Journal of Chromatography, 594 (1992) 291–295
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 838

Correlation between column surface area and retention of polar solutes in packed-column supercritical fluid chromatography

Terry A. Berger* and Jerome F. Deye

Hewlett-Packard Co., P.O. Box 900, Avondale, PA 19311-0900 (USA)

(First received May 14th, 1991; revised manuscript received October 30th, 1991)

ABSTRACT

The retention of organic acids, amines, aminophenols, and amides was directly proportional to the surface area of Diol coated silica particles. Surface area was measured *in situ* using the method of Hong and Parcher [*Anal. Chem.*, 62 (1990) 2313–2317]. Linear regression analysis was performed on the results from four pore diameters (100, 300, 500 and 4000), plus the origin. Correlation coefficients between retention and surface area were as high as 0.9991. Both the surface area of the chromatographic support and solute retention changed by more than 14 times. All the solutes tested behaved similarly, indicating that changing the surface area is a viable means of retention control.

The smallest pore diameter tested (100 Å) produced the same efficiency as some of the larger pore sizes (*i.e.*, 300 and 500 Å) suggesting that still smaller sizes, like 60 Å, might be useful for supercritical fluid chromatography. Particles with 4000 Å pores produced poor efficiency, due to peak tailing, which suggests that deactivation was inadequate.

INTRODUCTION

In adsorption chromatography (liquid–solid chromatography, LSC), retention is a linear function of surface area [1]. However, in bonded-phase chromatography, multiple retention mechanisms are more likely (solute interactions with both bonded phase and silanols), leading to poor peak shapes. Little attention is, subsequently, paid to the effects of surface area on retention. Studies on the physical, chemical and retention characteristics of the silica particles [*i.e.* ref. 11] used in liquid chromatography (LC) are preoccupied with the effects of silanols and other active sites. Little attention is paid to the effects of surface area. Further, it is widely accepted that bonded phases, particularly long alkyl phases like (C_{18}), tend to block access of solutes to the full surface area inside smaller pores.

In supercritical fluid chromatography (SFC), only one report has examined the relationship between the pore structure of silica gel and retention [2]. The retention of low-molecular-weight polymer oligomers appeared to be unrelated to the C_{18} phase loading, but was related to pore diameter (but not surface area). Polar molecules produced substantially different behavior [2]. Phenol was nearly unretained and retention was independent of the pore diameter (or surface area). However, the retention of pyridine changed as much as 20 times when the nominal surface area was changed by 6.2 times. The difference was attributed to interactions between the basic pyridine and acidic residual silanols on the packing surface. Since both phenol and silanols are acidic, it was reasoned that there should be little interaction between the two. However, if the retention of acidic solutes was unrelated to the characteristics of either the bonded stationary phase, or the silanols, then there was no controlled interaction. Similarly, the retention of the basic solute appeared to depend on characteristics of the uncontrolled silanols, not on the bonded stationary phase. In either case, octadecyl phases must be considered inappropriate

0021-9673/92/$05.00

for the separation of these polar solutes and only marginally appropriate for the separation of the polymers investigated.

The separation of polar solutes is best accomplished on polar stationary phases. Phenols [3], hydroxybenzoic acids [4] polycarboxylic acids [5] and benzylamines [6] all yield the best peak shapes on polar phases like Diol when separated with modified fluids. Low-polarity phases, like C_{18}, tend to produce little retained but tailed or asymmetric peaks. Pure carbon dioxide failed to elute most of these solutes from either standard or deactivated low polarity stationary phases.

The effect of the packing surface area on retention in polar systems has not been studied. In this work, retention of polar solutes on Diol packings with methanol-modified carbon dioxide is reported as a function of measured surface area. The relative efficiencies of packings with different pore diameters is also briefly examined.

EXPERIMENTAL

The chromatographic system was described previously [3–6] and included two Hewlett-Packard (HP) Model 1050 high pressure pumps. One pump was modified with a passive inlet check valve and the head was chilled to 4.0°C to pump liquified carbon dioxide. The other pump was an unmodified isocratic unit for delivering liquid modifiers. Both pumps were operated in the flow control mode. A "tee" and a 200 × 2 mm column packed with stainless-steel balls were used to dynamically mix the two fluids. A Rheodyne Model 7520 valve with a 0.2-μl loop was used as the injector. An HP Model 5890 GC acted as the column oven. Detection was via an HP photodiode array UV–VIS detector with a high-pressure 8-μl flow cell. The signal wavelength was 210 nm with a 4-nm bandwidth. The reference wavelength was 450 nm with an 80-nm bandwidth. Pressure was monitored both upstream and downstream of the column and controlled at the detector outlet with an electronic backpressure regulator built in-house.

In situ surface areas were measured using the technique of Hong and Parcher [7] and the results are presented in Table I. The columns were 100 × 2 mm, containing 7 μm diameter Nucleosil Diol packings, purchased from Keystone Scientific, Bellefonte, PA, USA. The pore diameters were 100, 300, 500 and 4000 Å, corresponding to nominal surface areas of 350, 100, 35 and 10 m^2/g, respectively. The Diol coating was applied by the silica manufacturer. No information on phase loading was available.

The carbon dioxide was supercritical grade purchased from Scott Specialty Gases, Plumsteadville, PA, USA. Methanol was high-purity grade, purchased from Burdick & Jackson, Muskegon, MI, USA. The methanol concentration is presented as mol%. The methanol contained 0.1 *M* citric acid or 0.6% v/v isopropylamine (IPAm) as a very polar additive [8]. The IPAm was 99% pure, purchased from Aldrich Chemicals, Milwaukee, WI, USA.

TABLE I

MEASURED AND CALCULATED VALUES FOR THE SURFACE AREA ON FOUR DIOL PACKINGS, ALONG WITH CALCULATED PACKING WEIGHT, AND TRANSIT TIMES

Pore diameter[a] (Å)	Nominal area[a] (m^2/g)	Measured area[b] (m^2)	Calculated packing weight[b] (g)	Transit time, t_0 (min)
100	350	43	0.120	0.103
300	100	15	0.150	0.122
500	35	8.6	0.250	0.192
4000	10	3.0	0.300	0.179

[a] Manufacturer's value.
[b] From ref. 7.

RESULTS AND DISCUSSION

The retention times of a wide range of solutes were measured on columns containing each of the four different pore size silica based Diol particles. Although each of the column packing materials is characterized by the manufacturer with a bulk measurement of the surface area per unit weight, the density of silica particles varies greatly with the pore diameter making such nominal comparisons relatively meaningless. Retention times of 4-hydroxybenzoic acid using five methanol concentrations in carbon dioxide were plotted against nominal surface areas as shown in Fig. 1. The results are nonlinear due to the difference in densities of the particles and, thus, the weight of packing in the different columns. The modifier contained 0.1 *M* citric acid.

The polar additive was included in the mobile phase to improve peak shapes and suppress unwanted interactions [3–8]. Temperature was fixed at 40°C. Pressure was varied slightly to maintain [9] a constant inlet density of 0.84 g/cm^3 and an outlet density of 0.75 g/cm^3.

For a more realistic correlation, the solute partition ratio (k') should be plotted against the actual column surface area. To convert raw retention times (t_R) into partition ratios [$k' = (t_R - t_0)/t_0$], a value for the column transit time of an unretained peak (t_0) is required. In packed column SFC, the transit time can almost never be directly measured when modified fluids are used, because the modifier is usually significantly retained and solutes seldom elute before the modifier.

Column transit times were estimated based on the volume of the empty columns minus both the calculated interstitial and pore volumes of the particles together with the likely average mobile phase density in the column [9]. It was recently verified that both the interstitial and pore volumes contribute to the column void volume [10]. The pore volumes were obtained from the manufacturers measured values expressed in ml/g together with the weight of packing in each column. The weight was obtained by dividing the nominal surface area of the packings (in m^2/g) by the measured surface area (in m^2) for each column.

Partition ratios using the calculated transit times and measured retention times of 4-hydroxybenzoic acid are plotted against measured surface area in Fig. 2. All the plots suggest a linear relationship between area and retention.

The retention of other solutes was also studied as a function of column surface area. Plots of partition ratios *vs.* measured surface area for representative solutes is presented in Fig. 3. Solutes presented include acids, bases, and amides. In addition several amphoteric solutes containing both weakly acidic and weakly basic functional groups were included. As with 4-hydroxbenzoic acid, the plots were linear. The retention data for these and other solutes was subjected to a linear regression analysis with the origin included in the data sets. The results are summarized in Table II.

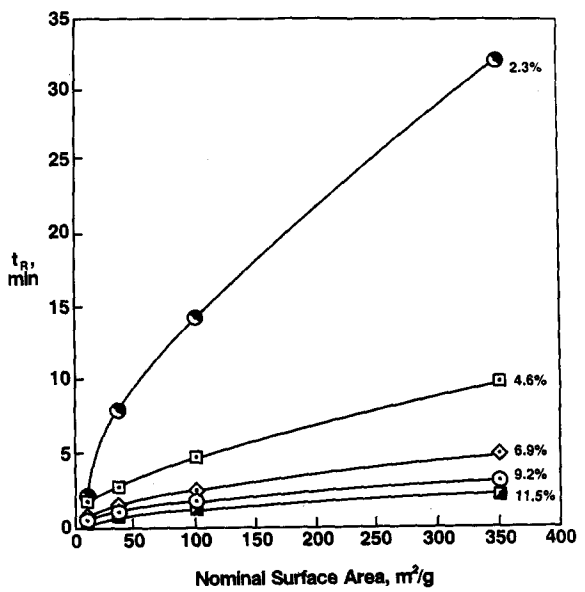

Fig. 1. Retention times of 4-hydroxybenzoic acid plotted against surface area at 5 different concentrations of methanol (containing 0.1 *M* citric acid) in carbon dioxide all at constant density. Areas are nominal values from manufacturer. Columns: 100 × 2 mm, particle diameter 7 μm, Nucleosil Diol; flow, 1.0 ml/min; average column density, 0.76 g/cm^3; column temperature, 40°C.

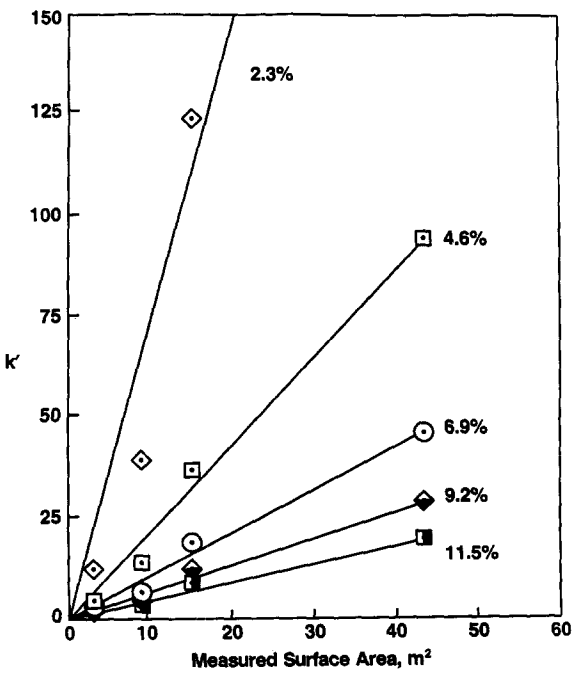

Fig. 2. Retention data for 4-hydroxybenzoic acid from Fig. 1 reduced to partition ratios plotted against *in situ* measured surface areas. Other conditions as in Fig. 1.

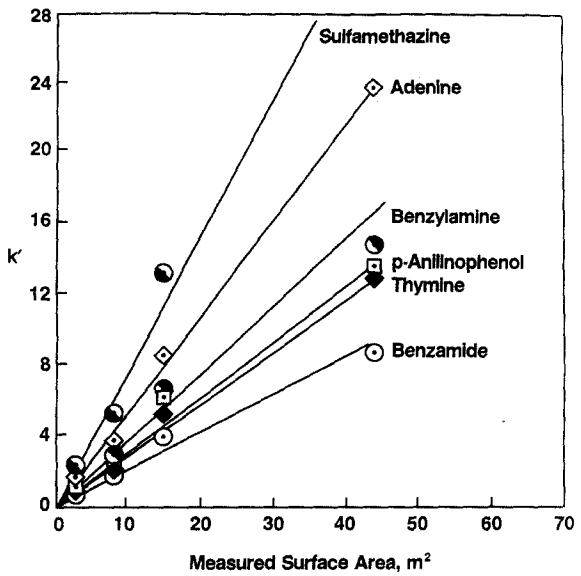

Fig. 3. Plots of the partition ratio of various solutes against the *in situ* measured surface area of the Nucleosil Diol columns used in Fig. 1. Conditions: 1.0 ml/min of 10% (v/v) methanol (containing 0.6% isopropylamine) in carbon dioxide at 40°C, 182 bar outlet pressure.

All solutes (except N,N-diethylaminophenol, $r = 0.9831$) produced correlation coefficients greater than 0.99. Such high correlation coefficients indicate that, despite some scatter in the data, the relationship between retention and surface area is, indeed, linear. The scatter in the data is largely due to the uncertainty in both the surface area measurements and in the accuracy of the mobile phase composition, particularly at low modifier concentration.

The slopes from the linear regressions were further used to calculate the idealized ratio of retention times of each solute at the highest and lowest surface areas. The mean and standard deviation of these ratios for twelve different solutes were then determined. For a measured surface area change of 14.3 times, retention of the solutes changed 14.4 ± 6 times. Thus, surface area and retention were directly proportional.

Since the retention of both acidic and basic solutes is linearly related to the surface area of the packing, one can conclude that the separation process is under control. The same mechanism appears to operate on all pore sizes. In addition, the

TABLE II

PARTITION RATIOS OF SOLUTES ON FOUR DIFFERENT SURFACE AREA COLUMNS AND THE CORRELATION COEFFICIENT, *r*, FROM LINEAR REGRESSION INDICATING THE DEGREE OF FIT TO A STRAIGHT LINE

The origin was included in the calculation of the correlation coefficient. Columns: 100 × 2 mm, 7 μm Nucleosil Diol, 1 ml/min, 10% methanol (containing 0.6% isopropylamine), 40°C, 182 bar outlet pressure.

Solute	Partition ratios, k'				Correlation coefficient, r
	Pore diameter (Å)/measured surface area (m^2)				
	4000/3	500/8.6	300/15	100/43	
Thymine	1.17	2.08	5.09	12.66	0.9967
Adenine	1.41	3.39	8.64	24.07	0.9979
Cytosine	4.66	10.90	23.27	68.77	0.9991
Benzylamine	1.65	2.64	6.62	14.97	0.9934
Benzamide	0.95	1.46	3.79	8.78	0.9938
Nicotinic acid	3.62	10.58	18.47	41.91	0.9957
p-Anilinophenol	1.41	2.57	6.22	13.77	0.9931
N,N-Diethylaminophenol	0.90	1.27	3.20	6.19	0.9831
Sulfamethazine	2.11	4.97	12.90	34.92	0.9976
Sulfanilamide	3.70	8.48	18.24	51.97	0.9991
Sulfisomidine	2.96	14.75	18.78	46.49	0.9912
Sulfapyridine	2.77	6.35	15.75	43.17	0.9980

chemistry employed to produce the bonded stationary phase appears to be equally effective on all the pore sizes producing the same loading per unit area.

None of the separation problems associated with acids and bases in a previous report [2] were encountered.

Efficiency

Efficiency measurements were not the primary objective of this study. However, changing retention by varying surface area is useful only if efficiency is similar on all the columns.

Extracolumn effects tended to limit the measured efficiencies, particularly at short retention times. The columns were all 100 × 2 mm I.D., operated with a flow of 1 ml/min (approximately 2 times optimum). Components contributing to post-column band broadening included: approximately 1 m of 125 μm I.D. connector tubing, several zero dead volume (ZDV) fittings, and an 8-μl detector flow cell. Despite these limitations, a few general comments can be made. At very long retention times the columns produced up to 6720 theoretical plates [$N = 6.28\ (t_R/W_c)^2$] on 7-μm particles, corresponding to 94% of the theoretical maximum (assuming $h_{min} = 2d_p$). Peaks were symmetrical even at partition ratios approaching 200 ($k' = 188$). Since the flow rate is up to twice the optimum value, these results are quite satisfactory. There was no significant difference in efficiency between the 100 and 300 Å pore diameter packings. The 500 Å column produced 83% of theoretical efficiency. The 4000 Å columns produced no more than 32% of theoretical efficiency, due to significantly tailed peaks. However, retention on both the 500 and 4000 Å packings was much lower than on the smaller pore sizes, making extracolumn effects more important on the former.

CONCLUSIONS

Unlike previous results with C_{18} packings [2], the retention of polar acids and bases was linearly proportional to the packing surface area. No difference in behavior was noted between acids or bases. Linear regressions of better than 0.99 were typical. Statistical analysis showed that retention changed 14.4 ± 6 times for a surface area change of 14.3 times. The linear, proportional relationship between surface area and retention indicates that the Diol phase was equally applied (the same loading) to a wide range of pore sizes. These results also suggest that the inside of all the pore sizes were equally accessible to the solutes. These observations are in contrast to reversed-phase LC, where long-chain (*i.e.*, C_{18}) bonded phases appear to be more difficult to apply to smaller pores (more residual activity) and decrease accessibility of solutes to the interior of pores.

Changing pore diameter appears to be an effective, and predictable means of adjusting the retention of a wide range of solutes. The high efficiency on the smaller pore diameters suggest that even smaller diameters might be useful in packed-column SFC.

ACKNOWLEDGEMENTS

The authors wish to thank Jon Parcher and his graduate students at the University of Mississippi for making the surface area measurements on the columns used in this study. They also thank Bill Wilson of this laboratory for helpful discussions and review of the manuscript.

REFERENCES

1 L. R. Snyder, *Principles of Adsorption Chromatography*, Marcel Dekker, New York, 1968, Ch. 6.
2 A. Nomura, J. Yamada and K.-I. Tsunoda, *J. Chromatogr.*, 448 (1988) 87–93.
3 T. A. Berger and J. F. Deye, *J. Chromatogr. Sci.*, 29 (1991) 54–59.
4 T. A. Berger and J. F. Deye, *J. Chromatogr. Sci.*, 29 (1991) 26–30.
5 T. A. Berger and J. F. Deye, *J. Chromatogr. Sci.*, 29 (1991) 141–146.
6 T. A. Berger and J. F. Deye, *J. Chromatogr. Sci.*, 29 (1991) 310–317.
7 H. Song and J. F. Parcher, *Anal. Chem.*, 62 (1990) 2313–2317.
8 T. A. Berger and J. F. Deye, *J. Chromatogr.*, 547 (1991) 377–392.
9 T. A. Berger and J. F. Deye, *Anal.Chem.*, 62 (1990) 1181–1185.
10 H. G. Janssen, H. M. J. Snijders, J. A. Rijks, C. A. Cramers and P. J. Schoenmakers, *J. High Resolut. Chromatogr.*, 14 (1991) 438–445.
11 J. Nawrocki and B. Buszewski, *J. Chromatogr.*, 449 (1988) 1–24.