Journal of Chromatography, 360 (1986) 353–369
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 571

CHARACTERIZATION OF BONDED-PHASE SILICA GELS WITH DIFFERENT PORE DIAMETERS

B. W. SANDS*, Y. S. KIM and J. L. BASS
The PQ Corporation, R & D Center, P.O. Box 258, 280 Cedar Grove Road, Lafayette Hill, PA 19444 (U.S.A.)
(First received November 5th, 1985; revised manuscript received February 18th, 1986)

SUMMARY

Three silica gels, manufactured in a similar fashion and with nominal pore diameters of 100, 200 and 250 Å, were cleaned of contaminants (< 100 ppm total), their surface was adjusted to *ca.* pH 4.5, and their particle sizes and particle size distributions were adjusted as closely as possible. Surface areas (S_{BET}), pore volumes ($V_{p(cum)}$) and median pore diameters (D) were measured and compared.

The silica gels were reacted with *n*-alkyldimethylchlorosilanes (C_nH_{2n+1})-$(CH_3)_2SiCl$ where n = 1, 4, 8 and 18. S_{BET}, $V_{p(cum)}$, D and [silane] of the bonded silica gels were measured. It was found that the concentration of any one bonded silane increases as the pore diameter of the silica gel increases, and that the level of bonding decreases linearly as n increases. S_{BET} and $V_{p(cum)}$ are reduced the greatest for a smaller diameter bonded silica gel, especially as n increases.

The C_{18}-bonded silica gels were used to evaluate the effects of pore diameter on the high-performance liquid chromatography of small molecules (phenol, acetophenone, benzene) and large protein molecules (ribonuclease, insulin, lysozyme, serum albumin, ovalbumin). Capacity factor (k') and N/m for small molecules slightly decreased (for non-polar molecules) as a function of the pore diameter and the pore volume of the bonded or bare silica gel (and increased as a function of the surface area of the bare silica gel). While resolution of small molecules decreased sharply as a function of both pore diameter and pore volume, the resolution of protein separation increased as the pore diameter and pore volume of the silica gel increased, the increase being the greatest for the higher molecular weight proteins.

INTRODUCTION

There has been a considerable amount of effort on the part of researchers of silica packings for high-performance liquid chromatography (HPLC) to describe their results in terms of the properties of the bare silica gel. Comparisons of performance are often made on silica gels that are from different manufacturers, are manufactured differently and may contain different levels of unknown contaminants. There is limited information from manufacturers of silica gel on the chromatographic

0021-9673/86/$03.50

implication of varying (increasing) the pore diameter of a series of silica gels whose properties (D, $V_{p(cum)}$, S_{BET}) before and after bonding are measured and known.

There have been few examples of the characterization by porosimetry of silica before bonding and after bonding[1], especially where both the silica pore diameter and the hydrocarbon chain length of the silane are varied. Other authors are also currently studying these effects[2]. Most authors relate silica performance to either the pore diameter or surface area before bonding.

Small organic molecules are fairly well known to be best separated on reversed-phase, small pore-diameter, high surface area silica gels[3]. Large molecules (specifically proteins) however, have frequently been shown to separate best on reversed-phase, large(r) pore-diameter silica gels[4]. We intend to show the relationships of pore diameter, pore volume and surface area (before and after bonding) on the separation of small and large molecules and to identify more clearly which properties of the bare silica gel and bonded silica gel are the most important.

EXPERIMENTAL

Chemicals and materials

All solvents were HPLC grade. All silanes were obtained from Silar Labs. and used as supplied. Gas chromatographic (GC) analysis indicated *ca.* 99% purity except for $(C_{18}H_{37})(CH_3)_2SiCl$ which contained a mixture of C_x isomers with $C_{18} > 75\%$.

Uracil, phenol, acetophenone and benzene, analytical reagent grade, were purchased from Fisher Scientific. Ribonuclease A, insulin, lysozyme, bovine serum albumin and ovalbumin were purchased from Sigma.

Silicas

The silica gels, which are identified by their nominal median pore diameters (see Table I for measured D) of 100, 200 and 250 Å, were manufactured by the PQ Corporation. In order to ensure low soluble surface ion concentration, the silica gels were washed and decanted with 18×10^6 ohm water and dried at 378 K and at a reduced pressure of 25 mmHg for 16 h. All silica gels had total residual soluble anions and cations < 100 ppm, and their surface pH was *ca.* 4.5 [10%(w/w) in water].

Porosimetry

Nitrogen sorption measurements were performed on a Micromeritics 2100 Accusorb® Analyzer, modified with a Nexus Associates Accu Mate® automated con-

TABLE I

PROPERTIES OF SILICA GELS

Silica gel	*100 Å*	*200 Å*	*250 Å*
S_{BET}, N_2 (m^2/g)	423	261	204
$V_{p(cum)}$ (ml/g)	1.13	1.30	1.36
D(Å)	105	188	250

trol and data processing system. Standard static adsorption and desorption procedures were followed to develop the sorption isotherm at 77 K. Surface areas were calculated using the BET equation[5] from the adsorption isotherm below 0.2 times the vapor pressure of liquid nitrogen at the isotherm temperature. Pore volume distributions were calculated from the desorption isotherm for pores < 600 Å diameter, assuming a cylindrical shape, using the method of Barrett *et al.*[6].

Silylation procedure

A 25-g amount of silica gel was activated at 423 K and at a reduced pressure of 0.5 mmHg for 4 h. The material was cooled to room temperature and 80 ml of chlorosilane were added. All reactions were performed at 150°C, or reflux if lower, for 16 h under nitrogen. The reaction products were washed, in order, with 0.1 l of toluene, tetrahydrofuran (THF), THF–water, water, THF and hexane. Possible fines were decanted during each stage of the wash procedure to yield particle sizes of 20 $\pm$ 2 μm and volume distributions, d_{90}/d_{10}, of 1.8–2.0. The products were dried at 378 K and at a reduced pressure of 25 mmHg for 16 h. This procedure is similar to that of Unger *et al.*[7].

The carbon analyses and concentrations of silane (based on the surface area of the unbound silica) are given in Table II. The carbon analyses were performed by Alpha Labs. via CO_2/IR. The α values were calculated according to Berendsen and De Galan[8], where adjustment is made for the added weight of the silane.

Chromatographic equipment

The liquid chromatograph was a Waters Model 510, with a U6K sample injector, Model 481 UV detector and HP3390A integrator.

Column packing procedure

The bonded silica to be packed, ≈ 3 g, was suspended and sonicated in 40 ml of isopropyl alcohol. The supernatant was decanted after standing for 10 min. The packing material was again resuspended in 40 ml of isopropyl alcohol and transferred

TABLE II

CARBON ANALYSES AND SILANE CONCENTRATIONS OF BONDED SILICA GELS

Silane	*% Carbon* $\pm \sigma_{n-1}$ (concentration of bonded silane, μmol/m^2)*		
	100 Å	*200 Å*	*250 Å*
$(CH_3)_3Si-$	4.68 ± 0.01 (3.39)	3.06 ± 0.02 (3.47)	2.44 ± 0.04 (3.48)
$(C_4H_9)(CH_3)_2Si-$	8.66 ± 0.02 (3.30)	5.92 ± 0.02 (3.48)	–
$(C_8H_{17})(CH_3)_2Si-$	12.52 ± 0.07 (3.00)	9.11 ± 0.07 (3.34)	7.37 ± 0.02 (3.36)
$(C_{18}H_{37})(CH_3)_2Si-$	19.20 ± 0.02 (2.52)	15.47 ± 0.13 (3.09)	13.20 ± 0.02 (3.25)

* In triplicate.

to the packing reservoir. The packing pump used was a Chemco Model 124A. Packing was performed at 57 MPa with methanol as the pressurizing and washing solvent.

Chromatography procedure

Small molecules. Chromatography of the small molecules (uracil, phenol, acetophenone, benzene) was performed by an isocratic mode of elution with acetonitrile–water (70:30) (η = 0.59 Pa · s at 298 K) at a flow-rate of 1 ml/min. Column pressures were 0.8–1.1 MPa. Sodium nitrate was used to determine t_0.

Proteins. A sample consisting of 40 μg of insulin (I), 100 μg of ribonuclease (R), 5 μg of lysozyme (L), 200 μg of ovalbumin (O) and 200 μg of serum albumin (S) was used for each injection. A linear gradient was used [0.1% trifluoroacetic acid (TFA)] from 100% water (A) to 80% (A)–20% acetonitrile (B) over the first 5 min, continuing to 70% (B) over 30 min and returning to 100% (A), with a flow-rate of 1 ml/min.

Performance. The column performance, N, was determined according to Foley and Dorsey[9]:

$$N = \frac{41.7\ (t_R/W_{0.1})}{(A/B\ +\ 1.25)}$$

where t_R = peak retention time, $W_{0.1}$ = peak width at 10% of peak height and A/B = peak asymmetry factor.

Also,

$$\alpha = k_2/k_1$$

And,

$$R_s = \frac{t_{R2} - t_{R1}}{(W_2 + W_1)/2}$$

where R_s = resolution and W = the baseline peak width.

RESULTS AND DISCUSSION

Silica gels

All silica gels used in this study are irregular, produced by a typical gel–hydrothermal treatment process, milled and narrowly sized. We produced silica gels with nominal pore diameter, D, of 100, 200 and 250 Å. The silica gels were treated as noted in the experimental section.

The BET surface area, S_{BET}, cumulative pore volumes $V_{p(cum)}$, and median pore diameters D, are given in Table I. Two observations are obvious: $V_{p(cum)} \propto D$ and $1/S_{BET} \propto D$. The 100-, 200- and 250-Å silica gels were manufactured deliberately to yield a narrow pore size distribution. These distributions are shown in Fig. 1. Also, as shown in Fig. 2, there is a fairly good linear relationship between $V_{p(cum)}$ and D.

The particle size of each silica was made to be 20 ± 2 μm and the particle size

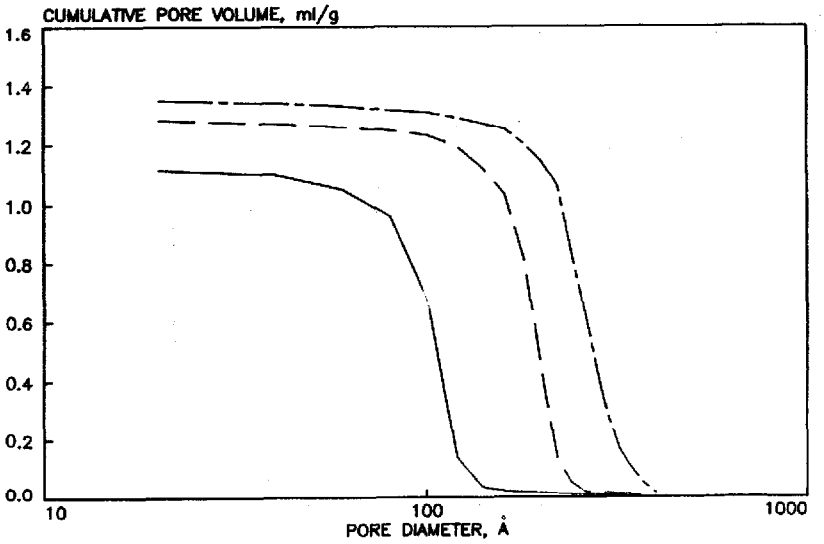

Fig. 1. Pore size distributions of unbonded silica gels. 100 Å (———), 200 Å (– – –), 250 Å (– – –).

volume distribution 1.8–2.0 in order to eliminate any significant particle size effects upon N/m or R_s in chromatography.

Silylation

Carbon analyses and the calculated silane concentration, μmol/m^2, are given in Table II. Several trends are apparent.

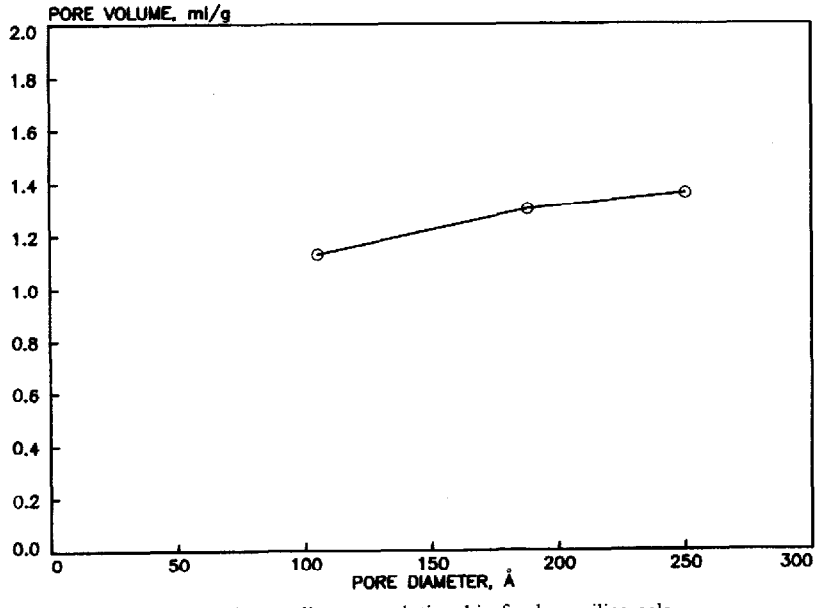

Fig. 2. Pore volume and pore diameter relationship for bare silica gels.

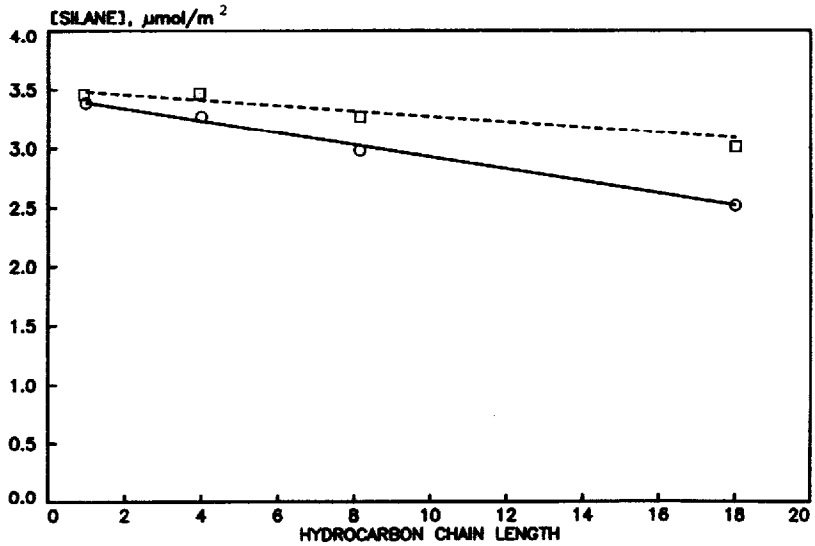

Fig. 3. Concentration of bonded silane *vs.* hydrocarbon chain length, *n*, of $C_nH_{2n+1}(CH_3)_2Si$ for the 100-Å (□) and 200-Å (○) silica gels.

Within a silica gel with a given pore diameter, the concentration of silane apparently decreases linearly as the *n*-alkyl chain length increases, consistent with expectation and literature[3,10,11]. This is shown in Fig. 3 for the 100- and 200-Å silica gels.

The concentration of a given bonded silane increases as the pore size of the silica gel increases, as shown in Fig. 4 for $(C_{18}H_{37})(CH_3)_2Si$–. Extrapolation of the data suggests that the concentration of silane should cease increasing at some pore diameter at or slightly higher than 250 Å, where the total available silanol content has been bonded. Also, it would be expected that the concentration of $(C_{18}H_{37})(CH_3)_2Si$– would approach but not exceed the value of 3.48 for $(CH_3)_3Si$– observed as a result of neat silylation with chlorosilanes.

Properties of bonded silicas

It seems that the expected (literature) chromatographic performance of a silica gel is often based upon its pore structure and surface area before bonding. It is shown here that these properties are definitely altered (reduced) by bonding, and the chromatographic performance may be related to these altered properties.

The remaining, or residual, surface areas of the bonded silica gels decrease significantly as the bonded silane chain length increases (Table III, Fig. 5). The residual surface area for the 100-Å, C_{18}-bonded silica gel is 41% of the bare silica's surface area, and for the 250-Å, 67%. There is greater retention of surface area as the pore diameter of the bare silica gel increases. Actually, the residual surface areas of all three C_{18}-bonded silica gels are very close in value, a reflection of the large C_{18} molecule blocking most of the small pores that contain the highest surface area.

Similar trends are seen for the residual cumulative pore volumes of the bonded silica gels (Table IV, Fig. 6).

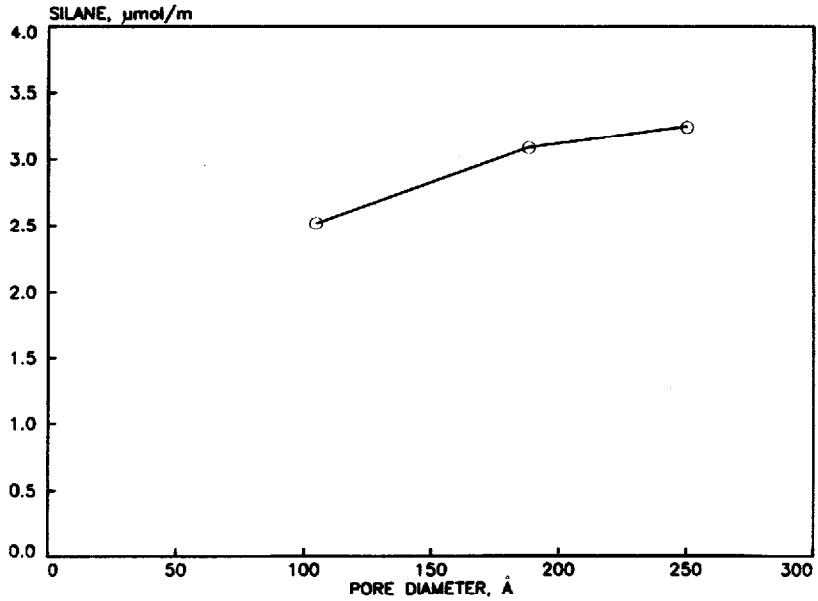

Fig. 4. Concentration of bonded $C_{18}H_{37}(CH_3)_2Si$ *vs.* pore diameter of the bare silica gel.

The residual $V_{p(cum)}$ and S_{BET} for the C_{18}-bonded silica gels are plotted in Fig. 7 as a function of the median pore diameter of the silica gels. As might be predicted, the residual pore volume increases and the residual surface area decreases (almost linearly) as a function of pore diameter.

A reported chain length for $(C_{18}H_{37})(CH_3)_2Si-$ is 24.5 Å in an "upright" configuration[1]. Any pore less than 49 Å in diameter would be blocked and the larger pores reduced in diameter by 49 Å. This would explain the greater decrease in the surface area and pore volume of the 100-Å silica gel *vs.* the 200-Å and 250-Å pore diameter silica gels. These larger pore diameter silica gels also have very few micropores or few pores as small as 49 Å in diameter. Thus, the decreases in S_{BET} and $V_{p(cum)}$ for these silica gels would be mostly a result of pore shrinkage and not blockage.

The median pore diameters of the bonded silica gels do not decrease appre-

TABLE III

RESIDUAL BET SURFACE AREAS OF BONDED SILICA GELS

Silane	S_{BET} *(m²/g) of bonded silica gel*		
	100 Å	*200 Å*	*250 Å*
–	423	261	204
$(CH_3)_3Si-$	325	212	166
$(C_4H_9)(CH_3)_2Si-$	285	204	–
$(C_8H_{17})(CH_3)_2Si-$	258	182	149
$(C_{18}H_{37})(CH_3)_2Si-$	176	147	136

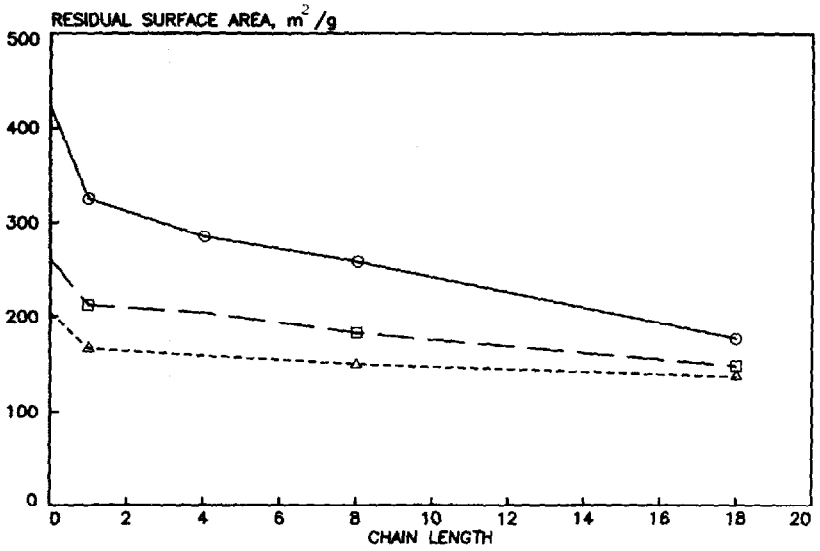

Fig. 5. Residual surface areas of the bonded silica gels [100 Å (○), 200 Å (□), 250 Å (△)] as a function of hydrocarbon chain length, n, of $C_nH_{2n+1}(CH_3)_2Si$.

ciably from the bare silica gels, as shown in Table V. There is a linear relationship when comparing D (bonded) *vs.* D (unbonded) with a correlation of 0.99 and slope of only 0.9. The median pore diameters of the bonded silica gels are a reflection of the blocking of many small pores while larger pores are being reduced. Fig. 8 illustrates that there is no major change in the relative pore size distribution of the bonded silica gels, but only in the cumulative pore volume. There seems to be no real significant change in the narrowness of the pore size distribution of the bonded silica gels, as shown in Fig. 9.

Chromatography of small molecules

Typically, it has been thought that liquid chromatography of small molecules is best performed using a small-pore silica gel. The results here do not seem to dispute this. Uracil, phenol, acetophenone and benzene were separated on the C_{18}-bonded 100, 200 and 250 Å silica gel (Fig. 10). Uracil is the most polar and its rapid elution

TABLE IV

RESIDUAL CUMULATIVE PORE VOLUMES OF BONDED SILICA GELS

Silane	$V_{p(cum)}$ *(ml/g of bonded silica gel)*		
	100 Å	*200 Å*	*250 Å*
–	1.13	1.30	1.36
$(CH_3)_3Si$–	0.91	1.20	1.22
$(C_4H_9)(CH_3)_2Si$–	0.80	1.10	–
$(C_8H_{17})(CH_3)_2Si$–	0.70	1.00	1.07
$(C_{18}H_{37})(CH_3)_2Si$–	0.53	0.80	0.93

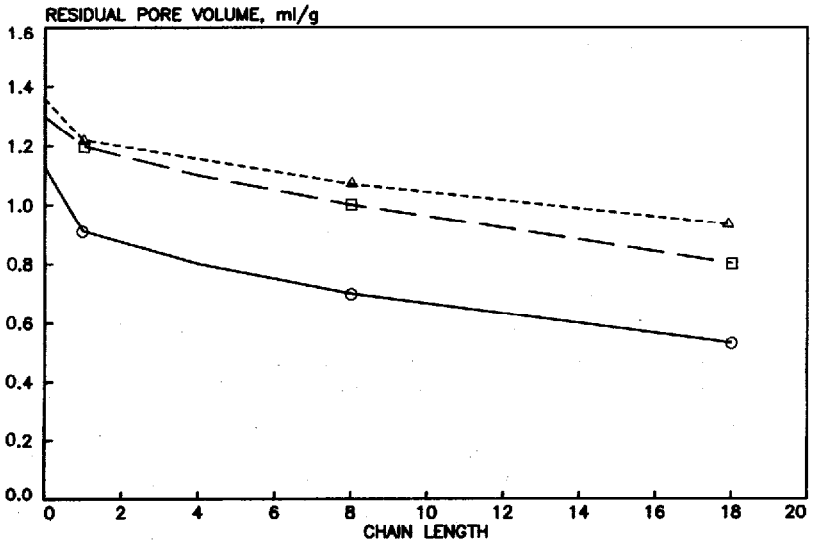

Fig. 6. Residual pore volumes of the bonded silica gels [100 Å (○), 200 Å (□), 250 Å (△)] as a function of hydrocarbon chain length, n, of $C_nH_{2n+1}(CH_3)_2Si$.

is expected, close to t_0. A lack of peak tailing and good asymmetries indicate inaccessible silanols. The results for phenol, acetophenone and benzene are given in Tables VI and VII.

It is found for the chromatography of small molecules that k', N/m, α and R_s can all be related to the values of D and $V_{p(cum)}$ of both the bare silica gels and the respective residual values of the C_{18}-bonded silica gels. The residual S_{BET} values of all the C_{18}-bonded silicas are very close and bear little relationship with the S_{BET}. D

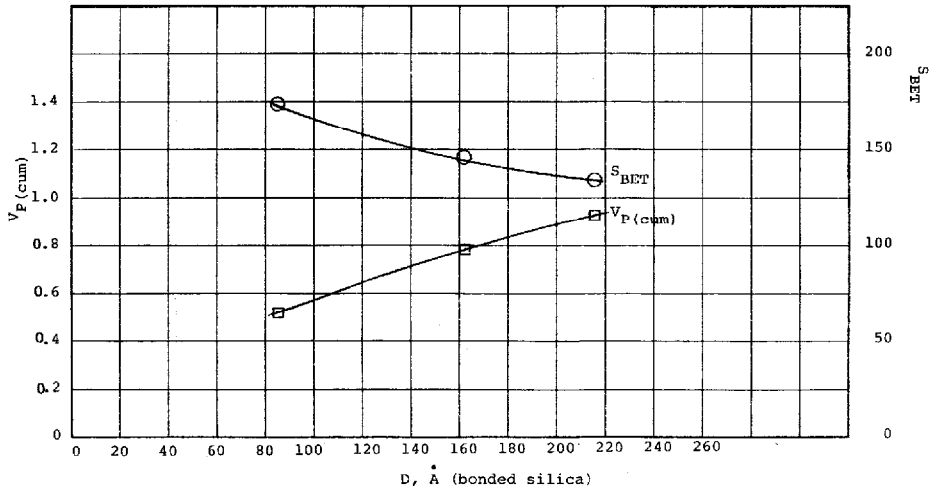

Fig. 7. Residual pore volume (□) and surface area (○) of the C_{18}-bonded silica gels as a function of the pore diameter of the unbonded silica gel.

TABLE V

PORE DIAMETERS OF C_{18}-BONDED SILICA GEL

Silica gel	*D(Å) (unbonded)*	*D(Å) (bonded)*
100 Å	105	85
200 Å	188	162
250 Å	250	216

or $V_{p(cum)}$ of the bare silica gel. Thus, given the above, the relationship of k', N/m, α and R_s may be shown *vs.* D or $V_{p(cum)}$ almost interchangeably.

The relationship between k' and D (silica) is illustrated in Fig. 11. In general, k' decreases slightly for the non-polar molecules with an increase in D (or $V_{p(cum)}$). Also, as shown in Fig. 12, k' increases slightly for the non-polar molecules with an increase in S_{BET} (silica) in agreement with the findings of Roumeliotis and Unger[10]. There is little relationship between k' and residual S_{BET} for the bonded silicas, such that if k' (*e.g.* benzene) is divided by the residual S_{BET}, the values are 1.3, 1.4 and 1.3 ($\cdot 10^{-2}$), respectively.

The increase in k' for non-polar molecules with an increase in the carbon content of the bonded silica gel is also noted. It is felt that the present study cannot easily distinguish between the effects of carbon content and surface area, and k' appears to be directly proportional to both.

Column efficiency (theoretical plates) decreases slightly for all molecules as a function of D, as shown in Fig. 13, and resolution decreases with an increase in D and $V_{p(cum)}$, as illustrated in Fig. 14.

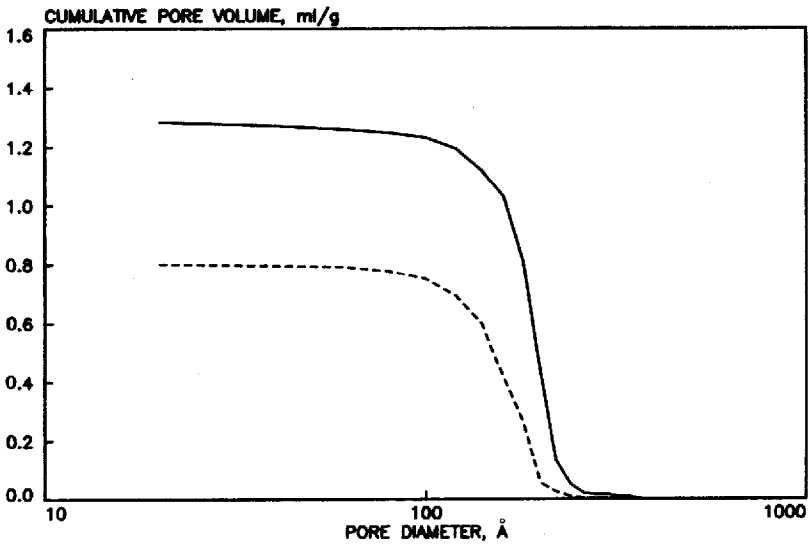

Fig. 8. Pore size distribution comparison between the unbonded (———) and C_{18}-bonded (– – –) 200-Å silica gel.

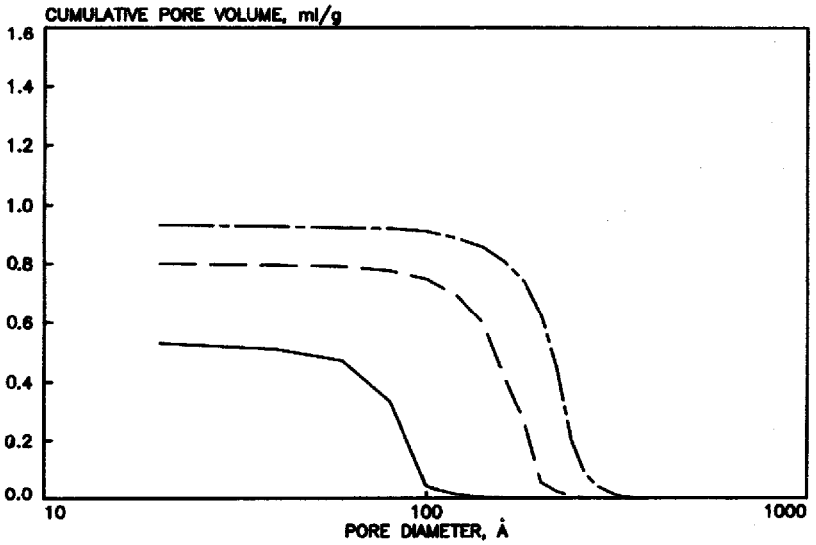

Fig. 9. Pore size distributions of the C_{18}-bonded silica gels. 100 Å (———), 200 Å (– – –), 250 Å (– — –).

TABLE VI

CHROMATOGRAPHIC PERFORMANCE WITH SMALL MOLECULES

Silica gel	*Molecule*	*k'*	*A/B*	*N/m*
100 Å	Phenol	0.98	1.1	8000
	Acetophenone	1.54	1.1	11300
	Benzene	2.24	1.0	12400
200 Å	Phenol	1.09	1.1	8100
	Acetophenone	1.46	1.0	9000
	Benzene	1.99	1.0	11600
250 Å	Phenol	1.04	1.5	5800
	Acetophenone	1.32	1.0	7900
	Benzene	1.72	1.1	9100

TABLE VII

CHROMATOGRAPHIC PERFORMANCE WITH SMALL MOLECULES

Silica gel	*Separated molecules*	α	R_s
100 Å	Phenol–acetophenone	1.57	3.12
	Acetophenone–benzene	1.45	3.22
200 Å	Phenol–acetophenone	1.34	1.89
	Acetophenone–benzene	1.36	2.56
250 Å	Phenol–acetophenone	1.27	1.35
	Acetophenone–benzene	1.30	1.87

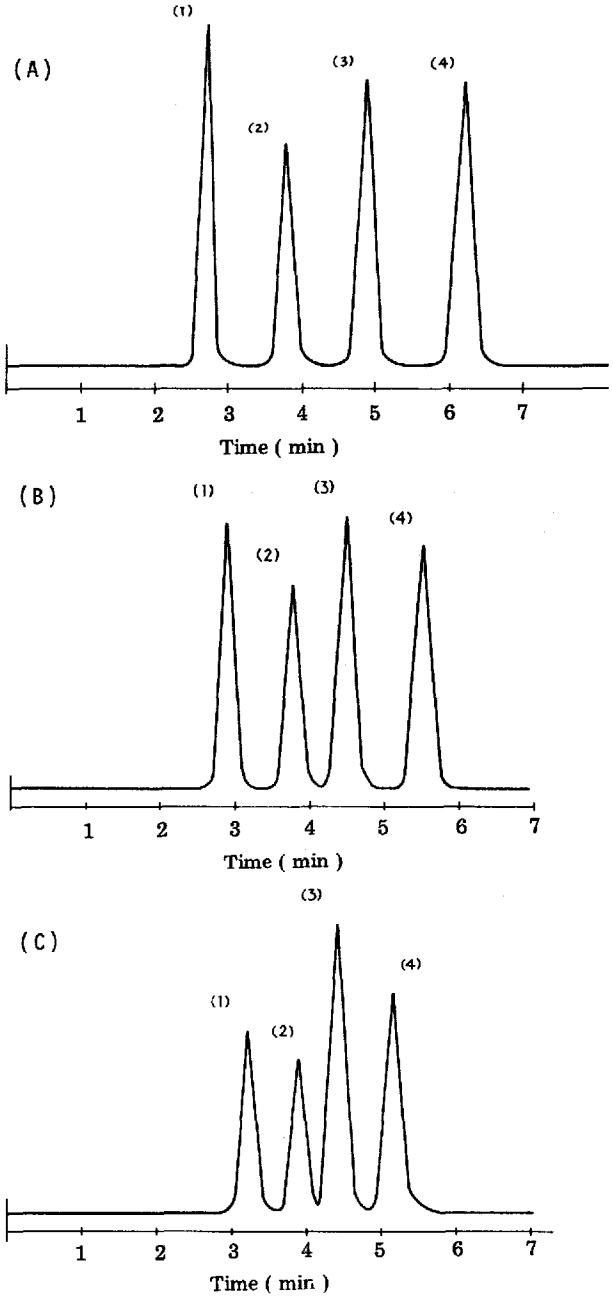

Fig. 10. Chromatograms of small molecules on silica gels A (100 Å), B (200 Å), C (250 Å). Elution order: 1 = uracil, 2 = phenol, 3 = acetophenone, 4 = benzene.

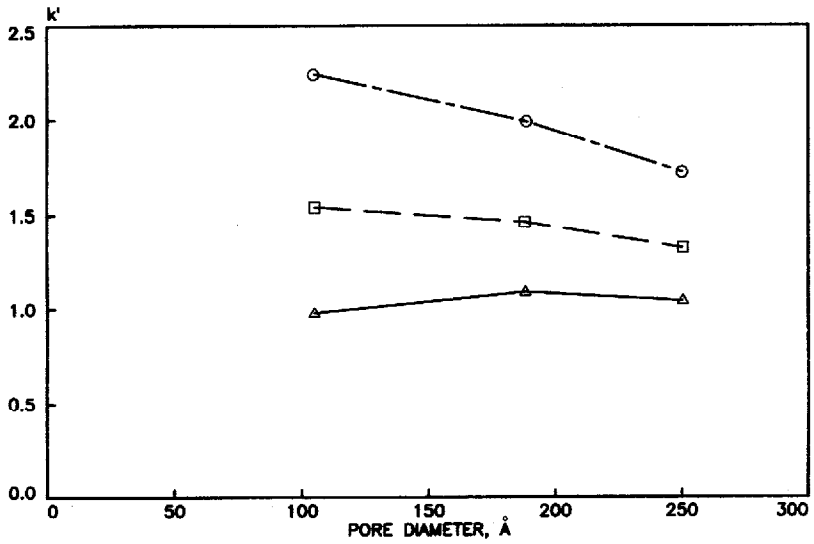

Fig. 11. Capacity factors, k', for phenol (△), acetophenone (□) and benzene (○) *vs.* the pore diameter of the unbonded silica gels.

Thus, it appears that small molecule separation and resolution are inversely proportional to D and $V_{p(cum)}$. The overall performance of a silica in separating small molecules increases to some extent with an increase in S_{BET} and decreases with an increase in D and $V_{p(cum)}$ of the bare silica and the residual D and $V_{p(cum)}$ of the bonded silica. The residual S_{BET} does not predict performance, however.

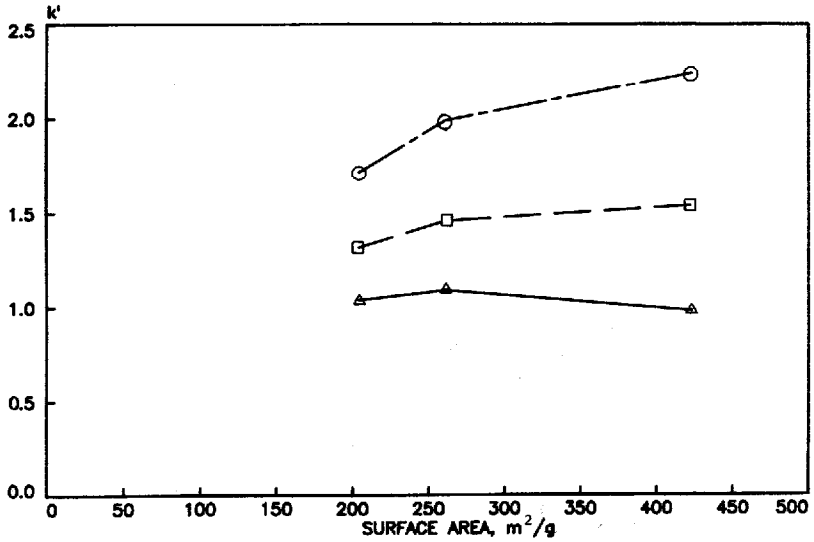

Fig. 12. Capacity factors, k', for phenol (△), acetophenone (□) and benzene (○) *vs.* the surface area of the unbonded silica gels.

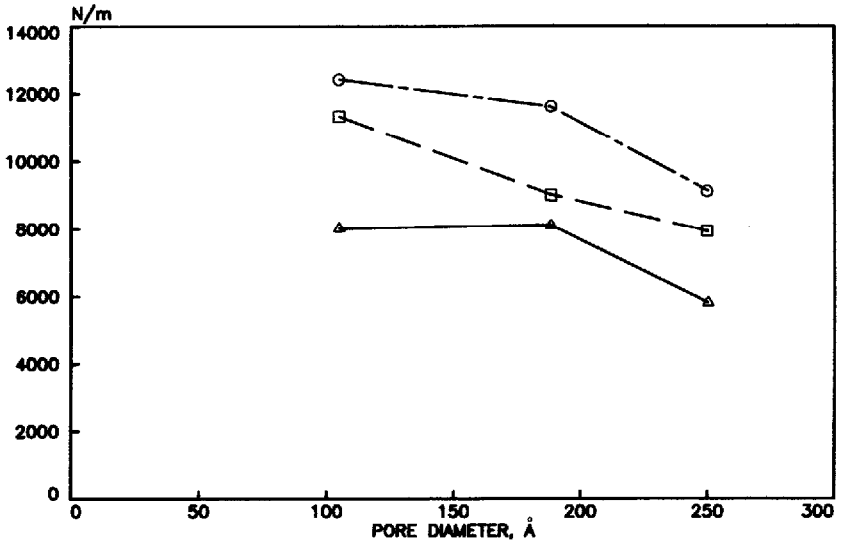

Fig. 13. Efficiency (plates/meter) for phenol (△), acetophenone (□) and benzene (○) *vs.* the pore diameter of the unbonded silica gels.

Chromatography of proteins (large molecules)

Insulin, ribonuclease, lysozyme, ovalbumin and serum albumin were separated on the C_{18}-bonded 100-, 200- and 250-Å silica gels (Fig. 15). The order of elution is the same on all silicas and seems to be in a somewhat general order of protein molecular weight. The calculated resolutions are given in Table VIII.

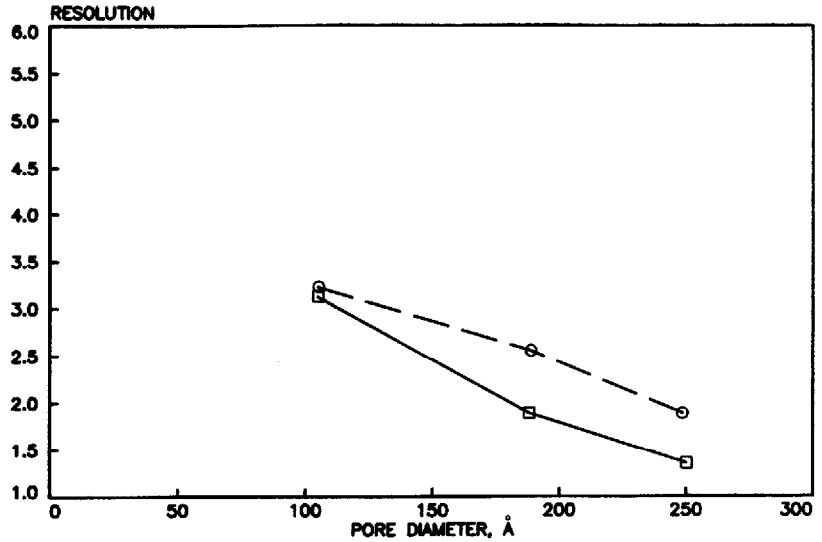

Fig. 14. Resolution of phenol–acetophenone (□) and acetophenone–benzene (○) *vs.* the pore diameter of the unbonded silica gels.

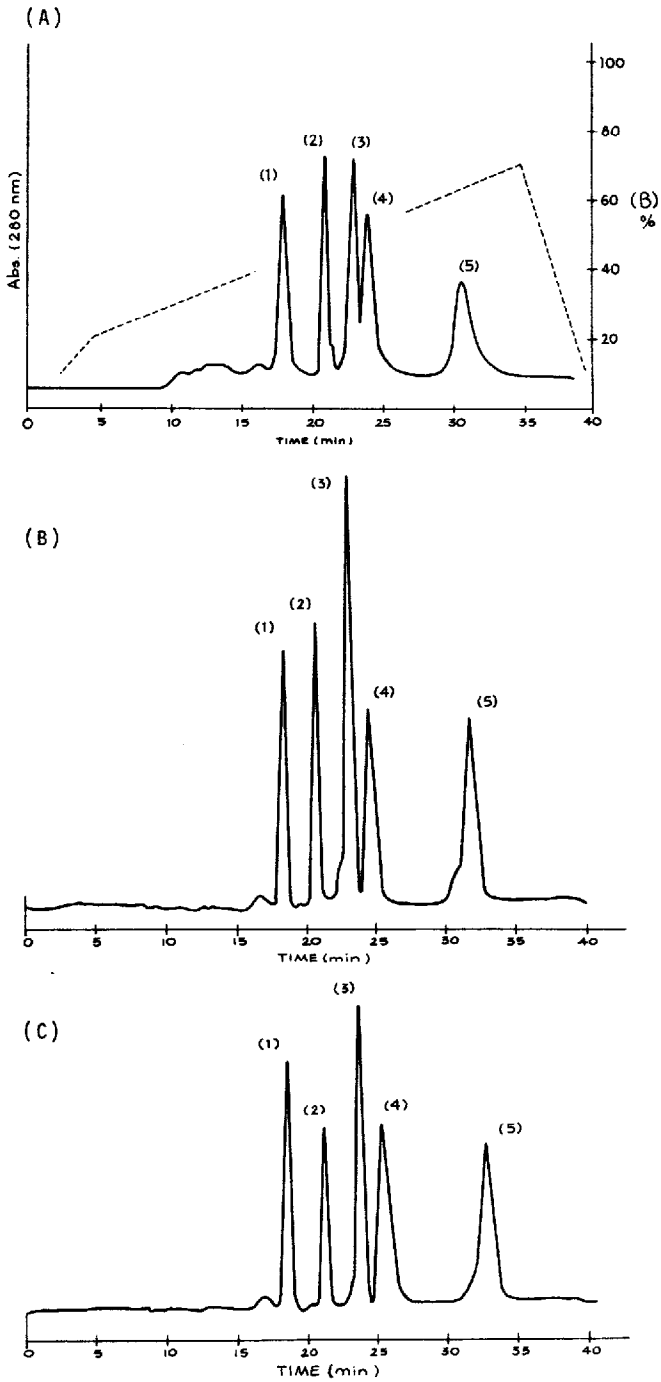

Fig. 15. Chromatograms of proteins on silica gels A (100 Å), B (200 Å), C (250 Å). Gradient shown for % B (acetonitrile). Elution order: 1 = ribonuclease, 2 = insulin, 3 = lysozyme, 4 = serum albumin, 5 = ovalbumin.

TABLE VIII

CHROMATOGRAPHIC PERFORMANCE WITH PROTEINS

Silica gel	*Separated proteins**	R_s
100 Å	R–I	3.28
	I–L	2.18
	S–O	3.23
200 Å	R–I	3.76
	I–L	3.60
	S–O	4.92
250 Å	R–I	3.70
	I–L	3.61
	S–O	5.39

* R = ribonuclease, I = insulin, L = lysozyme, S = serum albumin and O = ovalbumin.

One of the striking results is that for the same gradient, the same particle size and the same flow-rate, the retention times of the proteins (see Fig. 15) are practically identical for all three silicas. The differences in surface area, pore size and pore volume of the three silicas seem to have little or no effect on the retention.

The resolution of protein separation increases as D (Fig. 16) or $V_{p(cum)}$ increase. There is shown, however, to be a diminishing effect between 200 and 250 Å, consistent with the findings of Chang *et al.*[12]. The changes in resolution seem entirely due to an increase in efficiency. (Unfortunately, efficiency cannot be measured easily in gradient elution.)

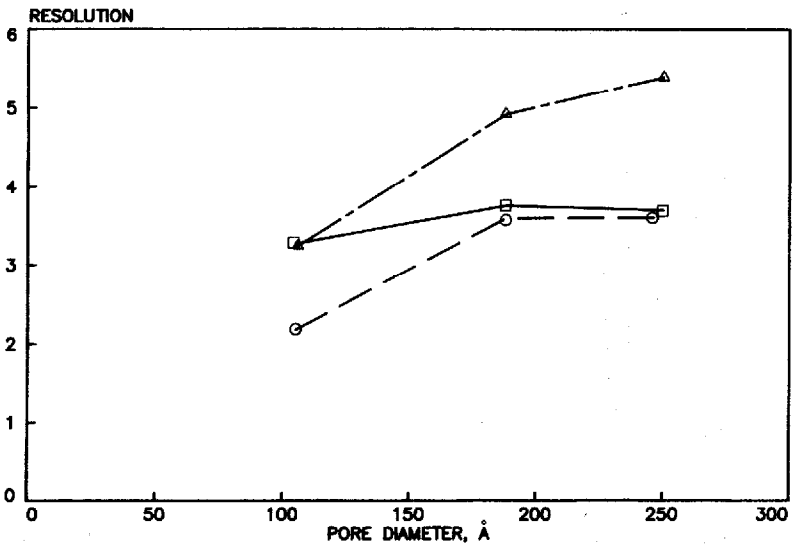

Fig. 16. Resolution of ribonuclease–insulin (□), insulin–lysozyme (○) and serum albumin–ovalbumin (△) *vs.* the pore diameter of the unbonded silica gel.

Thus, we have established that the separation of the proteins studied can be better performed on a silica with a large pore diameter and pore volume. This pore diameter is indicated to be between 200 and 250 Å.

CONCLUSIONS

(1) The surface concentration of a bonded silane increases as the mean pore diameter of the silica gel increases.

(2) Silylated silica gel has a reduced S_{BET}, $V_{p(cum)}$ and D as compared with the unbonded silica gel. The larger the D of the silica, the less is the effect of silylation. For instance, the 100-Å silica gel is reduced in S_{BET} by 58% and $V_{p(cum)}$ by 53% when bonded with $(C_{18}H_{37})(CH_3)_2SiCl$. The 250-Å silica gel, however, is reduced in S_{BET} by only 33% and $V_{p(cum)}$ by 32%.

(3) For any given D, the residual S_{BET} and $V_{p(cum)}$ decrease essentially linearly as the hydrocarbon chain length of the silane increases from 1 to 18.

(4) The overall HPLC performance of a C_{18}-bonded silica gel can be related to the median pore diameter of the unbonded silica gel. However, performance can also be related to $V_{p(cum)}$. The residual S_{BET} for all of the C_{18}-bonded silica gels is essentially the same value.

(5) For the separation of small molecules, k', N/m and R_s generally decrease with an increase in D. Thus, small molecules are better separated on small pore diameter silica gels.

(6) The proteins studied are separated better as the pore diameter of the silica gel increases, with a diminishing improvement as the pore diameter increased from 200 to 250 Å.

ACKNOWLEDGEMENTS

We wish to thank Virgil Swadley for the processing of the silica gels and Beverly Tait for her efforts in silylation. We wish also to thank Dr. Richard Hartwick of Rutgers University and Dr. Georges Guiochon of Georgetown University for their comments on this work.

REFERENCES

1 G. E. Berendsen, K. A. Pikaart and L. de Galan, *J. Liq. Chromatogr.*, 3 (1980) 1437.
2 L. C. Sander and S. A. Wise, in *Chemically Modified Surfaces*, Gordon and Breach, New York, in press.
3 K. K. Unger, *Porous Silica — Its Properties and Use as Support in Column Liquid Chromatography*, Elsevier, Amsterdam, New York, 1979.
4 J. D. Pearson, N. T. Lin and F. E. Regnier, *Anal. Biochem.*, 124 (1982) 217.
5 S. Brunauer, P. H. Emmett and E. Teller, *J. Am. Chem. Soc.*, 60 (1938) 309.
6 E. P. Barrett, L. G. Joyner and P. H. Halenda, *J. Am. Chem. Soc.*, 73 (1951) 373.
7 K. K. Unger, N. Becker and P. Roumeliotis, *J. Chromatogr.*, 125 (1976) 115.
8 G. E. Berendsen and L. de Galan, *J. Liq. Chromatogr.*, 1 (1978) 561.
9 J. P. Foley and J. G. Dorsey, *Anal. Chem.*, 55 (1983) 730.
10 P. Roumeliotis and K. K. Unger, *J. Chromatogr.*, 149 (1978) 211.
11 W. Cheng and M. McCown, *J. Chromatogr.*, 318 (1985) 173.
12 S. H. Chang, R. Noel and F. E. Regnier, *Anal. Chem.*, 48 (1976) 1839.
13 E. Pfannkoch, K. C. Lee and F. E. Regnier, *J. Chrom. Sci.*, 18 (1980) 430.