Fig. 2). Backpressures could generally be restored to original levels (*ca.* 1200 p.s.i.) by backflushing the column with a solution of aqueous sodium hydroxide (0.1 *M*) for 10 min at a flow-rate of 2 ml/min. No increases in column backpressure were observed during successive injections of the ribonuclease A solution on the ODS column or successive injections of the octapeptide standards on either column.

Calculations

Peak capacity (*PC*) was calculated for each chromatographic peak in the chromatograms shown in Figs. 1 and 4 by the equation $PC = (t_1 - t_0)/4\sigma$, where t_1 = elution time in minutes of the last peak in the chromatogram, 4σ = width of the peak of interest at baseline, and t_0 = column dead time (ODS: 0.96 min; PBDA: 1.62 min). The value of t_0 was determined by injection of a sample of pure water. Since *PC* is

inversely proportional to stationary phase particle diameter [16], equivalent peak capacity, PC', for a phase with an 8-μm particle diameter was calculated by the equation $PC' = PC \cdot d/8$, where PC = the experimental peak capacity and d = the particle diameter of the phase (ODS: 5 μm; PBDA: 8 μm). Chromatographic resolutions were calculated by the method described by Snyder and Kirkland [17]. Corrected retention times, t', were calculated by the formula $t' = t - t_0$, where t = experimental retention time. Gradient dwell time, t_d, was determined to be 2.37 min using the procedure described by Snyder and Dolan [18]. Critical mobile phase concentrations (*i.e.*, the percentage of acetonitrile in the mobile phase at elution time of the solute) was calculated by eqn. 1:

$$CC = C_i + (C_f - C_i)\,[(t' - t_d/t_g] \quad (1)$$

where CC = the critical concentration, C_i = the initial percentage of acetonitrile in the mobile phase gradient, C_f = the final percentage of acetonitrile in the mobile phase gradient and t_g = gradient time [16].

RESULTS AND DISCUSSION

The structure and physical characteristics of the PBDA phase compares favorably with other polymeric and polymer-coated phases that have been successfully used for the HPLC analysis of proteins and peptides. Its pore size of 24 nm and particle size of 8 μm are similar to those of other phases used for peptide and protein separations [19,20]. Like most polymer-coated materials, PBDA is also stable in acidic mobile phases which are generally used in protein and peptide HPLC separations [4,12,13].

Separations on the PBDA column were compared with those obtained on a column packed with a polymeric octadecylsilane phase, Vydac ODS, that has been widely used for the HPLC analyses of peptides and proteins [8,19]. Two mixtures of protein and peptide standards were chosen for the comparisons. The first mixture consisted of four natural proteins in the molecular weight range of 12 000–30 000: ribonuclease A, lysozyme, carbonic anhydrase and cytochrome *c*. The second mixture consisted of the eight synthetic octapeptides listed in Table I which have molecular weights over an order of magnitude lower. These octapeptides were originally synthesized for studies of enzyme-substrate interactions [14,15,21], and were ideal for the present study, since they contained the amino acid residue *p*-nitrophenylalanine that strongly absorbs UV radiation, allowing for easy detection. Additionally, these octapeptides varied only in the identities of two amino acid residues, allowing for a controlled analysis of the factors affecting the retention of peptides of similar size and structure.

Comparisons of protein and peptide separations, peak capacities and general chromatographic properties

Chromatograms of the protein test mixture on the PBDA and Vydac ODS columns are shown in Fig. 1. Separations of these compounds on the PBDA column are inferior to that obtained on the ODS column. The smaller pore size (24 *vs.* 30 nm) and the larger particle size (8 *vs.* 5 μm) of the PBDA phase cannot entirely account for

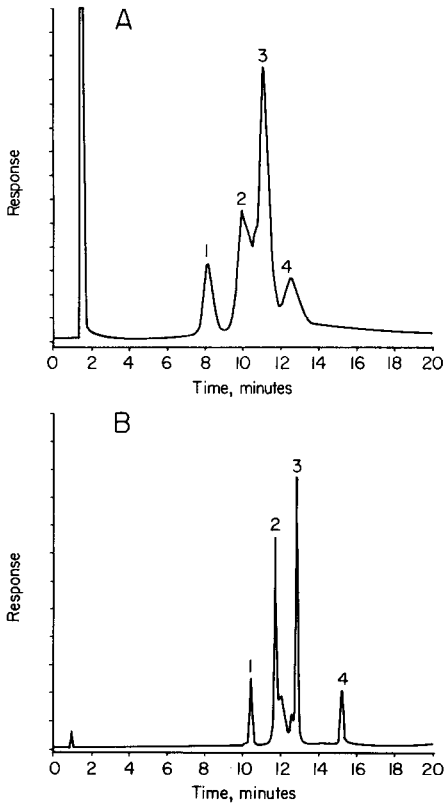

Fig. 1. Chromatograms of mixture of protein standards on PBDA (A) and ODS (B) columns. Peaks: 1 = ribonuclease A; 2 = cytochrome *c*; 3 = lysozyme; 4 = carbonic anhydrase. Other peaks correspond to impurities. Eluent described in Experimental.

the 5-fold differences in average peak capacity (a measure of column efficiency [22]) and chromatographic resolution (a measure of both efficiency and selectivity [17]) between the PBDA and Vydac ODS columns (Table II). Phases with particle dimensions similar to PBDA have produced *PC* and resolution data for similar proteins which are much more comparable to that obtained on the Vydac ODS phase [19,20]. Nor can the dimensional differences alone account for the increases in column backpressure observed during replicate injections of a protein sample on the PBDA column (see Fig. 2), or for the consistantly smaller cytochrome *c* peak areas obtained on the PBDA phase compared to those obtained on ODS (over 50% lower at all solute concentrations; see Fig. 3A). These observations suggest poor mass transfer and incomplete recovery of the proteins on the PBDA column, ultimately resulting in substantial irreversible protein adsorption on the stationary phase. Similar adsorption problems have also been observed for proteins on porous phases consisting of polystyrene–divinylbenzene (PRP) copolymers [6]; as in the present study, adsorbed protein material could be removed by backflushing the column with a protein-hydrolyzing solution [6].

TABLE II

RETENTION TIMES, PEAK CAPACITIES AND RESOLUTIONS FOR PROTEIN STANDARDS (FIG. 1)

t = Retention time in min; PC = peak capacity; PC' = equivalent peak capacity for a phase with 8-μm particle diameter; R_s = chromatographic resolution between the indicated protein and the protein eluting immediately before it.

Protein	Column	t	PC	PC'	R_s
Ribonuclease A	ODS	10.43	26.36	16.47	–
	PBDA	8.20	8.64	8.64	–
Cytochrome c	ODS	11.71	52.71	32.94	3.16
	PBDA	10.04	7.26	7.26	1.33
Lysozyme	ODS	12.84	75.70	47.31	5.35
	PBDA	11.14	10.89	10.89	0.88
Carbonic anhydrase	ODS	15.19	19.97	12.48	6.42
	PBDA	12.51	5.16	5.16	0.88
Mean	ODS	12.54	43.68	27.30	4.98
	PBDA	10.47	7.98	7.98	1.03

Separations of the lower-molecular-weight peptides on the PBDA phase are generally better than corresponding separations of proteins. Fig. 4 shows chromatograms of a mixture of three octapeptides on the PBDA and ODS columns. Table III displays peak capacity and resolution data calculated from these chromatograms. Although the average peak capacity (corrected for differences in particle size between the two phases [22]) obtained for the Vydac ODS column is higher than that obtained for the PBDA phase, the difference (less than a factor of 2) is not nearly as great as that observed with the higher-molecular-weight protein separations. In fact, the chromato-

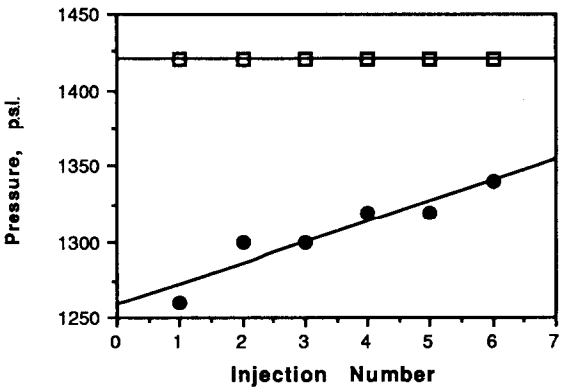

Fig. 2. Plot of column backpressure at the start of mobile phase gradient *vs.* injection number for successive analyses of ribonuclease A. □ = ODS column; ● = PBDA column.

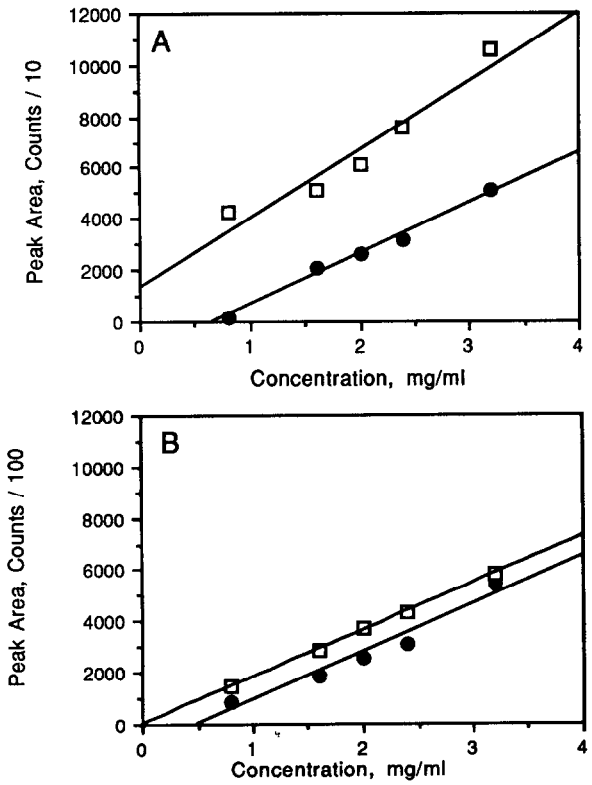

Fig. 3. Plots of chromatographic peak area *vs.* concentration of sample analyzed. (A) Plot for cytochrome *c*; (B) plot for Leu-Pro-R. □ = ODS column; ● = PBDA column.

graphic resolution between the first two peaks in the chromatogram of the octapeptide mixture is actually higher on the PBDA column than it is on the ODS column (Table III). In contrast to that observed with protein analyses on the PBDA column, backpressure did not increase with replicate octapeptide injections. Additionally, representative peptide peak areas obtained on the PBDA phase at various analyte concentrations are only about 15% lower than those obtained on the ODS phase (Fig. 3B), in contrast to the 50% reduction observed for cytochrome *c* peak areas on PBDA (Fig. 3A). Clearly, these data indicate that solute–stationary phase mass transfer resistance and irreversible solute adsorption on the PBDA phase are much higher for the proteins than for the octapeptides.

The higher solute adsorption and resistance to solute–stationary phase mass transfer observed for proteins than for the lower-molecular-weight octapeptides on the PBDA stationary phase indicates that the chromatographic efficiency of the PBDA phase is dependent upon solute size. Peak broadening and high solute mass transfer resistance has been observed previously during separations of small molecules on some polymer and alumina-based stationary phases, and was attributed to interactions of the π orbitals of solutes with those of the stationary phases [5], and acid–base

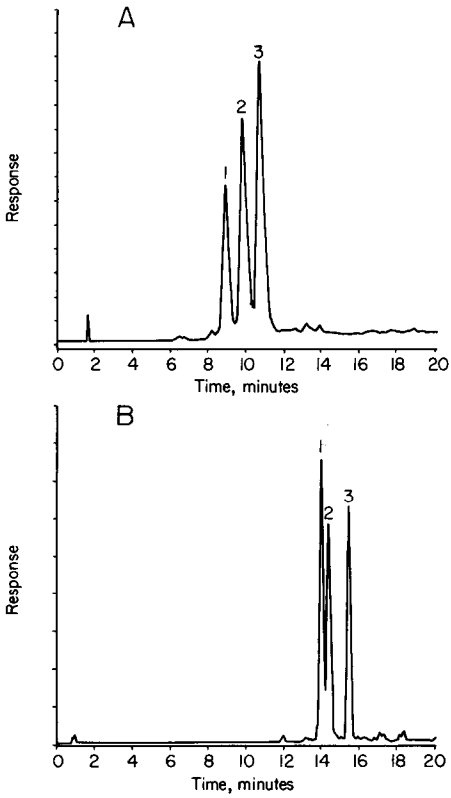

Fig. 4. Chromatograms of mixtures of octapeptide standards on PBDA (A) and ODS (B) columns. Peaks: 1 = Ala-Pro-R; 2 = Ser-Pro-R; 3 = Leu-Pro-R. Eluent described in Experimental.

TABLE III

RETENTION TIMES, PEAK CAPACITIES AND RESOLUTIONS FOR THREE OCTAPEPTIDE STANDARDS (FIG. 4)

Column heading abbreviations are the same as those used in Table II.

Peptide	Column	t	PC	PC'	R_s
Ala-Pro-R	ODS	13.98	39.01	24.38	–
	PBDA	8.79	18.31	18.31	–
Ser-Pro-R	ODS	14.34	38.89	24.30	0.98
	PBDA	9.71	9.75	9.75	1.32
Leu-Pro-R	ODS	15.39	21.95	13.72	2.18
	PBDA	10.59	11.08	11.08	1.02
Mean	ODS	14.57	33.28	20.80	1.58
	PBDA	9.70	11.08	11.08	1.17

interactions of solutes with the alumina support [10]. However, neither of these effects can be the controlling factor for solute–stationary phase mass transfer resistance on the PBDA phase for the separations discussed in the present study. π-Orbital interactions can be eliminated as a controlling factor because they would be expected to cause similar mass-transfer problems in the separations of proteins and peptides on other stationary phases containing unsaturated bonds, such as polystyrene-coated silica. These were not observed in a recently published study [3]. Interactions of solutes with exposed alumina sites can also be eliminated as a major factor controlling solute mass transfer. If present, such interactions would lead to greater peak broadening for the peptides than for the proteins, since, being smaller, the peptides could more readily access these sites than the proteins. The observed increases of chromatographic peak width and solute adsorption with increasing solute size on the PBDA phase can more reasonable be attributed to factors related to the interaction of solutes with the uniquely shaped PBDA particles. The larger protein molecules may become entrapped in the crevices between platelets of the PBDA particles more readily than the smaller peptide molecules, resulting in the observed greater mass transfer resistance for the proteins than for the peptides on PBDA.

It has been suggested that the unique shape of the PBDA particles allows for more efficient solvent flow through this material than that which can be obtained for standard spherical silica particles, which would result in lower column backpressures, especially at high mobile phase flow-rates [12,13,23]. The two upper curves in Fig. 5 are graphs of the normalized column backpressure (*i.e.*, pressure divided by column length) *vs.* mobile phase flow-rate for the ODS and PBDA columns under isocratic conditions. While column backpressures are indeed lower for the PBDA column, this comparison does not take into account the differences in the particle diameters of the two phases. Since column backpressure is inversely proportional to the square of particle diameter [16], a more accurate comparison can be made if the normalized backpressures for the ODS column are corrected to correspond to the same particle diameter as the PBDA phase by multiplying the experimentally-obtained pressure values by the factor 25/64, which is the ratio of the squares of the diameters of the ODS and PBDA phases. The lowest curve in Fig. 5 shows that these corrected backpressures

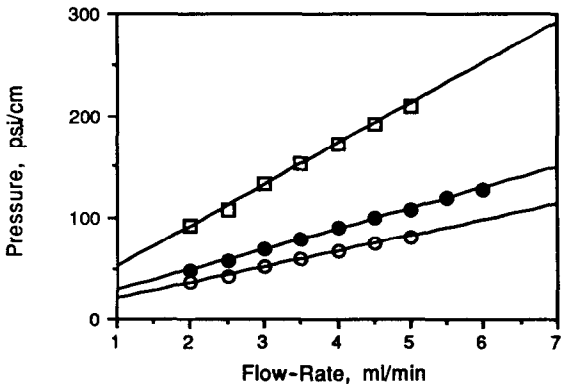

Fig. 5. Plot of the normalized column backpressure *vs.* the mobile phase flow-rate for the ODS and PBDA columns, using a mobile phase of 15% acetonitrile and 85% aqueous TFA (0.1%). □ = ODS column; ● = PBDA column; ○ = ODS column, corrected to correspond to an 8 μm particle size.

are in fact lower than those obtained on a PBDA column of equivalent length and particle diameter. Contrary to that which was previously speculated, column backpressure is not inherently lower for the PBDA phase particles, at least when compared with a phase consisting of highly porous silica-based spherical particles such as Vydac ODS.

Quantitative comparisons of retention mechanisms on ODS and PBDA

Although the incomplete resolutions, large peak widths and substantial peak area reductions for proteins on the PBDA phase clearly indicate a strong dependence of solute size on mass transfer resistance for the uniquely shaped PBDA particles, the retention of solutes on the PBDA phase can be governed by other factors. Comparison of the retention mechanisms of low-molecular-weight organic solutes on ODS and other phases has been accomplished by determining the degree of correlation of the isocratic capacity factors (k') of a large number of compounds on columns packed with ODS and other phases. For example, deviation of phenolic compounds from general correlations between capacity factors of other compounds on ODS and octadecyl-bonded alumina (ODA) has recently been interpreted as indicating the presence of solute-accessible basic alumina sites on the ODA phase [24]. Although a similar comparison has never been reported for such compounds on ODS and PBDA phases, appropriate retention data are available from an earlier study [11]. Fig. 6 shows a logarithmic graph of the capacity factors of 31 low-molecular-weight compounds on the PBDA column *vs.* their capacity factors on an ODS column. The degree of correlation between these retention parameters is high ($R = 0.961$), and there are no apparent deviations of the capacity factors of any specific class of compounds. Other studies have also shown high correlations between capacity factors of small solutes on PBDA and ODS phases and the octanol–water partition coefficients of these solutes [9–11]. The results from these two correlations indicate that retention of small solutes on the ODS and PBDA phases is governed by very similar hydrophobic interaction mechanisms.

Owing to their large size, higher-molecular-weight compounds such as peptides and proteins are retained on some reversed-phase columns by mechanisms other than

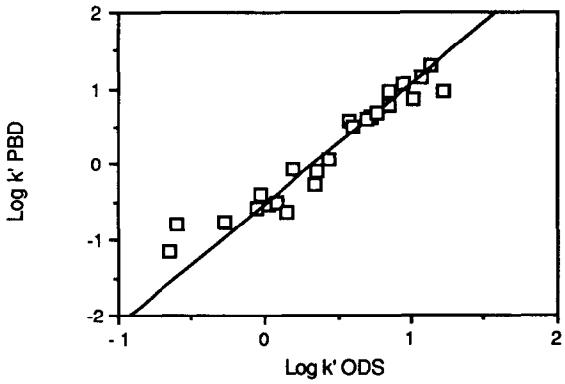

Fig. 6. Plot of the logarithm of the capacity factors of low-molecular-weight compounds on the PBDA column *vs.* the logarithm of their capacity factors on the ODS column. Linear correlation coefficient = 0.961. Data are from ref. 11.

hydrophobic interactions, such as solute size exclusion [22]. However, previous studies have indicated that size-exclusion effects are minimal on the Vydac ODS phase, even for the retention of proteins with molecular weights as high as 50 000 [19]. To determine the extent to which these size-exclusion effects are present on the PBDA phase, a quantitative comparison of the retention of peptides and proteins on the PBDA and Vydac ODS columns analogous to that described earlier for smaller solutes is desirable.

Since the elution of peptides and proteins on both the ODS and PBDA columns could not be performed in an isocratic mode without extensive peak tailing, correlation of their isocratic capacity factors in a manner similar to that discussed earlier for the low-molecular-weight solutes was not possible. Alternatively, the "critical concentrations" of acetonitrile, *CC* (eqn. 1), of the peptides and proteins on each column were correlated with each other. The critical concentration corresponds to the volume fraction of organic modifier at the time of solute elution, and has been shown to be

TABLE IV

CORRECTED RETENTION TIMES AND CRITICAL CONCENTRATIONS FOR PROTEIN AND OCTAPEPTIDE STANDARDS

t' = Corrected retention time (min); *CC* = critical mobile phase concentration, as defined in the text.

Peptide	Column	t'	*CC*
Ribonuclease A	ODS	9.47	31.30
	PBDA	6.58	22.62
Cytochrome *c*	ODS	10.75	35.14
	PBDA	8.42	28.14
Lysozyme	ODS	11.88	38.53
	PBDA	9.52	31.45
Carbonic anhydrase	ODS	14.23	45.59
	PBDA	10.89	35.55
Ala-Pro-R	ODS	13.02	25.97
	PBDA	6.02	15.48
Leu-Pro-R	ODS	14.43	28.09
	PBDA	8.44	19.10
Ser-Pro-R	ODS	13.38	26.51
	PBDA	7.13	17.14
Lys-Ala-R	ODS	11.92	24.33
	PBDA	5.01	13.96
Lys-Arg-R	ODS	11.92	24.33
	PBDA	5.67	14.95
Lys-Asp-R	ODS	11.58	23.81
	PBDA	3.63	11.89
Lys-Leu-R	ODS	13.90	27.30
	PBDA	8.48	19.16
Lys-Ser-R	ODS	11.58	23.81
	PBDA	7.79	15.70

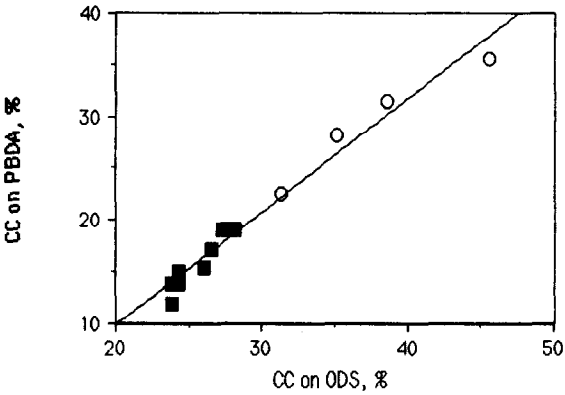

Fig. 7. Plot of critical acetonitrile concentrations of protein and octapeptide standards on PBDA column *vs.* their critical acetonitrile concentrations on the ODS column. ○ = Protein standards; ■ = octapeptide standards. Linear correlation coefficient for all data = 0.986.

roughly independent of column dimensions, gradient time, or stationary phase particle size for large molecules [19,22]. The critical concentration is thus a measure of the relative degree to which a protein or peptide is retained by the stationary phase; a lower critical concentration indicates a lower degree of protein retention.

CC values for the protein and peptide standards on the two columns are listed in Table IV. *CC* values for all solutes on the PBDA stationary phase are significantly lower than those on the ODS phase, indicating weaker protein and peptide hydrophobic interactions with PBDA. Nevertheless, the correlation between solute critical concentrations on the two columns, shown graphically in Fig. 7, is high ($R = 0.986$), indicating very similar retention mechanisms for peptides and proteins on both columns. Remarkably, the correlation between critical concentrations on the PBDA and ODS columns is identical for both peptides and proteins, as demonstrated by the excellent fit of the data for both sets of compounds on the same linear regression line (Fig. 7). Since solute size exclusion would be expected to have a greater effect on the proteins than the octapeptides, the absence of any significant difference in the CC correlations obtained for the proteins and octapeptides confirms the absence of size exclusion as a significant retention mechanism on either column.

CONCLUSIONS

In this study, the retention of peptides and proteins on the PBDA and ODS stationary phases is demonstrated to be predominantly controlled by the same hydrophobic interaction mechanisms which govern the retention of smaller molecules on these phases. Although the PBDA phase was shown to be somewhat less hydrophobic than ODS, the general selectivities of the two phases are quite similar toward compounds of all molecular weights. However, mass transfer resistance, peak broadening and irreversible solute adsorption is more dependent on solute size for the PBDA phase than it is for ODS, which results in low column efficiency for separations of proteins on PBDA columns. To at least some degree, this is attributable to

size-dependent interactions of solutes with the uniquely-shaped PBDA particles. On a practical basis, the results of this study indicate that the novel PBDA phase and other phases based upon similar fused microplatelet particles may be more effectively used for separations of lower-molecular-weight organic compounds and peptides than for separations of proteins and other large polymers.

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