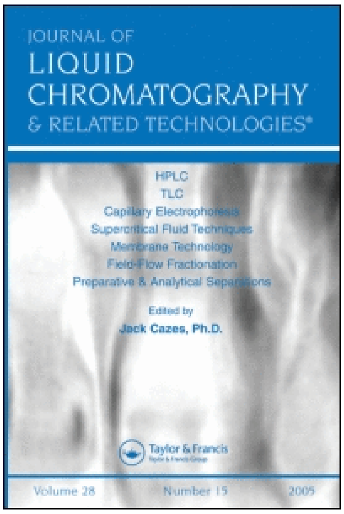

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ASSESSMENT OF MOBILE PHASE FLOW RESISTANCE OF FUSED MICROPLATELET ALUMINA-BASED STATIONARY PHASES FOR REVERSED PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

The mobile phase flow resistances (ϕ's) of several stationary phases consisting of surface-modified Unisphere™ fused microplatelet alumina particles were determined and compared with corresponding values obtained from stationary phases based upon more conventional spherical and angular silica and alumina particles. Although the ϕ value obtained for a fused-microplatelet alumina-based phase was slightly lower than the value obtained on a spherical alumina-based phase with similar particle dimensions and surface modification, factors other than particle shape were found to have a substantial effect on mobile phase flow resistance. Larger ϕ values were obtained with phases with smaller average particle diameters. These were attributed to the presence of greater numbers of microparticles with diameters less than 5 microns in such phases. Larger ϕ values obtained for wide pore (21 nm) and octyl-bonded alumina-based phases over narrow pore (11 nm) and octadecyl-bonded phases were attributed to the former's ablities to entrap larger volumes of stagnant mobile phase within their pores.

Relatively high flow resistance values were obtained for polybutadiene-coated alumina, and were attributed to strong inter-particle attractions due in part to π-π bonding interactions. It is concluded that stationary phase particle shape is often not the single determining factor in establishing the mobile phase flow resistance for stationary phases consisting of spherical or fused-microplatelet particles.

INTRODUCTION

The permeability of the stationary phase determines the column inlet pressure at a given mobile phase flow rate. Permeability is important in the selection of proper conditions for a high performance liquid chromatographic (HPLC) analysis, since column inlet pressure is quantitatively related to a column's overall efficiency (1, 2). Additionally, since it is generally recommended that column backpressure be kept low to prevent premature wearing of pump and injector components (generally less than 2500 psi for standard columns) (1), the permeability of a stationary phase can establish the maximum mobile phase flow rate at which a column can be operated.

A useful parameter in comparing the permeabilities of stationary phases is the flow resistance, ϕ. This dimensionless parameter is a relative measure of the inherent opposition of a stationary phase to the flow of mobile phase through it, and is corrected for differences in column length, stationary phase particle size and mobile phase viscosity (2-4). ϕ values for various normal and reversed phase columns have been determined (5-8). Typical values range from 200 to over 1200 for well-packed columns. It has been generally established that flow resistance is lowest for pellicullar (i.e. non-porous) phases with spherical particles, intermediate for porous phases with spherical particles, and highest for porous phases with angular (i.e., irregularly-shaped) particles (3, 4). Column packed with phases that have ϕ's higher than 5000 develop backpressures which are too high to be used with normal HPLC mobile phase flow rates of 0.5-2.0 mL/min (2).

We have been recently investigating the properties of some new stationary phases which are based upon alumina rather than the more-commonly used silica. We have shown that these octadecyl-bonded alumina (ODA) and polybutadiene-coated alumina (PBDA) phases are useful for a number of applications, including the separations of alkaloids (9), the determination of octanol-water partition coefficients (10), and the chromatographic analysis of peptide and protein mixtures (11, 12). These phases are based upon uniquely-shaped porous alumina particles consisting of microplatelets bound together in a symmetrical, spheroidal manner (13, 14).

It has been suggested that the unique shape of the alumina particles can allow more efficient solvent flow through the material than that which can be obtained with standard spherical particles, resulting in lower mobile phase flow resistance and lower column backpressures (15). However, we have obtained mixed experimental results related to this issue. While column backpressures (corrected for particle size differences) for an ODA column were indeed found to be to lower than those obtained for an octadecylsilane (ODS) column consisting of spherical particles (12), the corrected backpressures obtained for a PBDA column were found to be higher than those obtained for the same ODS column (11).

In an effort to resolve the differences in the experimental results described above and to better understand the relationships between stationary phase types, particle shape and mobile phase flow resistance, we have determined ϕ values for several polymer-coated and alkyl-bonded alumina phases which are based upon the unique fused microplatelet particles. In this report, we compare these values with each other as well as with those obtained for other phases with more conventional spherical and angular particle shapes.

EXPERIMENTAL

Materials and Apparatus

All solvents used were glass distilled, obtained from E.M.Science (Cherry Hill, NJ, USA.). Trifluoroacetic acid (TFA) was 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. The wavelength monitored for dead volume determinations (see below) was 220 nanometers. Chromatographic retention data were recorded and processed on a Perkin-Elmer Omega data system.

Columns

Ten chromatographic columns were used in these studies, as described in Tables 1 and 2. Column 1 was obtained from Biotage, Inc. (Charlottesville, VA, USA).The experimental (not available for purchase) columns 2-6 were all prepared at Alcoa Laboratories (Alcoa Center, PA). Columns 1-6 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 (13, 14). Columns 7-8 consist of surface-modified Spherisorb™ A5Y spherical alumina. The Spherisorb alumina for column 7 was purchased from Phase Separations, Inc. (Norwalk, CT USA). Its surface was

TABLE 1

Stationary Phase Descriptions

Column	Abbreviation	Substrate	Surface Modification	Particle Shape
1	PBDA	Al_2O_3	PBD	FMP
2	ODA8NP	Al_2O_3	C-18	FMP
3	ODA5NP	Al_2O_3	C-18	FMP
4	ODA5WP	Al_2O_3	C-18	FMP
5	OCA5NP	Al_2O_3	C-8	FMP
6	OCA5WP	Al_2O_3	C-8	FMP
7	C18A5Y	Al_2O_3	C-18	SPH
8	PODA5Y	Al_2O_3	POD	SPH
9	ECONSIL	SiO_2	C-18	ANG
10	VYDAC	SiO_2	POD	SPH

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

TABLE 2

Column Dimensions

Column	Length (mm)	Avg. Particle Diam. (microns)	Avg. Pore Diam. (nm)	Surface Area (m^2/g)
PBDA	250	8.0	24	40
ODA8NP	250	8.0	11	103
ODA5NP	150	5.6	11	105
ODA5WP	150	5.6	21	46
OCA5NP	150	5.6	11	105
OCA5WP	150	5.6	21	46
C18A5Y	150	5.0	13	93
PODA5Y	250	5.0	13	93
ECONSIL	250	10.0	6	450
VYDAC	150	5.0	30	90

modified with monomeric octadecyl groups at Alcoa Laboratories by methods described below. Column 8, packed with polymeric octadecyl-coated alumina was also obtained from Phase Separations, Inc. Column 9, consisting of angular monomeric "Econosil" octadecylsilane particles, was obtained from Alltech Associates (Deerfield, IL, USA). Column 10, consisting of spherical "Vydac" polymeric octadecylsilane particles, was obtained from The Separations Group (Hesperia, CA, USA).

The polymer-coated alumina stationary phases in columns 1 and 8 were prepared by processes similar to those described by Bien-Vogelsung et al (16). Monomeric alkyl groups (octyl or octadecyl) were covalently bonded to the Unisphere and Spherisorb alumina particles packed in Columns 2-7 using processes described by Wieserman, et al. (17) and Haky et al.(9).

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 (4).

Determinations of Column Inlet Pressure, Column Dead Volume and Mobile Phase Viscosity

Columns were equilibrated at 25 degrees C at each flow rate for at least 20 minutes using a mobile phase consisting of 85% acetonitrile and 15% water, each containing 0.1% TFA. This mobile phase was chosen because it had been previously used for related studies with ODA and PBDA columns (11, 12). Generally, pressure readings were recorded in psi directly from the pressure monitor of the HPLC system, at mobile phase flow rates ranging from 0 - 5.0 mL/min.

Column dead volume times, t_o, were determined for each column in minutes by injecting a sample of pure water at a mobile phase flow rate of 1.0 mL/min, and noting the time of the first baseline deflection in the resulting chromatogram. Dead volume times determined in this manner are numerically equivalent to the column dead volumes, V_o, in mL (1).

The viscosity of the mobile phase was determined to be 0.95 centipoise at 25 degrees C by the classic method described by Salzburg et al. (18).

Calculations

Total porosities, ε_{tot}, were calculated by equation 1, described by Bristow and Knox (2):

$$\varepsilon_{tot} = 21.22 \frac{f_v t_o}{d_c^2 L} \qquad (1)$$

where f_v is the mobile phase flow rate at which t_o was determined (1.0 mL/min), d_c is the column inner diameter (4.6 mm) and L is the column length in mm. Mobile phase resistance parameters, ϕ, were calculated using equations 2 and 3, discussed below.

RESULTS AND DISCUSSION

Relationship Between Flow Rate, Permeability, and Column Backpressure

The flow resistance parameter ϕ is generally defined through an extension of Darcy's Law (19), which describes laminar flow of gases and liquids through enclosed systems. ϕ is often calculated for a given stationary phase from pressure and dead-volume time measurements using equation 2,

$$\phi = \frac{100\ \Delta p\ t_o\ d_p^2}{\eta\ L^2} \qquad (2)$$

where Δp is the column inlet pressure in bar, t_o is the column dead time in seconds, d_p is the average particle diameter in microns, η is the mobile phase viscosity in centipoise, and L is the column length in millimeters (2). This equation can easily be rearranged to define a relationship between column inlet pressure and mobile phase flow rate. If F is the mobile phase flow rate in mL/min and V_o is the column dead volume in mL, $t_o = V_o / 60F$. Substitution of this relationship into equation 2, conversion of the units of Δp into the more commonly used psi (1 psi = 0.0689476 bar) and rearrangement of the resulting equation gives the following equation 3:

$$\Delta p = \frac{\eta\ L^2\ F\ \phi}{413.69\ V_o\ d_p^2} \qquad (3)$$

Equation 3 predicts that for a given column and mobile phase, a plot of Δp vs F will give a straight line with a slope of $(\eta\ L^2\ \phi)\ /\ (413.69\ V_o\ d_p^2)$, from which the value of ϕ can be calculated if all other parameters are known. The linear relationship between column inlet pressure and mobile phase flow rate is confirmed experimentally in this study by the graphs of Δp vs F for three representative

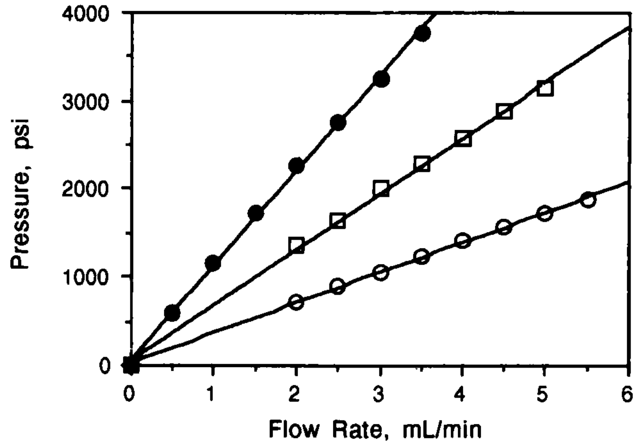

FIGURE. 1. Plots of column inlet pressure vs mobile phase flow rate for three representative HPLC columns
Key: ● ECONSIL; □ VYDAC ; ○ ODA8NP

columns in Figure 1, and also by the excellent linear regression correlation coefficients for these and the rest of the columns shown in Table 3.

Flow resistance values calculated from the slopes of the plots of Δp vs F for each of the columns used in this study are shown in Table 4. Since they are derived from several pressure measurements, the values of ϕ calculated in this manner are inherently more accurate than those calculated by equation 2, which involves a single pressure measurement. The nonzero intercepts which were obtained for each column (Table 3) cannot be predicted from equation 3. Compared to the range of values of inlet pressures measured during these experiments (up to 4000 psi), these intercept values (averaging less than 50 psi) are insignificant. Nevertheless, these intercept values probably reflect extra-column contibutions to backpressure, such as the pressure drop accross the inlet and outlet lines of the chromatographic

TABLE 3

Linear Regression Parameters From Plots of Flow Rate vs. Column Inlet Pressure

Column	Slope	Intercept	R^2
PBDA	529.0	99.8	0.997
ODA8NP	342.5	29.6	0.999
ODA5NP	697.7	54.4	0.999
ODA5WP	717.9	28.9	0.999
OCA5NP	663.3	34.2	0.999
OCA5WP	766.4	27.5	0.998
C18A5Y	904.0	13.3	0.999
PODA5Y	1144.0	53.3	0.998
ECONSIL	1073.8	63.3	0.998
VYDAC	630.0	63.0	0.998

TABLE 4

Stationary Phase Dead Volumes, Mobile Phase Flow Resistances, And Total Porosities

Column	V_o	ϕ	ε_{tot}
PBDA	3.24	764.2	0.78
ODA8NP	3.38	516.2	0.81
ODA5NP	1.79	758.0	0.72
ODA5WP	2.02	880.1	0.81
OCA5NP	2.07	833.4	0.83
OCA5WP	2.08	967.6	0.83
C18A5Y	1.96	857.3	0.79
PODA5Y	2.82	561.9	0.68
ECONSIL	3.17	2371.7	0.76
VYDAC	1.92	585.3	0.77

V_o = column dead volume in mL; ϕ = mobile phase resistance; ε_{tot} = total porosity system, or contributions to backpressure due to turbulent flow, as discussed by Giddings (20). At any rate, the correspondence of higher values of ϕ with higher experimental inlet pressures for the columns investigated confirms that the ϕ values calculated by the method described above are precise and useful parameters for the comparison of the permeabilities of different types of stationary phases.

Comparison of ϕ's for Spherical, Angular, and Fused Microplatelet Phases

Generally, the resistance parameter values obtained for all columns in this study (Table 4) are of the same order of magnitude as those obtained previously for other reversed-phase columns packed with porous phases, both experimentally (4, 21) and theoretically (21). In accord with previous studies, the flow resistance of the column packed with angular-shaped particles (ECONSIL) was found to be substantially higher than that obtained with columns packed with phases of other particle shapes (4, 5). Total porosities are similar and within acceptable ranges (2) for all columns (see Table 4), indicating that this parameter is independent of particle shape, and thus is most likely controlled by the volume between stationary phase particles (i.e., interparticle porosity), in contrast to the volume within the particles themselves (i.e, intraparticle porosity) (22).

Comparison of flow resistance values for columns packed with spherical and fused-microplatelet particles is a complicated process. For example, as shown in Table 4, ϕ for the spherical silica-based VYDAC column is significantly lower than ϕ for the fused-microplatelet PBDA column, but also is somewhat higher than that obtained for the fused-microplatelet ODA8NP column. As discussed later, these data indicate that factors other than particle shape, such as pore size, type of surface modification and ease of packing are also important in establishing column flow resistance. A valid comparison of the resistance of spherical and microplatelet-based particles is therefore best accomplished by keeping these other parameters as constant as possible. In the current data set, the columns which most meet these criteria are the microplatelet alumina-based narrow pore monomeric octadecyl-bonded ODA5NP column and the spherical alumina-based narrow pore monomeric octadecyl-bonded C18A5Y column, since both columns were packed with alumina-based particles of similar dimensions whose surfaces were modified by the same chemical process. The lower flow resistance value for the ODA5NP column (Table 4) indicates that a microplatelet-based alumina particles may indeed offer less resistance than similarly-derivatized spherical alumina particles. However, it should be emphasized that the even lower resistance values obtained for columns packed with some of the spherical silica and spherical alumina-based phases (e.g., VYDAC, PODA5Y) indicate that other factors are often more important than particle shape in establishing the mobile phase resistance of a particular column.

Comparison of ϕ's For Fused Microplatelet Alumina-Based Phases

The flow resistance data for columns packed with the fused microplatelet alumina-based phases reinforces the importance of factors other than particle shape in determining column inlet pressure. For example, the ODA8NP column has a considerably lower ϕ than the PBDA column, despite the fact that both phases have identical 8 micron particle diameters. The higher resistance of the PBDA column may be caused by its larger pore diameter (see Table 2 and discussion below) and also by the greater affinity of its particles towards each other due to π-π bonding interactions, which could cause an increased resistance to penetration of the fused particles by the mobile phase. Such π-π interactions have been recently shown to be important in establishing the chromatographic selectivity of the PBDA and other unsaturated polymer-coated phases (23).

The higher resistance of the ODA5NP column than the ODA8NP phase (Table 4) most probably reflects the presence of greater numbers of microparticles with diameters less than 5 microns in the ODA5NP phase. Such microparticles are known to cause higher-than-expected column backpressures with some pellicular phases (7). Assuming similar particle size distributions in both the ODA5NP and ODA8NP phases, these small particles must be present in greater quantities in the ODA5NP phase, due to its smaller average particle diameter.

The greater fragility and difficulty in packing wide pore over narrow pore phases (24) may account for some of the increased resistance of the wide pore ODA5WP and OCA5WP columns over corresponding narrow pore ODA5NP and OCA5NP columns (see Table 4). However, at least some of the higher flow resistance of the wide pore ODA5WP and OCA5WP phases over their narrow pore homologs can be attributed to their entrapment of larger amounts of relatively stagnant mobile phase in their larger pores, which require greater pressures to be moved out and through the column. This explanation is consistent with the low resistances which have been found for phases consisisting of pellicullar particles (6,8), which have no pores and thus cannot entrap any stagnant mobile phase. The entrapment of larger amounts of stagnant mobile phase by the smaller-chain OCA5NP and OCA5WP phases may also account for their higher flow resistances over corresponding longer-chain ODA phases (ODA5NP and ODA5WP, respectively), whose surface functionalities require more space than the octyl-bonded phases and thus exclude larger volumes of mobile phase from their pores.

The substantial differences in resistance values obtained for different types of fused micoplatelet alumina-based phases indicates that particle shape is not the single determining factor for the flow resistance of these types of phases. Other factors clearly have an important effect..

CONCLUSIONS

In this study, both the total porosities and flow resistance parameters of columns packed with phases based on unique alumina-based fused microplatelet particles have been shown to be comparable in value with those of phases based on more conventional spherical particles. This allows the fused microplatelet alumina phases to be employed using mobile phase compositions and flow rates which are similar to those used for stationary phases with spherical particles.

When all other conditions are held exactly the same, the mobile phase resistances of the fused-microplatelet shaped particles may be slightly lower than that obtained with spherical particles. However, this study has shown that particle shape is only one of several factors determining the flow resistance of reversed-phase HPLC columns. In many cases, factors such as particle pore diameter and the type of surface modification are more important than particle shape in establishing differences in the column inlet pressures of different stationary phases at a given flow rate. Therefore, stationary phase particles need only be constructed in shapes that do not give rise to excessively high inlet pressures which overshadow the contributions of these other factors. Spherical and fused microplatelet-based particle shapes both meet these criteria.

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